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FOREWORD

I am pleased to put into the hands of readers Volume-4; Issue-6: Nov-Dec 2019 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",** an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
Date: Jan, 2020

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Assessment of the local varieties of the fig (*Ficus carica L*) tree in Moukrisset area of Morocco

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***Abstract*— In Morocco, the cultivation of the fig tree has an ancestral character, the villagers of some production areas claim that its culture is very old and that the dried fruit traded with cereals, coming from Gharb. Its cultivation, which once covered a number of flat lands, is currently limited to hills, sloping land and housing. The aims of our study were to identify and characterize the richness of fig tree. For that the prospection of fig tree plants was carried out in Moukrisset region of morocco during the period from 2015 to 2016. A total of 300 accessions were sampled, studied and identified by used the biometric analyses. As a result of the study a total of 30 different fig varieties were identified. Several synonymies and homonymies were detected. Comparison of the ecotype shows the high significatif difference.*

***Keywords*— fig tree, genetic resources, biometric analysis, Moukrisset region, Morocco.**

I. INTRODUCTION

Fig is one of the oldest known fruit trees in the world. It's considered as sacred fruit in all sacred books and it played an important role in the mythology of many societies. Botanically identified as *Ficus carica* L., the common fig belongs to the family of *Moraceae*. The somatic chromosome number is 2n = 2x = 26 [1], [2]. Fresh and dried figs formed a large part of human food [3]. Figs may be processed or cooked in various ways. Commercial canning and drying of figs are actually industries of great importance.

In Morocco, the fig is considered a minor fruit species although it socioeconomic and historical importance. The regions in which the fig tree assumes economic importance are Taounate (22230 ha), Chefchaouen (7050 ha), Al Hoceima (5000 ha), Ouazzane (3150 ha), Tetouan (2000 ha) [4]. In Morocco, the production of fresh figs in 2018 growing season for fresh consumption was approximately 57000 tons with a total area of 46000 hectares (ha) of fig plantations [4]. Particularly, in rural area, fig production assumes economic importance mainly in Moukrisset area. This area is rich in figs with much diversified varieties, but with a lack of knowledge of caprification techniques. But a lot of problem hinders the maintenance of the fig tree growing in this region. In the area of Moukrisset where the shale dominates, land erosion is widespread. The steep slopes are overgrown with water throughout the winter season, resulting in mass movements of entire fields uprooting fruit trees. In addition, the introduction of the plum tree in the Zoumi and Moukrisset areas has driven the fig tree out of the main irrigated and fresh fields. This rapid extension of the plum tree shows that they have met climatic and edaphic conditions favorable to their development.

Evaluation of genetic variations within cultivated crop species is central to plant breeding strategies and genetic resource conservation [5]; [6]. Morphological and agronomic characters are useful in surveys of plant species diversity but these characters are highly influenced by environmental conditions. To overcome this, a large array of molecular markers is increasingly used to assess genetic polymorphism. Unfortunately, there has been little research dealing with the genetic diversity in fig germplasm [7], [8]; [9], [10]; [11]; [12]. In morocco, surveys done in different regions of Morocco contributed to identify and describe numerous cultivars [13]; [14]; [15]; [16].

In morocco, the fig tree plays a significant economic role in the diet of the population of the North region. However, the marketing of the product is little developed in our time and this after having had a much greater importance in the past. In order to analyze the constraints on the production and marketing of figs, important factors for the development of this sector, particularly the knowledge and characterization of local varieties, must be taken into account. In this context, a study identifying these different aspects of the subject was carried out in the Moukrisset region in the North West of Morocco.

II. MATERIAL AND METHODS

Prospection and sampling have been carried out at different localities in Moukrisset region in Northwestern of morocco (fig. 1). In total 300 accessions were gathered. In many cases, either isolated plants or plants located at old fig plantations areas were sampled (Table 1).

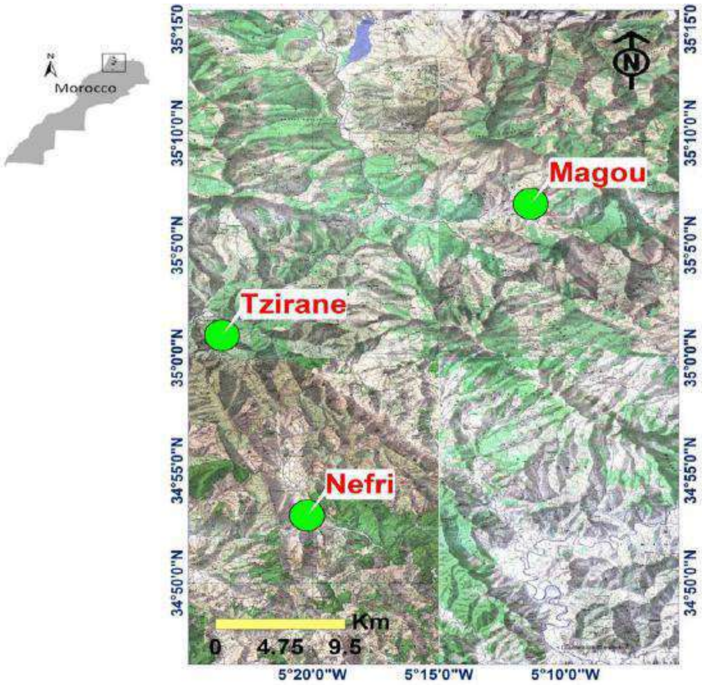

Fig. 1. Collection sites.

Table 1: Location of prospecting sites

Number of ecotype	Douar	Caîdat	Locatlity	Altitude	Geographic coordinate
100	Magou	Bab taza	Bab taza	789m	35°N - 06,847' 0,05°W - 11,377'
100	Tzirane	Moukrisset	Moukrisset	386m	35°N - 01,004' 0,5°W - 23,574'
100	Nefri	Zoumi	Zoumi	572m	34°N - 52,9971' 0,5°W - 20,213'

Plant material

Fig fruits from thirty cultivars (Lkourti, Silfafn Lqouti, Rhouddane, Lharchi lkhal, Aounq hmam, Zenfoukh, Maalmnous, Lqouti, Herich, Lmdar, Lgouhri, Hmir, Tabli, Lkhoumsi, kouzi, Lmdar lkhal, Hafr lbghal, Lmdar lebied, Kharar, Smouni, Sabaa wrkoud, Lkouhli, Lferzaoui, Hafr Lbral, Snani, Ournaksi, Achir, Lemti) were harvested from the three respective areas during cropping seasons 2015 and 2016. Cultivars were selected for their large distribution and their commercial value in the three regions. Samples of 300 homogenous fruits were chosen for each ecotype. Fruits were selected ripe and free from diseases.

Pomological characters

Biometric Approach

To examine the characteristics of the fruit for each ecotype, it was considered useful to approach a biometric study of the fruit based on the evaluation of the weight, caliber, dimensions and ostiole of the fruit. For each ecotype, a sample of twenty four fruits was randomly collected from different branches of the tree. Fruit weight was measured using a laboratory precision balance. Dimensions of the fruits such length, width, height and ostiole width were measured using a caliper (Fig. 2). The descriptors used were adapted list drawn up by European program GEN LMBO 029 [14].

General appearance of the fruit:

The general appearance of the fruit corresponds to its external form. In this aspect, we were interested in the shape and size of the fruit.

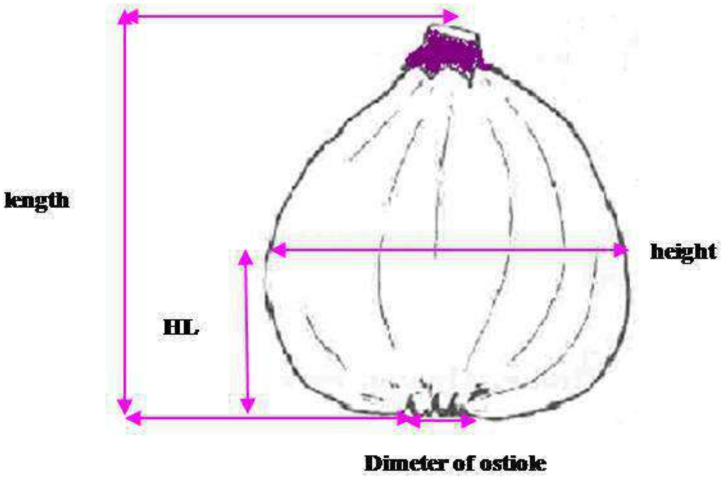

Fig 2: Representative diagram of measurements made on fig fruit

Fruit Shape

The fruits are of variable shape within the same tree and during the same season [15]. To avoid this hazard, we often rely on the presence or absence of neck. Other authors rely on three dimensions of the fruit to differentiate the varieties: the length C, the diameter D, and the distance A separating the base from the center of the circle of diameter D. The study of the shape of the fruit is important because it is related to the treatment that is applied to it. This is how the flattened shape with a short neck is ideal for canning. Other forms require certain precautions during transport; and others may facilitate the marketing of fresh fruits.

Statistical analysis

Comparison between the ecotype was made by statistical analysis of collected data. Statistical analyzes are performed with SPSS software version 21.

III. RESULTS AND DISCUSSION

300 accessions were identified in Moukrisset region. Many of the sampled accessions were collected either as unidentified or with local names. As result of the biometric approach the prospected plant material was characterized.

The average weight of the varieties studied varied from 13.7 g to 71.5 g (Table 2). The Zenfoukh variety shows the highest weight while the Kouzi variety shows the lowest weight. The analysis of variance showed fifteen homogeneous groups from the weight fruit (Table 2).

The average length of the fruit varied between 5.26 cm and 3.01 cm (Table 2), the variety Saidi showed the height value of the length of the fruit whereas the variety Kouzi showed the lowest value of the length of the fruit. We distinguished 16 groups significantly different from the Length of fruit (Table 2). The variety Smouni, Lgouhri, Maalmnous, Lemti, Kharar, Herich are not significantly different. The variety Kouzi, Silfaf, Hmir, Snani, Hafr lbral Rhouddane, Aounq hmam, Sabaawrkoud, Lkourit, Saidi each one formed one group significantly different to the other group (Table 2).

The width of the fruit varied between 2.98 cm and 5.73 cm (Table 2), the Gouzi variety shows the weaker value of width fruit while the variety Zenfoukh shows the greatest value of the width of the fruit (Fgi.5). The analysis of variance shows eighteen groups significantly different from the width of fruit. The varieties Lkouhli, Larchi, Lkhal, Lkhoumsi, Snani are significantly not different for the parameter Width of fruit (Table 2).

The value of HL varied between 1.53 cm and 5.15 cm (Table 2), the variety Lkourti shows the greatest value while the variety Gouzi shows the lowest value of HL. The analysis of variance shows twenty one groups significantly different from the parameter HL (Table 2).

The diameter of the ostiole varied between 0.310 cm and 1.40 cm (Table 2) the variety Lkouhli shows the smallest value of the diameter of the ostiole whereas the variety Lgouti shows the greatest value of the diameter of the ostiole. The analysis of variance shows that the variety Lkourti, Lharchi lkhal, Zenfoukh, Maalmnous, Lqouti, Herich, Lgouhri, Aounq hmam, Lkhoumsi, Lmdar lkhal, Smouni, Sabaawrkoud, Lferzaoui, Hadr lbral, Ournaksi are not significantly different (Table 2).

The length/ Width ratio of the fruit varies between 0.72 and 1.25 (Table 2), the greatest value of the length / width ratio of the fruit was observed in the Saidi variety, while the lowest value was observed in the variety Zenfoukh. The analysis of variance shows five groups significantly different. The variety Rhouddane and Saidi are significantly different from the ratio Length/width (Table 2).

The length / HL of the fruit varied between 1.37 and 2.75 (Table 2), it is found that the variety Lferzaoui showed the lowest value of the ratio length / HL while the variety Saidi showed the most great value. Table 2 shows fifteen groups significantly different (Table 2).

In these results, we find that the variety Kouzi (Fig 3) showed the greatest value of the weight of the fruit, the Length and the width of the fruit. However the Saidi variety (Fig 4) showed the greatest value of the Length, Length/Width and Length/HL, when the variety Lemti (Fig 5) show the high value of Diameter of ostiole.

Table 2: Biometric characteristics of fig cultivars harvested from the region

	Weight (g)	Length (cm)	Width (cm)	HI (cm)	Diameter of ostiole (cm)	Length/Width	Length / HL
LKourti	59,5 i	4,87ij	5,15 m	5,15 m	0,83 abcd	0,93 abcd	1,74 abcdefg
Silfaf	20,5 abc	3,2 ab	3,50 bcd	2,04 bcdef	0,414 ab	0,92 abcd	1,57 abcdef
L'Qouti	35,1 def	3,82 bcdefg	4,31 hijk	2,26 efghi	1,40 e	0,893 abcd	1,68 abcdefg
Rhouddane	26,9 bcd	4,40 fghi	3,57 bcde	2,31 fghij	0,342 ab	1,24 cd	1,92 defgh
Lharchi lkhal	25,7 abcd	3,76 bcdef	3,86 cdefgh	1,91 bcd	0,63 abcd	0,97 abcd	1,63 abcdefg
Aounq hmam	28,8 bcde	4,41 ghi	3,71 bcdef	2,03 bcdef	0,50 abc	1,19 bcd	2,19 h
Zenfoukh	71,5 j	4,15 defg	5,73 n	3,01 l	0,70 abcd	0,72 a	1,37 a
Maalmnous	37,02 defg	3,67 bcde	4,50 ijkl	2,19 bcdefghi	0,570 abcd	1,15 bcd	1,68 abcdefg
Lqouti	25,3 abcd	3,85 bcdefg	3,72 bcdef	1,97 bcdef	0,57 abcd	1,02 abcd	1,91 defgh
Herich	35,7 defg	3,7 bcde	4,50 ijkl	2,24 defghi	0,68 abcd	0,84 a	1,66 abcdefg
Lmdar	26,7 bcd	3,4 abc	3,98 defghi	2,13 bcdefgh	0,425 ab	0,86 a	1,62 abcdefg
L'gouhri	31,08 bcdef	3,63 bcde	4,03 efghi	2,01 bcdef	0,64 abcd	,895 abcd	1,71 abcdefg
Hmir	34,2 def	3,5 abcd	4,43 ijkl	2,25 defghi	0,98 cde	0,82 a	1,57 abcdef
Tabli	42,4 fg	3,91 cefg	4,58 jkl	2,51 ijk	0,52 abcd	0,848 a	1,55 abcd
Lkhoumsi	26,6 bcd	3,9 cefg	3,90 cdefgh	2,06 bcdefg	0,51 abcd	1,01 abcd	1,80 bcdefgh
Kouzi	13,7 a	3,01 a	2,98 a	1,53 a	0,340 ab	1,01 abcd	1,97 fgh
Lmdar lkhal	40,1 efg	4,05 cefg	4,50 jkl	2,60 jk	0,70 abcd	,90 abcd	1,56 abcde
Hafr lbghal	32,4 cdef	4,13 defg	4,06 efghi	2,23 cdefghi	0,86 bcd	1,01 abcd	1,86 bcdefgh
Lmdar lbeid	47,7 h	3,9 cefg	4,70 klm	2,70 k	1,05 de	0,84 a	1,46 ab
Kharar	19,3 ab	3,7 bcde	3,40 abc	1,89 bc	0,350 ab	1,09 abcd	1,96 defgh
Smouni	42,9 fg	3,61 bcde	4,84 lmn	2,42 hijk	0,68 abcd	0,74 a	1,492 abc
Sabaa wrkoud	60,3 i	4,76 hij	4,75 klm	2,38 ghijk	0,70 abcd	1,01 abcd	1,62 abcdefg
Lkouhli	25,6 abcd	3,78 bcdefg	3,84 cdefgh	2,23 cdefghi	0,310 a	,97 abcd	1,67 abcdefg
LFrzaoui	47,1 h	3,4 abc	4,78 klm	2,52 ijk	0,58 abcd	0,722 a	1,37 a
Hafr lbral	60,07 i	4,23 efgh	5,11 m	2,52 ijk	0,61 abcd	0,82 a	1,67 abcdefg
Snani	28,3 bcde	4,2 defgh	3,90 cdefgh	2,08 bcdefg	0,460 abc	1,08 abcd	2,02 gh
Saidi	34,4 def	5,26 j	4,26 ghijk	1,93 bcde	0,490 abc	1,25 d	2,75 i
Ournaksi	25,9 abcd	3,84 bcdefg	3,77 bcdefg	1,95 bcde	0,67 abcd	1,02 abcd	1,96 defgh
Achir	18,7 ab	3,78 bcdefg	3,26 ab	1,86 b	0,490 abc	1,15 bcd	1,87 cdefgh
Lmeti	19,7 ab	3,6 bcde	3,31 ab	1,88 b	0,370 ab	1,09 abcd	1,97 efgh
Pα = 0.05	*0*	*0*	*0*	*0*	*0*	*0*	*0*

Significant differences within the same column and means followed by the same letter do not differ at Pα ≤0.05 according to Duncan test.

Fig 3. Variety Kouzi from Moukrisset Region

Fig 4: Variety Saidi from Mokrisset region

Fig 5: variety Lemti from Mokrisset region

IV. CONCLUSIONS

The results of this study give us knowledge of diversity and pomological characteristics of the fig tree in Moukrisset region of morocco. The fig tree in this zone does not show good performances. The size of the trees is very small, with less dense foliage. The yield is quite low. The skin of the fruit is thick and resistant. Fresh consumption of most varieties seems the most interesting. The fruit resists transport thanks to its firmness and the resistance of its skin. 30 local varieties were listed in this study and showed high biometric characteristic. The richness of fig tree in this region was very important and we suggest the program for the conservation of these resources genetic.

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Effect of different Gamma radiation doses on the growing of the Achmrar local fig variety *Ficus carica L.* in Morocco

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Abstract**—***This research was carried out in order to determine GR_{50} dose which reduces the growing of 50% as compared to control and give rises to mutation at the fig tree which were irradiated with five different Gamma doses (20, 40, 60, 80, and 100 Gy). The bud cutting irradiated with different doses were prepared and then rooted in greenhouse for three months. Results showed that chlorophyll leaf content decreased with increasing radiation doses, 44, 60 mg.g^{-1} to 24,53 mg.g^{-1} for control and 100 Gy doses respectively. Is was also found that average leaf area and stomatal conductance values deceased compared to the control its varied for the leaf area 65,75 cm^2 for control to 23,95 for the 100 Gy doses, and for stomatal conductance to 557,68 mmol/m^{2}s control to 75 mmol/m^{2}s for 100 Gy doses. As a result, GR 50, dose was found as 62 Gy for fig variety.*

Keywords**— *fig variety, Gamma radiation, GR $_{50}$ dose, chlorophyll, stomatal conductance.***

I. INTRODUCTION

The common fig is an emblematic and characteristic fruit species of the Mediteranean region. Is domestication may have been cotemporary to cereal crops (Poaceae) suggesting very early cultivation (9000 to 12,000 BCE) (Kislev et al., 2006). Fig diversity studies using morphological and molecular markers show that Morocco contains a great genetic diversity of the fig tree with geographic specificities (Oukabli et al., 2003; El oualkadi, 2004; Ater & al., 2008; Achtak et al., 2010). This fruit crop is widespread in the Mediterranean basin countries since it is well adapted either to different soils or climates (Mars, 2003). The fig tree has not been subjected to intensive breeding programs; therefore fig tree populations exhibit a rich genetic biodiversity. Fig propagation is usually carried out by stem-cuttings and this procedure has contributed to synonymy and homonymy, because missing identification and somaclonal variations are common events in sexually propagated species such as fig (do Val et al., 2013). In Morocco, the fig tree is the species where the greatest number of local varieties was listed (Tayou, 1985; El Oualkadi, 2004; Hmimsa, 2004). According to 2018 statistical data, 57.000 tons of fresh fig were produced in 46.000 hectares (ha) of fig plantations in Morocco (DPA Al Hoceima, 2016). Al Hoceima region, located at northwest part of Morocco, was classified in third rank in terms of national fig production on after Taounate-Chefchaouen and Tetouan region, with a surface of 4520 ha and a production of 2800 tons of figs (DPA Al Hoceima, 2016). Achmrar fig variety is a variety wich is of great importance in the annual production of the fresh fig varieties in the Al Hoceima region. It's considered as very old variety, the age of these trees date more than 80 years (DPA Al Hoceima, 2016). The most profitable production is obtained from the table fig varieties in the late growing season of the province. Limited number of fig varieties having some characteristics such as seedless, last season production and resistant to conservation and transportation restricts the profitability of fig culture in both the province and whole country. Therefore, it is thought that development of new fig variety using different breeding methods will contribute to national economy. The improvement of the varieties of crop plants is fundamental for the agricultural production and the horticulture. The use of model species, such as Arabidopsis (Phillips & al, 2008), is also leading to the more rapid development of new mutagenesis techniques. Batista, & al. (2008) reports that Gamma-ray mutagenesis in rice induces extensive transcriptome changes. Gamma and X rays were used to improve different characteristics of plants and to obtain new varieties and species (increasing productivity and quality, resistance to disease and pests and extreme conditions etc.) (Miah & al. 1966; Gokçora, 1973; Demir, 1980). Many researchers have made many studies using different mutagens (physical or chemical) on different plant (Donin, 1978; Yalçin, 1992; Aufhammer & al., 2000; Klu and Haarlent, 2000; Tayyar & al., 2003; El Oualkadi & al., 2018). In other study, it was found that increasing radiation dose on M1 generation decreases plant height, and plant numbers alive (Gaul, 1977). Other studies concerning this subject show that the radiation treatments are feasible to be applied on different parts of the plant such as tubers, cutting, seeds and stems (Gaul, 1977; Çoban, 1998). This research was carried out to determine the effects of different Gamma radiation doses on the cutting of Achmrar fig variety and to find out the GR 50 dose. Different doses were applied to bud cutting of Achmrar fig variety.

II. MATERIEL AND METHODS

This research was carried out at the National Institute of Agronomic Research of Tangier (INRA), Morocco. Achmrar fig variety was used as experimental crop in the study. Five lots of 20 buds cutting were irradiated separately by one of the following doses of radiation: 20 Grays, 40 Grays, 60 Grays, 80 Grays and 100 Grays. The control cutting was untreated.

A total of 120 cutting of Achmrar fig variety were cut in December 2018 and stored at 5°C for 2 months (Gerhardt and Cheng- Yung Cheng Schneider, 1971). All cutting were sown under a greenhouse in plastic bags and arrangers in a randomized plot experimental design with four replications. The cutting kept in a greenhouse for 3 months were observed weekly since the date of planting. After irradiation by Gamma radiation source, cutting was planted in each pot.

Necessary data were recorded on the growth, morphological and biochemical parameters. Total leaf area of the plant was measured with automatic electronic leaf area meter (model LI-3000, USA) and with imageJ software. Chlorophyll content of leaf was estimated by manual SPAD-502 Plus. The stomatic conductance was measured with Leaf Porometer. (Ap4; Delta-T, Cambridge, UK). The statistical analysis of variance was carried out by software SPSS ® version 21.0. The mean difference of the studies parameters among the treatments was adjusted by Ducan's Multiple Range Test (Gomez et al, 1984).

III. RESULTS AND DISCUSSION

The cumulative percentage of bud (CPB) followed a logarithmic progression for both control and irradiated buds (fig 1). The control showed a CPB (100%) higher than the irradiated buds. Irradiated buds showed decreasing CPB based on increasing doses of radiation. The lower CPB were noted for the high radiation doses; 55% for the radiation 100 Gy, 60 and 65% for the dose of 80 and 60 Gy respectively, 70 and 90% for the dose of 40 and 20 Gy applied on buds on Achmrar fig variety.

The correlation between radiation dose and cumulative percent of bud was r= -0.92***. The equation of related regression based on radiation dose is Y=77,72-0,42X (Fig. 2). Based on the minimum level of 50% of cuttings surviving compared to control, lead us to locate the optima radiation dose between 60 and 65 Grays for fig cuttings. The yield of cuttings surviving of these treatments was satisfactory since the rate of survival was ranged from 60 to 100%.

As a result, GR 50, dose for the application of mutation breeding of Achmrar fig variety was determined as 62 Gy. El oualkadi & al. (2018); Ponnusswani & al. (1992) found GR50 dose to be 20-25 Gy on woody cutting of grape variety.

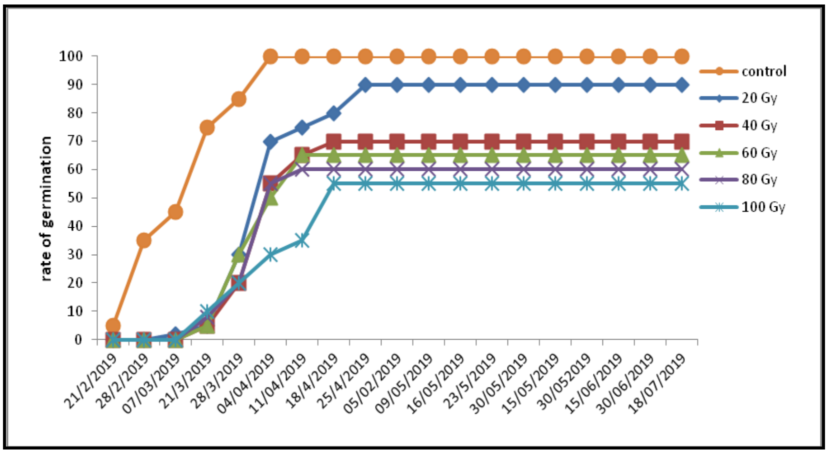

Fig 1. Cumulative rate of growing of Achmrar fig variety and irradiated with increased doses (20-40-60-80 and 100 Grays)

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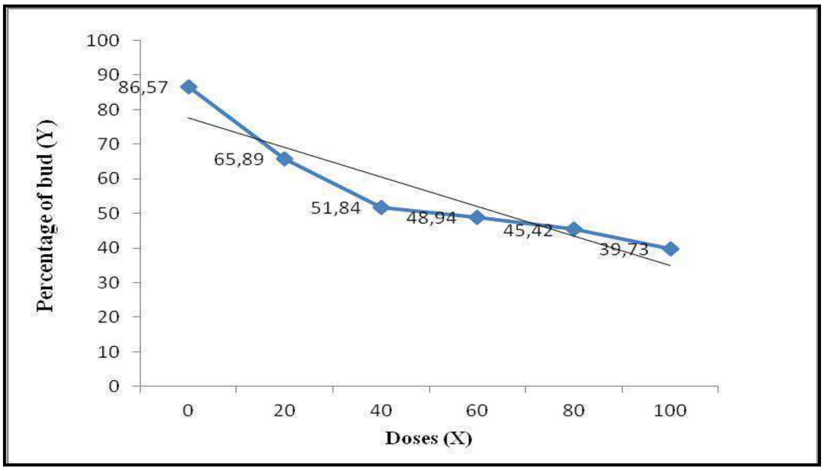

Fig.2: Average of the percentage of growing of the cuttings of the fig variety

Effects of five different Gamma radiation dose applied to the bud cutting of the achmrar fig variety on the average chlorophyll leaf, average leaf area and average stomatal conductance, are shown in table 1.

Table 1: Effects of different Gamma radiation doses on the bud cutting fig variety

Doses (Gy)	Chlorophyl of leaf (mg.g-^{1})	Leaf area (cm^2)	Stomatal conductance ($mmol/m^2s$)
Control	44,60 *b*	65,75 *c*	557,68 *d*
20	40,76 *ab*	59,26 *bc*	394,30 *cd*
40	34,72 *ab*	56,22 *abc*	307,63 *bc*
60	28,76 *a*	41,26 *abc*	108,63 *ab*
80	26,90 *a*	26,74 *ab*	96,63 *ab*
100	24,53 *a*	23,95 *a*	75,00 *a*
Pα =0.05	*0,002*	*0,025*	*<0,000*

**Significant differences within the same column and means followed by the same letter do not differ at Pα ≤0.05 according to Duncan test.*

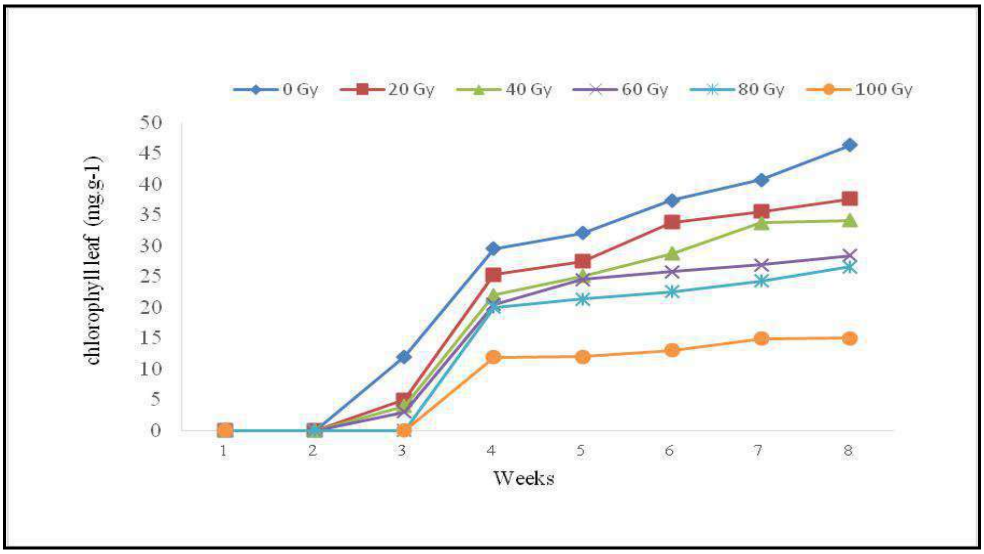

Fig 3: Rate of chlorophyll leaf of fig variety between 1st and 8 weeks

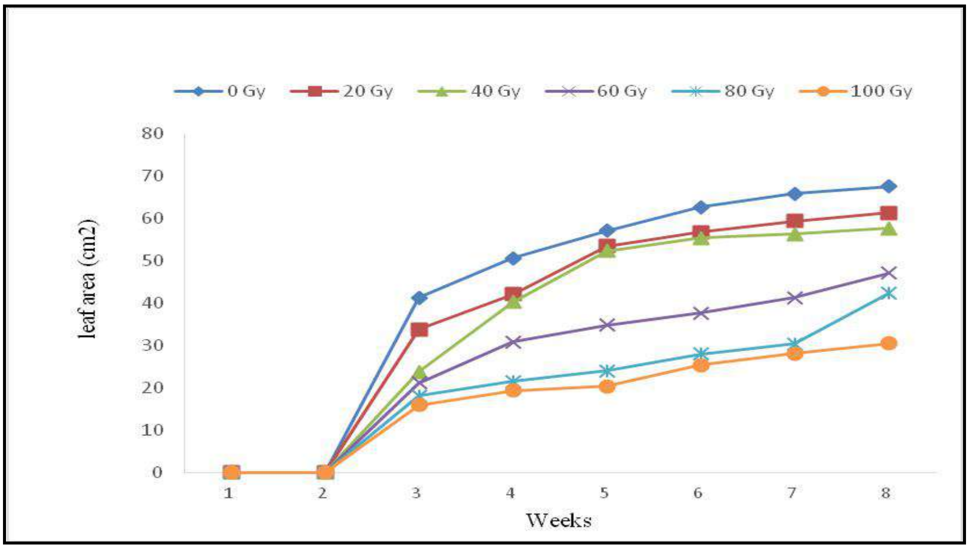

Fig 4 : Rate of leaf area of fig variety between 1 st and 8 weeks

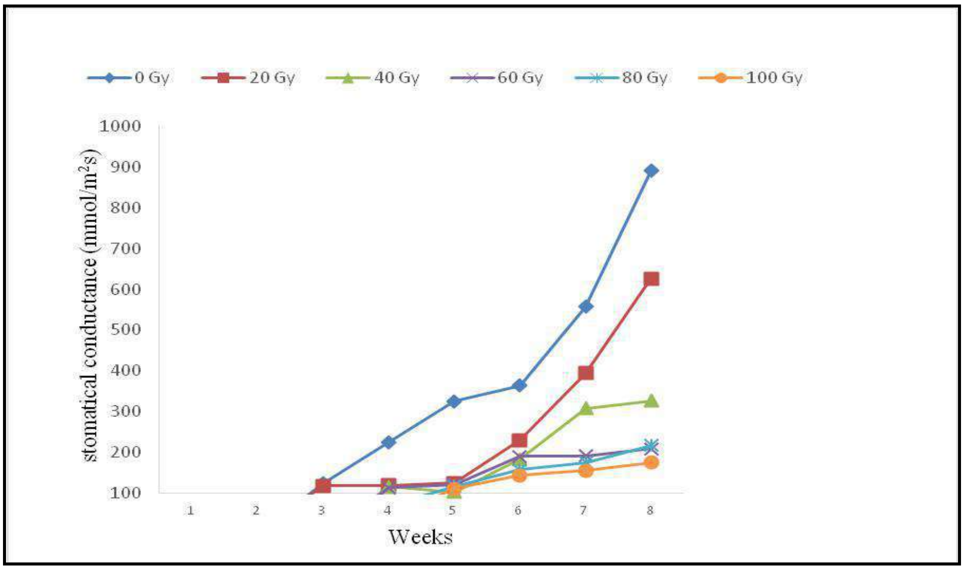

Fig 5: Rate of stomatical conductance (mmol m-^{2} s-1) of fig variety between 1st and 8 weeks

Effects of various Gamma radiation doses applied to Achmrar variety on the chlorophyll leaf, leaf area and stomatal conductance at the end of 6 weeks later are presented in Fig. 3; 4 and Fig 5.

Doses of 80 and 100 Gy Gamma radiation dose decreased the chlorophyll leaf, leaf area and stomatal conductance of the bud cutting significantly. All of the radiation dose increased chlorophyll leaf, leaf area and stomatal conductance period of the bud cutting compared to control (Fig.3, 4 and 5).

The effects of the cutting of Achmrar fig variety irradiated with different Gamma radiation on the bud cutting were determined. The control recorded a chlorophyll leaf content of 44 mg.g-1, while chlorophyll leaf at the 20, 40, 60, 80 and 100 Gy radiation dose decreased to 40.76, 34.72, 28.76, 26.90 and 24.53 mg.g-1, respectively. Depending on increasing radiation dose, leaf area values also decreased compared to control 65.75 cm2 and found to be 59.26, 56.22, 41.26, 26.74 and 13.95, respectively (Table 1).

Because of increasing radiation dose, the stomatal conductance in bud cutting of Achmrar fig variety decreased as well. The average stomatal conductance of control was 555.68 mmol/m2s, whereas it was 394.30, 307.63, 108.63, 95.63 and 75 mmol/m2s at 20,40, 60, 80 and 100 Gy, respectively (table 1). Leaf morphology and area are the main characters which are directly influenced by any mutagenic treatment. Lower doses were found to be stimulating on processes like nutrient uptake and photosynthetic activities (Al-Qudat, 1990; Antonov, 1985). Appearance of more number of smaller leaves with reduction in leaf area is also common (Sheppard and

Evenden. 1986). This effect may also cause compactness resulting in dwarf plants (Shin et al., 1988).

According to statistical analyses, various radiation dose treatments applied on Achmrar fig variety were highly significant for all the parameters as seen in Table 1 (P< 0,01).The results obtained from this research were in accordance with a study indicating that shooting delayed with increasing radiation dose in a round seedless grape variety (Çoban, 1988). We aslo found that shooting delayed with increasing radiation doses affected the development of shooting of the scions at different levels (Tayyar, 2003). These results were also in line with other various studies (Enset and Pratt, 1975; Donini, 1978; Donini, 1993; Hadju et al., 1995; Korosi et al, 1995; Lima de Silva and Daozon, 1995).

IV. CONCLUSION

It was evident from the results that different Gamma radiation doses applied had significant effects on the vegetative development parameters of the bud cutting. Increased Gamma radiation treatments resulted in decrease on vegetative development of bud cutting. It was determined that 62Gy Gamma radiation doses are suitable for Achmrar fig variety whereas 80 and 100 Gy radiation dose have many negative effects on the plants. These results give some information in determining appropriate dose levels on mutations breeding studies in other fig varieties.

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Application of ampelographic parameters to differentiate native *Vitis vinifera* L. cultivars

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***Abstract*— This study was conducted in three vegetation successive periods (2015, 2016) to determine ampelographic characteristics of the 39 grapevine accessions prospected in the Northwestern of Morocco by ampelographic criteria using OIV descriptors. The data were processed by multivariate statistical procedures. The integration of the obtained data with ampelographic data would be very important for the accurate identification of the Moroccan cultivars and can become a significant tool for the certification of quality grapevine produced in specific region.*
***Keywords*— Ampelography, Vitis vinifera, Morocco, OIV descriptors.**

I. INTRODUCTION

The viticulture domain has always received a significant interest and grapes are extensively used worldwide for fruit and wine production. [1] Estimated the existence of about 14,000 cultivars, with numerous synonyms and occasional use of the same or similar names for genetically different cultivars. In the last decades it has become imperative to handle the large germplasm of grapevine as well, and to properly identify the different cultivars. Traditionally, morphological and agronomical characteristics have been the main criteria for differentiating grapevine cultivars. Ampelography is the first step in grapevine selection, in establishing the relationship within and among grape cultivars and in solving different classification problems [2]. In grapevine collections an average of 5–10% of cultivars are misnamed, and even in commercial viticulture misnaming and confusions related to synonyms and homonyms do exist [3].The morphological descriptors may be easy to examine and therefore they are useful for the laboratory and field research work [4]. The morphometric characterization of *Vitis* varieties is carried out by the measurement of parameters in leaves, grapes and separated grapes. The measurement eliminates subjective evaluation of data. Systems to directly digitize phylometric parameters from leaves have been developed [5], [6].

Grapevine varieties have been characterized for identification purposes by ampelographic characters [7], [8], [9]. Study of plant morphology, mainly leaves, buds, and grape morphology (also called ampelography) it is the last means of detecting vine cultivars [10]. This method is still used for identification [11], [12], [13], particularly during the collection of data plants in situ.

In Morocco, the grapevine as well as the olive tree, the fig tree and the cereals are cultures well adapted to the natural climatic conditions of our country located at the end of the western Mediterranean. Before, the native grape varieties occupied areas whose extent or relative importance could not be assessed, mainly mountain vineyards [14]. However, during the last century, several factors have led to a progressive extinction for many local grape varieties and consequently to their genetic erosion. Currently, these varieties have become rare, little known, not inventoried, unexplored and threatened with extinction. Little study in Maghreb was carried to describe the cultivars of grapevine [14], [15], [16], [17].

As the identification of grapevine varieties by the use of classical ampelographic methods (morphological and morphometric characters) are sometimes afflicted by mis interpretations due to environmental influence [18], the integration of our data with ampelographic data would be of great importance to unambiguously identify the existing Moroccan cultivars and could also be used for legal protection of cultivars.

Our goal in this study was to complete the characterization of Moroccan grapevine prospected in north-west region using the ampelographic descriptors to confirm the existing synonymies and possible relationships among the plant materials in order to preserve the maximum amount of genetic variability for breeding and commercial purposes.

II. MATERIAL AND METHODS

The plant materials consisted of samples from the prospection sites located at the north-west of the country, all of them were classified as minor or endangered

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varieties. The total number of accessions studied was 39 and 10 grapes for each tree (Table 1). Ampelographic characters were described using OIV descriptors [19]. Sampling was done at the time of fruiting. In each site surveyed and with the help of the farmers we collected for each variety named and recognized samples of fruits. Principal Component Analysis (A.C.P) was done using SPSS Version 10 software.

Table 1. List of varieties studied

Feryal Khal1	Aferyal Byad1	Maticha Mferqa	Fekas khal
Feryal khal2	Aferyal Byad2	Maticha Mjemaa	Fekas Byad
Taferyalt kahla3	Taferyalt Byad3	Mouska	Fekas
Taferyalt Kahla4	Taferyalt Byad4	Mouska Bayda	Ineb Byad1
Taferyalt Kahla5	DiBI 1	Mouska hamra1	Ineb Byad2
Taferyalt Kahla6	Dibi 2	Mouska hamra2	Bezoul awda 1
Taferyalt kahla7	Echabel(Dibi)	Boukhanzir1	Bezoul awda 2
Taferyalt kahla8	Dibani 1	Boukanzir2	Rjiyil Dib 1
Sbiyae Bnat	Dibani 2	Boukhanzir3	Rjiyil Dib2
Ineb Nhal	Sanso	Zbarjel	

III. RESULTAT AND DISCUSSION

Average quantitative parameters of grapes

For each tree, we measured the following variables: weight, length, width, peduncle length and number of wings for each grape (Table.1). The results obtained show important variations between the grapes of the different sampled varieties. For example, the Dibi 1 tree showed the highest average weight with 872g per grape. On the other hand, the Maticha mferqa tree has on the contrary mounted grape with the lowest average weight with 71g. Also, the highest average grape length was observed in the Dibani 1 (30 cm) tree while the lowest average value was observed in the Taferyalt kahla 3 (6.5 cm) tree. Regarding the average width of the grapes, we noticed that the greatest average width of the grape was observed in the Ineb nhal tree (16 cm), while the lowest average value was observed in the Fakkas tree (4 cm). The average Length/Width ratio shows more or less homogeneous values in most grapes, the highest Length/Width ratio was observed in the Fekas tree (2.75), while the lowest Length/Width ratio was found in the Boukhanzir tree 3 (1.2). The average number of wings ranges from 29 in the Bezoul aouda tree 2 to 4 in the Taferyalt kahla 3 tree (Table 1).

Table.1. quantitative parameters of grapes

Variety	Total weight (grape)	Length (cm)	Width (cm)	long/width	Peduncle length	Number of wings
Feryal Khal1	435	28	17	1.64	3.5	18
Feryal khal2	129	17	10	1.70	3	16
Taferyalt kahla3	21	6.5	4.5	1.44	1.5	4
Taferyalt Kahla4	43	10.5	8	1.31	1.8	11
Taferyalt Kahla5	151	18	11	1.63	3.4	13
Taferyalt Kahla6	283	19	11.5	1.65	2	20
Taferyalt kahla7	333	21	11	1.90	2	21
Taferyalt kahla8	296	20	12	1.66	2	15
Aferyal Byad1	551	27	14	1.92	3.5	20
Aferyal Byad2	463	23.5	14	1.67	3.5	26
Taferyalt Byad3	74	17	11.5	1.47	4	12
taferyalt Byad4	17	8	5.5	1.45	4	5
DiBI 1	872	25	17	1.47	3.2	24
Dibi 2	400	30	17	1.76	4	27
Echabel(Dibi)	348	24.5	13.5	1.81	5.5	17
Dibani 1	359	30	14	2.14	4.5	23
Dibani 2	139	15	11	1.36	2	16
Maticha Mferqa	71	15.5	8.5	1.82	2.7	14
Maticha Mjemaa	138	14.5	9	1.61	3.4	19

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Mouska	358	19	12	1.58	2.5	16
Mouska Bayda	184	18.5	11	1.68	2.6	11
Mouska hamra1	20	15	7	2.14	2.2	16
Mouska hamra2	142	18.5	9	2.05	2.5	15
Ineb Nhal	263	26	15.5	1.67	2.8	24
Fekas khal	445	26	16	1.62	4.5	20
Fekas Byad	222	14.5	7.5	1.93	3.2	23
Fekas	10	11	4	2.75	2	10
Ineb Byad1	367	25	17	1.47	3.2	16
Ineb Byad2	157	20	8.5	2.35	2.3	14
Bezoul awda 1	43	14	5.5	2.54	1.5	14
Bezoul awda 2	239	15	8.5	1.76	3.2	29
Boukhanzir1	352	21	8.5	2.47	3.4	20
Boukanzir2	145	19	11	1.72	3.7	18
Boukhanzir3	163	13.5	11.2	1.20	2.2	9
Sbiyae Bnat	93	10	6.5	1.53	3.5	10
Rjiyil Dib 1	277	20	12.5	1.6	2.2	16
Rjiyil Dib2	311	18.5	10	1.85	3.4	18
Zbarjel	90	11.5	6	1.91	2.5	12
Sanso	251.28	19.44	11.01	1.76	3.047	17.605

Principal component analysis

We used the means of the quantitative parameters of the grapes to perform a principal component analysis. Table 2 shows the percentages of the variance associated with each axis.

Table 2: Variances associated with the axes of the ACP of the characters of the grape

Total variance explained			
component	Total	% de la variance	% cumulative
1	4.829	40.242	40.242
2	1.944	16.198	56.440
3	1.653	13.775	70.214
4	1.033	8.605	78.820

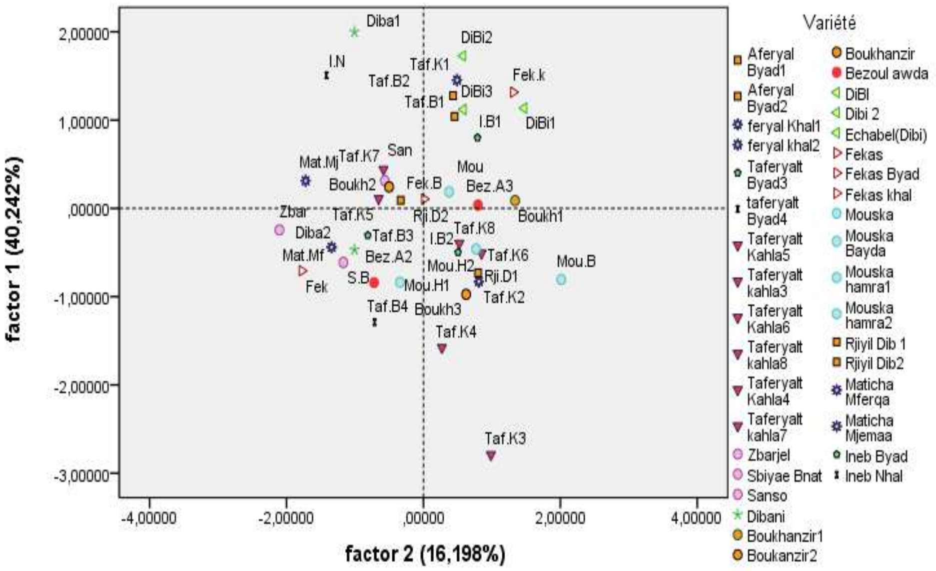

Fig 1. Projection in the plane (1,2) of the principal component analysis

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From the result obtained in Figure 1 we see a compact structure of all trees, no structuring is visible. To better see the structure we eliminated the 12 trees of the "variety" Taferyalt and redo the analysis with only the other varieties. Figure 2 shows the result obtained.

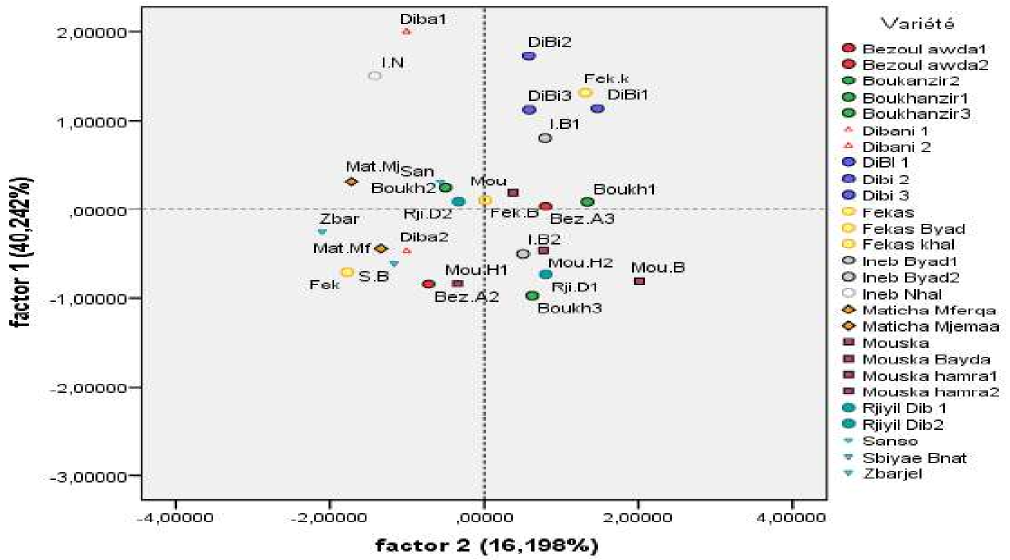

Fig. 2: Projection in the plan (1,2) of the principal component analysis (without Taferyalt cultivar)

In axis 1, the representation shows that the weight variables, the length, the width of the bays are well correlated in the positive direction. Grape parameters: weight, length, width, number of wings and length of peduncle are moderately correlated with axis 1. In the negative sense, the length-on-cluster variable is well correlated with the axis 1. From the analysis of Figure 2, we notice a variability resulting from the remarkable dispersion of the feet studied at the plane formed by the two axes (1 and 2). Axis 1 encompassing 40.24%, of the total information, is defined positively and mainly by the lengths, the widths, the weights and by the numbers of the wings. Therefore, axis 1 opposes the three feet of the variety Dibi, Fekas khal, and Ineb byad 1 in the positive side. As for axis 2, which represent 16.19% of information. It is negatively defined by the sugar content encompassing the feet Maticha mjamaa and mfera, Dibani, Rjiyil Dib (Figure 2). In axis 1, the representation shows that the weight variables, the length, the width of the bays are well correlated in the positive direction.

Therefore, none of these characters distinguish varieties between them, at least from data obtained from our sampling. Indeed, from the results of the factorial analysis (A.C.P) of the quantitative and semi-quantitative parameters of the grapes and berries we note an absence of a net structuring of the feet of the different varieties. And this, despite the discriminating power of the parameters used between different feet without reference to the variety. It is then thought that there is a problem in this study which resides in the number of insufficient feet sampled and the lack of repetitions within the same variety. The only variety that has a large number of feet is the variety Taferyalt with 12 feet. On the other hand, we must not forget the problem of synonymy and homonymy that prevents a good characterization of varieties. For this, molecular characterization is essential for good discrimination of varieties.

IV. CONCLUSION

Morocco is a very rich country in terms of homonymous grape varieties which results from the old tradition of grape cultivation in north-west of country, which began in romain period. We are of the opinion that it is crucial to preserve this genetic potential by describing a reasonable nomenclature and determining the relationships among these varieties through DNA-based markers.

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Use the qualitative parameters from grapes and grapes berries to identify the native *vitis vinifera* in Morocco

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Abstract— *We have used qualitative parameters to characterize and identify the native cultivars vitis vinifera in Northwestern of Morocco. Thirty nine accessions of locale grapevine were selected for this study and for each tree fifteen grape and fifteen grape berries were describe using ampelographic descriptors OIV. The table of ampelographic modalities shows a variation within the parameters studied between the different tree and inside the tree of the same variety. This variation gives us a preliminary idea of the diversity of the tree from local accessions of grapevine how deserves to be preserved and protected. Our results showed a significant variation of the shape of berries not only between the varieties but also inside the same variety.*

Keywords— ***qualitative parameters; vitis vinifera, local accessions, Morocco.***

I. INTRODUCTION

Grapevine (*Vitis vinifera L.*) is one of the oldest and most important perennial crops in the world. [1] estimated the existence of about 14,000 cultivars, with numerous synonyms and occasional use of the same or similar names for genetically different cultivars. The Mediterranean region overlaps with the area of distribution of *Vitis vinifera subsp. sylvestris*, the species from which the cultivated grapevine was domesticated [2]. The history of grapevine cultivation parallels the history of civilization along the Mediterranean basin. From the earliest events of grape and wine production, dating back to before 6000 BC in the Transcaucasian region [3] to the present, there has been an important trade of grapevines and their products, mainly wine and raisins, promoted by different cultures like the Phoenicians, Greeks, Romans, and Muslims. Furthermore, as observed all along the Mediterranean basin, human activities are rapidly eroding the number and size of existent local grapevine populations. A morphological characterization of these grapevine resources can help identify the genotypes that should be preserved and partially prevent or delay this genetic erosion.

Traditional ampelography (from the Greek ampelos-grapevine and graphos-description), analysing and comparing morphological characters to identify cultivars, is not sufficiently reliable and consistent due to environmental factors, individual plant biology, and plant growth stage [4]; [5], [6]; [7]. More recently, microsatellite markers have extensively been used for varietal characterization [8]; [9]; [7]; [10]; [11] and for rootstock identification [12]. Pedigree [13]; [5] and parantage analysis [14]; [15] has also been reported. Since SSRs have been revealed fully informative and solid markers, they have definitely been involved in mapping studies [16]; [17]; [18]; [19]. Moreover, these markers have been used for identification of chimaeras of grapes [20]; [21]; [22]; [9]. A novel SSR application has finally concerned the authentication of varietal wines [23].

Viticulture in Morocco was likely introduced by the Phoenicians, who brought with them their taste for wine [24]. Much later, with the expansion of the Muslim culture, wine production was forbidden and grapevines were grown to produce table grapes and raisins. Currently, grapevines are grown throughout Morocco mainly for fruit production. Many grapevine cultivars, recognized as local cultivars, are still cultivated. However, their substitution by newly bred cultivars is causing a rapid reduction in the number of local genotypes. Up-to-now in Morocco, varietal identifications have been carried out with ampelographic studies [24], [25], [26], [27]. The goal of this work was to use the qualitative parameters to identify and to characterize the Moroccan native *vitis vinifera* cultivars prospected from the north west of morocco.

II. MATERIAL AND METHODS

The study was conducted on a set of clusters and varieties of prospective varieties in northwestern of Morocco. A total of 39 vines were selected for this study and for each tree 15 grapes and 15 berries were selected for the study.

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The choice of vines was made according to their age in the orchards. A set of qualitative parameters has been used for the description and characterization of local grape fruits according to the OIV codes [28], [29]. Sampling was done at the time of fruiting. In each site studied and with the help of farmers, we collected samples of named and recognized fruits.

Principal Component Analysis (A.C.P) was done using SPSS Version 10 software. For the purposes of statistical analysis we have coded the different states of each parameter. Table 1 illustrates this coding of the parameters with their meanings.

Table 1: The coding table for qualitative parameters of bunches and berries

	Parameter	Value	Signification
Grape	Easy detachment of the pedicel	1	Very loose
		2	Loose
		3	Medium
		4	compact
		5	Very compact
Berrie grape	Easy detachment of the pedicel	1	Very easy
		2	Easy enough
		3	Difficult
	Shape	1	Rounded
		2	Flattened at the ends
		3	Elliptical
		4	egg-shaped
		5	Troncovoïde
		6	Obovoid
	Succulence of the pulp	1	Very little juicy
		2	A little juicy
		3	Juicy
	Intensity of pigmentation of the pulp	1	Very slightly colored
		2	Slightly colored
		3	Colorful
		4	Highly colored
		5	Very strongly colored
	Color of the epidermis	1	Green yellow
		2	Pink
		3	Red
		4	Red purple
		5	Dark blue

III. RESULTS AND DISCUSSION

The qualitative parameters of the cluster and the berries

The qualitative (semi-quantitative) results obtained are shown in Table 2. The table of ampelographic modalities shows a variation within the parameters studied between the different tree and inside the tree of the same variety. This variation gives us a preliminary idea of the diversity of the tree under study. For example, the tree of the Taferyalt variety expresses a rounded-to-flattened shape at Taferyalt kahla2, Taferylat Kahla3, Taferylat Kahla4, Taferylat Kahla5, Taferyalt Kahla7 and Taferyalt Khala8. On the other hand, the Taferylat Kahla1 tree has an ovoid shape. Thus, the tree Taferylat Bayda1, Mouska Hamra1 and Mouska Hamara2 show an elliptical shape. The tree of the variety Bezoul El awda shows the most distinctive form (troncovoid) among the studied tree. Similarly, the Maticha variety has mounted berries that resemble tomatoes with a difference in compactness that varies from compact in Maticha mjemaa and loose in Maticha mferqa (Table.2).

Table 2: List of quality parameters of grapes and berries

Variety	Compactness	facility of detachment of the pedicel	Shape	Succulence of pulp	Intensity of the pigmentation of pulp	Color of the skin	Firmness of pulp
feryal Khal1	3	2	4	3	2	4	3
feryal khal2	2	2	1	3	2	4	3
Taferyalt kahla3	2	3	2	3	4	5	3
Taferyalt Kahla4	2	2	1	3	4	4	3
Taferyalt Kahla5	3	2	2	3	2	4	2
Taferyalt Kahla6	4	2	5	3	2	4	3
Taferyalt kahla7	3	3	2	3	3	2	3
Taferyalt kahla8	4	2	2	3	4	2	3
Aferyal Byad1	3	3	3	3	2	1	3
Aferyal Byad2	3	2	2	3	3	1	3
Taferyalt Byad3	2	2	1	3	2	2	3
taferyalt Byad4	3	2	1	3	2	2	3
DiBI 1	2	3	2	3	3	2	3
Dibi 2	2	2	2	3	2	2	3
Echabel(Dibi)	1	2	1	3	2	2	3
Dibani 1	2	3	1	3	2	1	3
Dibani 2	2	2	1	3	2	1	3
Maticha Mferqa	2	2	2	2	2	4	2
Maticha Mjemaa	4	2	2	3	3	1	3
Mouska	4	3	1	3	2	1	3
Mouska Bayda	3	3	5	3	2	2	2
Mouska hamra1	1	2	3	2	3	2	2
Mouska hamra2	2	2	3	2	2	1	2
Ineb Nhal	4	2	1	3	3	5	3
Fekas khal	4	2	1	3	3	4	3
Fekas Byad	5	3	6	3	2	2	3
Fekas	3	2	2	2	4	5	3
Ineb Byad1	3	2	1	3	3	1	3
Ineb Byad2	2	2	2	3	2	1	3
Bezoul awda1	1	2	4	2	2	1	2
Bezoul awda2	4	2	3	3	3	2	3
Boukhanzir1	5	2	1	3	3	3	2

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Boukanzir2	2	2	2	3	4	2	3
Boukhanzir3	4	3	1	3	3	1	3
Sbiyae Bnat	4	1	1	3	2	1	3
Rjiyil Dib 1	3	2	5	3	3	4	3
Rjiyil Dib2	3	1	4	2	3	4	3
Zbarjel	4	2	1	1	4	4	1
Sanso	4	2	2	3	2	4	2

Principal component analyses of the semi-quantitative parameters of the bunches and berries

We used after coding (transformation of the qualitative variables into semi-quantitative variables) the means of the parameters qualitative of bunches and berries to carry out an analysis in principal component. Table 3 shows the percentages of the variance associated with each axis.

Table 3: Variance associated with PCA axes with semi-quantitative berries variables

Explained original variance			
Component	Total	% of the variance	% cumulated
1	1.93	27.64	27.64
2	1.36	19.44	47.14
3	1.16	16.60	63.74
4	.926	13.23	76.98
5	.815	11.64	88.62
6	.582	8.31	96.93
7	.214	3.06	100.00

We carried out the analysis in component of principal starting from the semi characters quantitative of the grapes and of berries of the various trees. The results are shown in figures 1 and 2.

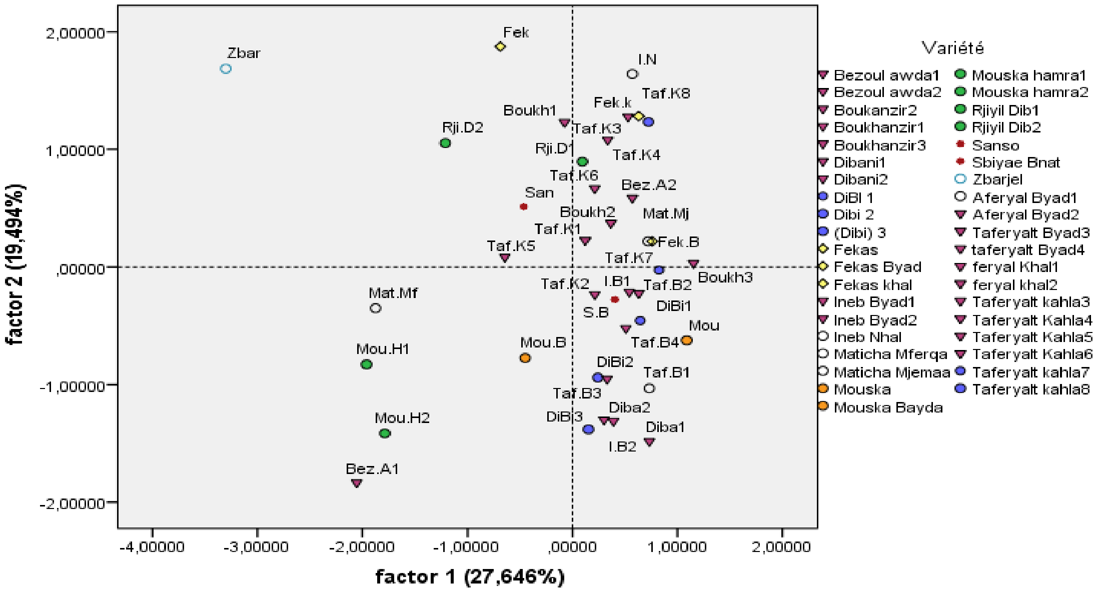

Fig. 1: Projection on the plan (1, 2) of the ACP of the semi-quantitative parameters

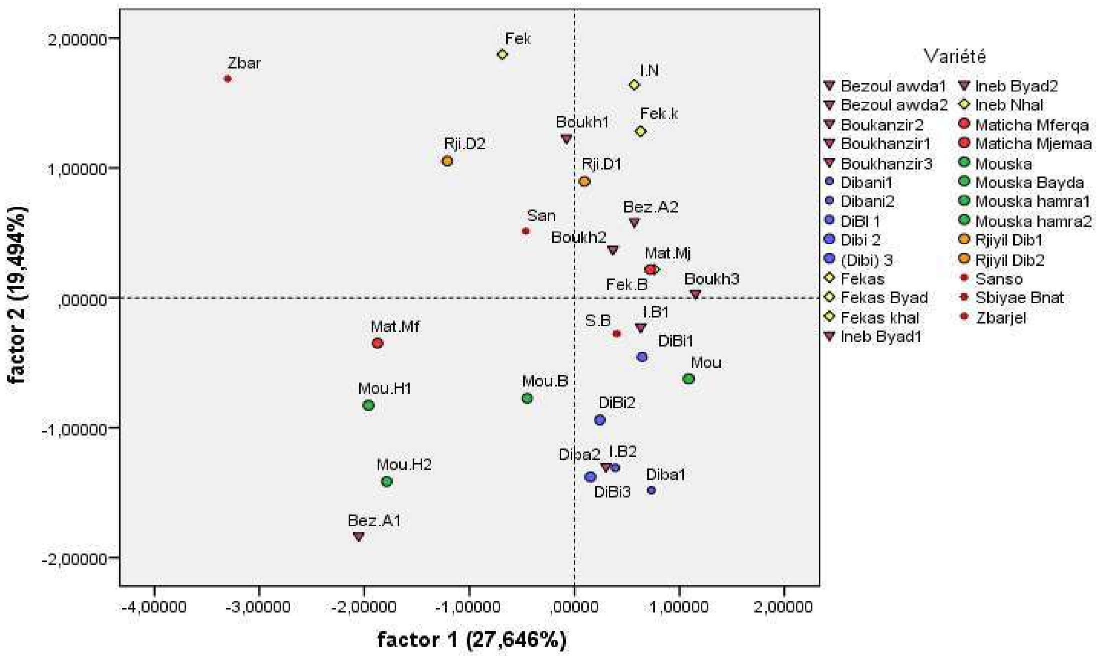

Fig. 1 Projection on the plan (1, 2) of the ACP of the semi-quantitative parameters (without Taferyalt)

According to the results obtained we notice the absence of a clear structure. On the other hand formation of a homogeneous group on the positive side of axis 1 and insulation of a few tree (Maticha mferqa, Mouska hamra1, Mouska hamra2, Mouska bayda, Bezoul aouda1, Zbarjel, Sanso, Boukhanzir1, Taferylat kahla5 and Fekass) at the negative side. One notices also the regrouping of the tree of each variety. As an example, tree of the variety Dibi, Mouska, Dibani and Boukhazir.

Indeed, our results showed a significant variation of the shape of berries not only between the varieties but also inside the same variety. It is the case of the tree of the Taferyalt variety whose shape of berries varies round form (feryal Kahl2 and TaferyaltKahla4) with flattened at the ends (Taferyalt Kahla5, Taferyalt Kahla7 and Taferyalt Kahla8) with elliptic berries(Taferyalt Byad1) or ovoid (Feryal Khal 1) and troncovoïde (Taferyalt Kahla6). The sugar content (Brix), is variable within the feet of the same variety but, we notice sometimes similar values at tree of various varieties such as for example at Taferyalt Kahla7, Maticha mjemaa, Bezoul El awda1, Boukhanzir3 and Sanso.

IV. CONCLUSION

The study of the morphology of plants, mainly leaves, buds, and the grape morphology (also called ampelography) was until recently the only means of identifying vine cultivars. This method is still used for identification particularly during in situ plant collection. In ours study the utilization of the qualitative parameters help us to more identify and characterize the local variety of grapevine in morocco. Indeed, a significant variation of the shape of berries not only between the varieties but also inside the same variety was observed. This study must be completed in the future by use the molecular markers for the best identification.

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Screening of Indigenous Rhizospheric Cyanobacteria as Potential Growth Promotor and Biocontrol of *Ralstonia* syzygii subsp. *indonesiensis* on Chili

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Abstract— *The use of microbial inoculants as biofertilizers and/or antagonists of phytopathogens provides a promising alternative to chemical fertilizers and pesticides. Cyanobacteria are a remarkable group of prokaryotes, which are known to exist independently and in symbiotic/facultative associations with a diverse range of members of the plant. Cyanobacteria inoculation had been reported to enhance the growth, nitrogen fixation and yields. Although, their establishment and role in plant growth promotion and biocontrol activity is poorly known. This research purposed to isolate and screen the best indigenous cyanobacteria from chili rhizosphere to promote growth rate and control R. syzygii subsp. indonesiensis on chili. The study consisted of three parts: (i) isolation and multiplication of the cyanobacteria from soil rhizosphere, and screening through Hypersensitive response (HR) on Mirabilis jalapa for pathogenicity test, (ii) in planta screening of selected cyanobacteria isolates (from second's step) to increase growth of chili seedlings, and (iv) in planta evaluation of selected cyanobacteria isolates for the control of bacterial wilt disease and promote growth of chili. Cyanobacteria isolated with BG-11 medium and incubated in room temperature with 12/12 h light/dark cycle. 49 cyanobacteria had isolated from chili rhizosphere. All isolates also showed suppression of disease development caused by R. syzygii subsp. indonesiensis. BCBY 3.1.3, and CBY 5.1 showed suppression of symptom appear (60.00 day post inoculation (dpi) compared to control (.38.667 dpi) and also suppressed disease severity (1.67) compared to control (3.00). The two strains which have best ability to increased growth rate also have best ability to fully suppressed disease development with no symptom appear until last day of observation.*

Keywords— ***Cyanobacteria; PGPR; screening; chili;***

I. INTRODUCTION

Ralstonia syzygii subsp. *indonesiensis* [1], previously named *Ralstonia solanacearum* phylotype IV, is a soil-borne gram-negative bacterium that causes bacterial wilt disease in over 200 families of plants, including chili [2,3]. This pathogen causes wilt by infecting plants through roots and colonizing stem vascular tissue and the vascular tissues in the lower stem of the wilted plants usually show a brown discoloration [4]. Attention has been paid to minimize the disease infestation through cultural practices, development of resistant varieties and use of chemicals, but most of them have a limited success [5].

Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers, forcing the search for alternative ways to improve crop yield in sustainable agriculture [6]. Biological systems are therefore preferred over chemical fertilizers, as they are not only ecofriendly and economical in approach, but are also involved in improving the soil quality and maintenance of natural soil flora.

The use of microbial inoculants as biofertilizers and/or antagonists of phytopathogens provides a promising alternative to chemical fertilizers and pesticides. Plant microbe interactions involve beneficial and detrimental relationships and the type of microorganisms involved determines the final outcome of the relationship, ranging from pathogenesis to symbiosis. Such interactions can influence plant growth and development, modulate nutrient dynamics, and alter a plant's susceptibility to abiotic /biotic stress [7]. Plant growth promoting rhizobacteria (PGPR) include beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms [8]. The use of PGPR is steadily increasing in agriculture, as it offers an attractive way to reduce the use of chemical fertilizers, pesticides and related agrochemicals.

Cyanobacteria are cosmopolitan microorganisms, which play significant roles in diverse ecosystems. Cyanobacterial inoculation is known to enhance the growth, nitrogen fixation and yields in the rice-wheat cropping sequence [9]. The favorable conditions provided by the rice fields for nitrogen fixation by these organisms leads to enhanced plant-available N in soil and yield improvement of rice [10, 11]. Cyanobacteria liberate extracellular substances and modulate pH, temperature and redox activity, besides playing a role in the volatilization of ammonia and methane generation; therefore, are directly of indirectly implicated in the management and productivity of rice ecosystem [12]. Cyanobacterial inoculation is also known to improve the stability of soil due to excretion of polysaccharides, lipids which aid in enhancing aggregation [13]. Their influence on other crops besides rice, e.g. wheat, tomato, chili and pulse and vegetable crops were also documented [14-16].

Cyanobacteria are potentially contributed towards biological nitrogen fixation [17], phosphate solubilization [18] and mineral release to improve soil fertility and crop productivity [19]. Cyanobacteria were also known to add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants [20-21].

Cyanobacterial inoculation is known to enhance the growth, nitrogen fixation and yields in the rice-wheat cropping sequence [22, 23]; however, very few reports are available on their role in disease reduction and protection against fungal diseases [24,25]. Cyanobacteria inoculation had been reported to enhance the growth, nitrogen fixation and yields. Although, their establishment and role in plant growth promotion and biocontrol activity is poorly known. This research purposed to isolate and screen the best indigenous cyanobacteria from chili rhizosphere to promote growth rate and control *R. syzygii* subsp. *indonesiensis* on chili.

II. METHODS

2.1. Study Area

This research has been done in Laboratory of Microbiology, Department of Plant Protection, and greenhouse, Faculty of Agriculture, Andalas University, Padang, Indonesia during January to July 2019.

2.2. Procedures

The study consisted of three parts: (i) isolation and multiplication of the cyanobacteria from soil rhizosphere, and screening through Hypersensitive response (HR) on *Mirabilis jalapa* for pathogenicity test, (ii) in planta screening of selected cyanobacteria isolates (from second's step) to increase growth of chili seedlings, and (iv) in planta evaluation of selected cyanobacteria isolates for the control of bacterial wilt disease and promote growth of chili.

2.3. Isolation of potential cyanobacteria isolates

Rhizosphere samples were collected from healthy chili's rhizosphere in endemic area of bacterial wilt in Tanah Datar, Solok and Agam District, West Sumatra Province, Indonesia. Soil and plant samples were given identification tags indicating the location, date of collection and type of crop, and were brought to the Microbiological Laboratory at Faculty of Agriculture, Universitas Andalas, Padang, West Sumatra, Indonesia. Samples were isolated one day after transport to the laboratory.

1 g of rhizospheric soil sample of chili were homogenized with 10 mL of sterilized tap water and serial diluted up to 10^{-5}. From this suspension, 1 mL of the suspension then suspended separately to 10 mL of BG-11 media, Yeast extract mannitol agar (YEMA) and Jensen's Medium agar and then separately placed in a Petri dish and growth in 2 days at incubated at 27 ºC in an incubator with light/dark cycles (16:8 h) with white light (50–55 mmol photons m [26]. Thereafter, dominant bacterial colonies were purified on the previous growth medium. A single colony of bacteria then transferred aseptically to microtube that contain 1 mL of sterilized aquadest as stock and stored in refrigerator. The isolates of cyanobacteria morphological character were noted based on morphological character of colonies, gram test and hypersensitive reactions (HR) assayed all according to methods of Klement et al., [27]. The positive results of HR indicated that the assayed isolates were pathogen to plants and not used for further studies.

2.4. In planta screening of selected cyanobacteria isolates to increase growth of chili seedlings

The selected isolates multiplied with preculture and main culture in BG-11 medium according to modified methods of Yanti et al., [28], and its density adjusted to 10^{8} CFU/mL with McFarland solutions scale 8.

Chili seeds used are varieties of Laris. Seeds sterilized before used with consecutively sterilized aquadest, NaOCl 1%, three times rinsed with sterilized water each for 2 minutes and then wind dried.

Sterilized chili seeds dipped to cyanobacteria suspensions and control dipped to sterilized aquadest for 10 minutes, wind dried and planted to pot-tray contained sterilized soil and cow dung manure mixture (2:1 v/v). Nurseries done in 3 weeks with parameter observed were germination rate, seedlings' height and number of leaves. All treatments used 25 seeds.

2.5. In planta evaluation of selected cyanobacteria isolates to promote growth and yield of chili

All chili seedlings from previous stage were planted to polybag contain same soil mixtures and reintroduced with cyanobacteria isolates with dipping methods for 15 minutes before. Parameter observed height, number of leaves, first flowering and yields.

III. RESULTS AND DISCUSSIONS

The cyanobacterial isolates were collected from chili field area located in Solok, Tanah Datar and Agam Regency, West Sumatra Province, Indonesia. The strains were purified and characterized morphologically and physiologically as given in Table 1. For morphological characterization of the plant growth promoting cyanobacterial were recorded. From 49 cyanobacteria isolates, 10 isolates were found to had positive results to Hypersensitive (HR) test. The HR results indicate the possivilities of the isolates as plant pathogens and were not used further.

Table 1. Indigenous Cyanobacteria isolated from Rhizosphere of Chili

No	Isolates	Gram	HR	Colony Shape				
				Colour	Shape	Elevation	Margin	Size (cm)
1	CBY 1.2.1	+	+	Yellowish cream	Circular	Convex	Entire	0.1
2	CBY 1.2.2	+	-	white	Circular	Convex	Entire	0.2
3	CBY 1.3	+	+	white	Irreguler	Umbonate	Undulate	0.2
4	CBY 2.2.1	+	-	Yellowish cream	Circular	Convex	Entire	0.2
5	CBY 2.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
6	CBY 2.3.1	+	-	white	Irreguler	Umbonate	Undulate	0.2
7	CBY 2.3.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
8	CBY 2.2.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
9	CBY 3.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
10	CBY 3.1.3	+	-	Yellowish cream	Circular	Convex	Entire	0.3
11	CBY 3.3	-	-	brown	Irreguler	Umbonate	Undulate	0.4
12	CBY 3.1.3	+	-	Yellowish cream	Circular	Convex	Entire	0.1
13	CBY 3.2	+	-	brown	Irreguler	Umbonate	Undulate	0.4
14	CBY 3.3.3	-	+	Yellowish cream	Circular	Convex	Entire	0.4
15	CBY 4	+	-	greenish	Circular	Convex	Entire	0.3
16	CBY 4.2	+	-	white	Irreguler	Umbonate	Undulate	0.2
17	CBY 44	+	-	Yellowish cream	Circular	Convex	Entire	0.2
18	CBY 5.2	+	+	white	Irreguler	Umbonate	Undulate	0.6
19	CBY 5.1	+	-	greenish	Circular	Convex	Entire	0.3
20	CBY 6.1.3	+	+	white	Irreguler	Umbonate	Undulate	0.3
21	CBY 6.1.1	+	-	Yellow	Circular	Convex	Entire	0.3
22	CBY 6.1.2	+	-	Yellowish cream	Circular	Convex	Entire	0.1
23	CBY 7.1	+	-	Yellowish cream	Circular	Convex	Entire	0.1
24	CBY 7.2	-	-	white	Circular	Convex	Entire	0.2
25	CYB 2.5.2	-	-	Yellowish cream	Circular	Convex	Entire	0.3
26	CYB 4.3	-	-	grey	Circular	Convex	Entire	0.1
27	CYB 5.3	+	-	grey	Circular	Convex	Entire	0.1
28	CYB6.4	+	-	Yellowish cream	Circular	Convex	Entire	0.2
29	CYB 7.3	+	+	white	Circular	Convex	Entire	0.5
30	CYB 7.4	+	-	white	Circular	Convex	Entire	0.5
31	CBY 8.2.2	+	-	greenish	Circular	Convex	Entire	0.4
32	CBY 8.2.1	+	-	greenish	Circular	Convex	Entire	0.3
33	CBY 8.2.3	+	-	greenish	Circular	Convex	Entire	0.2

34	CBY 9.1.3	+	-	Grey	Irreguler	Raised	Undulate	0.1
35	CBY 10.1.1	+	-	Yellowish cream	Circular	Convex	Entire	0.2
36	CBY 10.2.1	+	+	Yellowish cream	Circular	Convex	Entire	0.1
37	CBY 10.1.2	+	-	Yellowish cream	Circular	Convex	Entire	0.3
38	CBY 10.2.2	+	-	Yellowish cream	Circular	Convex	Entire	0.3
39	CYB 8.3	+	-	grey	Circular	Convex	Entire	0.2
40	CYB 8.4	+	-	yellow	Circular	Convex	Entire	0.2
41	CYB 9.3.1	+	-	white	Circular	Convex	Entire	0.2
42	CYB 9.4	+	-	Yellowish cream	Circular	Convex	Entire	0.3
43	CYB 9.3	+	-	white	Circular	Convex	Entire	0.2
44	CYB 9.3.2	+	+	white	Circular	Convex	Entire	0.2
45	CBY 11.2.2	+	-	Yellowish cream	Irreguler	Umbonate	Undulate	0.4
46	CBY 11.1.1	+	-	Yellowish cream	Circular	Convex	Entire	0.1
47	CBY 11.2.1	+	-	Yellowish cream	Circular	Convex	Entire	0.1
48	CBY 11.1.2	-	+	Yellowish cream	Circular	Convex	Entire	0.1
49	CBY 12.2	-	+	Yellowish cream	Circular	Convex	Entire	0.1

Ten of 39 cyanobacteria isolated from chili rhizosphere could increase height and germination rate of chili seedlings compared to control. Total of leaves also showed variated (Table 1). CBY 3.1.3 were the best isolates among all the cyanobacteria assayed with seedlings' height 11.06 cm and total leaves 5.33 with effectivity 105%. The 10 cyanobacteria strains (CBY 3.1.3, CBY 5.1, CBY 9.1.3, CBY 3.2, CBY 44, CBY 4.2, CYB 9.4, CBY 2.3.1, CBY 10.2.2 and CBY 3.3were were the best isolates to promote growth rate (seedlings' height and total of leaves) of chili and used for further studies.

Table 2. Height and total leaves of seedlings introduced with cyanobacteria isolates (21 days after introduction (DAI)

Isolates	Seedlings' height (cm)	Total of leaves
CBY 3.1.3	11.10 a	5.33 a
CBY 5.1	10.30 ab	5.00 ab
CBY 9.1.3	10.23 ab	5.00 ab
CBY 3.2	9.50 bc	5.33 a
CBY 44	9.50 bc	5.33 a
CBY 4.2	9.27 bcd	5.33 a
CYB 9.4	8.67 cde	5.00 ab
CBY 2.3.1	8.63 cde	4.33 bc
CBY 10.2.2	8.50 cdef	5.00 ab
CBY 3.3	8.40 cdef	4.33 bc
Control	8.20 cdefg	4.66 abc
CBY 2.2.2	8.20 cdefg	5.00 ab
CYB 4.3	8.16 cdefgh	4.66 abc
CYB 9.3.1	8.13 cdefgh	4.66 abc
CBY 4	8.00 cdefghi	4.66 abc
CYB 8.4	8.00 cdefghi	5.00 ab
CYB 5.3	7.93 defghi	4.33 bc
CBY 2.3.2	7.90 defghi	4.33 bc
CYB 8.3	7.90 defghi	4.66 abc
CBY 6.1.1	7.83 defghi	4.33 bc
CBY 8.2.3	7.80 defghi	4.33 bc

CBY 10.1.2	7.66 efghi	5.00 ab
CBY 2.2	7.63 efghij	4.00 c
CBY 2.2.1	7.53 efghijk	4.66 abc
CBY 3.1.3	7.50 efghijk	4.33 bc
CBY 3.2	7.36 efghijkl	4.33 bc
CBY 11.2.1	7.33 efghijklm	4.66 abc
CBY 10.1.1	7.20 efghijklmn	4.33 bc
CBY 8.2.1	7.16 efghijklmn	4.33 bc
CYB 2.5.2	7.06 fghijklmn	4.33 bc
CBY 1.2.2	7.03 fghijklmn	4.33 bc
CBY 7.2	7.03 fghijklmn	4.00 c
CYB 9.3	6.73 ghijklmn	4.00 c
CBY 6.1.2	6.66 hijklmn	4.00 c
CBY 11.1.1	6.56 ijklmn	4.33 bc
CYB6.4	6.13 jklmn	4.00 c
CBY 8.2.2	6.10 klmn	4.66 abc
CYB 7.4	5.93 lmn	4.33 bc
CBY 11.2.2	5.83 mn	4.00 c
CBY 7.1	5.73 n	4.00 c

Note: Means with the same letter are not significantly different by LSD test at $p < 0.05$

Phosphorus and nitrogen are very essential nutrients for plant growth and inoculation with phosphate solubilizing and nitrogen fixing cyanobacteria has been shown to improve plant growth by increasing the availability of phosphate and nitrogen content [29-31]. Cyanobacterial strains may protect plants from phytopathogens due to hydrogen cyanide production [32]. Phytohormone producing cyanobacteria are also involved in the promotion of plant growth. Need further checked for to determined cyanobacteria with ability to promote growth.

Beneficial effect of cyanobacteria introduction in chili plants were also noticed in generative stage of chili growth. The result proved that 7 of 10 isolates was able to increase growth of chili plants compared to control (Table 3). The strains also promote flowering time and increase yields of chili. The 10 best cyanobacteria strains were CBY 3.1.3, CBY 5.1, CBY 9.1.3, CBY 3.2, CBY 44, CBY 4.2 and CYB 9.4.

Table 3. Plant growth promotion activity of cyanobacteria isolates on generative phase of chili.

Isolates	**Plant height (cm)**	**Total of leaves**	**First flowering time**	**Yields (g)**
CBY 3.1.3	76.667 a	20.000	44.500 a	168.32 a
CBY 5.1	75.667 a	19.000	43.500 a	145.12 b
CBY 9.1.3	73.333 a	19.000	42.600 ab	141.60 b
CBY 3.2	70.000 a	19.000	41.200 ab	135.29 bc
CBY 44	66.000 ab	18.667	41.200 ab	129.78 c
CBY 4.2	62.000 ab	17.333	40.000 b	124.63 c
CYB 9.4	60.667 ab	16.667	38.600 c	115.26 d
control	59.000 ab	16.667	36.400 cd	113.44 d
CBY 2.3.1	58.000 ab	15.667	34.600 d	108.46 de
CBY 10.2.2	58.000 ab	15.000	34.400 d	93.988 e
CBY 3.3	50.333 b	14.000	33.000 de	86.452 f

Note: Means with the same letter are not significantly different by LSD test at $p < 0.05$

The increase in shoot lengths and number of leaves on chili plants, and furthermore the yields could be due to the action of one or more of the growth promoting substances especially seeds with cyanobacterial cultures. The results of inoculated and non-inoculated plants were recorded in order to make comparison. Many researchers have reported that the co-cultivation of crops with cyanobacteria caused a considerable increment in growth and biochemical parameters, both in control and field conditions [33-35].

Another study also found that the cyanobacterial inoculations could also promote growth rate of plants such as wheat [16, 38], *Lupinus termis* [37], Pea [34] and rice [38,39]. The phyto-stimulatory potential of cyanobacteria was also attributed to the atmospheric nitrogen fixation, making it available to the associated plants [16]. Increase in growth affects the overall development and growth of the plants by stimulating the water and nutrient uptake from soil. From the present study, we concluded that both cyanobacterial strains stimulated the growth of plant and they can be effectively used for biofertilization and plant growth improvement of different crops.

All isolates also showed suppression of disease development caused by R. *syzygii* subsp. *indonesiensis*. BCBY 3.1.3, and CBY 5.1 showed suppression of symptom appear (60.00 day post inoculation (dpi) compared to control (.38.667 dpi) and also suppressed disease severity (1.67) compared to control (3.00). the two strains which have best ability to increased growth rate also have best ability to fully suppressed disease development with no symptom appear until last day of observation.

Table 4. R. syzygii subsp. indonesiensis disease development on chili plant inoculated with Cyanobacteria Indigenos

Isolates	**First Symptom Developed**	**Severity**
CBY 3.1.3	60.000 a	1.67 a
CBY 5.1	60.000 a	1.67 a
CBY 44	50.667 ab	1.67 a
CBY 9.1.3	49.000 abc	1.33 ab
CBY 2.3.1	43.000 abc	1.00 abc
control	38.667 abc	0.67 abc
CYB 9.4	37.333 bc	0.67 abc
CBY 3.2	36.333 bc	0.33 bc
CBY 4.2	32.000 bc	0.00 c
CBY 10.2.2	29.667 bc	0.00 c
CBY 3.3	27.667 c	0.00 c

Note: Means with the same letter are not significantly different by LSD test at $p < 0.05$

Cyanobacterial strains showed biocontrol activity against *R. syzygii* subsp. *indonesiensis*. However further studies about their mechanisms in in vitro conditions were necessary to know the isolates abilities. The potential activity of cyanobacteria to inhibit certain soilborne diseases such as *R. syzygii* subsp. *indonesiensis* needs further investigation before they can be accepted as biocontrol agents for agriculture. Although the reports of cyanobacteria ability as biocontrol were not much, cyanobacterial strains had been reported to produce a wide range of plant growth regulators such as abscisic acid, ethylene, jasmonic acid, auxin, and cytokinin-like substances as well as the cytokinin isopentenyl adenine [39,40]. The cyanobacteria abilities as biocontrol agents of plant pathogens were still not well studied. Other have been evaluated the antifungal activity of terrestrial cyanobacterium *Nostoc commune* against the *Candida albicans* [41,42]. These antifungal activities are very interesting in the perspective of cyanobacterial research and possibly are important in commercial. Nevertheless, the antifungal activities of cyanobacterial metabolites were rarely studied.

IV. CONCLUSION

From the present study, we concluded that the cyanobacterial isolated from Chili Rhizosphere in West Sumatera could stimulated the growth of chili plant, Increase yields and control *R. syzygii* subsp. *indonesiensis.* Out of all the strains assayed in this study, isolates CBY 3.1.3 and CBY 5.1. were the best isolates to both promote growth, increase yields and control *R. syzygii* subsp. *indonesiensis.* Further studies are need to determine the strains ability.

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Discrimination the accessions of grapevine cultivars Taferyalt with phyllometric parameters

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***Abstract*— *The northwestern region of Morocco is rich in native vine cultivars where the problem of synonymy and homonymy is very common and causes the spread of varieties or the color of the fruits. The name of the grape cultivar Taferyalt has become inseparable from the name of the northwestern region where the cultivar is met under different names. In front of this situation, we tried to classify the accessions which have the same ampelographic character but with the different names (synonyms) or which showed several names which are given to the same variety (homonyms). The results of the phyllometric analyzes confirmed that these accessions are different.*

Keywords*— *Taferylat cultivar, Phyllometric parameters, Morocco, synonymy, homonymy.

I. INTRODUCTION

Vitis vinifera L., the commonly cultivated grapevine, is one of the most widely grown fruit plants in the world [1]. It has subspecies with West Asiatic and European origins, and ranges from Central Asia to the Mediterranean Basin [2]. The cultivated grape *V. vinifera subsp. sativa* has played an important economic and cultural role throughout human history in different parts of the world. Researchers from East and West European countries initiated efforts to collect and preserve germplasm from a wide range of countries, including regions where autochthonous germplasm had not been investigated by genetic and ampelographic methods [3, 4]. Traditionally, morphological and agronomical characteristics have been the main criteria for differentiating grapevine cultivars, but it is well known that many of those characters are strongly influenced by environmental conditions. In grape, synonymies are very common [5, 6, 7, 8, 9] and convergences of names for distinct cultivars (homonyms) also occur because of some peculiar features of the varieties or because they grow in different regions [10]. That is a major problem in the management of grapevine germplasm collections [11, 12].

Ampelometric methods can also be a useful tool in cultivar identification, as they are less expensive, do not require special equipment and can give relevant results if managed properly and the resulting data are analyzed appropriately. Leaf descriptors have generally been used as effective tools for characterization of grapevine cultivars [13], [14], [15], [16], [17], [18], [19], [20]. Study of plant morphology, mainly leaves, buds, and cluster morphology (also called ampelography) until it is the last means of detecting vine cultivars [21]. This method is still used for identification [22], [23], [24] particularly during the collection of data plants in situ. Many ampelographic studies have been made of grapevine cultivars from all over the world, but only a few have described those of Algeria and Morocco [25], [26], [27], [28]. Recently, many of the Maghrebi cultivars have now been profiled by nuclear and chloroplast microsatellite analysis [29], [30], [31], [32], [33].

In Morocco, the grapevine is one of the oldest crops, it is known from the past important wine growing areas despite this decline, and viticulture occupies an important place in the Moroccan socio-economic fabric. Actually in Morocco the most modern area of grapevine sector was constituted only by foreign introduced grapevine but these plant need adaptation cultural, simultaneously this exist the old local type of grapevines but the most of them not express all their potential of production and offer consequently a weak yield because the plantation of theses local grapevine was without grafting. In spite of this situation, the old grapes of grapevine remain much appreciated by the Moroccan consumer and during our prospection [30], the farmers claimed to make it begin research of improvement and valorization on its old grapevines. In this study we try to identify the native cultivar Taferyalt by used the phyllometric parameters.

II. MATERIAL AND METHODS

Twelve trees of Taferyalt accessions were selected in these study and thirty leaves of each tree was used. Several studies show that this is a sufficiently large and representative number of samples [34], [35], [36], [37],

[38], [39]. The leaves selected were between the 7th and 12th nodes, counted from the base of the primary branch following the recommendations of the [40].The leaves were dried in herbarium. Indeed, the measurements were made on the sheets scanned using Super Ampelo software [41]. This software measures different quantitative characteristics of the sheet and also calculates different parameters such as distances, angles, ratios, and descriptors of the OIV (International Office of Vine and Wine) (Fig 1). The software provides a total of 125 numeric values for each sheet. But only 86 characters were used, eliminating redundancy between characters. Principal Component Analysis (A.C.P) was done using SPSS Version 10 software.

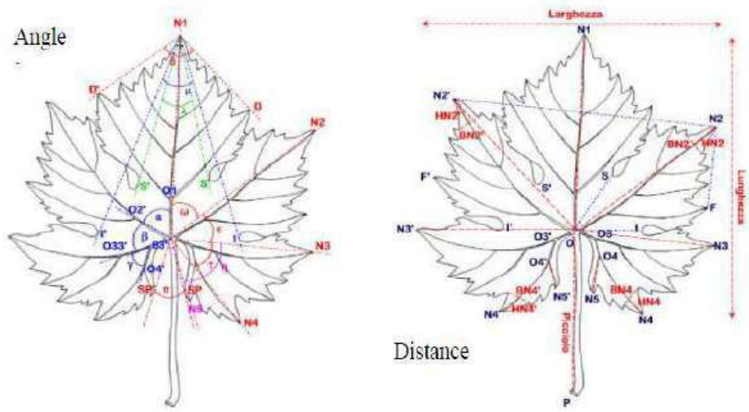

Fig.1. Point of the sheet requested by the information system to calculate the parameters (software Super Ampelo)

III. RESULT AND DISCUSION

The leaves of the vine represent five main veins. The relative dimensions of the nerves are certain elementary relations: wedge-shaped, cordate, pentagonal, circular and reniform. But the enormous variability of characters such as lobes, teeth, petiolar sinus, hairiness, pigmentation, are the rights of choice for the differentiation of varieties [42]. So, the shape of the leaf varies according to the varieties, it can also vary even on each foot. Indeed, it is not uncommon to see on one foot, leaves almost whole to another so cut up. The shape of the leaf is determined by the length of the nerves (NI, N2, N3, N4 and N5), as well as the angles between the veins (α, β, ε, δ, ʎ, μ). The size of the leaf is determined from its length (LU) and its width (LA). We distinguish a petiolar sinus (OP), an upper and lower lateral sinus, separating the lobes (Fig.1).

Average distances

We calculated the average for the leaf parameters of the different trees from the automatic measurements performed by the SuperAmpelo software (Table 1). Originally, the software measures 86 parameters for each leaf. This total number seems to be high compared to previous studies on grapevine cultivars as [43] (43 descriptors); [44] (50 descriptors); [45] (61 descriptors) and [46] (71 descriptors). We performed an analysis of variance to find the most discriminating parameters, we have shown that only 20 most discriminating parameters for distances and angles whereas only 16 parameters are the most discriminating for angles (Table 1, 2 and 3).

From Table 1 it can be seen that the tree Taferyalt khal 2 presents the means of the distances of the different points of the highest leaves compared to other trees of the Taferyalt variety. In general, and from the results of Table 1 we can say that the different tree of the variety Taferyalt does not represent the same average values of the different points of the leaves.

Table 1: Averages of the most discriminating parameters among the distances

Tree	OP	OS	OS1	OI	OI1	HN2	HN21	HN4	HN41	BN2	BN21	BN4	BN41	OO31	OO3	SPSP1	N4N41	FN21	O4N5	O41N51
Aferyal khal 1	18.39	12.38	11.13	12.4	11.07	1.92	1.79	2.04	1.75	3	2.55	2.96	2.77	2.71	4.57	24.11	17.85	12.93	4.76	2.61
Feryal khal 2	**14.17**	**14.61**	**13.86**	**13.84**	**12.93**	**3.48**	**2.38**	**3.16**	**2.69**	**3.88**	**3.31**	**3.95**	**3.35**	**4.25**	5.82	**28.95**	**26.19**	**15.83**	5.69	**4.10**
Taferyalt	16.14	11.75	11.18	11.57	10.48	2.03	1.86	2.12	1.85	2.91	2.39	**2.69**	2.28	2.46	4.22	23.59	15.61	12.05	4.36	2.47

Khal3																				
Taferyalt Khal4	16.92	11.8	10.87	11.68	10.91	**1.79**	1.65	1.93	1.63	2.86	**2.5**	2.78	2.39	2.52	4.35	23.62	17.11	11.69	4.29	2.14
Taferyalt Kahla5	14.61	**10.08**	**9.261**	**10.31**	**8.93**	1.87	**1.65**	**1.91**	**1.56**	**2.73**	2.38	2.86	**2.24**	2.09	4.16	**19.9**	**10.62**	**10.16**	**4.28**	**1.83**
Taferyalt Kahla	24.06	13.29	12.46	12.38	12.48	2.11	2.27	2.57	2.54	2.81	2.69	2.91	3.11	2.65	**6.1**	26.25	19.49	14.64	**7.18**	2.03
Taferyalt Kahla7	23.73	11.3	10.4	11.06	9.754	2.03	2	2	1.95	2.78	2.68	2.79	3.12	2.59	5.54	24.33	16.13	13.5	5.73	2.10
Taferyalt Kahla8	**25.51**	13.31	12.05	13.49	12.09	2.11	1.84	2.19	1.81	3.13	2.83	2.97	2.59	**1.88**	5.96	25.12	15.5	12.21	5.59	1.94
Aferyal Byad1	**18.39**	12.38	**11.13**	12.4	**11.07**	**1.92**	**1.79**	**2.04**	**1.75**	3	**2.55**	2.96	2.77	**2.71**	**4.57**	**24.11**	17.85	**12.93**	**4.76**	**2.61**
Aferyal Byad2	14.07	12.88	12.38	**12.87**	**12.33**	2.64	2.13	2.76	1.98	2.76	**3.39**	2.88	**3.31**	2.77	**7.13**	**25.82**	16.47	**15.44**	**7.52**	2.83
Taferyalt Byad3	17.94	11.81	11.39	12.25	11.95	2.61	2.27	**2.98**	**2.26**	3.08	2.59	**3.54**	2.75	**3.67**	4.82	25.44	19.9	13.38	4.79	3.42
Taferyalt Byad4	17.47	**12.97**	**13.16**	12.62	11.99	**2.78**	2.18	2.62	2.13	**3.33**	2.82	3.09	2.71	3.55	4.74	25.34	**21.31**	14.23	4.87	**3.59**

Average angles

The average values of the angles of the points of the different trees are very variable from one tree to another (Table 2). Note that the tree Aferyal bayad 2 shows the average values of the different points of very large angles compared to the average values of the angles of other trees.

Table 2: Averages of the most discriminating parameters among the angles

Tree	AL (α)	AL1 (α′)	BE (β)	BE1 (β′)	GA (ɤ)	(GA1 (ɤ′)	ET (ε)	ET1 (ε′)	TA	TA1	PI (π)	DE (δ)	LAM (λ)	MU (μ)	ALBE (α+β) (μ)	ALBE1 (α′+β′)	ALBEGA (α+β+ɤ)	ALBEGA1 (α′+β′+ɤ′)	ANGA (η)	ANGA1 (η′)
Aferyal khal 1	50.76	55.59	48.73	11.07	50.78	50.54	47.2	48.45	42.6	42	59.14	113.8	45.45	49.15	99.48	66.67	150.3	117.2	59.07	58.54
Feryal khal 2	51.55	54.38	45.32	10.81	51.89	55.79	44.3	49.3	41.6	41.9	69.12	103	41.3	48.42	96.85	65.2	148.8	121	59.17	60.07
Taferyalt Khal3	54.71	51.68	48.81	9.7	51.93	49.4	52.1	45.91	43.1	41.9	60.54	107.4	42.68	48.46	103.5	61.36	155.5	110.8	57.72	56.79
Taferyalt Khal4	47.88	47.06	45.43	8.64	49.24	48.83	51.7	44.6	40.5	41.6	73.22	108.3	40.13	49.19	93.33	55.7	142.5	104.5	55.78	55.35
Taferyalt Kahla5	54.29	56.68	50.5	11.48	56.4	59.06	52.9	50.81	48.1	50.1	36.23	108.2	44.83	46.65	104.8	68.16	161.2	127.2	65.91	68.56
Taferyalt Kahla	51.56	55.93	43.51	11.56	54.16	52.95	44.2	45.59	45.4	46.9	35.75	98.71	42.43	50.06	95.05	67.49	149.2	120.4	73.73	69.64
Taferyalt Kahla7	51.48	51.27	46.45	10.92	53.27	54.29	47.8	50.22	46.8	48.1	41.94	105.2	36.03	43.32	97.93	62.19	151.2	116.5	68.15	68.48
Taferyalt Kahla8	49.8	47.54	43.74	10.02	48.94	45.22	45.6	50.16	49.6	48	37.46	89.75	44.36	48.41	93.55	57.56	142.5	102.8	70.08	73.51
Aferyal Byad1	50.76	55.59	48.73	11.07	50.78	50.54	47.2	48.45	42.6	42	59.14	113.8	45.45	49.15	99.48	66.67	150.3	117.2	59.07	58.54
Aferyal Byad2	**56.33**	**75.96**	**58.85**	**17.74**	**57.84**	**56.44**	**51.1**	**53.83**	**48**	**49.2**	**17.58**	**109.9**	**45.81**	**47.57**	**115.2**	**93.69**	**173**	**150.1**	**74.87**	**73.89**
Taferyalt Byad3	52.01	57.72	43.95	8.07	53.35	52.57	50.5	48.56	40.4	39.3	76.13	103.6	37.15	46.89	95.96	65.79	149.3	118.4	53.05	53.2
Taferyalt Byad4	56.35	54.11	44.62	7.53	51.75	53.06	49.5	45.08	39.8	39.8	74.15	105.9	44.55	51.01	100.9	61.65	152.7	114.7	54.38	56.54

Average ratios

Table 3 shows the reports of different measurements made by the SuperAmpelo software. We note that the different rations are variable between the trees of the variety Taferyalt.

Table 3: Averages of the most discriminating parameters among the ratios

Tree	RS	RS1	HD	LULA	RI	HBD	HBD1	HD1	RP	RI1	ALBEOSOI	ALBEOSOI1	HBN2	HBN41	HBN21	HBN4
Aferyal khal 1	0,52	0,51	1,23	0,91	0,62	0,66	1,32	2,50	0,73	0,61	0,08	0,05	0,67	0,64	0,71	0,70
Feryal khal 2	0,50	0,46	1,57	0,88	0,62	0,63	1,42	3,18	0,45	0,56	0,06	0,05	0,90	0,8	0,73	0,82
Taferyalt Khal3	0,49	0,50	1,27	0,95	0,65	0,71	1,54	2,43	0,63	0,64	0,09	0,05	0,71	0,86	0,76	0,81
Taferyalt Khal4	0,50	0,50	1,15	0,93	0,63	0,63	1,31	2,35	0,65	0,64	0,08	0,05	0,63	0,70	0,66	0,73

Taferyalt Kahla5	0,50	0,52	1,08	0,95	0,66	0,62	1,28	2,18	0,68	0,64	0,1	0,07	0,68	0,71	0,71	0,68
Taferyalt Kahla	0,50	0,48	1,4	0,97	0,62	0,72	1,44	2,52	0,85	0,65	0,07	0,05	0,75	0,82	0,87	0,88
Taferyalt Kahla7	0,46	0,45	1,31	0,94	0,58	0,69	1,38	2,58	0,9	0,55	0,08	0,05	0,74	0,63	0,76	0,72
Taferyalt Kahla8	0,52	0,55	1,46	0,97	0,64	0,73	1,53	2,92	0,92	0,66	0,07	0,04	0,69	0,71	0,67	0,74
Aferyal Byad1	0,52	0,51	1,23	0,91	0,62	0,66	1,32	2,50	0,73	0,61	0,08	0,05	0,67	0,64	0,71	0,70
Aferyal Byad2	0,50	0,50	1,64	0,94	0,62	0,74	1,54	3,34	0,5	0,61	0,09	0,07	0,96	0,6	0,62	0,98
Taferyalt Byad3	0,46	0,48	1,39	0,95	0,61	0,61	1,30	2,89	0,63	0,61	0,08	0,05	0,85	0,80	0,86	0,82
Taferyalt Byad4	0,51	0,51	1,35	0,93	0,67	0,63	1,31	2,78	0,63	0,63	0,07	0,05	0,82	0,78	0,78	0,83

Principal component analysis

Using different leaf phyllometric measurements, we performed a principal component analysis of the different trees of the Taferyalt variety to see if the trees cluster together. The result is shown in Figure 2. From the figure we see that the trees of the taferylat variety form a group together except one tree Taferyalt Beyad 2 which is very far from other trees. Also the tree Taferyalt khal 1 and tree Taferyalt Byad 1 are superimposed. According to the result of the principal component analysis it can be said that it is a single vine variety Taferylat presented by several accessions, except the tree Taferyalt Beyad 2.

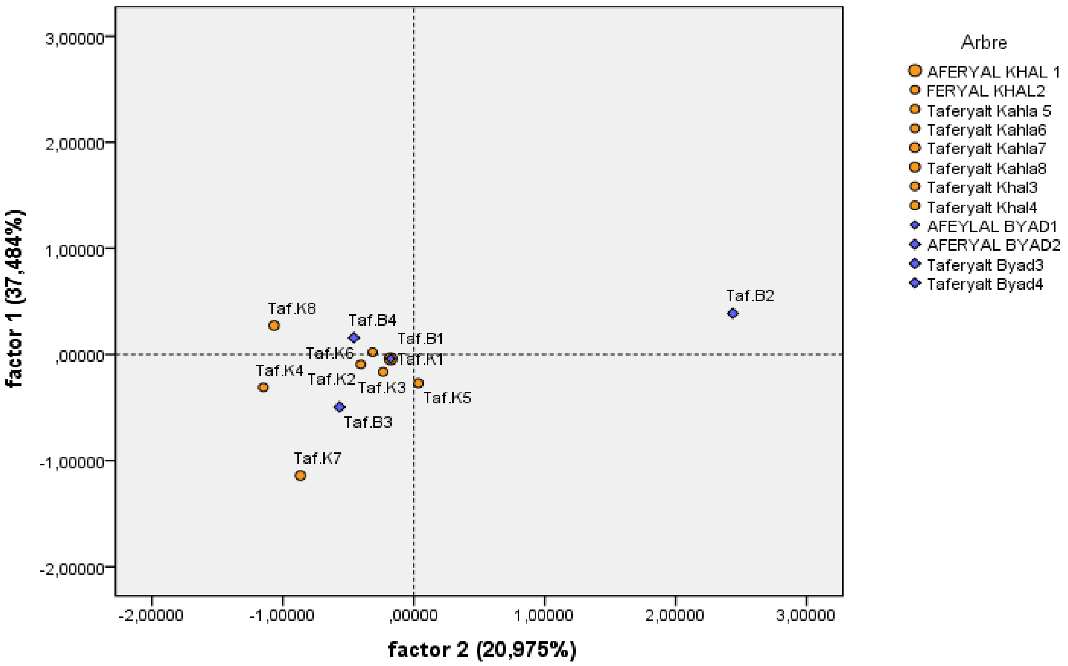

Fig. 2: Representation of the 12 trees of the "variety" Taferyalt

This result can be explained by the fact that there are problems of nomenclature due mainly to synonymy (several names are given to the same variety) and homonymy (the same name is given to several varieties). This problem is very common among local varieties. Synonymies are particularly related to the dissemination of varieties, which have been widely circulated and which have often been named differently in the different growing areas. As for the homonymy very common in the culture of the Moroccan vine, it is particularly determined by the color of the fruit as for the varieties "EL Biod and EL Kehal". The names El Biod and El Kehal are given to varieties for which the morphological characters are different but whose fruits have a white or black color, hence the Arabic name of EL Biod (White) or EL Kehal (Black). In other study, [47] presumed the variety 'Kéknyelu˝' for the same as the Italian 'Picoletto bianco' (= 'Picolit'). [48] commented that Italian ampelographers approve of the similarity of the two cultivars. He suggests importing 'Picolit' to Hungary to clarify this issue. Nowadays molecular markers are useful tools detect homonymies and synonymies [49].

IV. CONCLUSION

In this study, the different parameters phyllometric was used to identifiy the accessions of one of the must answered cultivars in the north western of morocco, the result indicate the clear difference between the two studied accessions. On the basis of these results it can be established, that as we supposed the studied cultivars are different.

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Diversity of the Local Varieties from grapevine Tree in the Northwestern of Morocco

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***Abstract*— *The culture of grapevine in Morocco is ancestral and preserves a particular importance in the traditional agroecosystems of the Riffian mountains. But the importance of varietal diversity remains ignored. These study was carried out to evaluate this diversity, for that a investigations and investigations were carried out in north-west of the country. The stations were selected according to the importance of the orchards of grapevine tree in the agroecosystem. A complete description morphological, physiological and pomological relating the tree and the fruit was carried out. The culture of grapevine in the area of study show a great diversity with 21 listed native varieties. One notes a great confusion of denomination on the level of the prospected varieties. This problem can be with several causes and mainly with the errors of appreciation of the characters or the differences of local names which vary from one locality to another.*

***Keywords*— Grapevine, varietal diversity, traditional agroecosystem, Morocco.**

I. INTRODUCTION

Vitis vinifera L., the commonly cultivated grapevine, is one of the most widely grown fruit plants in the world [1]. The cultivated grape *V. vinifera subsp*. sativa has played an important economic and cultural role throughout human history in different parts of the world. However, its ancestor the European wild grape V. *vinifera subsp. sylvestris*, is close to extinction. In Morocco, the grapevine as well as the olive tree, the fig tree and the cereals are cultures well adapted to the natural climatic conditions of our country located at the end of the western Mediterranean. The culture of the grapevine is practiced in a traditional way. It uses a vegetable material not selected based on local varieties with localized denomination and distribution. This culture remains especially in the traditional agroecosystem like the agroecosystem of mountains. It is the case of the Riffian mountains in the areas north of Morocco where this 90-93% national orchard concentrate. These agroecosystem was characterized valence of the micro property (<0.5 ha) and small property (<5 ha) and a weak useful agricultural area [2], [3]. The Riffian field is very heterogeneous (climate, ground, relief, occupation of the ground), and the diversity is much contrasted nature sometimes of the agroecosystems of this area [4]. Arboriculture like grapevine and fig is one of the principal productions in these agroecosystems. The culture of grapevine is in clear regression, historically it's occupied a dominating place, the grapevine fruit and dried grapes were exchanged against cereals coming from the plains. Many ampelographic studies have been made of grapevine cultivars from all over the world, but only a few have described those of Algeria and Morocco [5], [6], [7], [8]. Recently, many of the Maghreb cultivars have now been profiled by nuclear and chloroplast microsatellite analysis [9], [10], [11], [12], [13]. Grapevine varieties have been characterized for identification purposes by ampelographic characters [14], [15], [16]. Study of plant morphology, mainly leaves, buds, and cluster morphology (also called ampelography) until it is the last means of detecting vine cultivars [17]. This method is still used for identification [18], [19], [20] particularly during the collection of data plants in situ. Into this article, one proposes to present some aspects of the culture of the grapevine in the mountains agroecosystem and show the importance of varietal diversity in these areas.

II. MATERIAL AND METHODS

Work was based on prospecting and investigations carried out in 3 stations in Northwestern of the country. These stations were selected according to the importance of the orchards of the grapevine tree in the agroecosystem. The orchards where the samples were collected were localized using a GPS (Table.1). The investigations are based on the use of a questionnaire bearing into socio-economic and agronomic aspects of the culture of the grapevine. During the prospecting a complete description of the varieties was assured, relating the tree and the fruit. The descriptors used were adapted list drawn up OIV descriptors of the OIV (International Office of Vine and Wine) and phenological stages of [21]. The evaluation of the plant health state of the trees related to the diseases whose symptoms are clearly defined and who are easily observable in situ on the tree. The data processing was carried out by using the NTSYSpc software for the analyses of similarities and algorithm UPGMA for the construction of the clusters.

Table 1: Location of prospecting sites

Locality	Altitude	Geographic coordinate
BNI HAMDILLAH	303 m	35°N -02,009' 0,5° W 19,870'
MOKRISSAT	562 m	35°N - 54,627' 0,05°W -20,687'
ZOUMI	648 m	34°N - 49,690' 0,5°W - 16,783'
ELaachayich	359 m	35°N - 12,3377' 0,05°W - 19,568'

III. RESULTS AND DISCUSSIONS

Socioeconomics Aspects

Most of the orchard owners (80%) have found that vine growing is just additional activity for which they hold hardly the 1/10 of the cultivated surface. Often the orchard is for subsistence farming. We note the regression of orchards that are abandoned in favor of other crops considered more profitable, this also due to the lack of commercial sector infrastructure. Most of the production is consumed on site or transformed into traditional grape juice called Samet.

Agronomic Aspects and Plant Health State

According to the investigations carried out at the vineyards traditional, the farmers affirm that the vine is well adapted on the climatic ground and conditions and it does not require any particular care. The Scandinavian Western exposure and of north however are preferred because they offer a certain freshness which compensates for the poverty of the ground. Cultivating techniques like cutting, the agricultural work, the fertilization or pollination are seldom practiced. The lack of care leads to an exaggerated development of the tree which supports the development of the diseases. The study of health of factory proves that the majority of the orchards are touched by the various diseases of which most frequent are gray rot and the mildew. However, the farmers affirm that in the majority of the cases which the diseases do not affect the productivity of the trees.

Varietal Diversity and Richness of Local Varieties

The prospection carried out in the area assures us the seniority of the varieties. The farmers affirm that the majority of the vines are local and very few varieties were introduced and confirm the nature of the vegetable material of the local varieties. The prospection showed a great morphological diversity, thus we counted not less than 21 local varieties or denominations (Table.2).

Among the listed varieties, it is Taferylat which is drawn aside and more appreciated. We finds denominations identified in other sectors of region, like Dibi, Boukhanzir, Ineb Byad, Bezoul awda, Sbiyae bnate, Mouksa bayda and mouska hamra. But, ther are also varieities as for example whose names are quoted for the first time, Sans, Zbarjel, Maticha Ineb nhal…

The variety Dibi showed the higher weight of grappe (872g), the low value was observed in the variety Mouska hamra (20g). The most sweet fruit was Taferylat bayd with the maximum value of brix (49), but the acidic fruit was Feryal khal with the minimum value of brix (29) (Table.1).

Table.1. Principal Characteristics of local varieties listed in this study.

Variety	Weight grappe(g)	Brix	Compactness	Shape of the fruit	Color of the epidermis
Feryal Khal1	435	29	Medium	Ovoid	Red purple
Taferyalt kahla8	296	46	Compact	Flattened at the ends	Pink
Aferyal Byad1	551	42	Medium	Elliptical	Green yellow
Taferyalt Byad3	74	49	Loose	Rounded	Pink
Dibi 1	872	45	Loose	Flattened at the ends	Pink
Echabel(Dibi)	348	37	Very loose	Rounded	Pink
Dibani 1	359	35	Loose	Rounded	Green yellow
Maticha Mferqa	71	47	Loose	Flattened at the ends	Red purple
Maticha Mjemaa	138	40	Compact	Flattened at the ends	Green yellow
Mouska Bayda	184	38	Medium	Troncovoid	Pink
Mouska hamra1	20	46	Very loose	Elliptical	Pink
Ineb Nhal	263	35	Compact	Rounded	Dark blue
Fekas khal	445	32	Compact	Rounded	Red purple
Fekas Byad	222	37	Very compact	Obovoid	Pink
Ineb Byad1	367	35	Medium	Rounded	Green yellow

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Bezoul awda 1	43	40	Very loose	Ovoid	Green yellow
Boukhanzir1	352	42	Very compact	Rounded	Red
Sbiyae Bnat	93	39	Compact	Rounded	Green yellow
Rjiyil Dib 1	277	55	Medium	Troncovoid	Red purple
Zbarjel	90	35	Compact	Rounded	Red purple
Sanso	251,28	40	Compact	Flattened at the ends	Red purple

By the use the ampelometric data we realized a hierarchical classification (Fig.2). This classification is based on distances measured by a similarity index. This analysis consists of gradually aggregating the individuals according to their similarity. The hierarchical ascending classification requires the definition of a measure of similarity (distance) or a criterion of aggregation of objects (samples). According to [22] she produces a suite of nested partitions of the set of objects to classify. In our study, this analysis was carried out by introducing the mean values of each variable for each tree.

The analysis of the dendrogram of the hierarchical classification (Fig. 1) shows the existence of two main groups (classes) divided in turn into several subgroups. The first class has 12 trees: it can be subdivided into two subclasses, Subclass 1 with Taferyalt Byad2 and Maticha Mferqa. Subclass 2 with Bezoul El awda1, Mouska, Taferyalt Kahla5, Sanso, Fekas Byad, Ineb Nhal, Ineb Byad2, Maticha Mjemaa, Ineb Byad1 and Fekas Khal. We notice the strongest similarity is observed in Fekas Byad and Sanso. The second class has 27 trees. The Subclass 1 represented by the single tree: Zbarjel distinguished mainly from other tree studied.

Subclass 2 with Fekass, Boukhanzur1, Taferyalt Kahla8, Taferyalt Kahla6, Rjiyil Dib2, Rjiyil Dib1 and Taferyalt Kahla .Mouska Hamra2 and Sbiyae El bnat. Boukhanzir3, Dibani2, Taferyalt Kahla2, Taferyalt Byad4, Taferyalt Byad3, Taferyalt Khal4, Boukhanzir2. Mouska Bayda, Dibani1, Dibi2, Mouska Hamra1, Dibi1, Dibi3, Taferyalt Kahla3, Taferyalt Byad1 and Taferyalt Khal1.

In this group the tree with the strongest similarity are Taferyalt Khal7 and Rjiyil Dib1, Taferyalt Byad3 and Taferyalt Byad4, Taferyalt Kahla4 and Boukhanzir2, Dibi2 and Dibani1, Taferyalt Khal1 and Taferyalt Byad1.

The varieties which bear the same name have fruits with similar pomological characters, but it is far from being a general case. Indeed, of the notable differences can be observed within the same variety so much so that individuals supposed to belong to the same variety can be different and separate on the analysis (Fig. 1).

We think that they can be in certain cases different varieties but bearing the same name (homonymy) or on the contrary same varieties but carrying different local names (synonymy).

It is thus necessary to record the importance of the problems of denomination of the varieties listed in the prospected area. These problems are due to local names which sometimes vary from one locality to another and the absence of the syntheses and varietal characterization.

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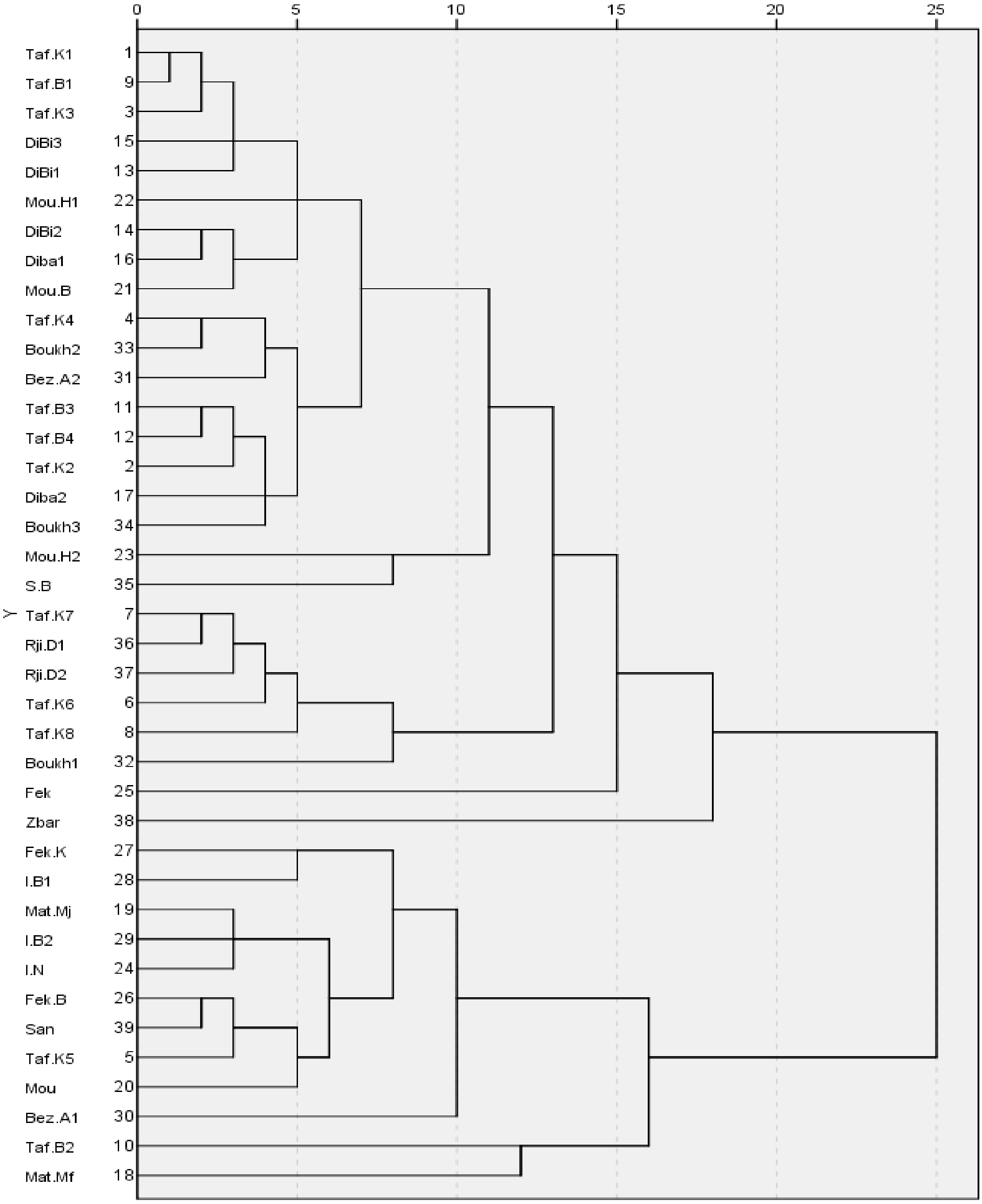

Fig. 1. Representation in cluster of individuals of the north region of Morocco.

IV. CONCLUSION

The culture of the grapevine in the north Morocco is practiced in a traditional way. The vegetable material used corresponds to very old varieties or local denominations. There are no introductions or uses of new or selected varieties. Although the prospecting was made on a limited study they showed the existence of a great varietal diversity. Indeed, Twenty one “local varieties” were listed in this study. However, in the absence of a pomological and morphological characterization, the value of the varietal denominations poses problems. One is in the presence of an important genetic inheritance little known who the evaluation is and the characterization is not carried out yet. The regression of this culture and the transformations socio-economic that the area knows constitute a serious threat of erosion and of loss of this phytogenetic resource.

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Use phyllometric parameters to discriminate the Moroccan Native *vitis vinefera* cultivars

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Abstract— *The aims of this study is to use the phyllometric parameter to identify the autochthonous cultivars in north oust of morocco. Eighty six phyllometric parameters were measured/ calculated on 585 leaf samples from thirty nine grapevine cultivars, gathered during several years, from different traditional vineyard in Northwestern of Morocco. The leaves were scanned and images were analyzed using SuperAmpelo. Principal component analysis was performed to discriminate different accessions of cultivars. The results show a heterogeneous distribution of different accessions. This can be explained by the existence of the problem of synonymy and the homonyms frequently encountered in local varieties. In this study we tried to value these native cultivars by using the phyllometric approach. These varieties could be a valuable tool for improving local economies.*

Keyword— ***Phyllometry, Vitis vinifera, Morocco, discriminant analysis, cultivar identification.***

I. INTRODUCTION

The grapevine is one of the oldest known fruit species in the world and therefore researchers have always wanted to learn more [1], [2]. It is important to mention that it has an economic importance, with a total area of 7.726 mha and global production up to 750 mql over the world (http://www.oiv.int/en/databases-and-statistics/statistics).
According to [3], the origin of the genus Vitis is located in Eurasia and then spread to the rest of the world.
In North Africa Viticulture became consolidated under Roman influence [4], [5]. The grapevine has always occupied an important place in the traditional Mediterranean landscape, with its presence under these two wild and cultivated forms [6]. The culture of grapevine is well rooted in the traditions of the Maghreb populations in general and Moroccan in particular. In Morocco, according to the statistics of the Ministry of Agriculture, the culture of grapevine has an area of 33 787 ha with a production of 346656 T of the grape table in 2017/2018. Study of plant morphology, mainly leaves, buds, and cluster morphology (also called ampelography) until it is the last means of detecting vine cultivars [7]. This method is still used for identification [8], [9], [10] particularly during the collection of data plants in situ.
Many ampelographic studies have been made of grapevine cultivars from all over the world, but only a few have described those of Algeria and Morocco [11], [12], [13], [14]. Recently, many of the Maghrebi cultivars have now been profiled by nuclear and chloroplast microsatellite analysis [15], [16], [17], [18], [19]. Ampelometric methods can also be a useful tool in cultivar identification, as they are less expensive, do not require special equipment and can give relevant results if managed properly and the resulting data are analyzed appropriately. Leaf descriptors have generally been used as effective tools for characterization of grapevine cultivars [20], [21], [22], [23], [24], [25], [26], [27]. For these reasons, they can still be the method of choice, especially for research groups without the resources or knowledge required for molecular analyses. Computer-aided survey systems for phyllometry, such as SUPERAMPELO [28] and GRA.LE.D [29] and some others have been developed as solutions for the time-consuming limitations of phyllometric research. The aim of this research is the use the phyllometric parameters for valorization of autochthonous cultivars, which could represent a valuable instrument for improving the local economies.

II. MATERIAL AND METHODS

Plant material

The plant materials consisted of samples prospected in Northwestern of Morocco (Fig 1 and Table 1), all of them were classified as minor or endangered varieties. The total number of accessions studied was 39 (Table 1). Each accession consisted of five replications.

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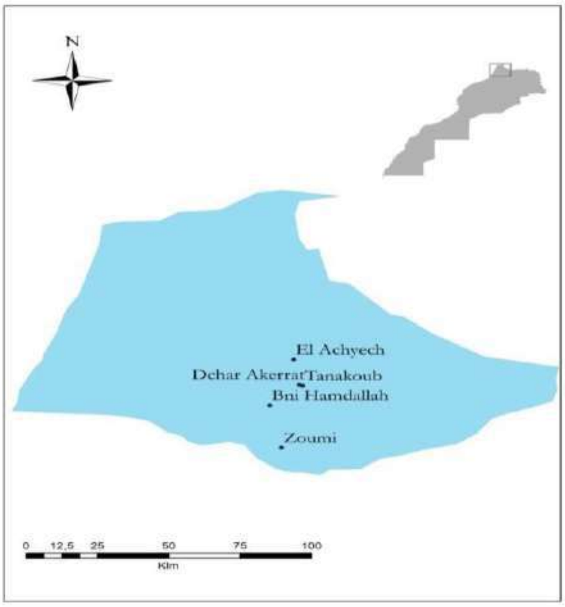

Fig.1 Location of the stations prospected on the map

Table 1: Geographical coordinates of sampling sites

Station	Locality			Varieties
	Longitude (N)	Latitude (W)	Altitude (AL)	
EL Aachayich	3512377	00519568	359 m	Echabel; Ineb Byad; Mouska; Bezoul El Awda; Maticha; Taferyalt khal; Taferyalt Byad; Sbiyae Bnat; Fekass
Bni Hamdillah	3502009	00519870	303 m	Ineb Byad; Dibani; Ineb Nhal; Fekas Khal; Aferyal Byad; Aferyal Khal; Fekas Byad; Dibi
Mokrissat	3554627	00520687	562 m	Dibani; Sanso; Boukhanzir; Taferyalt Khal
Zoumi	3449690	00516783	648 m	Bezoul El Awda; Boukhanzir; Rjiyil Dib; Zbarjel ; Taferyalt Khal

Based on the phenological stages of [30], sampling of adult leaves was done between fruit set and veraison. We selected 15 leaves per varietals. Several studies show that this is a sufficiently large and representative number of samples [31], [32], [33], [34], [35], [36]. The leaves selected were between the 7th and 12th nodes, counted from the base of the primary branch following the recommendations of the [35].

The leaves were dried in herbarium. Indeed, the measurements were made on the sheets scanned using Super Ampelo software. This software measures different quantitative characteristics of the sheet and also calculates different parameters such as distances, angles, ratios, and descriptors of the OIV (International Office of Vine and Wine) (Figure 2). The software provides a total of 125 numeric values for each sheet. But only 86 characters were used, eliminating redundancy between characters. Principal Component Analysis (A.C.P) was done using SPSS Version 10 software.

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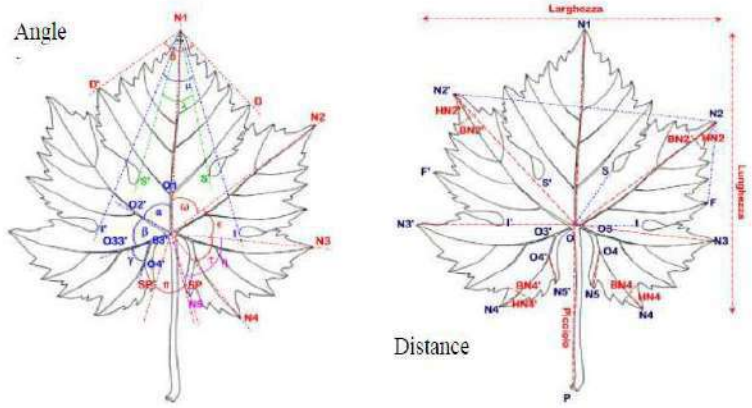

Fig.2. Point of the sheet requested by the information system to calculate the parameters (software Super Ampelo)

III. RESULTS AND DISCUSSION

Averages of the distances between remarkable points

We calculated the average for the leaf parameters of the different trees from the automatic measurements performed by the SuperAmpelo software. Originally, the software measures 86 parameters for each sheet. This total number seems to be high compared to previous studies on grapevine cultivars as [37] (43 descriptors); [38] (50 descriptors); [39] (61 descriptors) and [40] (71 descriptors).

The averages per tree and per variety were calculated. Based on the measurements made using the SuperAmpelo software, the variables relating to the distances between remarkable points of the sheet were 31 variables. The averages obtained show significant variations not only between trees of different varieties but also between trees of the same variety. For example, the parameter OP (petiole length) showed in the tree Taferyalt Kahla 8 has the highest value compared with Taferyalt Kahla 5 and Feryal khal 2 which showed a lower value in trees of this variety.

The Fekas tree showed a value of the lowest petiole length among all trees. The Feryal Khal 2 tree showed the highest values of OS distances (Petiolar sinus distance at the upper right chest) and OS1 (Petiolar sinus distance at left chest) and BN2 (tooth base located at the end of N2) and BN21 (Base of the tooth at the end of N2 '). View the symmetry of the leaf of the vine one can think that certain distances like (OS, OS1, OI, OI1, HN2, HN21, HN4, HN41, BN2, BN21, O4N5 and O41N51) will bring the same information. Thus, the parameters OS, OI, HN2, HN4, BN2, OO3 and O4N5 on the right side would be respectively symmetrical with the parameters OS1, OI1, HN21, HN41, BN21, OO31 and O41N51 on the left side. However, we note that the values on the right side are different from the values on the left side.

Mean angles

We calculated the average values of the angles for the leaves of the different trees according to the SuperAmpelo software data. The number of angle variables measured is very important, 20 variables. The averages obtained show significant variations not only between trees of different varieties but also between trees of the same variety. Thus, for example, the parameter AL (Angle (α) between N1 and N2 measured at the first bifurcation), it has shown that the value of the highest angle (α) is observed in the tree Taferyalt Khal 3 and on the contrary, the Taferyalt Kahla 8 tree shows the lowest value in this variety.

The Ineb Byad1 tree recorded the largest angle value (α) of all varieties, whereas the Zbarjel tree showed the lowest angle value (α). The sum of the angles showed a slight difference between the trees. Like the distances, the parameters of the angles also have their symmetrical parameters. This is the case of angles (α', β', ɤ'and ε'). The study of the characters relating to the angles (α', β', ɤ'and ε') shows a wider opening in Aferyal Byad2 with 75.96°. For the angle (β '), the maximum is observed in the Maticha mferqa tree with 20.73° followed by Aferyal Byad2 with 17.74° and Ineb Nhal with 17.72°. As for the angle (ɤ '), its maximum is reached at Bezoul El awda1 with 62.089°. However, the minimum value of the variable (ɤ ') varies between 40.91° for Boukhanzir1 and 44.562° for Boukhanzir3. We obtained an angle π (angle of opening of the petiole measured between SP and SP ') very open in trees Bezoul El awda2 and Zbarjel with respectively 88.56° and 82.8° which reflects on the degree of opening of the petiolar sinus. The low values are those obtained by Ineb byad1 with 16.05° and Aferyal Byad2 with 17.58°.

Averages of the ratios between variables

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We calculated the average ratios for the leaves of different trees. The ratio variables were calculated automatically by the software used. The number of ratios calculated is very important, 16 ratios. The averages obtained show significant variations not only between trees of different varieties but also between trees of the same variety. The parameter RS (The ratio of the petiole sinus distance to OS in the upper right and the length of the N2 rib) showed the highest value in the Dibani1 tree and the Fekass tree. On the other hand, the Maticha Mferqa tree and Mouska Hamra2 showed the lowest value. The parameter ALBEOSOI (The ratio of the sum of the angles AL + BE and the sum of the distance between the sinus of the petiole and OS at the top right and the sinus of the petiole and bottom right OI) showed the same value (0.08) in 11 trees. Likewise for parameter ALBEOSOI1 (Ratio between the sum of the angles AL1 + BE1 and the sum of the distance between the sinus and the petiole OS1 upper left and the sinus of the petiole and lower left OI1) which shows a value of 0.05 at 18 trees and usually the values of the ratios are closer to each other.

Principal component analysis

We conducted a principal component analysis on the 39 trees surveyed. The result is shown in Figure 3. From the figure we see a very heterogeneous distribution of accessions. Some accessions of the same variety are grouped together, as is the case for some accessions of the Taferyalt variety. However the other accessions are not grouped around the same variety. This result is explained by the existence of the problem of synonymy and homonymies frequently encountered in local varieties. Synonyms, homonyms, similar names, and possible sampling and / or errors collection, proper identification can be complicated.

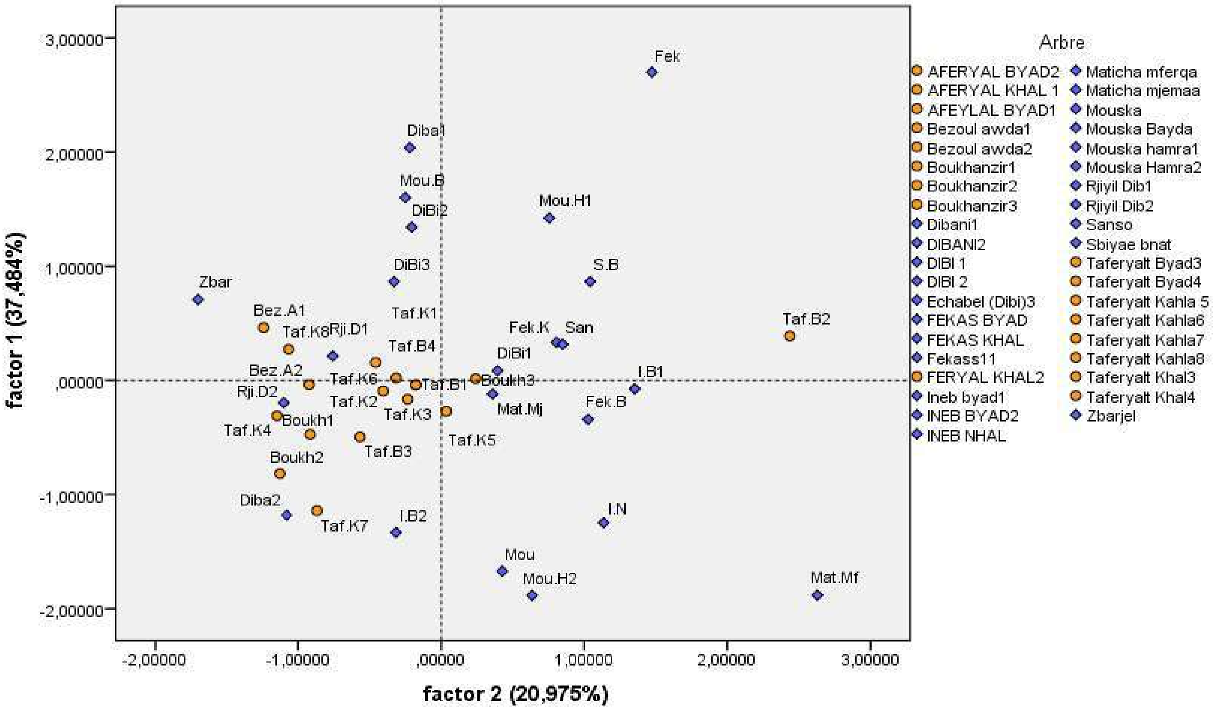

Fig.3: Graphical representation of the principal component analysis

The leaves of the vine have five main veins that start from the petiolar point. The relative dimensions of the ribs relative to each other and the angles that separate them are at the origin of a number of elementary forms of limbs: wedge-shaped, cordate, pentagonal, circular and reniform. But the enormous variability of other traits such as lobes, teeth, petiole sinus, hairiness, pigmentation, makes the leaves the organs of choice for variety differentiation [41].

In a similar study [42], on the analysis of the efficacy of discrimination of certain phyllometric parameters of indigenous *V. vinifera* cultivars in Croatia and with a similar methodological approach, they obtained results which go in the same direction as those we got. According to [43], the size of the leaf reflects the vigor of the plant. Among the feet studied, the foot Ineb byad1 has the longest leaf with a value of 62.43 cm followed by Fekas khal and Mouska with respectively 58.77 and 58.58 cm. The rest of the feet express intermediate values. Ampelography remains the main and unavoidable tool for the identification of grape varieties [44], but the morphological characters are influenced by environmental factors, such as soil properties [45], water availability and salinity [46], the nature of the rootstock, the level of nutrition [47] and the health status of the plant [48]. Thus, it must be remembered that this method of morphological characterization is not sufficient but must be completed by molecular characterization.

IV. CONCLUSION

This study represents a model for the analysis of discriminant of certain phyllometric parameters for the discrimination of native *Vitis vinifera* cultivars. The result of the Principal component analysis has been satisfying for some accessions that group together author of the same variety. The use of morphological parameters remains the first step in cultivar characterization. But we need to be further elucidated by studying the Moroccan grapevine with molecular methods.

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Characterization of grape berries of same local varieties in Morocco

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***Abstract*— Ampelography is the first step in grapevine selection, in establishing the relationship between grape cultivars. In this study grape berries collected from grapevine grow in the Northwestern of Morocco. A total of 15 grape berries from 39 accessions were characterized using OIV descriptors. We calculate the Average quantitative parameters of the all berries collected. The analysis of variance for the quantitative parameters of the berries shows that the ratio Long/Width of the berries is the only character that shows the significant variations. A highly significant correlation of length with width and weight can be observed with respectively 0.860 ** and 0.873 **. Principal Component Analysis with Quantitative Berry Parameters show some trees of the same variety are grouped together but they are no a clear structuring of trees according to their varieties; this may be due to the problem of synonymies and homonymies generally very common in local varieties.*

***Keywords*— Vitis vinifera cv, Grape berries, synonymy, homonymy, Morocco.**

I. INTRODUCTION

The cultivated grape is believed to have been domesticated around 4000 BC from a perennial wild grape originally classified as V. sylvestris C.C. Gmelin occurring from north-eastern Afghanistan to the southern borders of the Black Sea and the Caspian Sea [1], [2]. However, based on a recent archaeological finding in the Zagros mountains of Iran, [3] suggested 5400–5000 BC as the probable period of domestication of the grape. Currently, over 6000 cultivars are documented, including wine, table and raisin types [4]. Moreover, the wide distribution and long cultivation history of the grape have led to the development of numerous cultivars that have many synonyms, a problem that plagues germplasm collections [5], [6]. Traditional methods of describing grape vine varieties based on the plant’s vegetative and reproductive traits (ampleography) have contributed greatly to establishing the identity and relationships among *V. vinifera* cultivars [7], [8], [9], [10]. Nevertheless, ampelographic traits are often plastic, with a large genotype–environment interaction component rendering them less useful in classifying closely related cultivars. Nevertheless, cultivar names are often ambiguous owing to transliteration, the substitution of local or regional names for the original cultivar names, the presence of variants within cultivars (clones) and poor documentation of passport data, which includes ecogeographical, climatological and ethnographic information associated with germplasm accessions. The number of methods to classify and identify grape vine varieties has increased rapidly in the last two decades. Ranging from classical IUV-IBPGR-UPOV charts [11] (e.g. Anonymous 1983) to isoenzymatic markers [12], [13] or molecular characterization by DNA analysis [14], [15], [16], numerous methods to distinct between the different grape vine genotypes have been proposed. In Morocco, the grapevine as well as the olive tree, the fig tree and the cereals are cultures well adapted to the natural climatic conditions of our country located at the end of the western Mediterranean. During the last century, several factors have led to a progressive extinction for many local grape varieties and consequently to their genetic erosion. Currently, these varieties have become rare, little known, not inventoried, unexplored and threatened with extinction. Little study in Maghreb was carried to describe the cultivars of grapevine [17], [18], [19], [20]. This is not a study on the characterization of local berries of vines has not been made. Although the grape berries play an important role. The epicuticular wax layer of grape berries not only plays an important physiological role during berry development, but also impacts on the economic aspects of all viticultural commodities. The wax bloom scatters light and imparts a frosted appearance to the berry [21], which is considered attractive and desirable by consumers of table grapes [22].

After a short introduction to *vitis vinifera* cv and the methods of her characterization, the present work shows results obtained by using the grape berries for characterization of native cultivars of grapevine from Morocco.

II. MATERIAL AND METHODS

Grape berries (*Vitis vinifera L. cv*) were collected at frequent intervals from vines grown in the North –West of Morocco. All of the plants were classified as minor or endangered varieties. The total number of accessions studied was 39 and 15 grapes berries for each tree. Ampelographic characters were described using OIV descriptors [23], [24]. Sampling was done at the time of fruiting. In each site surveyed and with the help of the farmers we collected for each variety named and recognized samples of fruits.

Principal Component Analysis (A.C.P) was done using SPSS Version 10 software.

III. RESULTAT AND DISCUSSION

Average quantitative parameters of the berries:

We measured for each berry: length, width, length-to-breadth ratio, weight, number of pips and Brix (sugar content).

In berries average lengths vary between 20.33 mm in Bezoul Elawda tree 2 and 11.48 mm in Zbarjel tree. Average widths varied between 17.89 mm in the Boukhanzir2 tree and 10.85 mm in the Fekas tree. The length-to-width ratio that gives an idea of the shape of the berry is greater than 1 in all trees except Taferyalt khal4, Maticha mferqa and Maticha mjemaa. For the average weight of the berries it varies from 0.70 g among the accessions of the variety Fekkas to 4.55 g at Muscat bayda. The number of pips is one in the feet Mouska Hamra1 and Fekas, one to two in the feet Taferyalt Kahla5, Aferyal Byad2, Taferyalt Byad4, Maticha Mferqa, Muska, Mouska Hamra2, Ineb Nhal, Fekas Khal, Ineb Byad2 and Boukhanzir2, from three to four pips at Bezoul El awda2 and Dibani2 and finally from two to three in the remaining feet. Sugar or brix ranges from 55% in Rjiyil dibi 1 and Taferyalt Kahla 3 vines to 29% in Feryal khall trees (Table.1).

Table 1. List of the quantitative parameters of the berries

Variety	Long (mm)	Width (mm)	Long/Width.	Weight (g)	Number of seeds	Brix
Feryal Khal1	17.494	16.081	1.087	3.5	2.67	29
Feryal khal2	17.444	15.665	1.113	3.214	2.06	51
Taferyalt kahla3	15.839	15.658	1.011	3.016	2.4	55
Taferyalt Kahla4	14.546	14.588	0.997	2.616	2.9	47
Taferyalt Kahla5	14.179	13.706	1.034	1.849	1.94	38
Taferyalt Kahla6	17.371	16.132	1.076	3.094	2.27	50
Taferyalt kahla7	14.8	14.393	1.028	2.156	2.6	40
Taferyalt kahla8	16.41	15.87	1.034	2.83	2.67	46
Aferyal Byad1	17.617	15.284	1.152	3.002	2.6	42
Aferyal Byad2	17.667	16.305	1.083	3.264	1.8	35
Taferyalt Byad3	13.061	12.361	1.056	1.718	2	49
Taferyalt Byad4	13.301	12.628	1.053	1.616	1.5	45
Dibi 1	18.302	16.908	1.082	3.64	2.06	45
Dibi 2	18.09	16.573	1.091	3.442	2.06	37
Echabel(Dibi)	17.028	16.857	1.011	3.51	2.8	37
Dibani 1	14.119	13.975	1.011	1.966	2.13	35
Dibani 2	13.643	11.773	1.158	1.491	3.13	46
Maticha Mferqa	11.536	12.381	0.931	1.362	1.2	47
Maticha Mjemaa	11.617	13.461	0.863	1.415	2.8	40
Mouska	16.975	14.873	1.141	2.627	1.87	35
Mouska Bayda	19.665	17.521	1.122	4.557	2.4	38
Mouska hamra1	14.792	12.532	1.183	1.848	1	46
Mouska hamra2	18.692	15.222	1.227	3.079	1.27	39
Ineb Nhal	13.246	12.506	1.059	1.478	1.8	35
Fekas khal	19.519	18.007	1.083	4.313	1.87	32
Fekas Byad	16.574	14.591	1.135	2.679	2.73	37
Fekas	11.535	10.85	1.063	0.708	1.1	38
Ineb Byad1	18.046	16.595	1.087	3.484	2.27	35

Ineb Byad2	16.697	15.557	1.073	3.203	1.93	43
Bezoul awda 1	16.195	11.035	1.467	1.709	2.6	40
Bezoul awda 2	20.338	15.712	1.294	3.305	3.27	42
Boukhanzir1	20.093	17.899	1.122	4.447	3.2	42
Boukanzir2	14.51	12.984	1.117	1.626	1.33	42
Boukhanzir3	16.868	15.997	1.054	3.128	2.6	40
Sbiyae Bnat	12.959	12.166	1.065	1.595	2.6	39
Rjiyil Dib 1	17.133	15.152	1.132	2.687	2.6	55
Rjiyil Dib2	14.557	13.968	1.042	1.997	2.47	47
Zbarjel	11.487	10.968	1.047	0.99	3	35
Sanso	15.327	13.289	1.153	1.967	2.47	40

Analysis of the variance of the quantitative variables of the berries:

We performed an analysis of variance for the quantitative parameters of the berries. The result obtained (Table 2) showed that the ratio Long / Width. of the berries is the only character that shows significant variations. The Long / Width ratio expresses the shape of the berries.

Table.2: Results of the analysis of the variance of the quantitative parameters of the berries

ANOVA à 1 facteur		
	F	Signification
Long/Larg. (B)	7.550	.000
long	2.016	.063
poids	1.962	.071
larg.	1.611	.147
Brix	.978	.502
Nbre de pépins	.825	.638

Analysis of the correlations of the quantitative parameters of the berries

We performed a correlation analysis for the quantitative parameters of the berries. Table.3 *shows* the results obtained.

Table.3: The correlation between the quantitative parameters of the berries

	Long (mm)	Width (mm)	Long/Width	Weight (g)	Number of seeds	Brix
Long	1					
Width.	.860**	1				
Long/Width.	.438**	-0.038	1			
Weight	.873**	.898**	0.172	1		
Number of seeds s	0.107	0.13	0.051	0.174	1	
Brix	-0.101	-0.078	-0.053	-0.087	-0.065	1

The correlation is significant at the 0.01 level (bilateral)

Thus, a positive and highly significant correlation of length with width and weight can be observed with respectively 0.860 ** and 0.873 **. Also a positive and highly significant correlation of 0.898 ** in width with weight.

Principal Component Analysis with Quantitative Berry Parameters

We used the means of the quantitative parameters of the grapes berries to perform a principal component analysis (Fig.1 & 2). Table 4 shows the percentages of the variance associated with each axis.

Table 2: Variances associated with the axes of the ACP of the characters of the grape berries

Total variance explained			
Component	Total	% of variance	% cumulated

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1	2.862	47.697	47.697
2	1.071	17.845	65.542
3	1.015	16.909	82.452
4	.931	15.523	97.975
5	.103	1.720	99.695
6	.018	.305	100.000

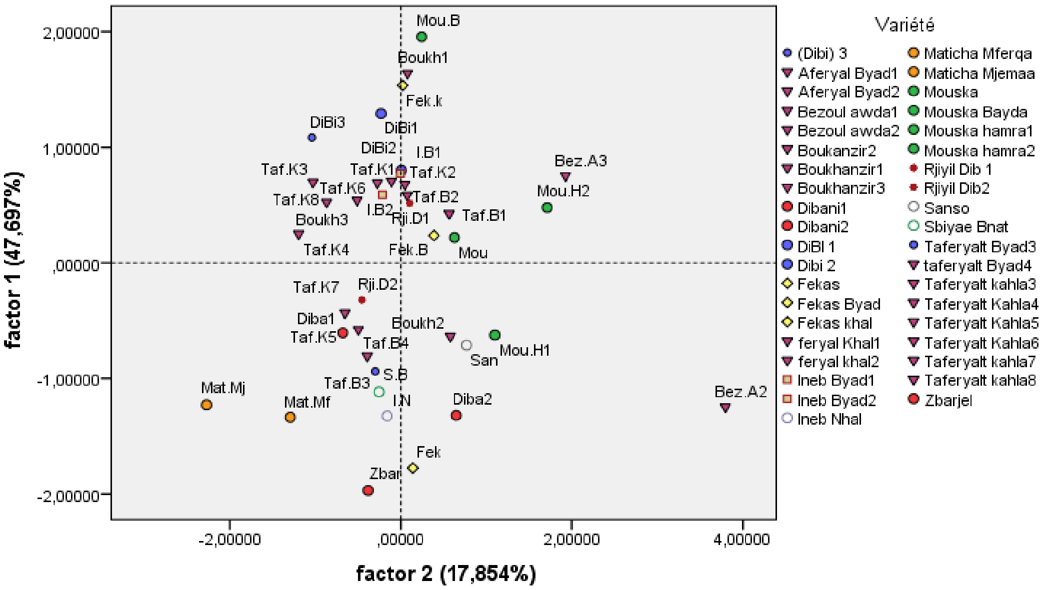

Fig.1: Projection in the plane (1,2) of the PCA of the quantitative parameters of the berries.

The figure .1 shows a not clear enough structuring of the various trees, In the trees of the Taferyalt variety we notice the formation of two groups like that of the previous ACP, but the points are almost all in the negative side of the axis 1 except the Taf tree. B1. We found it useful to eliminate the different trees of the Tarferylt variety and repeat the analysis without them, the result shown in figure .2.

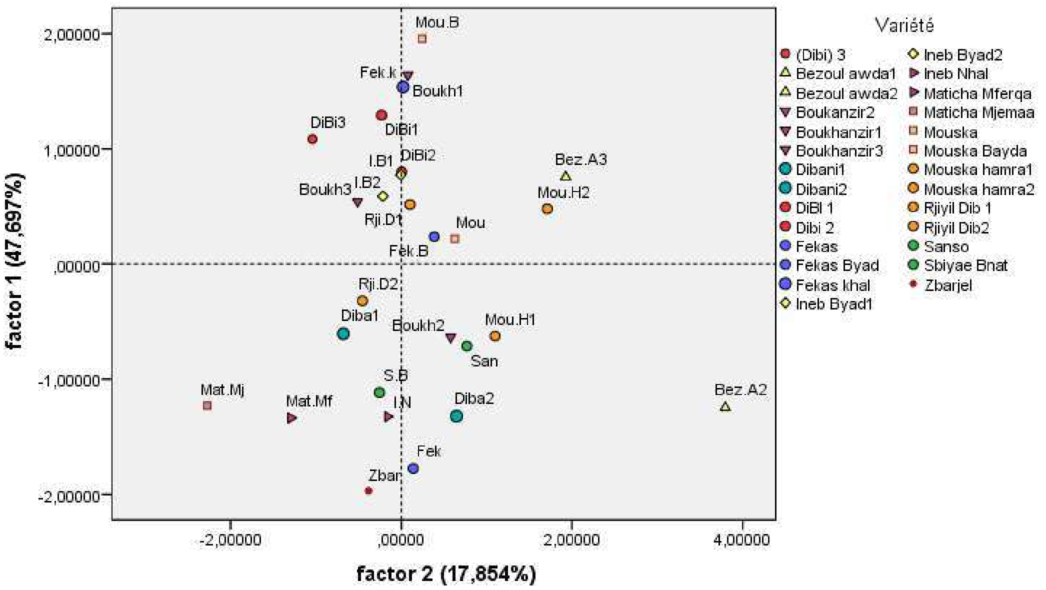

Fig.2: Projection in the plane (1,2) of the PCR of the quantitative parameters of the bays. (Without Taferyalt)

From Fig. 2. We Note that some trees of the same variety are grouped together. This is the case, for example, of trees of the Dibi variety. Also we noticed that the two trees of the two varieties Maticha are a little closer. In general we do not see a clear structuring of trees according to their varieties; this may be due to the problem of synonymies and homonymies generally very common in local varieties. Grape (berry) has different shapes depending on the variety: it can be globose, flattened, elliptical, ovoid, elongated, etc. The shape of each grape is characteristic of the variety, the flesh or pulp is generally colorless, only some varieties have a tinted flesh; this flesh contains important percentages of sugar, various acids, and minerals. In general, the seeds, or seeds of the vine, are generally one or two, sometimes three, and exceptionally four.

IV. CONCLUSION

The use of berries for the characterization of trees of different local varieties proved to be a good means for the distinction between local varieties, however the low number of trees studied as well as the problem of synonymy and homonymies frequently encountered in local varieties leave this identification not weak. In the future this study must be completed by molecular analysis for better identification.

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Influence of Drying Methods on the Drying Characteristics and Nutritional Quality of Fermented Locust Beans

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***Abstract*— *This study was designed to give the drying characteristics of fermented locust beans using different drying methods. The production of good quality of fermented locust beans is essential, having known its rate of spoilage. Different methods have been used over time and this has probably affected the quality of stored fermented locust beans. This study is meant to guide the producer in selecting the best drying method to be used for processing of fermented locust bean. Three different drying methods were used: the mechanical (convective) dryer, indirect (cabinet) solar dryer and sun drying. The mechanical (convective) drying experiments were conducted for four air drying air temperatures at 45°C, 55°C, 65°C and 75°C at air velocity of 0.9 m/s. During the drying process, the weight loss was measured at 30 min intervals for all the drying methods. The result was used to determine the moisture content, moisture ratio and the drying rate. The lowest moisture content was at the mechanical drying (75°C) due to the high air temperature than the other drying methods. The constant rate and the falling rate were observed at the early hours of the drying for the mechanical drying method. The drying rate also varies with the drying methods. The mechanical drying at air temperature of 55°C at air velocity of 0.9 m/s of the fermented locust beans gives the best quality.*

***Keywords*— Locust bean, Nutritional Value, Moisture content, Drying characteristics.**

I. INTRODUCTION

The African locust bean, *Parkia biglobosa,* is an important tree crop in Africa. The tree is a perennial one and its life cycle is for many years. It is grouped under the leguminous crops family called the *leguminosae*, now called *fabaceae,* and further under the sub-family *mimosoideae*. It is a natural nutritious condiment which is common in the traditional diet of both rural and urban dwellers in West-African countries including Nigeria. Locust bean seed is the matured fruit seed that comes from the parkia tree. It is harvested and processed into a fermented product. *Parkia biglobosa* seeds serve as a source of useful ingredients for consumption. It has been reported that the husks and pods are used for feeding livestock, and the floury pulp can be used as a refreshing drink, which is rich in vitamin C and sugar. Apart from its excellent caloric and nutrient value, its wide adaptability, drought resistance, and multifunctional usage makes it a sustainable source of its by-products. It is a multipurpose tree of which most parts are useful.

The most important part of the tree is found in its seeds which are processed into food condiment. Apart from the flavouring attribute of the processed locust bean, it also contributes significantly to the intake of protein, carbohydrate, calcium, phosphate, iron content and essential fatty acids [1,2,3,4,5]. The seed has high protein content (40%), high vitamin, moderate fat content (35%), carbohydrate and macronutrients such as potassium, sodium, magnesium, calcium, nitrogen and phosphorus [6]. The processing of locust bean into food condiment is mainly done manually which makes it tedious for large scale production. The daily demand for this condiment is on the rise because of its nutritive and medicinal benefits. The raw African Locust beans are nutritionally deficient but when fermented into food condiment, Iru, the physical, chemical and nutritional characteristics of the seeds improves [7]. Fermentation improves the digestibility of many foods, increases nutritional values, and provides important living enzymes and beneficial microorganisms to our diet. There is a breakdown of certain constituents, reduction of anti-nutritional factors in grains and the synthesis of B-vitamins [8].

Drying is a method of food preservation which removes moisture from the food to avoid the growth of bacteria, yeasts and molds. It also helps in slowing down the action of enzymes because it removes moisture. People have

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been preserving food through drying for thousands of years, because dried food yields maximum quantity for the least volume [6,7]

II. MATERIALS AND METHODS

Processing Procedures of Locust Bean Fruits

The fermented locust bean was processed following the procedure presented on the flow chart in Figure 1.

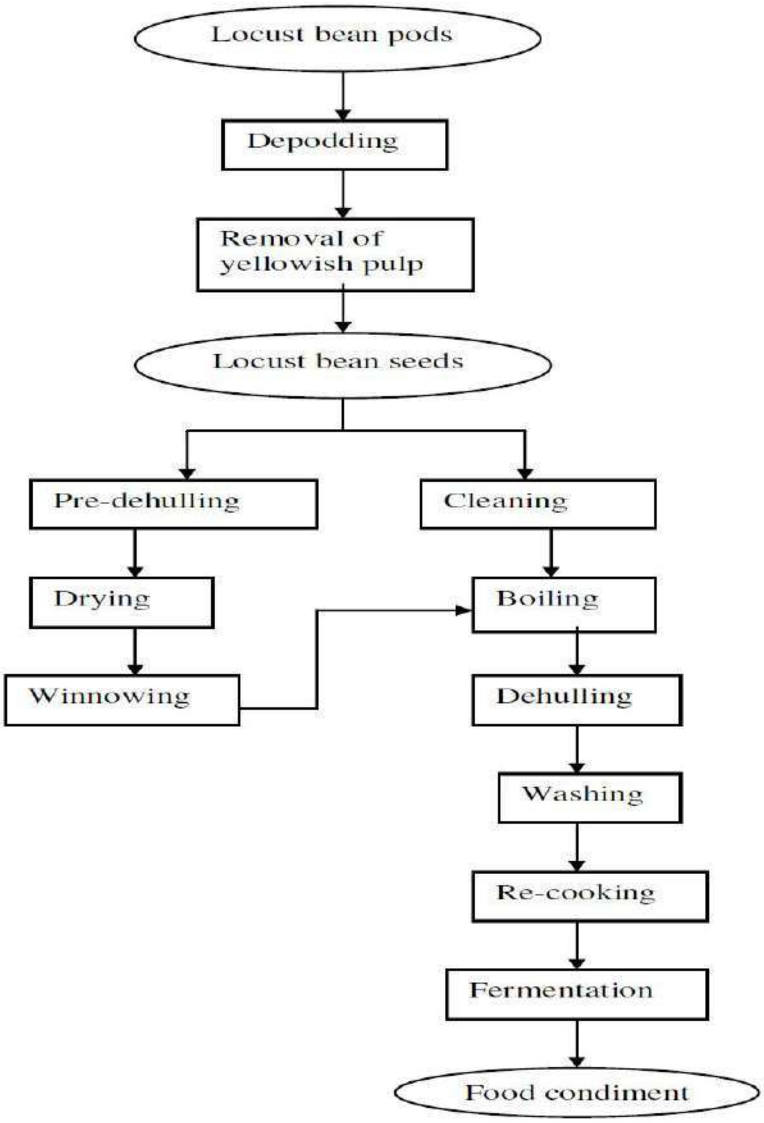

Fig.1: Flow chart for traditional processing of locust bean fruits to food condiment [9]

Types of experimental dryer

Two different experimental dryers were used, they are, indirect solar dryer and mechanical cabinet dryer

Drying with mechanical dryer

100 g of fermented locust beans sample was placed in a perforated tray of the cabinet dryer as shown in plate 3.3. The samples were subjected to drying at 45°C, 55°C, 65°C and 75°C at air velocity rate at 0.9m/s. And the weight loss were measured at an interval of 30mins until there is no further reduction in weight, the moisture content of the sample was determined by an oven dry method.

Open sun drying

100 g of fermented locust beans was loaded in a net and was placed inside a tray. The initial weight of the condiment was noted and the subsequent weights were taken at the intervals of 30 mins. A sensitive weighing balance was used for the weight measurement.

Indirect solar drying

100 g of fermented locust beans was placed inside the tray that is inside the solar dryer. The initial weight of the sample was taken and subsequent ones were taken at intervals of 30mins. A sensitive weighing balance was used for weight measurement and the temperature

regulator was used to determine the temperature inside the dryer.

Temperature measurement

The dry air and ambient temperature were taken using a thermometer and were recorded in the data book.

Determination of initial moisture content

10 g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was transferred into the oven set at 102°C to dry for 24 hour, the crucible plus the sample was removed from the oven and transferred to desiccators, cooled for ten minutes and weighed. The moisture content was calculated as percentage moisture (AOAC 2005)

Proximate Composition

The crude protein content, crude ash, crude fat, crude fibre and carbohydrate content of the samples were determined using standard methods as reported by [10].

III. RESULTS AND DISCUSSION

Moisture content of the locust beans

The average initial moisture content of the fresh fermented locust beans was 61.1 % (wb). This value agreed well with reports from literature [11].

Weather Condition

During this experiment, the weather condition was slightly favorable for both the indirect solar drying and the sun drying. The solar radiation energy is maximum at midday and minimum at evening in the days of experiment. During the experiment, the ambient temperature ranged from 20- 38°C.

Performance of the Drying Methods

Table 1 shows the drying time, final moisture content and the weight of water in the sample after drying for all the drying methods. The mechanical dryer at air temperature of 75°C gave the least drying time. This indicates that, it takes a lesser time to dry at higher temperature.

Table.1: The drying time and moisture content at sun, solar and air temperature of 45, 55, 65 and 75◦c at air velocity of 0.9m/s.

Drying Methods	**Fresh locust beans(g)**	**Weight of water after drying (g)**	**Final molsture content**	Drying time (hour)
SUN	100.30	5.71	12.76	23
SOLAR	100.09	6.56	14.38	23
MEC 45°C	100.00	6.90	14.74	17
MEC 55°C	100.39	7.01	15.34	15
MEC 65°C	100.39	6.88	15.09	13
MEC 75°C	100.47	5.78	13.02	6

The indirect solar dryer

The maximum temperature obtained for the indirect solar dryer was 48°C and it took 23 hours to reach a final moisture content of 14.38% (dry basis (db)).

Mechanical drying characteristics of fermented locust beans

The plot of the drying characteristics curve of fermented locust beans is shown in Figures 1 to 3. The moisture content was 14.74, 15.34, 15.09 and 13.02 % at air temperature of 45, 55, 65 and 75°C respectively after the drying process. Figure 4.1 shows that the moisture content reduces with drying time. The mechanical dryer at air temperature of 75°C gave the least drying time while air temperature of 45°C gave the highest drying time. Figure 4.2 also shows that the higher the drying air temperature, the faster the drying process. The falling rate follows after the constant rate for all the samples at air velocity of 0.9 m/s. The constant-rate period occurred in a very short time in comparison with the whole drying time.

Figure 2 and 3 show that the drying rate reduces with increasing drying time. The highest drying rate was observed during the early hour for all the drying air temperatures. The final drying rates are 0.18, 0.02, 0.3 and 0.03 kg/hr for drying air temperature of 45, 55, 65 and 75°C respectively. However, the peak drying rate was observed at air temperature of 75°C.As moisture is further removed, the falling rate curve is observed.

As the moisture reduces, more energy is needed to remove the inner moisture which gives rise to the falling rate. Since the energy supplied for drying is constant, there was a reduction in the drying rate. The initial drying rate was high because moisture for evaporation came from regions near the surface. As drying continues, the drying rate gradually decreased because water to be evaporated must be transported from the inner layers to the surface of the material. Therefore, the falling rate region expressed a resistance to heat and mass transfer inside the material [12]. This result is similar to the observations on the drying of garlic slice [13] and onions slices [14]

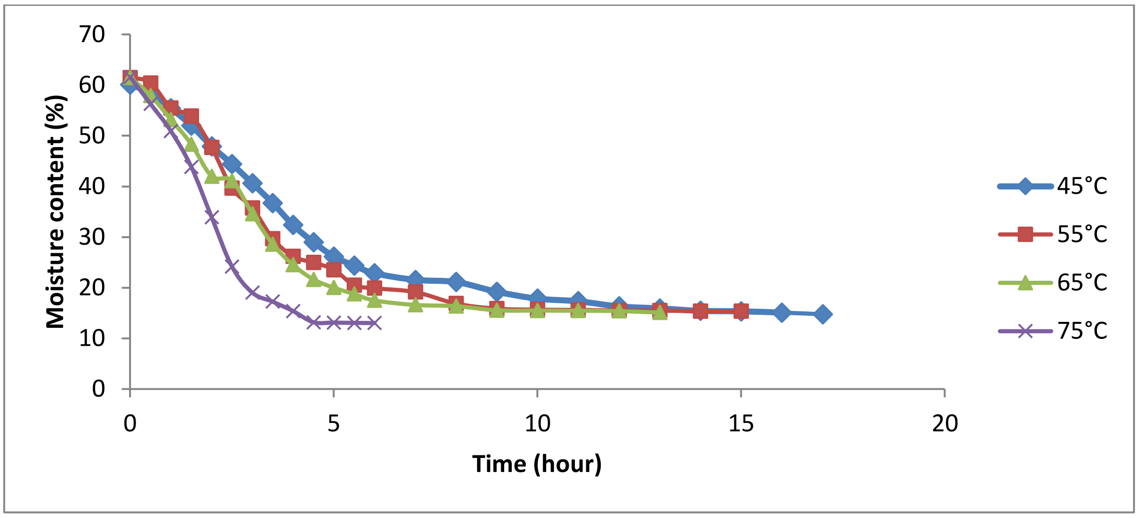

Fig.1: Variation of moisture content with drying time for mechanically dried fermented locust beans.

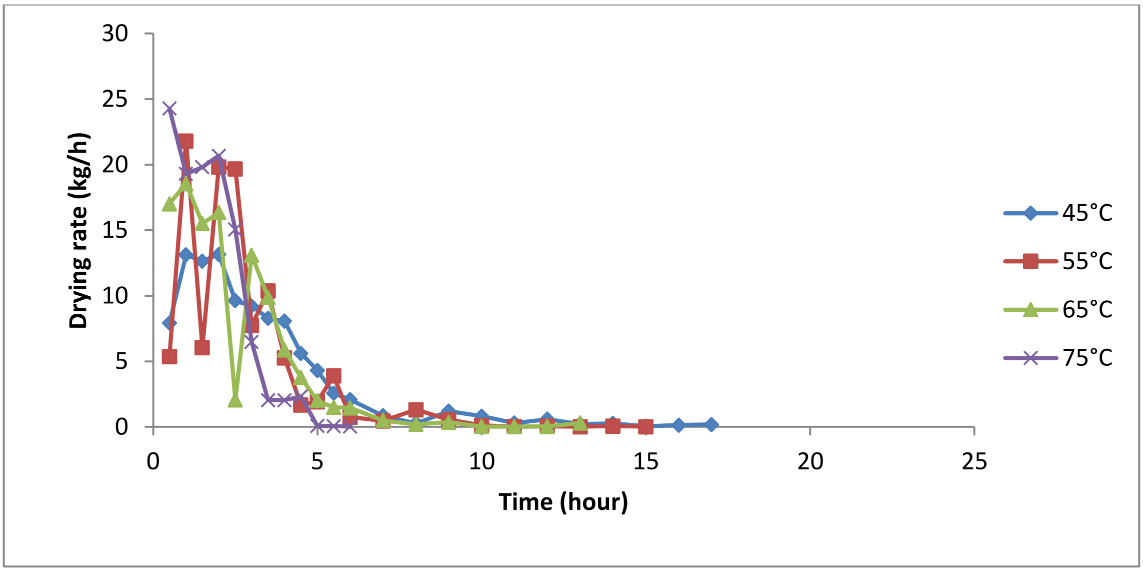

Fig.2: Variation of drying rate with drying timefor mechanically dried fermented locust beans.

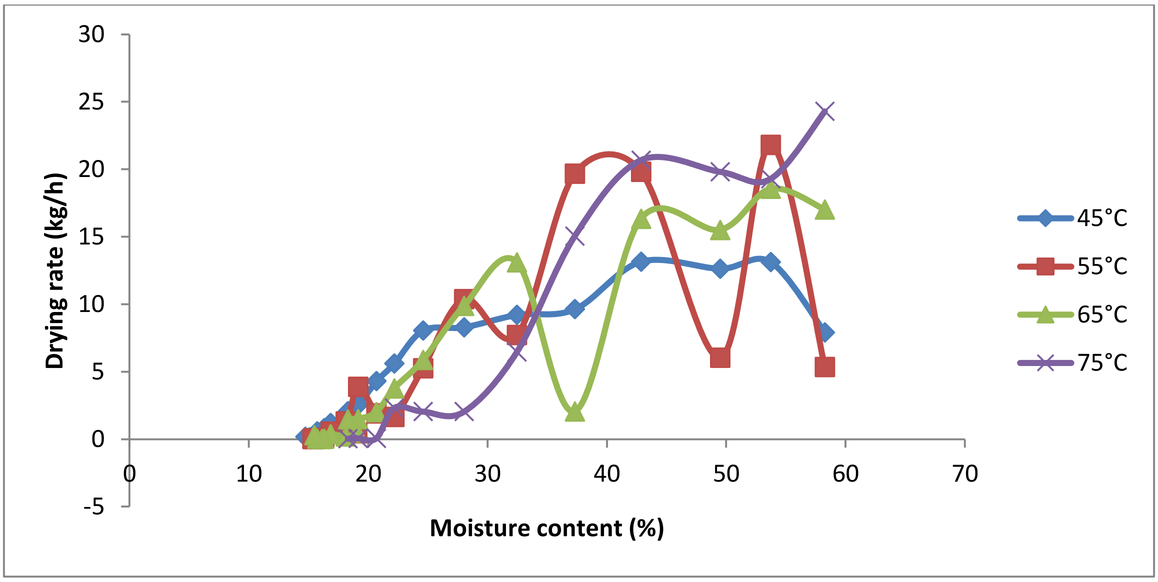

Fig.3: Variation of drying rate with moisture content for mechanically dried fermented locust beans.

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Solar drying characteristics of fermented locust beans

The plot of the drying characteristics curve for solar drying is shown in Figures 4 to 6. The moisture content curve against time is shown in figure 4. It shows a reduction in moisture content of the wet fermented locust beans with time.

There was fluctuation in the drying rate against time as shown in figure 5. This may be due to the rise and fall of the solar radiation due to the weather condition. The drying process occurred mainly at the falling-rate drying period because there was almost no constant-rate drying period. This indicates that the mode of mass transfer in the material was a moisture diffusion process [15]. It was also noted that after undergoing a short heating period, the drying rate significantly increased. Moisture for evaporation has to come from the regions near the surface. As drying continues, the drying rate decreases as water to be evaporated must be transported from inner layers of the material to the surface layer. Therefore, the falling rate region expressed a significant increase in resistance to heat and mass transfer inside the material [12]. The drying rate observed in this research was similar to the results reported on the thin layer solar drying process of grapes [16] and apricots [17, 18].

Sun drying characteristics of fermented locust beans

The plot of the drying characteristics curve for sun drying is shown in Figures 7 to 9. The moisture content curve against time is shown in Figure 7. The curve shows a reduction in moisture with time.

There was fluctuation in the drying rate against time. This may be due to the rise and fall of solar radiation due to the weather condition. The drying process also occurred at the falling-rate drying period because there was almost no constant-rate drying period. During the drying process, water is removed from the wet fermented locust beans to the environment along time, till equilibrium is reached. According to several authors, air drying of food always have a short or no constant rate period, followed by a long falling-rate period This may be to the fact that most foods are dense solids with low porosity, and so molecular diffusion of the liquid through the product is limited.Moreover, the continuous evaporation of surface moisture could lead to formation of a hard surface layer which reduces the moisture removal from the material, thus reducing the drying rate [19, 20, 21, 22].

The drying rates were higher at the beginning of the drying process and later decreased with decreasing moisture content. [23] had similar occurrence in the study of the drying kinetics of open sun drying of fish. As indicated in the curves of Fig. 6, the drying occurred mainly under falling rate of drying period. During the falling rate period, the predominant mechanism of mass transfer in the sample is that of internal mass transfer. This result also agree with the study of thin layer drying of olive fruit [24].

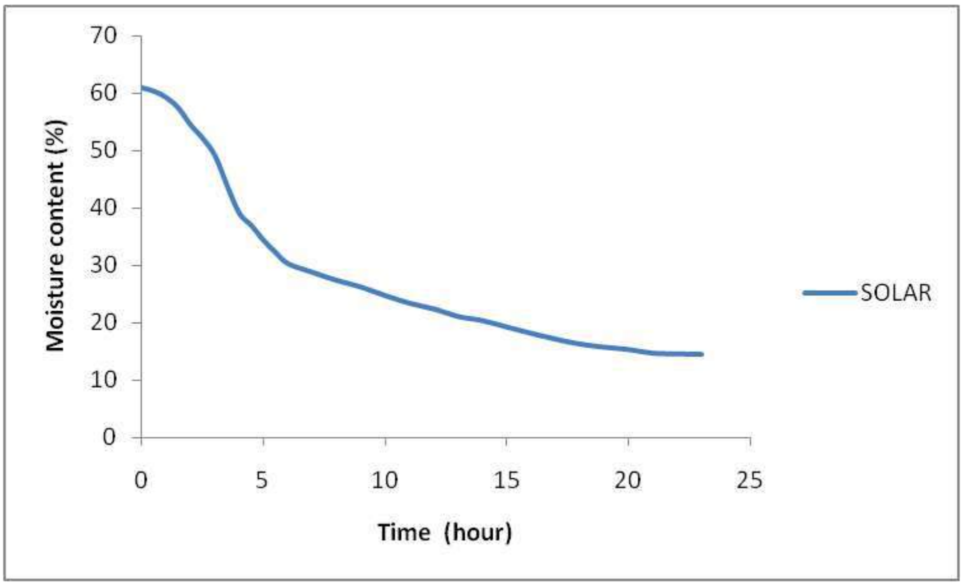

Fig.4: Variation of moisture content with drying time for solar dried fermented locust beans.

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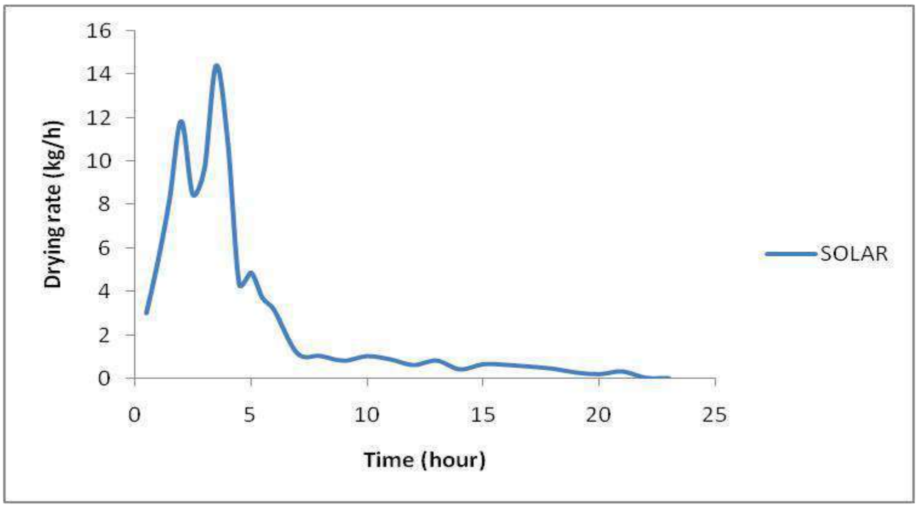

Fig.5: Variation of drying rate with drying time for solar dried fermented locust beans.

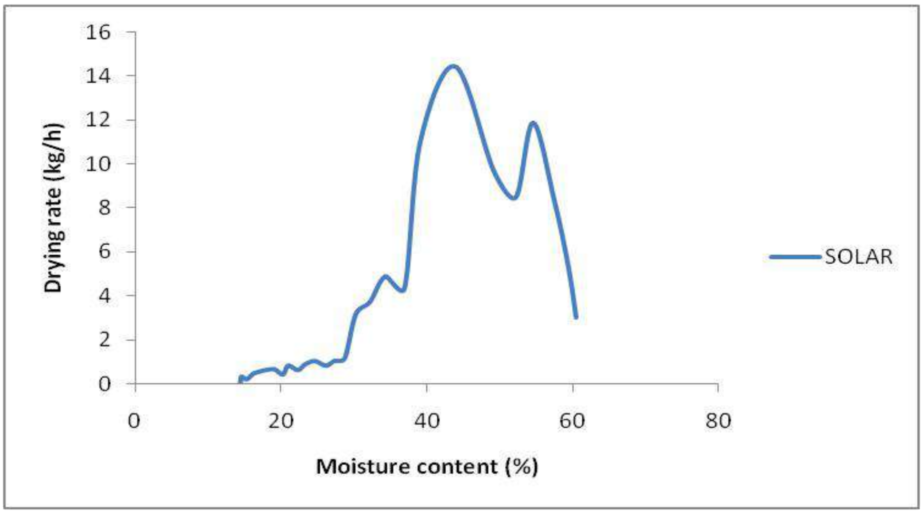

Fig.6: Variation of drying rate with moisture content for solar dried fermented locust beans.

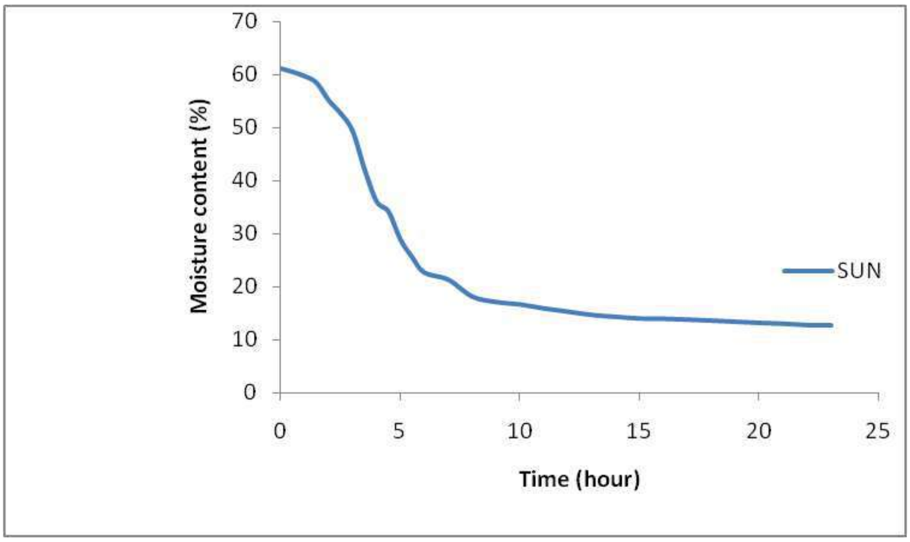

Fig.7: Variation of moisture content with drying time for sun dried fermented locust beans.

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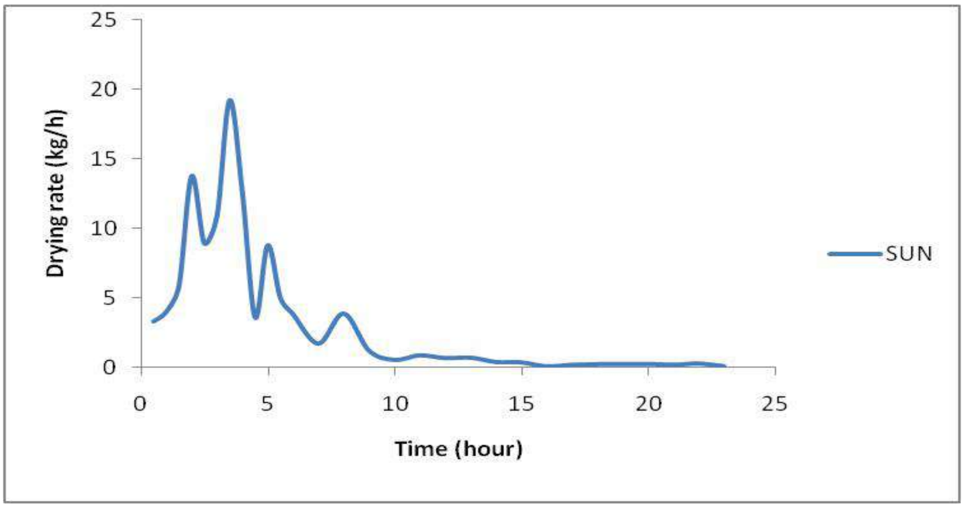

Fig.8: Variation of drying rate with drying time for sun dried fermented locust beans.

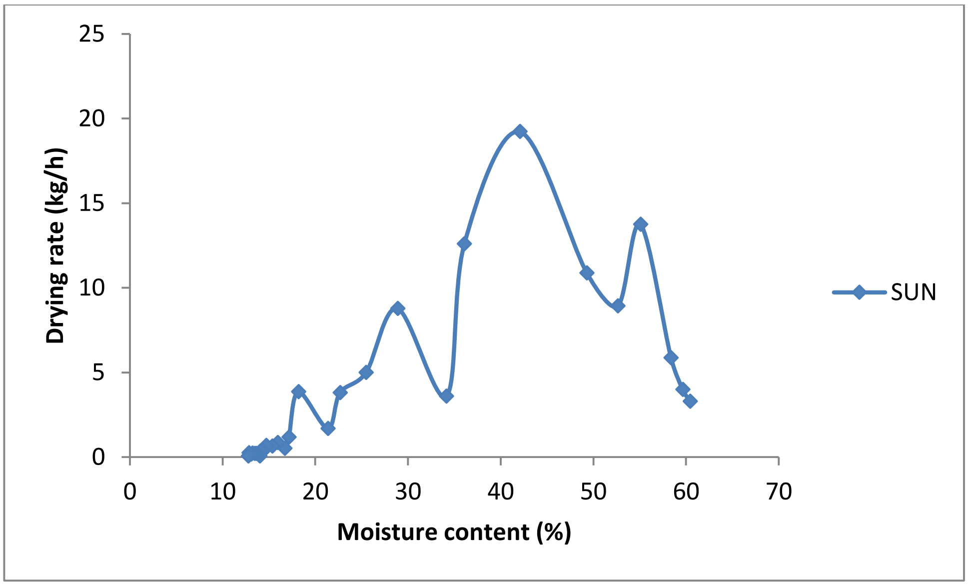

Fig.9: Variation of drying rate with moisture content for sun dried fermented locust beans.

NUTRITIONAL QUALITY OF DRIED FERMENTED LOCUST BEANS

Quality of mechanically dried fermented locust beans

Table 2 shows the result obtained from the proximate analysis of the wet and dried fermented locust beans. The moisture content of the dried fermented locust beans are 14.71, 15.30, 15.09 and 13.00% at 45, 55, 65 and 75°C respectively. The results showed that there were significant ($P < 0.05$) differences between moisture content of the wet fermented locust beans and the dried ones. The result shows the ash content of the samples ranges from 2.68 to 2.95%.Differences in ash may be due to variation in drying air temperature. The carbohydrate content of the samples increased from 24.08 to 43.65%. The result agreed with the result of the proximate composition of fermented locust beans reported by [25]. The variation may be due to differences in air temperature. It was reported that African locust beans is rich in plant protein and essential fatty acids [26]. The results showed that there were significant ($P < 0.05$) differences between the crude protein of the wet fermented locust beans and the dried ones. The condiment at air temperature of 55°C has the highest crude protein content of 27.81% and crude fat of 27.4% The difference in the levels of protein content obtained in this study may be attributed to differences in air temperature. [27] reported that crude protein contents of 29.00%, 32.00% and 37.34% were obtained for the raw, boiled and fermented locust beans respectively. The decrease in protein of the dried fermented locust beans may be due to the absence of proteolytic enzyme which is present in the wet fermented locust beans as reported by [28] There was a significant reduction in the proximate composition of all the mechanical air temperature dried samples compared to the wet fermented locust beans.

Quality of solar dried fermented locust beans

The proximate composition of the solar dried fermented locust beans is shown in Table 3. The following data were obtained, % moisture (14.33), % protein (26.44), % fat (27.55), % carbohydrate (38.38), % fibre (4.48) and % ash content (2.81). This data showed that, the solar drying has effect on the nutritional composition of the fermented locust beans. The nutritional composition of the beans was reduced due to the solar drying process. There was a significant reduction of the proximate composition of the dried locust beans compared to the wet fermented locust beans. [29] Reported a decrease in the nutritional composition in the solar drying of fresh amaranthus (tete), vernonia (ewuro) and fluted pumpkin (ugu).

Quality of sun dried fermented locust beans

The proximate composition of the sun dried fermented locust beans is shown in Table 3. The following data's were obtained, % moisture (12.78), % protein (25.75), % fat (27.41), % carbohydrate (39.82), % fibre (4.11) and % ash content (2.80). The condiment at the sun drying gave the least protein content. The proximate composition of the sun dried fermented locust beans showed that, the sun drying has effect on the nutritional composition of the fermented locust beans. There was a significant reduction in the proximate composition compared to the wet fermented locust beans. Similar report was given by [30] in the sun drying of fish.

Table.2: Nutritional value and physical characteristics of mechanically dried fermented locust beans

Samples	% MC	% ASH	% Crude Fat	% Crude Protein	% CHO	%Crude Fibre	**Aroma**
Fresh	55.22 ± 0.02^{e}	3.37 ± 0.02^{d}	35.19 ± 0.02^{d}	32.07 ± 0.02^{e}	24.08 ± 0.02^{a}	5.18 ± 0.02^{d}	Very Pronounced
MECH 45	14.71 ± 0.04^{b}	2.68 ± 0.03^{a}	27.39 ± 0.09^{c}	27.61 ± 0.07^{c}	39.03 ± 0.06^{c}	3.03 ± 0.03^{c}	Moderately Pronounced
MECH 55	15.30± 0.07^{d}	2.73± 0.06^{a}	27.4± 0.35^{c}	27.81± 0.01^{d}	38.51± 0.01^{b}	3.29± 0.01^{c}	Very Pronounced
MECH 65	15.09 ± 0.03^{c}	2.82 ± 0.01^{b}	25.82 ± 0.06^{b}	27.53 ± 0.02^{b}	40.35 ± 0.03^{d}	33.33 ± 0.29^{b}	Pronounced
MECH 75	13.00 ± 0.02^{a}	2.95 ± 0.04^{c}	23.7 0± 0.02^{a}	27.04 ± 0.03^{a}	43.65 ± 0.02^{e}	3.56 ± 0.01^{a}	Slightly Pronounced

Mean in each column bearing different letter are significantly different at 5% level of probability

Table.3: Nutritional value and physical characteristics of sun and solar dried fermented locust beans

Samples	% MC	% ASH	% Crude Fat	% Crude Protein	% CHO	%Crude Fibre	**Aroma**
FRESH	55.22 ± 0.02^{c}	3.37± 0.02^{c}	35.19± 0.02^{c}	32.07 ± 0.02^{c}	24.08 ± 0.02^{a}	5.18± 0.02^{c}	Very Pronounced
SUN	12.78± 0.10^{a}	2.8 0± 0.01^{a}	27.41± 0.02^{a}	25.75 ± 0.03^{a}	39.82 ± 0.05^{c}	4.11 ± 0.02^{a}	Pronounced
SOLAR	14.33 ± 0.05^{b}	2.81 ± 0.02^{b}	27.55 ± 0.04^{b}	26.44 ± 0.07^{b}	38.38 ± 0.11^{b}	4.48± 0.10^{b}	Pronounced

Mean in each column bearing different letter are significantly different at 5% level of probability

IV. CONCLUSION

The drying process of the fermented locust beans for air temperature of 75°C was the shortest being the highest air temperature.The moisture content and the drying rate decreases with the drying time for all the drying methods. The drying method has a significant effect on the proximate composition and the organoleptic properties of the fermented locust beans. To have maximal nutritional values from fermented locust beans, it is best to consume it fresh. However, for storage purposes, the mechanical drying method with drying temperature of 55°C at air velocity of 0.9m/s gave the best quality condiment.

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Effect of Relay-Planting Several Legume Species at Various Ages of Rice on Growth and Yield of Red Rice Grown Together with Legume Crops under Aerobic Irrigation System

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Abstract— *This study aimed to examine the effects of relay-planting several species of legume crops at various ages of rice plants on growth and yield of red rice grown together with legume crops in pot culture under aerobic irrigation systems. The pot experiment was carried out in a plastic house from August to December 2018, designed according to the Complete Randomized Design with three replications and two factorial treatment factors, namely legume species (s1= peanut var. Hypoma-3, s2= mungbean var. Kenari, s3= soybean var. Dering-1), and ages of red rice when the legume crops were relay-planted (u1= relay-planting at 1 week after seeding rice (WASR), u2= 2 WASR, u3= 3 WASR, and u4= 4 WASR). The results indicated that the ages of rice when the legume crops were relay-planted showed more significant effects on growth and yield components of the red rice compared with the legume species did, but there were no interaction effects between the two treatment factors tested. Among the various ages examined, relay-planting legume crops when the red rice age was 3 weeks resulted in the highest growth and yield components of the red rice examined, in which the mean values of tiller number (26.2 tillers/clump), panicle number (20.4 panicles/clump), filled grain number (1030 grains/clump), and grain yield (26.88 g/clump) were highest compared with relay-planting legume crops at other times. Among the species of legume crops examined, mungbean var. Kenari grown together with the red rice resulted in the highest average of red rice grain yield (25.99 g/clump) as well as the highest harvest index (47.01%) compared with the other legume species.*
Keywords— ***red rice, aerobic rice system, legume crops, relay-planting, intercropping***

I. INTRODUCTION

Red rice can be used as a high health value food source due to its higher contents of anthocyanins, fibers, and vitamins B and E, compared with white rice [1]. In addition, its economic values are also high so that increasing the productivity of red rice can be used to overcome various food, nutrition and economic problems, especially in Indonesia. Over the past few years, the need for red rice has continued to increase in accordance with the increasing people's awareness on the importance of health and recognition of the health benefit from consuming red rice instead of white rice. However, most of the existing red rice varieties are upland rice varieties, which are generally low in their average productivity [2]. Therefore, it is necessary to increase the productivity of red rice through application more productive rice cultivation technologies, one of which is by growing red rice under aerobic irrigation techniques, which was called as aerobic rice systems (ARS) [3]. One of the advantages of growing rice under ARS is that it can be intercropped with legume crops [4], and legume crops have the potentials to fix atmospheric nitrogen to increase nitrogen content of the soil, which can increase productivity and sustainability of crop production systems [5]. In relation to intercropping, Inal *et al.* [6] reported that maize-peanut intercropping could induce an increase in concentration of nutrients in their rhizospheres, and increased concentrations of nutrients in the shoots of the maize and peanut plants.

In relation to benefits of intercropping rice plants with legume crops in aerobic irrigation systems, results of previous researches have shown that growing-together rice plants in one pot or intercropping rice plants on raised-beds with legume crops could increase growth and yield of rice plants, either without or with application of mycorrhiza bio-fertilizers. For example, Dulur *et al*. [7] reported that various promising lines of red rice grown in pot culture under aerobic irrigation systems resulted in significantly higher numbers of leaves, tillers and filled panicles per clump compared with rice plants grown under conventional or flooded system. Grain yield of two genotypes of red rice was also significantly higher under aerobic irrigation than under flooded system in pot culture, either in intercropping with soybean or without soybean [8]. Wangiyana *et al*. [4] also reported that various rice genotypes including upland, amphibious and paddy rice genotypes grown together with soybean in pot culture under aerobic irrigation system produced greener leaves and higher percentages of tillers producing filled panicles compared with those grown in monocrop under aerobic irrigation system.

Those greener leaves of rice plants in rice-soybean system could be due to some transfer of biologically fixed-N from soybean to rice, as those reported by Chu *et al*. [9], who measured significant amount of N transfer from peanut to rice plants grown in intercropping under aerobic system, which resulted in higher N accumulation, chlorophyll content, filled tiller number, dry matter, and 1000-grain weight of rice plants in intercropping with peanut than in monocrop. Under permanent raised-bed growing conditions, Dulur *et al.* [10] also reported higher panicle number and higher filled grain number per clump in red rice intercropped with peanut than those in monocrop rice or conventional rice. Wangiyana *et al.* [11] also reported that additive intercropping various genotypes of red rice with soybean var. Dering-1 on raised-beds significantly increased rice grain yield, especially when inoculated with arbuscular mycorrhial fungi (AMF), compared with those in monocrop.

In addition to the effects of different varieties or species of legume crops intercropped with rice or cereal crops, the timing of relay-planting the legume crops relative to the ages of the cereal crops grown in cereal-legume intercropping systems also reported to have some effects on growth and yield of both cereal and legume crops in the intercropping systems. Farida *et al*. [12], for example, reported that delaying the relay-planting of peanut between double or triple rows of red rice plants in aerobic irrigation system on raised-beds increased panicle number per clump, compared with relay-planting one week after seeding the red rice. In intercropping maize with two legume cover crops (Mucuna and Canavalia) planted at 0, 2, 4, and 6 weeks after planting maize (WAPM), Lawson *et al*. [13] reported that planting the legume cover crops 6 WAPM resulted in the highest maize grain yield compared with planting legume earlier, but grain yields of the legume cover crops were highest in the earliest planting time and lowest in the in the latest planting time. In addition, Armstrong *et al.* [14] also reported that delayed planting of lablab bean, velvet bean and scarlet runner bean in intercropping with maize from 2 to 4 weeks increased maize grain yield, but decreased yield of the bean crops. In these two cases, both in [13] and in [14], it could mean that there was stronger competition by the legume crops for maize yields in the earlier planting time of the legume crops, or vice verse. Therefore, the timing of relay-planting of legume crops in intercropping with cereal crops may be very important in order to increase the crop’s yield through intercropping systems.

This research was aimed to examine growth and yield of red rice as affected by different relay-planting dates of several species of legume crops in intercropping or growing together with red rice in pot culture under aerobic irrigation systems in a plastic house.

II. MATERIALS AND METHOD

Design of experiment

The pot experiment in this study was conducted in a plastic house located in the experimental farm of the Faculty of Agriculture, University of Mataram, which is located in Narmada District of West Lombok, Indonesia, from September 2018 to January 2019. The experiment was designed according to the Completely Randomized Design, with three replications and two treatment factors, namely: species of legume crops grown together (intercropped) with red rice in a pot culture (s1= peanut var. Hypoma-3; s2= mungbean var. Kenari; s3= soybean var. Dering-1), and relay-planting dates (u1= relay-planting legume crops at 1 week after seeding rice (WASR); u2= 2 WASR; u3= 3 WASR; u4= 4 WASR). To be able to calculate the land equivalent ratio (LER) of grain yield per clump, pot culture of monocrop red rice and each legume crop was also established each with three replications.

Implementation of the experiment

The plastic pots used in this pot experiment were from a previous pot experiment of intercropping various red rice genotypes with soybean [4]. Each pot has 4 circumference side-holes of 9 mm diameter at 1 cm above the pot base for use as sub-irrigation holes. For this experiment, each pot was filled with 7 kg air-dried soil that has been sieved with 2 mm opening sieve, and watered up to the field capacity. The red rice genotype used in this study was the promising amphibious red rice line “F2BC4A86-3” (or “AM-G4”) selected from results of a previous research [2]. For planting the red rice, seeding the pre-germinated AM-G4

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seeds was done in a seedling tray filled with a mixture of soil and rice husk ash (1:1), which was thinned after one week to allow only two seedlings to grow per hole of the seedling tray. The same procedure was also applied to seeding of the pre-germinated legume seeds, but they were seed-coated with *Rhizobium* inoculant before seeding, and the timing of seeding was in accordance with the treatment of relay-panting dates.

Transplanting those seedlings (rice and legume crops) to the prepared pots was done at 10 days after seeding of each species. In an intercropping pot, red rice seedling and legume seedling were transplanted at 10 cm apart in the center of the soil surface in the pot. For fertilization of the crops, fertilizer type and doses, and timing of fertilization were the same as in the previous research [4]. Irrigation was also done through sub-irrigation using those side-holes of the pots by placing all pots in wooden tank of 10 cm height covered with plastic sheet and filled with water with water surface maintained around 1-2 cm above the side holes, as described in previous research [4]. Other crop maintenance activities were also as described in previous research [4].

Observation variables and data analysis

Observation variables included maximum plant height, number of leaves per clump, number of tillers per clump, average growth rates (AGR) of plant height, leaf number, tiller number, panicle number per clump, filled grain number per clump, percentage of unfilled grain number (calculated from percent of unfilled grain number to total spikelet number per clump), dry straw weight per clump, grain yield (filled grain weight) per clump, weight of 100 grains, and harvest index. Data were analyzed with analysis of variance (ANOVA) and Tukey's HSD tests at 5% level of significance, using the statistical software CoStat for Windows Ver. 6.303.

III. RESULTS AND DISCUSSION

Based on the results of data analysis, the ANOVA results show that there was no significant interaction between the relay-planting dates and species of legumes grown together with rice in pot culture on growth and yield components of the red rice. Compared with the species of legumes grown together with the red rice, the relay-planting dates, i.e. rice ages at which legume crops were inserted, showed more dominant effects, which had a significant effect on the maximum plant height, number of leaves per clump, number of tillers per clump, AGR of leaf number of per week, AGR of tiller number per week, panicle number per clump, filled grain number per clump, percentage of unfilled grain number, weight of filled grains per clump (or grain yield per clump), and harvest index, while the legume species had significant affects only on the number of filled grains per clump, grain yield per clump and harvest index (Table 1).

Based on the average of growth variables, it can be seen in general that there was a tendency for the highest growth of the red rice to occur in the red rice plants grown together with legume crops that were relay-planted at three weeks after seeding rice (3 WASR) and lowest in rice plants relay-planted with legume crops at 1 WASR (Table 2). This indicated that earlier relay-planting those legume crops for intercropping or growing together with the red rice crop in pot culture will result in higher degree of competition for rice growth by the legume crops, and lower degree of competition in later relay-planting time of legume those crops.

Table 1. Summary of ANOVA results of the effects of relay-planting dates and species of legume crops grown together with red rice in pot culture on growth and yield components of the red rice

Observation variables	Relay-planting dates	Legume species	Interaction
Maximum plant height	s	ns	ns
Leaf number per clump	s	ns	ns
Tiller number per clump	s	ns	ns
Average growth rate (AGR) of plant height	ns	ns	ns
AGR of leaf number per clump	s	ns	ns
AGR of tiller number per clump	s	ns	ns
Panicle number per clump	s	ns	ns
Filled grain number per clump	s	s	ns
Percentage of unfilled grain number	s	ns	ns

Dry straw weight per clump	s	ns	ns
Grain yield (filled grain weight) per clump	s	s	ns
Weight of 100 filled grains	ns	ns	ns
Harvest index (%)	ns	s	ns

Remarks: s = significant, ns = non-significant

Table 2. Average plant height, leaf number per clump, tiller number per clump, and average growth rates (AGR) of plant height, leaf number per clump, and tiller number per clump for each level of treatment factors

Treatments	**Maximum plant height (cm)**	**Leaf number per clump at anthesis**	**Tiller number per clump at anthesis**	**Average growth rate (AGR)**		
				Plant height (cm/week)	**Leaf number per clump (leaves/week)**	**Tiller number per clump (tillers/week)**
u1: 1 week	102.11 b	77.44 b	15.00 b	19.74 a	19.07 b	4.04 b [1)]
u2: 2 week	108.89 ab	114.44 a	24.00 a	21.48 a	31.72 a	6.59 a
u3: 3 week	111.89 a	121.11 a	26.22 a	21.98 a	33.30 a	6.98 a
u4: 4 week	109.00 ab	109.00 a	24.89 a	20.53 a	29.60 a	6.61 a
HSD 0.05	7.18	21.91	5.13	2.40	6.70	1.54
s1: Groundnut	108.75 a	106.83 a	22.67 a	21.38 a	28.56 a	6.09 a
s2: Mungbean	108.33 a	108.67 a	22.92 a	20.75 a	29.54 a	6.28 a
s3: Soybean	106.83 a	110.00 a	22.00 a	20.68 a	27.17 a	5.80 a
HSD 0.05	5.63	17.18	4.02	1.88	5.25	1.21

[1)] Mean values followed in each column by the same letters are not significantly different between levels of a treatment factor based on its Tukey's HSD at 5% level of significance

In relation to competition between legume and non-legume crops in an intercropping system, especially in response to different dates of relay-planting the legume crops, previous researches also reported similar results. Armstrong *et al.* [14] showed that delaying the date of relay-planting lablab bean, velvet bean, and scarlet runner bean between maize rows relative to the maize planting date significantly reduced the bean fodder yield but significantly increased maize fodder yield. Lawson *et al.* [15] also reported that delaying the date of relay-planting soybean, cowpea and groundnut from 0 to 2 and 4 weeks after planting maize reduced leaf area index and shoot biomass of the legume crops.

In relation to yield components of the red rice in this research, the average number of filled panicle per clump, filled grain number per clump, and grain yield per clump also tended to be highest in the pots where the legume crops were relay-planted at 3 weeks after seeding the red rice (3 WASR), and lowest in the pots where the legume crops were relay-planted at 1 week after seeding the red rice (Table 3). These data show significant increases with the increasing delay of the dates of relay-planting the legume crops in intercropping (growing together) with the red rice plants in pot culture. These could mean that earlier dates of relay-planting those legume crops may have imposed higher degree of competition with the red rice plants. In relation to different timing of relay-planting legume crops between cereal and legume crops, many have reported similar yield trends in relation to delaying the dates of relay-planting legume crops in intercropping systems, as those reported by Armstrong *et al.* [14]. Lawson *et al.* [13] also reported that maize grain yields increased with delaying the dates of relay-planting Mucuna and Canavalia cover crops between rows of maize in intercropping systems, but the delay decreased grain yield of the legume cover crops.

Table 3. Average panicle number per clump, filled grain number per clump, percentage of unfilled grain number, dry straw weight per clump, grain yield per clump, weight of 100 filled grains, and harvest index for each level of treatment factors

Treatments	Filled panicle number per clump	Filled grain number per clump	Percentage of unfilled grain number (%)	Dry straw weight (g/clump)	Grain yield (g/clump)	Weight of 100 filled grains (g)	Harvest index (%)
u1: 1 week	13.89 b	722.44 b	18.75 b	22.85 b	18.78 b	2.59 a	44.04 a[1)]
u2: 2 week	18.77 a	870.89 ab	29.37 a	34.19 a	22.85 ab	2.63 a	41.06 a
u3: 3 week	20.44 a	1030.00 a	27.00 ab	31.93 ab	26.88 a	2.59 a	45.57 a
u4: 4 week	17.11 ab	989.89 a	20.77 ab	30.43 ab	26.17 a	2.64 a	46.56 a
HSD 0.05	2.97	187.77	7.03	7.15	4.84	0.11	6.28 a
s1: Groundnut	16.33 a	921.58 ab	20.36 a	28.61 a	24.51 ab	2.65 a	46.42 a
s2: Mungbean	18.42 a	998.83 a	25.35 a	29.80 a	25.99 a	2.61 a	47.01 a
s3: Soybean	17.92 a	789.50 b	26.21 a	31.13 a	20.51 b	2.58 a	39.50 b
HSD 0.05	2.57	160.01	6.09	6.19	4.19	0.09	5.44

[1)] Mean values followed in each column by the same letters are not significantly different between levels of a treatment factor based on its Tukey's HSD at 5% level of significance

In relation to yield of cereal crops in intercropping with legume crops, some reported lower yield of intercropped than monocropped cereal crops, such as those reported by Lawson *et al.* [13] and Lawson *et al.* [15] regarding to yield of maize in intercropping with some legume crops. However, many reported yield advantages for cereal crops when intercropped with legume crops, or no yield differences, compared with monocropped cereal crops. From an intercropping system of wheat-soybean, Li *et al.* [16] reported higher grain yield of wheat per ha in intercropping with soybean compared with in monocropped wheat, and yield differences were higher in higher doses of P fertilizer. However, from the results reported by Jeranyama *et al.* [17], maize grain yield was not reduced by intercropping with cowpea or *Crotalaria juncea* when maize was fertilized with 0-60 kg/ha N, but when it was fertilized with 120 kg/ha N, its grain yield was reduced, compared with monocropped maize. This could mean that under low N fertilization, maize plants would get some N transfer from rhizosphere of legume crops in an intercropping with maize.

In relation to N contribution from legume to cereal crops in intercropping systems, Fujita *et al.* [18] reported that N transfer from soybean to sorghum, which resulted in higher dry matter production by sorghum in intercropping than in monocropping, was highest in the closest planting distance between both crops. Chu *et al.* [9] also reported N transfer from peanut to rice plants grown in intercropping under aerobic system, resulted in higher filled panicle number, spikelet weight and weight of 1000 seeds of the rice plants in intercropping than in monocropping system. Inal *et al.* [6] found that intercropping maize with peanut resulted in higher concentration of nutrients in rhizosphere and in the shoots of both crops. Previous researches using this red rice also reported that grain yield of red rice grown in aerobic irrigation system was higher in intercropping with legume crops than in monocropped or conventional red rice, for example those reported by Dulur *et al.* [8] and Wangiyana *et al.* [11] in intercropping red rice with soybean, and Dulur *et al.* [10] in intercropping red rice with peanut.

Based on the grain yield per clump in this research, however, the average grain yield was very low, i.e. the highest average of 25.99 g/clump in intercropping with mungbean, and the lowest average of 20.15 g/clump in intercropping with soybean (Table 3). These are much lower than those reported by Dulur *et al.* [8] under the same experimental site, i.e. under pot culture using soil from the same land in the same plastic house, with the highest average of 34.73 g/clump in intercropping with soybean. From field experiments conducted in other locations, by growing red rice on permanent raised-beds under aerobic irrigation systems, some researchers reported higher grain yields of red rice, i.e. 54.49 g/clump in intercropping with soybean [11], and 2632 filled grains per clump in intercropping with peanut [10]. The low grain yield of the red rice obtained in this research was probably due to prevalent attacks by stink bugs (*Leptocoriza acuta*) during the seed-filling stage, which resulted in a very high average of percentage of unfilled grain number, i.e. up to 29.37% (Table 3).

Among the legume crops examined for intercropping with red rice in pot culture, different species of those legume crops resulted in differences only in some yield components of the red rice tested, including filled grain number per clump, grain yield per clump, and harvest index (Table 3). In terms of their effects on grain yield of the red rice, mungbean of "Kenari" variety was found to be the best among those legume species intercropped with the red rice in pot culture, which resulted in the highest average of red rice grain yield (25.99 g/pot), while intercropping with soybean var. "Dering-1" resulted in the lowest average of red rice grain yield (20.51 g/pot). Wangiyana *et al.* [19] reported that among five varieties of mungbean tested for intercropping with red rice, the "Kenari" variety resulted in the highest average of red rice grain yield (27.37 g/pot).

However, between both treatment factors, the dates of relay-planting those legume crops had more dominant effects on growth and yield components of red rice in intercropping with legume crops under aerobic irrigation system because it seems to be related to the balances between competition and fixed-N contribution by legume crops to the red rice in intercropping with legume crops. The levels of N contribution by legume crops to non-legume crops in intercropping system also depend on levels of fertilizer doses applied to the crops, as reported by other researchers [16], [17]. Therefore, before a recommendation can be made, more extensive researches need to be done in different conditions of crop production systems to find out the best combination of rice and legume crops to be grown in intercropping systems.

IV. CONCLUSION

It can be concluded the dates of relay-planting legume crops for intercropping with red rice had significant effect on growth and yield of red rice, and relay-planting the legume crops 3 weeks after seeding the red rice was found to be the best timing.

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Spatial Distribution and Habitat Utilization of Reptiles in a Mediterranean Area (Castel di Guido, Rome, Italy)

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Abstract— *The purpose of this study is to describe, for the LIPU Castel di Guido Oasis, within the Castel di Guido Farm, on the Roman coast, the different spatial distribution of the different species of reptiles that coexist in the area. The work also defines correlations between the presence of animals and the various environmental and vegetation types present. In the study area, Testudo hermanni is a species that has the feeding areas, as well as the deposition areas, in the prairies adjacent the plant formation of the Mediterranean maquis(within 5 meters). Hemidactylus turcicus and Tarentola mauritanica seem to have a localization exclusively in the context of anthropic structures. Chalcides chalcides and Podarcis siculus appear to be confined to prairie areas, while Podarcis muralis is associated with the presence of trees of high-trunk plant associations. For Lacerta bilineata, a correspondence is outlined for the areas with arboreal shrubby vegetation, especially close to more humid habitats. Among the snakes Hierophis viridiflavus appears to be an ubiquitous species, with the frequentation of different habitats in the same percentage; more localized appear Vipera aspis and Elaphe quatuorlineata, the first more linked to wooded areas with the presence of bushy and shrubby vegetal coverings, the second is often associated with the simultaneous presence of forests and water collections. In general it is confirmed, as regards the snake community, the importance of ecotonal and transitional zones. The work also highlights how the study area is important for the conservation of reptile populations in the Roman area.*

Keywords— ***Reptiles, habitat, spatial distribution, Mediterranean, vegetational aspects.***

I. INTRODUCTION

The distribution of animals in their natural environment, and the selection of the habitat, are the result of the interaction between the species and the environmental factors characterizing a site (Heatwole, 1977; Orians, 2000).

It has been amply demonstrated, at the microhabitat level, that the persistence of most small terrestrial vertebrates in a particular site, strongly depends by specific factors, such as temperature, humidity, trophic availability and shelters against predators (Downes & Shine 1998; Oatway & Morris 2007; Peterman & Semlitsch 2013). This is especially true for heterothermic species, which have specific physiological needs and require suitable points for thermal exposure to the sun; furthermore, for small species, since they have limited spatial movements, the topography of the place also plays an important role (Capula *et al*.,1993; Grover 1996; Melville & Schulte 2001; Rittenhouse *et al*. 2003).

In this complex of interactions, the vegetation cover plays a decisive role in the composition of the environmental factors described above (Irschich & Losos 1999; Vanhooydonck *et al*. 2000; Hofer *et al*., 2002).

The purpose of this study is to describe, for the LIPU Castel di Guido Oasis, within the Castel di Guido Farm, on the Roman coast, the different spatial distribution of the different species of reptiles that coexist in the area and, at the same time, if there are well-defined correlations between the presence of animals and the various environmental and vegetation types present.

II. STUDY AREA

The present study was carried out within the Castel di Guido Farm, located in the Municipality of Rome, in the stretch between the 16th and 20th km of the Aurelia road (Topographic Paper IGM Sheet n°149 Tablet I - SO Maccarese, scale 1: 25.000) (Fig.1). Inside the farm, since 1999, the LIPU Castel di Guido Oasis has been realized. The farm has been under the direct management of the

Municipality of Rome since 1978 and has a production address of cereals and fodder as well as cattle breeding, both indoors (Italian Friesian cow) and the wild state (Maremma cow). The farm extends for 1966 hectares and is characterized by hilly nucleuses that degrade toward the coastal plain; the maximum altitude reached is 80 m asl, while the minimum altitudes reach about 10 m asl. The geological alternation of tufaceous layers superimposed on clay and sandy layers, with different behavior towards erosive exogenous agents, has determined over time the formation of a series of depressions and pianos. Human activity (reclamation works, agriculture, pastoralism) has further modified the territory, leading to the formation of hillocks and reliefs, interesting because they are characterized by relict vegetation (Chirici *et al*., 2001).

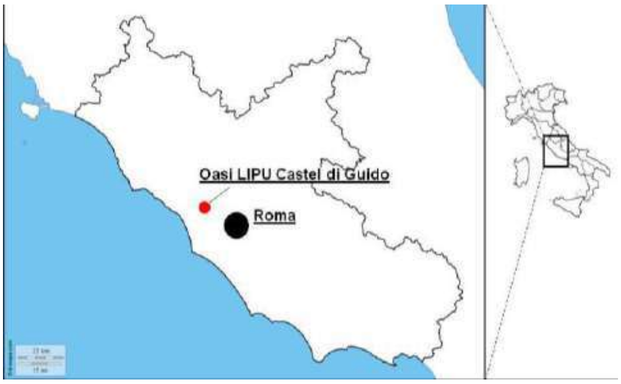

Fig. 1. Location of the study area.

The area is bioclimatically part of the transitional Mediterranean region, in the medium mesomediterranean thermotype unit and a superior sub-humid type (Blasi, 1994). The climate of the area is particularly mild by the proximity of the sea. The minimum temperatures are recorded in January (average value 3.9 °C), the maximum in July (average value 30.5 °C); rarely there are values below 0 °C and above 40 °C. In autumn the maximum rainfall occurs (over 275 mm), but also in spring there are frequent rains (175 mm) (Mangianti & Perini, 2001).

The birds of the LIPU Castel di Guido Oasis are represented by numerous permanent and migratory species (Cecere, 2006); among the most representative mammals we find *Hystrix cristata* Linneo, 1758, *Vulpes vulpes* Linneo, 1758, *Meles meles* Linneo, 1758, *Martes foina* Erxleben, 1777, *Erinaceus europaeus* Linneo, 1758 and *Muscardinus avellanarius* Linneo, 1758 (Imperio *et al*., 2007). Noteworthy is the recently proven presence of the wolf *Canis lupus* Linneo, 1758.

About the herpetofauna, reptiles are represented by 14 species (Table 1) (Pizzuti Piccoli *et al*., 2017a); in the area we found 5 species of Amphibians: common toad *Bufo bufo* (Linneo, 1758), Italian green toad *Bufotes balearicus* (Boettger, 1880), Italian tree frog *Hyla intermedia* Boulenger, 1882, green frog *Pelophylax bergeri* (Gunther, 1986)/ *Pelophylax kl. hispanicus* (Bonaparte, 1839) and smooth newt *Lissotriton meridionalis* (Boulenger, 1882) (Pizzuti Piccoli & De Lorenzis, 2015).

The area is established as an “Area of National Herpetological Relevance – AREN” by the *Societas Herpetologica Italica* ; it is also included within the State Natural Reserve of the Roman Coast and it’s included in the Special Conservation Zone "Macchia Grande di Ponte Galeria", according with the European Habitat Directive.

Table 1. The reptile species observed in the LIPU “Castel di Guido” Oasis.

Species observed	
European pond terrapin	*Emys orbicularis*
Hermann’s tortoise	*Testudo hermanni*
Mediterranean house gecko	*Hemydactilus turcicus*
European common gecko	*Tarentola mauritanica*
Italian slowworm	*Anguis veronensis*
Italian three-toed skink	*Chalcides chalcides*
Common wall lizard	*Podarcis muralis*
Italian wall lizard	*Podarcis siculus*
Western green lizard	*Lacerta bilineata*
Four-lined snake	*Elaphe quatuorlineata*
Western whip snake	*Hierophis viridiflavus*
Aesculapian snake	*Zamenis longissimus*
Grass snake	*Natrix helvetica*
Asp viper	*Vipera aspis*

VEGETATIONAL ASPECTS OF "CASTEL DI GUIDO" OASIS

The LIPU Castel di Guido Oasis is characterized by an evident vegetational complexity and a great floristic richness, that emerge in the different habitats present. According to data provided in 1999 by the farm, in which the Oasis is included, the 1966 ha was divided into several coltures. We have 17% (366 ha) occupied by crops such as durum wheat, corn, barley, olive groves, and medic grass, both old and new plants, 22% (430 ha) by natural woods with a prevalence of *Quercus ilex* L. and *Quercus pubescens* Willd., 22% (433 ha) is used for permanent pasturages, 28% (552 ha) it is covered by pine forests and reforestation areas, while the remaining part of the territory is occupied by roads, rural buildings and their neighboring lands, stables, irrigation canals and and other man-made structures (Filesi, 2001; Bartolomucci & De Lorenzis, 2004).

In particular, the vegetational formations present in the LIPU Castel di Guido Oasis, for the purposes of this research, have been classified into the following 4 categories: natural woods, grasslands, reforestation, arable lands. The first three categories, relevant to the investigation, are described below.

NATURAL WOODS

On the reliefs and slopes the vegetation consists, predominantly, of high stains and scrublands dominated by *Quercus pubescens* and *Quercus ilex* with a sclerophilic undergrowth characterized by the *Phillyrea* sp., *Rhamnus alaternus* L., *Erica arborea* L. and the very fragrant *Pistacia lentiscus* L.; in these areas there are also *Quercus suber* L., *Quercus cerris* L. and the hybrid *Quercus crenata* Lam. (Filesi, 2001; Bartolomucci & De Lorenzis, 2004).

On the sandy-gravelly soils of the numerous escarpments at the sides of the hills, we find bushes dominated by *Spartium junceum* L. with *Rubus ulmifolius* S., *Ulmus minor* Miller and, more localized, *Cercis siliquastrum* L., while in the valleys the vegetation is kept fundamentally equal to the potential one, being still made up of dense woods, especially of *Quercus cerris* and *Quercus frainetto* Ten.; *Malus sylvestris* Miller, *Crataegus monogyna* Jacq., *Cornus mas* L. and *Sorbus domestica* L. participate in the formation of the arboreal /shrub layer. In sites with a particularly fresh and humid microclimate, *Carpinus betulus* L. and *Quercus robur* L. are found together with the other oaks; along the ditches can be found remnants of riparian vegetation in *Salix alba* L., *Phragmites australis* (Cavill), *Thypa latifolia* L. and *Carex pendula* Hudson together with various horsetails (Filesi, 2001; Bartolomucci & De Lorenzis, 2004).

GRASSLANDS

At the base of the slopes, where the deforestation, excessive grazing and fires led to a rapid soil degradation (with consequent surfacing of debris and sands) vegetation consists of sparse evergreen bushes, some shrub species *Cistus monspeliensis* L., *Cistus* salviifolius L., Cistus creticus L. and herbaceous plants. Among the herbaceous plants *Compositae* prevail, such as *Helichrysum italicum* (Roth), *Anthemis tinctoria* L., *Senecio leucanthemifolius* Poiret, *Crupina vulgaris* Cass., *Hedypnois rhagadiloides* (L.), *Tragopogon hybridus* L., *Urospermum dalechampii* (L.), *Crepis zacintha* (L .), *Poaceae*, including *Cynosurus echinatus* L., *Briza maxima* L., *Dactylis glomerata* L., *Bromus madritensis* L., *Elytrigia atherica* (Link), *Aegilops geniculata* Roth, *Parapholis incurva* (L.), *Lagurus ovatus* L., *Phleum arenarium* L., *Bothriochloa ischaemum* (L.) and *Fabaceae*, among whose main species we mention *Lathyrus sphaericus* Retz., *Trifolium stellatum* L., *Lotus tetragonolobus* L., *Hymenocarpus circinnatus* (L.), *Onobrychis caputgalii* Scop., *Tripodium tetraphyllum* (L.). In this plant formation there are also many *Lamiaceae* such as *Teucrium capitatum* L. and *Salvia clandestina* L. and numerous orchids as *Anacamptis pyramidalis* (L.), *Serapias vomeracea* (Burm. F.), *Ophrys sphegodes* Mill., *Ophrys incubacea* Bianca, *Ophrys fuciflora* (F.W. Schmidt) (Filesi, 2001; Bartolomucci & De Lorenzis, 2004).

REFORESTATIONS

In the area there are many fairly fertile lowland areas, on which agricultural activities (crops and pasture) have always insisted; currently, part of these areas have been redeveloped through reforestation. Although, for this purpose, native plants have not always been used and adapted to the climatic characteristics of the place, this redevelopment operation is still very important (Chirici *et al*., 2000)

The first forestation within the area concerned specimens of *Pinus halepensis* Miller and *Pinus brutia* Ten., nowadays evolved into two mature forest fragments, one of these present inside the Oasis. In 1987 another reforestation campaign was restarted which led to the construction of other smaller-scale forest fragments; today we also find young reforestations (13-20 years) with *Quercus pubescens*, *Quercus ilex*, *Quercus suber*, *Malus sylvestris* and *Crataegus monogyna*, *Pinus pinea* L., *Pinus halepensis*. From 1987 to 1995, about 550,000 plants of different tree species were planted throughout the farm (Chirici *et al*., 2000).

III. MATERIALS AND METHODS

Data collection took place between January 2014 and December 2016; surveys were carried out weekly; the detection method adopted was that of the linear transept with "visual counts", V.E.S. = Visual Encounter Surveys. (Heyer, 1988; Crosswhite *et al*., 1999).

As a transept, a linear path of 2,200 meters, crossing all the representative environments and the ecotonal areas present, was chosen; were considered animal observations in the 5 meters to the right and to the left of the transept. The figures from n°2 to n°5 show the observations of the different reptiles carried out along the transept and superimposed on the vegetation map.

In the study, great attention was paid to ecotonal areas; the importance of ecotone is due to the fact that in it, generally, there is a greater biodiversity than in the biocenoses that it separates. For the reptile community, a the large number of localizations is concerned with the ecotones (Hofer *et al*., 2002) and this shows that snakes could cover a good part of their resource requirements in the transition zones present in the study area. It is assumed that much of the reptile population regularly uses the sunny side of transition environments to meet ecological needs (Hofer *et al*., 2002). In particular snakes, when moving, orient themselves along well-defined environment structures (Gregory *et al*., 1987). They move, over short distances, even along the ecotones whose texture facilitates the movement.

A sampling program, that takes this important function of ecotones into account, offers the possibility of detecting most of the reptiles residing in the study area during a season. During the transects, the animals were captured and marked where possible; this in order to obtain recapture rates that allow considerations on the mobility of individuals on the site.

In addition, for the study of snakes, metal coverboards positioned on the ground were used (Hofer *et al*., 2002).

The field work was carried out following the regulations and with all the authorizations necessary for this type of study. The animals, where captured, have always been studied on the field and released at the same capture site.

IV. RESULTS AND DISCUSSION

TESTUDINES

Emys orbicularis (Linneo, 1758)

The only specimens of *Emys orbicularis*, five in all, were observed in May 2015, at a ditch on the border of the reserve; given the limited nature of the data, the species was not considered for the purposes of this work. The species, which constitutes a new presence for the area, certainly needs in depth monitoring, to define its status in the area and the frequentation of the different habitats (Pizzuti Piccoli *et al*., 2017b).

Testudo hermanni Gmelin, 1789

The observed animals were 41 and were almost always within the distance of 5 meters from the edge of the wooded area, rarely (<5% of the observations) were they in open meadow areas; 70% of the specimens observed were found in ecotonal areas between meadow and natural wood (with a low arboreal-shrub layer composed of species of the Mediterranean maquis). The 30% was instead observed in the prairie areas adjacent to the areas with reforestation.

Nests were found in the prairie areas, close to the natural areas, both predated and with hatched eggs, always at about 50 cm from the bushes.

SAURIA

Hemidactylus turcicus (Linneo, 1758); *Tarentola mauritanica* (Linneo, 1758).

In the study area, *Hemidactylus turcicus* and *Tarentola mauritanica* are present exclusively in the artificial areas (village, rural buildings, stables) of the Castel di Guido Farm, where they appear in syntopy. They are also located in the two Oasis structures (Visitor Center and Bird Ringing Station) consisting of prefabricated wooden artefacts. In general, in the study area the two species appear numerous, even if located exclusively in the anthropic sites mentioned above.

Anguis veronensis Pollini, 1818

Only one specimen of *Anguis veronensis* was observed; the infrequence of findings can be related to the ecology of the species that makes it difficult to observe the species and the type of survey carried out in the field. The specimen was found in the morning hours (around 7.00 am) at a roadside area with boulders and brushwood

Chalcides chalcides (Linneo, 1758)

During the transects, 14 specimens of *Chalcides chalcides* were observed; all the specimens were found in the uncultivated grasslands with continuous turf.

Podarcis muralis (Laurenti, 1768); *Podarcis siculus* (Rafinesque Schmaltz, 1810); *Lacerta bilineata* Daudin, 1802

Lacerta bilineata is very common in the study area; during the survey, 104 individuals were observed. The species appears to be equally distributed in areas with natural forests and reforestation (respectively 57% and 43% of observations). It should be noted that 42% of the observations concern a very precise type of habitat, namely the areas close to small temporary ditches or drainage canals, with associated *Rubus* sp. vegetation and other shrubs. The two species *Podarcis muralis* and *Podarcis siculus* are very numerous in the territory. Of the 223 specimens observed of *Podarcis siculus*, 17% were found in wooded areas, while 83% of the observations were carried out in open meadow areas. For the species *Podarcis muralis*, 132 specimens were observed; of these, 6% were observed in lawn areas, while 94% were observed in wooded areas (33% of the specimens were found in areas with artificial reforestation, while 77% are present in areas with natural woods).

SERPENTES

Natrix helvetica (Lacépède, 1789), *Zamenis longissimus* (Laurenti, 1768), *Hierophis viridiflavus* (Lacépède, 1789), *Elaphe quatuorlineata* (Bonnaterre, 1790), *Vipera aspis* (Linneo, 1758).

For the snakes *Natrix helvetica* and *Zamenis longissimus* it is not possible to delineate a specific use of the habitat because of the few observations made.
Hierophis viridiflavus seems to be the most present species in the different environments of the Oasis, resulting equally distributed in meadow areas and uncultivated (in this case also close to the rural buildings present), in the reforestation areas and natural wood (with the following percentages: meadows and uncultivated = 27%, natural forests = 41%, reforestation = 31%).
As for *Vipera aspis*, 64% of the observations refer to wooded areas, while in 36% of the cases the species was found in marginal areas between wooded and uncultivated areas, with the presence of brambles and undergrowth, always in areas with the presence of abundant vegetation cover.
For *Elaphe quatuorlineata* 63% of the observations are attributable to a well-defined habitat; these is characterized by wooded areas (high-trunk trees) bordering on water collections (in particular, in the Oasis, there is an artificial pool with a very large surface). This site, based on field observations, constitutes a site that meets (at least temporarily) the ecological needs of the species.

V. CONCLUSION

The distribution of the species in the different habitats appears to be well defined for the species present, with the exception of *Emys orbicularis*, *Anguis veronensis*, Na*t*rix *helvetica* and *Zamenis longissimus* for which the exiguity of the observations does not allow to elaborate correlations with the habitat (Fig. 6).
Testudo hermanni is a species closely associated with the plant formation of the Mediterranean maquis, where it is often found; the feeding areas, as well as the deposition areas, consisting of the prairies are almost always adjacent (within 5 meters) to this type of vegetation.
Hemidactylus turcicus and *Tarentola mauritanica* seem to have a localization exclusively in the context of anthropic structures.
Chalcides chalcides and *Podarcis siculus* appear to be confined to prairie areas, while *Podarcis muralis* is associated with the presence of trees of high-trunk plant associations. For the *Podarcis muralis* it is remarkable the observation concerning the fact that, often, every individual occupies a single tree on which he finds an optimal exposure point, as well as, probably a refuge area from terrestrial predators. For *Lacerta bilineata*, a correspondence is outlined for the areas with arboreal shrubby vegetation, especially close to more humid habitats.
Among the snakes *Hierophis viridiflavus* appears to be a very vagile and ubiquitous species, with the frequentation of different habitats in the same percentage; more localized appear *Vipera aspis* and *Elaphe quatuorlineata*, the first more linked to wooded areas with the presence of bushy and shrubby vegetal coverings, the second is often associated with the simultaneous presence of forests and water collections. These observations, for snakes, are in agreement with other published studies for different areas **(**Gregory *et al*., 1987; Filippi & Luiselli, 2000; Hofer *et al*., 2002**)**; moreover, it appears evident, always in the case of snakes, that presence in the habitat is correlated, above all, to the trophic availability proper for the different species (this should allow an optimal utilization of the environmental resources, in mutual respect of the roles). In general it is confirmed, as regards the snake community, the importance of ecotonal and transitional zones, in agreement with the data in the literature (Capula *et al*., 1993; Hofer *et al*., 2002; Cattaneo, 2005; Corti *et al*., 2010).
In conclusion, the work is a first analysis of the correlation between species and habitat attended for the LIPU "Castel di Guido" Oasis; surely it will be important to deepen with further research, after this first preliminary analysis, the ecology of the species found. Given the number of species and individuals observed, the work also highlights how the study area is important for the conservation of reptile populations in the Roman area.

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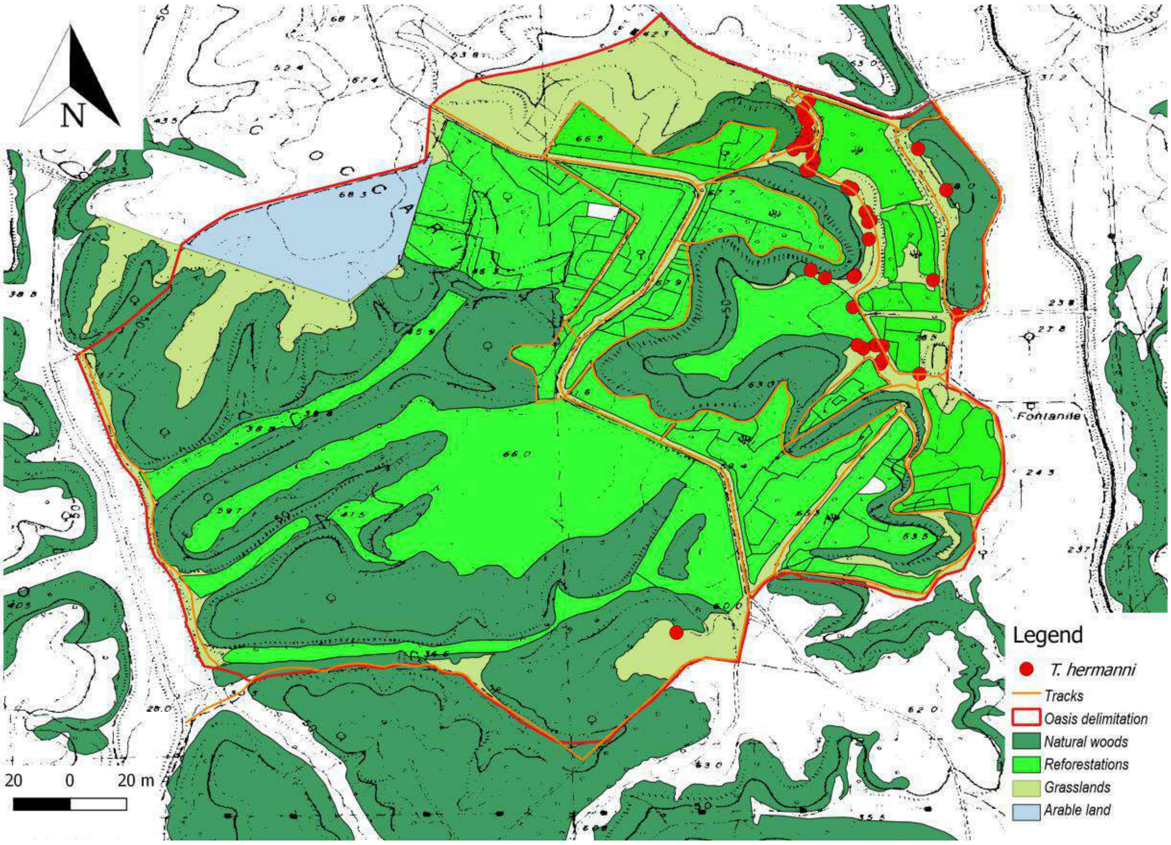

Fig. 2. Distribution and habitat utilization of Testudo hermanni in the study area.

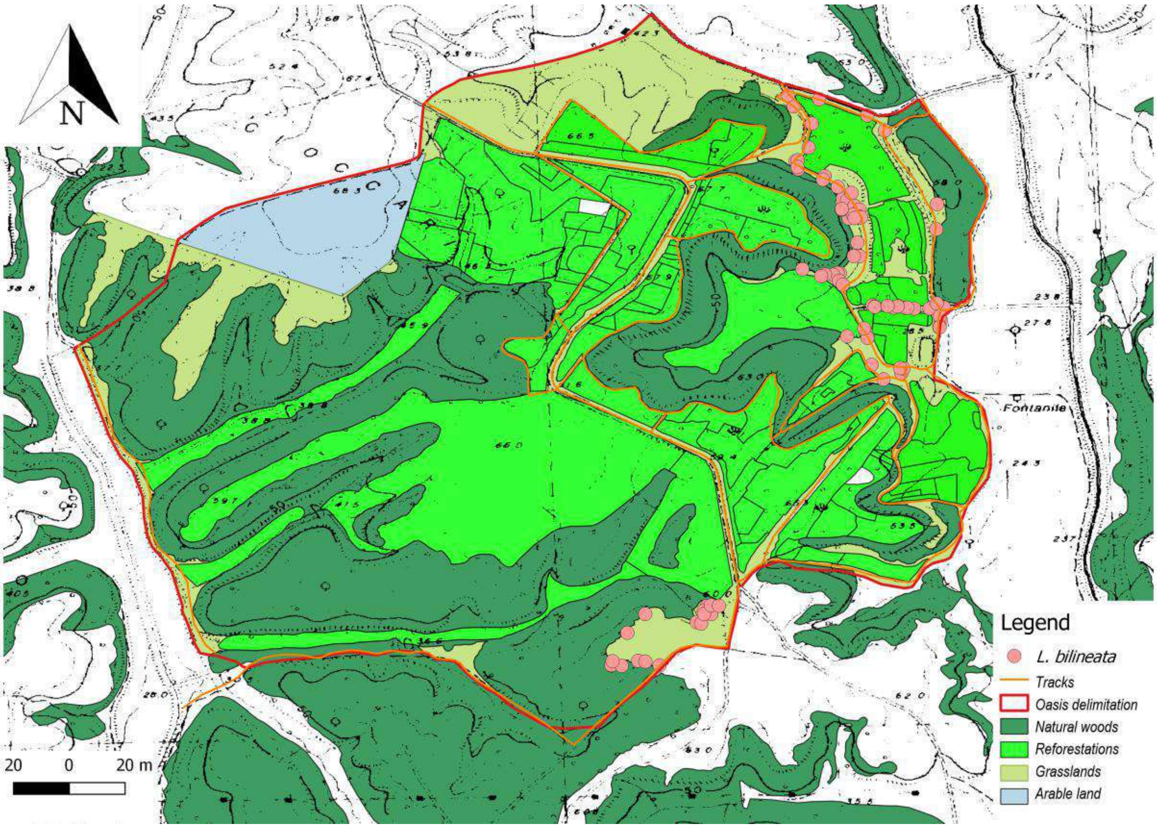

Fig. 3. Distribution and habitat utilization of Lacerta bilineata in the study area.

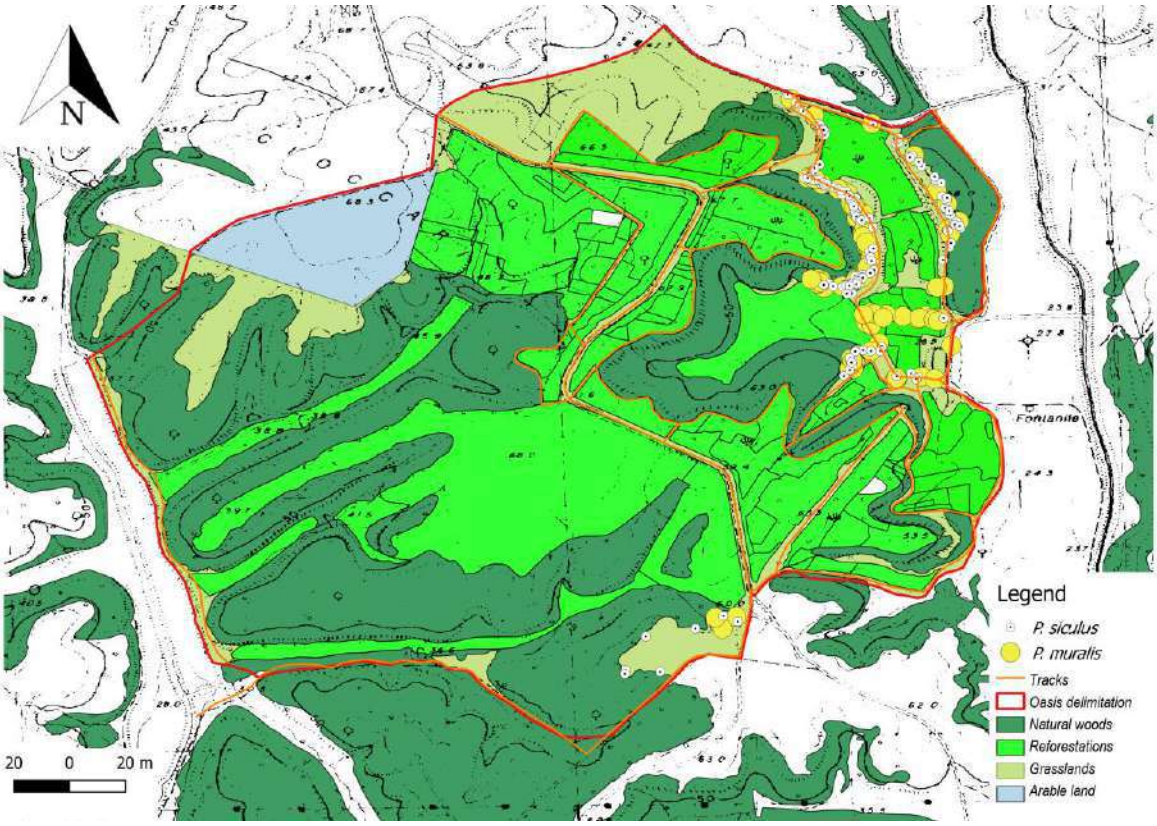

Fig. 4. Distribution and habitat utilization of Podarcis siculus and Podarcis muralis in the study area.

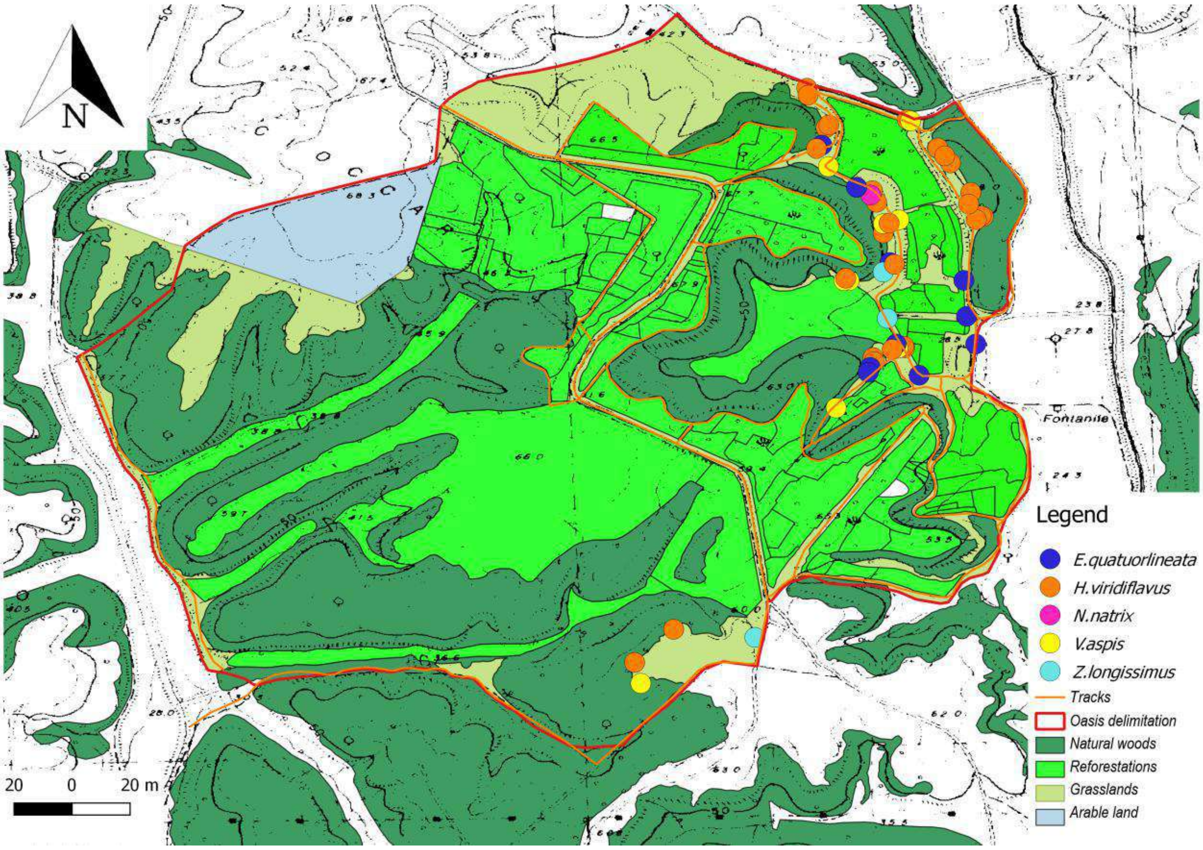

Fig.5. Distribution and habitat utilization of snake species in the study area.

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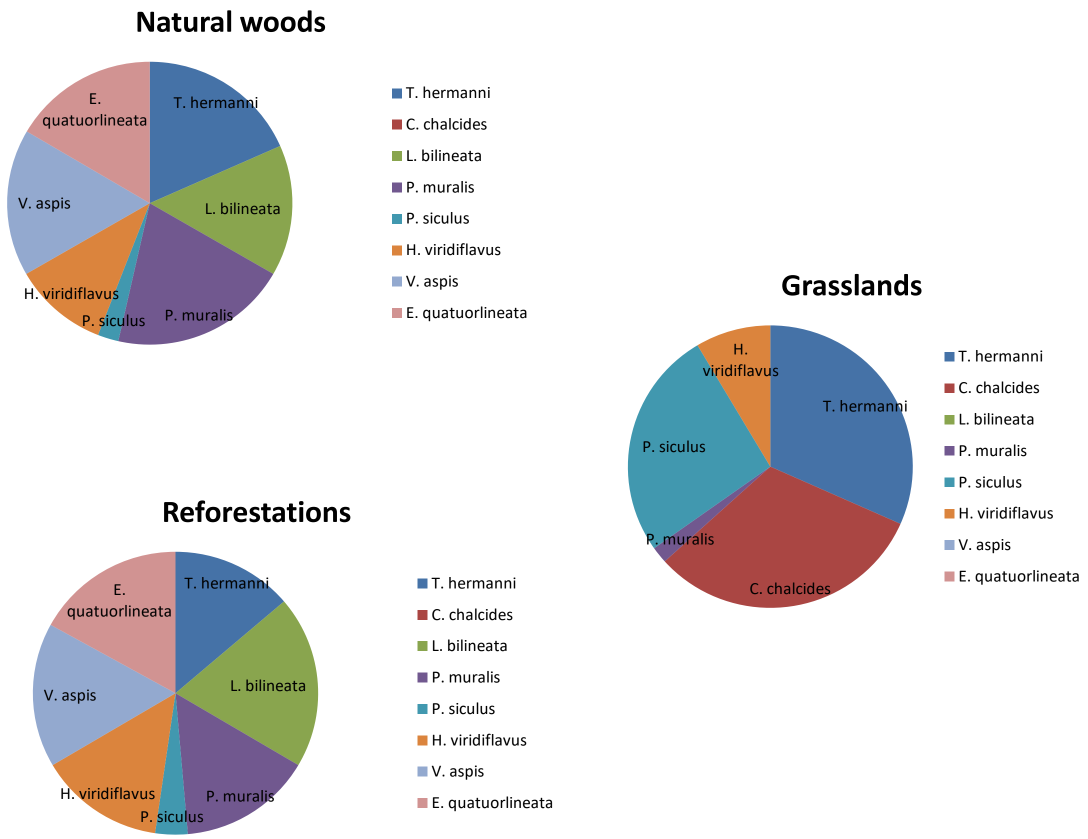

Fig.6. Reptiles observed in the study period and their presence percentage in the different habitats (some taxa are excluded; see text).

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Physicochemical, heavy metals and microbial pollution of surface and ground water in bodija Municipal Abattoir and its Environs

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***Abstract*— *Surface and ground water pollution is a major problem beclouding most developing nation and the poor method of abattoir effluent waste disposal system being one of the major causes of such pollution. The effluent disposal system of the abattoirs portends environmental and health hazards to the abattoir and its environs and therefore necessitating this study.*

This study was designed to assess physicochemical parameters, heavy metal concentrations and bacterial constituents of selected wells within Bodija municipal abattoir which serves as the main source of water used for meat processing. Samples of water from the wells in the abattoir and some wells in the neighborhood were collected and analyzed according to standard laboratory procedures.

The results obtained revealed the order of mean of heavy metal concentration in sampled abattoir wells (W_A) as Fe (0.67±0.26) > Mn (0.27±0.26) > Pb (0.16±0.08) and the order in sampled residential wells (W_R) as Pb (0.64±0.33) > Fe (0.54±0.22) > Cu (0.35±0.021) > Mn (0.20±0.03). Abattoir wells had significantly higher coliform and enterobactericeae counts than the residential wells. Virtually all the figures obtained were considerably higher than the permissible standard for drinking water.

The public health implication of these findings is especially important because of re-emergence of water borne disease. This survey also indicates that the health status, social and environmental qualities of residents of Bodija abattoir neighborhood will severely be affected.

Keywords*— Heavy metals, abattoir, Bodija, Pollution.*

I. INTRODUCTION

There is an ever increasing need for improved management of surface and ground water because they are the most readily available for human use, yet the most polluted as a result of anthropogenic activities (Ojekunle and Lateef, 2017). Groundwater is the commonest potable water source around the world and its elemental composition is an indicator of how safe it is for the consumption of humans, animals and plants (Batabyal and Chakraborty, 2015). Contamination and pollution of natural water bodies has emerged as a major challenge in developing and densely populated countries like Nigeria (Ezekoye *et al.,* 2013). Water pollution makes water to become unsuitable for human use and also it becomes more expensive in treatment for acceptable quality (Terrumun and Oliver, 2015). Human activities such as indiscriminate location of abattoirs in residential areas in developing countries impact negatively on natural water sources.

Abattoir can be defined as premises approved and registered by regulating authorities for safe and hygienic slaughtering, inspection, processing, effective preservation and storage of meat products for human consumption (Vershima *et al.*, 2015). The abattoir industry in Nigeria is an important section of the livestock industry that provides domestic meat supply to over 180 million people and also employment opportunities for a huge number of people (Nafarnda *et al.*, 2012). In Nigeria, there is lack of adequate waste management in all public abattoirs such that large solid wastes and untreated effluents are common (Adebowale *et al.,* 2010). Abattoirs usually releases wastewaters directly into the ecosystems without adequate treatment process thereby posing serious threats to surface water quality,

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general environmental safety and health (Tekenah *et al.*, 2014).

Pollutants from the abatoir commonly include blood, paunch manure, animal faeces, animal horns, bones, spent lubricants from machines like generators etc. (Ogbomida *et al.*, 2016). Also, there may be the presence of pathogenic microorganisms, such as *Salmonella*, *Escherichia coli* (including serotype 0157:H7), *Shigella*, parasite eggs and amoebic cysts (Adebowale *et al.*, 2010). Slaughtering process usually produces blood and paunch content from the intestine which may be flushed into open drains which are connected to surface water and may eventually enter into the groundwater (Adeyemo, 2002). Such contamination of water bodies from abattoir wastes could constitute major environmental and public health hazards (Osibanjo and Adie, 2007); it may also reduce oxygen in water, thereby endangers aquatic life.

Shallow wells surrounding abattoir sites are vulnerable to pollution from abattoir activities since the effluents may percolate into the soil and enter into the aquifer; whereas residents may not be aware of the health risk, as the water sometimes appears clean and potable for consumption. Assessing the water quality within the vicinity of abattoirs in residential areas will help to evaluate the risks on the health of residents who depend on the well waters for consumption and other domestic uses. However, this study analyzed the surface and ground water samples, by assessing the physiochemical properties, heavy metal concentrations and bacteriological constituents of selected wells within Bodija abattoir and its environs. This is very important because municipal/tap water is unavailable in the residential neighborhood of Bodija abattoir and people mostly dependent on wells as their readily water source.

II. MATERIALS AND METHODS

Description of study area

Bodija municipal abattoir, which id the study area is located in Ibadan North Local government Area of Oyo State, Nigeria. It is enclosed within latitude 7^0 441^1 and 7^0 426^1 and longitude 7^0 906^1 and 7^0 920^1 East. This study was carried out between June to July which falls within rainy season in Southwestern Nigeria.

Sampling design

The water samples were collected from ten wells designated W1-W10 selected in Bodija municipal abattoir and six (6) wells were selected systematically from the residential neighborhood R0-R5 which was at distance of 149m from the abattoir, thereafter samples wells were selected at 100m intervals. Global positioning system (GPS) was used to determine the coordinate points of the wells and the position of these sampled wells were located accurately on the map by using the 'add event theme' facility of the ArcGIS 9.3 software. 0.5 liters of water each were collected from all the wells in a clean, wide-mouthed sample bottles with tight screw dust proof stoppers for physiochemical parameters determination and 5ml in sterilized plastic for bacteriological examination.

Analyses

Determination of the parameters was based on the American Public Health Association (APHA) series of Standard Methods of Examination of Water and Effluent (APHA, 1998) while nitrate was determined using phenol disulphunic acid method (Marczenko, 1986). Heavy metals were determined using Atomic Absorption Spectroscopy (AAS) (Duada *et al.*, 2016).

III. RESULTS

Table 1: Physicochemical parameters of wells within the abattoir and outside the abattoir in the residential area.

Water Quality Parameters	Wells within the Abattoir	Wells outside the Abattoir
pH	6.15 ± 0.47	6.33 ± 0.26
Temperature	28.8 ± 0.31	29.1 ± 0.21
Conductivity	4.98 ± 2.00	1.60 ± 0.85
Dissolved Oxygen (DO) mg/l	4.79 ± 0.71	4.89 ± 0.70
Biochemical oxygen demand (BOD) mg/l	22.6 ± 13.1	18.8 ± 11.6
Total Solids (TS) mg/l	23.13 ± 19.8	173.0 ± 124.4
Total suspended solids (TSS) mg/l	94.9 ± 53.9	103.0 ± 52.1
Total dissolved solids (TDS) mg/l	137.4 ± 38.7	70.0 ± 25.5
Alkalinity (mg/l)	230.9 ± 128.3	142.5 ± 82.5

CO_2 (mg/l)	226.5 ± 62.5	207.5 ± 13.7
Hardness (mg/l)	572.8 ± 203.7	678.4 ± 442.1
Chloride (mg/l)	784.8 ± 442.1	665.0 ± 237.3
Nitrite (mg/l)	0.05 ± 0.03	0.04 ± 0.006
Nitrate (mg/l) (NO_3^-)	37.5 ± 8.2	51.3 ± 12.3

Table 2: Heavy metals detected in water samples from wells located within the abattoir (W_A)

Samples	Fe (mg/l)	Mn (mg/l)	Pb (mg/l)	Cd (mg/l)	Cu (mg/l)
W1	0.74	0.04	ND	ND	ND
W2	0.46	ND	ND	ND	ND
W3	0.73	0.38	1.06	ND	ND
W4	0.78	0.08	ND	ND	ND
W5	0.57	0.06	ND	ND	ND
W6	0.70	0.90	ND	ND	ND
W7	0.83	0.12	ND	ND	ND
W8	0.94	0.26	ND	ND	ND
W9	0.81	0.36	ND	ND	ND
W10	0.85	0.46	ND	ND	ND
Mean ± SD	0.67 ± 0.26	0.27 ± 0.26	0.16 ± 0.08	0	0

Table 3: Heavy metals detected in water samples from wells located in residential area close to the abattoir

Samples	Fe (mg/l)	Mn (mg/l)	Pb (mg/l)	Cd (mg/l)	Cu (mg/l)
RO (149m)	0.58	ND	ND	ND	ND
R1 (249m)	0.74	0.13	0.88	ND	0.02
R2 (349m)	0.52	0.62	0.40	ND	0.05
R3 (449m)	0.36	0.10	ND	ND	ND
R4 (549m)	0.20	0.14	ND	ND	ND
R5 (649m)	0.80	0.02	ND	ND	ND
Mean ± SD	0.54 ± 0.22	0.20 ± 0.03	0.64 ± 0.33	0	0.35 ± 0.021

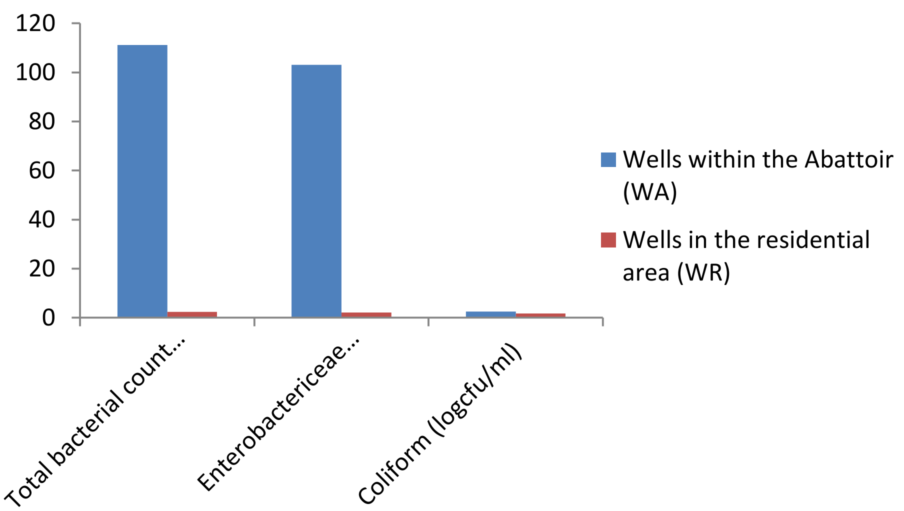

Fig 1: Bacteriological assessment of the water quality of wells within the abattoir and the residential area around the abattoir.

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Table 4: The difference in bacteria, enterobactericeae and coliform counts according to distance from the abattoir

Wells in residential areas (W_R)	**Total bacterial count (logcfu/ml)**	**Enterobactericeae count (logcfu/ml)**	**Coliform (logcfu/ml)**
RO (149m)	4.19 ± 0.99	3.96 ± 0.97	3.28 ± 1.78
R1 (249m)	1.58 ± 0.75	1.70 ± 0.87	2.23 ± 0.52
R2 (349m)	2.14 ± 1.39	2.33 ± 0.95	1.16 ± 0.41
R3 (449m)	1.21 ± 0.53	0.95 ± 0.00	0.95 ± 0.00
R4 (549m)	1.82 ± 1.03	2.12 ± 1.00	2.46 ± 1.11
R5 (649m)	3.22 ± 0.64	1.72 ± 0.92	1.04 ± 0.18
F ratio	5.868	5.586	4.54
	.002	.003	.004

IV. DISCUSSION

The physic-chemical properties of wells within the abattoir and outside the abattoir in the residential area in Table 1 revealed a pH of 6.15 and 6.33 respectively which is acidic. This support earlier report of Kara Abattoir effluents on the water quality parameters on River Ogun, Nigeria (Adesina *et al.,* 2018). The temperature of where both were 28.8 and 29.1. Temperature is one of the most important environmental features in waste water as it controls behavioural characteristics of organisms, solubility of gases and salts in water (Joanne *et al.,* 2011). The results of both wells were similar with the report of previous studies (Yogendra and Puttaiah, 2008; Magaji and Chup, 2012) which reported a range of 27.8–28.3 °C. However, the values were within the WHO standard of the permissible limit of < 40 °C (WHO, 2004). The total hardness for both wells was 572.8 ± 203.7 and 678 ± 442.1 respectively but exceed the limits of the WHO permissible limits of 150 mg/L. This support reports that abattoir wastewater contribute to total hardness values (Yogendra and Puttaiah, 2008). Nitrate in both water samples are 37.5 and 51.3 respectively and exceeded the standard of 10mg/l. This result was in line with (Akan *et al.,* 2010) findings on the chemical properties of abattoir wastewater samples in Maiduguri, Nigeria. Such high values of nitrate could result in the blue-eye syndrome in little children and pregnant women (Speijers, 1996).

From the result, the dissolved oxygen, Biochemical oxygen demand (BOD) is above the accepted standard for wastewater. High Biochemical Oxygen Demand values at the discharge point could be attributed to the low Dissolved Oxygen level, since low Dissolved Oxygen will result in high Biochemical Oxygen Demand and which is a strong indication of pollution (Tekenah *et al.,* 2014). The Total Suspended Solids (TSS) of the samples was 94.8 and 103 which exceeded the acceptable standard of 20mg/l (WHO, 2011). The high values of solids could be due to lack of sedimentation facility to separate the solid wastes and the liquid wastes before discharge High TSS can cause an increase in surface water temperature, because the suspended particles absorb heat from sunlight and this can cause dissolved oxygen levels to fall even further which can harm aquatic life (Giller and Malmqvist, 1998). Chlorides are important in the detection of sewage contamination of groundwater. The value for the chloride is 784.8 ± 442.1 and 665.0 ± 237.3 which is higher than the WHO permissible limits of 250 mg/L. The values were higher than the values from earlier study (Igbinosa and Uwidia 2018). Well water with high concentrations of chloride ions could damage plants if used for irrigation and it could also give drinking water an unpleasant taste (WHO, 2004).

In most Nigerian rivers, the heavy metals present are found in concentrations well above acceptable and permissible levels: lead, copper, zinc, nickel, chromium, cadmium and iron (Olayinka and Alo, 2004). The value for the copper is 0.35 ± 0.021 mg/L from wells located in the residential area. The values were within the WHO permissible limits of 2.0 mg/L. The value for the lead is 0.16 ± 0.00 and 0.64 ± 0.33 mg/L from the well sample within the abattoir and residential area respectively. The values were above the WHO permissible limits of 0.015 mg/L. Lead got into human body through food, water and air (Nazir *et al.,* 2015). In exposed individual, lead impacts the central nervous system leading to delayed mental and physical growth in children and could also affect the attention span and learning abilities of children (Omole *et al.,* 2018). Iron concentrations in the collected samples from within and outside the abattoirs are 0.67± 0.26 and 0.54 ± 0.22 which is above the maximum contaminant levels of the iron content based on WHO of 0.3 mg/L. This high iron content may probably be due to influx of waste blood that is carried by runoff and deposited into the shallow wells and nearby water source. Cadmium which is toxic to kidney and liver and causes poisoning in various tissues and organs of animals (Yapici *et al.,* 2006) was not detected in the sample waters.

Microorganisms are ubiquitous and are known for important functions which include; decomposition of organic materials, bioaccumulation of chemicals and biogeochemical cycling of elements. Factors that greatly influence their presence, abundance and growth in the environment are pH, temperature, pressure, availability of nutrients and salinity (Ogbonna and Ideriah, 2014). Coliforms were isolated from all the samples collected within the abattoirs and its environs (2.55logcfu/ml and 1.69logcfu/ml). The values were all above the WHO value of zero The presence of this physiologic group in these samples is an indication of feacal contamination of the samples (Prescott *et al.,* 2005). This is possibly due to indiscriminate deposition of cow dung within and around the abattoir. Through surface run-off, these are carried to the nearby water body and this leads to the presence of coliforms in such water body. *E. coli* is the most prevalent member of the fecal coliform group harboured by livestock. The presence of *E. coli* in water is considered a specific indicator of fecal contamination and the presence of enteric pathogens; similar findings have early been reported (Coker *et al.,* 2001; Igbinosa and Uwidia 2018). The microbiological analysis revealed that the abattoir wastewater samples were highly contaminated with bacteria. High organic content, organic biological nutrients, sufficient alkalinity of wastewater and its components such as blood, fat, manure, animal and undigested feeds in abattoir effluent may have contributed to the high microbial contents in the wells (Rajaram and Das, 2008; Nafarnda *et al.,* 2012).

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Statistical Prediction of Mineral Constituents in Cultivated Cucumber Crops Contiguous Cement Factory

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***Abstract*— *Cement dust influence soil characteristics as well as morphological, anatomical, bio-chemical and physiological characteristics of plants. The change in morphological characteristics of plants due to cement dust pollution directly affects bio-chemical and physiological characteristics of plants, which in turn indirectly express their effect on morphological changes. Calcium content in the cucumber cultivated soil are positively correlated $0.86^{*}(A_2\text{-}A_1)$ at 0.05 level of significant, 0.98^{**} $(A_3\text{-}A_4)$ showed 0.01 level of significant; cucumber leaves are positively correlated $0.86^{*}(A_2\text{-}A_1)$ at 0.05 level of significant, 0.98^{**} $(A_3\text{-}A_4)$ showed 0.01 level of significant and cucumber fruits are positively correlated $0.90^{*}(A_2\text{-}A_1)$ at 0.05 level of significant and 0.98^{**} $(A_3\text{-}A_4)$ showed 0.01 level of significant.*

***Keywords*— Cement pollution, cucumber, mineral constituents, bio-chemical, correlation.**

I. INTRODUCTION

Cement industry plays a vital role in the imbalances of the environment produces air pollution hazards. Cement pollution became a major threat to the survival of plants in the industrial areas. Toxic compounds such as fluoride, magnesium, lead, zinc, copper, beryllium, sulphuric acid and hydrochloric acid were found to be emitted with cement manufacturing plants. Rapid industrialization and addition of the toxic substances to the environment are responsible for altering the ecosystem (Clayton, 1982). Cement dusted plants showed significant effect on the growth of plant species compared with non-cement dust plants. Lerman and Darley, (1974); Oblisami *et al*., (1978); Krishnamurthy and Rajachidambaram, (1986); Nandi *et al*., (1987); Prasad *et al*., (1991) reported that the cement exhausts dust affects developmental morphology, physical and chemical properties of the soil, plant colouration, morphology and crop productivity (Sarabai and Vivekanandan, 1992).

II. MATERIALS AND METHODS

The present investigation was done at the experimental field during summer and monsoon seasons at Thalaiyuth of Tirunelveli District. The cultivated crops around the vicinity of the factory 3-10 kilometers were considered as affected plants and beyond 10 kilometers as control plants whereas, vegetation was not found up to 1-3 kilometers from the factory due to heavy dusting of Cucumber (Variety – Poinsettie).

Estimation of Mineral Content

The mineral contents Ca, Mg, K, Mn, Al, Fe, Si, Zn, Cu and S can be determined in acid digest of plant samples and soil with the help of Flame photometer or Atomic Absorption Spectrophotometer. Flame photometer is based on the principle that atoms of metallic elements which normally remain in ground state, under flame conditions, absorb energy when subjected to radiation of specific wavelength. The absorption of radiation is proportional to the concentration of atoms of that element. The absorption of radiation by the atoms is independent of the wavelength of absorption and temperature of the flame. First apply the respective cathode lamp for the particular nutrient bring determined and then feed the blank of the working standards to the flame photometer, Feed the aliquots (plant digest containing 0.5 g plant material in 50ml volume) directly or after a required dilutions of needed. Note down the readings (and dilution factor if dilution was done) on the flame photometer. Let the reading be 'M' compare the reading of

each element with their standard curves prepared already and calculate the concentration of each nutrient element in ppm.
Weight of plant material taken = 0.5 g
Volume made after acid digestion = 50 ml
Dilution factor = 50/0.5 = 100 times

III. RESULT AND DISCUSSION

Dust particles deposited on the plant surfaces and soil forms a crust on plant and land surface. A season with more wind velocity easily flows away through high velocity wind. So the deposition of dust particles became less. **Calcium** content in the cement polluted **soil** varied from 18.10 ± 0.017 in monsoon to 20.20 ± 0.141 in summer and control soil varied from 9.90 ± 0.717 in monsoon to 10.50 ± 0.131 in summer. Calcium content in the cement polluted cucumber **leaves** varied from 5.80 ± 0.002 in monsoon to 7.50 ± 0.071 in summer and control leaves varied from 4.30 ± 0.717 in monsoon to 4.80 ± 0.051 in summer. Calcium content in the cement polluted cucumber **fruits** varied from 6.30 ± 0.071 in summer to 6.50 ± 0.032 in monsoon and control fruits varied from 3.90 ± 0.091 in summer to 5.50 ± 0.232 in monsoon. The growth metabolic processes and yield of winter barley were found to be affected by the Duna cement (Borka, 1986).

Potassium content in the cement polluted **soil** varied from 13.50 ± 0.132 in monsoon to 16.50 ± 0.002 in summer and control soil varied from 5.20 ± 0.081 in summer to 5.30 ±0.112 in monsoon. Potassium content in the cement polluted cucumber **leaves** varied from 2.90 ± 0.112 in monsoon to 6.20 ± 0.007 in summer and control leaves varied from 0.30 ± 0.008 in monsoon to 2.60 ± 0.062 in summer. Potassium content in the cement polluted cucumber **fruits** varied from 4.20 ± 0.117 in summer to 4.80 ± 0.016 in monsoon and control fruits varied from 0.14 ± 0.003 in summer to 2.90 ± 0.017 in monsoon. **Magnesium** content in the cement polluted **soil** varied from 13.20 ± 0.232 in monsoon to 15.30 ± 0.071 in summer and control soil varied from 7.20 ± 0.121 in summer to 7.40 ± 0.112 in monsoon. Magnesium content in the cement polluted cucumber **leaves** varied from 2.90 ± 0.002 in monsoon to 5.20 ± 0.005 in summer and control leaves varied from 0.80 ± 0.008 in monsoon to 1.60 ± 0.003 in summer. Magnesium content in the cement polluted cucumber **fruits** varied from 3.90 ± 0.003 in summer to 5.20 ± 0.017 in monsoon and control fruits varied from 0.50 ± 0.005 in summer to 1.90 ± 0.001 in monsoon. Lal and Ambasht, (1981) reported changes in the accumulation of mineral plant nutrients as a result of cement dust.

Manganese content in the cement polluted **soil** varied from 18.10 ± 0.018 in monsoon to 19.90 ± 0.010 in summer and control soil varied from 8.90 ± 0.009 in summer to 9.80 ± 0.010 in monsoon. Manganese content in the cement polluted cucumber **leaves** varied from 3.60 ± 0.003 in summer to 4.60 ± 0.004 in monsoon and control leaves varied from 0.80 ± 0.001 in summer to 0.90 ± 0.009 in monsoon. Manganese content in the cement polluted cucumber **fruits** varied from 3.20 ± 0.002 in monsoon to 4.80 ± 0.012 in summer and control fruits varied from 0.70 ± 0.001 in summer to 1.00 ± 0.001 in monsoon. **Aluminum** content in the cement polluted **soil** varied from 10.20 ± 0.001 in monsoon to 12.40 ± 0.012 during summer and control soil varied from 5.00 ± 0.001 in summer to 5.50 ± 0.005 in monsoon. Aluminum content in the cement polluted cucumber **leaves** varied from 4.20 ± 0.004 in monsoon to 7.40 ± 0.014 in summer and control leaves varied from 2.80 ± 0.002 in monsoon to 4.80 ± 0.004 in summer. Aluminum content in the cement polluted cucumber **fruits** varied from 5.50 ± 0.015 in summer to 6.90 ± 0.006 in monsoon and control fruits varied from 2.70 ± 0.002 in summer to 5.10 ± 0.010 in monsoon. The increased level of Calcium, potassium, Magnesium, Manganese, Aluminum, Iron, Silica, Zinc, Copper and Sulphur in the soil, leaves, stem and fruits affect the vegetative growth, metabolism and yield of vegetables (Asubiojo *et al.*, 1991; Ade- Ademilua and Umebese, 2007).

Correlation co-efficient analysis

Correlation co-efficient analysis was carried out to evaluate the inter-relationship between different variables showed both positive and negative correlation. Calcium content in the cucumber cultivated soil are positively correlated 0.86^{*}(A_2-A_1) at 0.05 level of significant whereas 0.98^{**} (A_3 - A_4) showed 0.01 level of significant; cucumber leaves are positively correlated 0.86^{*}(A_2-A_1) at 0.05 level of significant whereas 0.98^{**} (A_3 - A_4) showed 0.01 level of significant and cucumber fruits are positively correlated 0.90^{*}(A_2-A_1) at 0.05 level of significant whereas 0.98^{**} (A_3 - A_4) showed 0.01 level of significant.Potassium content in the soil are positively correlated 0.90^{*}(A_2-A_1) at 0.05 level of significant whereas 0.99^{**} (A_3-A_4) showed 0.01 level of significant; cucumber leaves are positively correlated 0.85^{*}(A_2-A_1) at 0.05 level of significant whereas 0.97^{**} (A_3 - A_4) showed 0.01 level of significant and cucumber fruits are positively

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correlated 0.85^{*}(A_2-A_1) at 0.05 level of significant whereas 0.97^{**} (A_3 - A_4) showed 0.01 level of significant. Magnesium content in the soil are positively correlated 0.99^{**} (A_2-A_1) and (A_3 - A_4) at 0.01 level of significant; cucumber leaves are positively correlated 0.97^{**} (A_2-A_1) and (A_3 - A_4) at 0.01 level of significant and cucumber fruits are positively correlated 0.97^{**} (A_2-A_1) and (A_3 - A_4) at 0.01 level of significant.Manganese content in the soil are positively correlated 0.98^{**} (A_2-A_1) & 0.97^{**} (A_3-A_4) at 0.01 level of significant; cucumber leaves are positively correlated 0.96^{**} (A_2-A_1) and 0.97^{**} (A_3-A_4) at 0.01 level of significant and cucumber fruits are positively correlated 0.96^{**} (A_2-A_1) and 0.99^{**} (A_3-A_4) at 0.01 level of significant.Aluminum content in the soil revealed no correlation during summer and monsoon in cement polluted and control plants; leaves revealed no correlation during summer and monsoon in cement polluted and control plants and fruits revealed no correlation during summer and monsoon in cement polluted and control plants.

IV. CONCLUSION

The statistical prediction of mineral constituents and correlation regarding the studies on the effect of cement pollution revealed that the cultivated crops seriously core reduction in yield, marketable value and quality.

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Paraquat and glyphosate action on purple nutsedge (*Cyperus rotundus* L.) in Olive Orchards

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***Abstract*— *A weed control trial was conducted in order to investigate the effect of two herbicides, paraquat and glyphosate, on Cyperus rotundus infestation in olive orchards. Treatments consist on two glyphosate rates of application and two paraquat rates of application. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Each block contained five elementary plots, four plots of which were treated with the herbicides and the untreated plots serving as control. Observations were carried out at 30 and 90 days after application of herbicides. Results showed that treatment with glyphosate at 1080 g/ha and Paraquat at 400 g/ha recorded 100% of visual efficacy rating on C. rotundus at 30 days after treatment. At 90 days after treatment, glyphosate at 1080 g/ha showed the best efficacy recording 80% of visual efficacy rating on C. rotundus. However, paraquat at 800 g/ha showed weak control of C. rotundus recording only 50%. Glyphosate at 1080 g/ha recorded 78.6% of C. rotundus dry biomass reduction at 90 days after treatments. Paraquat at 400 g/ha, paraquat at 800 g/ha and glyphosate at 540 g/ha recorded weak efficacy varying from 34.7% to 53.5 % of C. rotundus dry biomass reduction at 90 days after treatments. Glyphosate at 1080 g/ha could be recommended to olive farmers when C. rotundus infestation is dominant.*

Keywords— ***Paraquat, glyphosate, purple nutsedge, Cyperus rotundus, Olive Orchards, Morocco.***

I. INTRODUCTION

Weeds compete with olive trees for water, nutrients, and sunlight. Orchard productivity is affected and young orchards may take longer to come into production (G. Steven Sibbett & Louise Ferguson). Weeds can also enhance the activities of other insects and diseases, and cause a fire hazard in the summer. Perennials are common weeds in olive orchards in Morocco. Their mode of multiplication is mainly vegetative by rhizomes, stolons, bulbs, suckers or tubers (Baudry, 2001). These methods of multiplication make their control difficult and limit the effectiveness of control methods. Purple nutsedge (*Cyperus rotundus* L.) is among the most harmful weed on tree plantations in Morocco (Hilali, 1995; Bensellam & *al*., 1997; Tahiri, 1997). It is a geophyte plant with ovoid tubers, blackish 10 to 15 mm long and 5 to 13 mm wide, with small stoloniferous rhizomes. Stems with triangular section, hairless, smooth, 15 to 60 cm high. Alternate leaves, arranged in rosette. Flattened spikelets, linear 1 to 2 cm long, composed of 10 to 20 fertile flowers. Trigon, brown, smooth seeds, 1 to 1.5 mm long and 0.8 to 1 mm wide (Tanji, 2005). Herbicides can control weed species in olive orchards provided are properly used. Paraquat and glyphosate are common herbicides used in olive tree in Morocco. Glyphosate is a non-selective broad-spectrum systemic herbicide. It belong to Amino-acids chemical family (Ezzahiri, 2017). It targets the enzyme 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS). It is effective for perennial weed control because it moves in phloem and kills all plants meristems. Paraquat is a non-selective broad-spectrum herbicide. It belong to Bipyridylium chemical family (Ezzahiri, 2017). It affects photosynthesis, by diversion of electrons from the iron-sulfur centres in chloroplast Phytosystem “I” and destructive reactive oxygen species are produced (Ezzahiri, 2017). Paraquat is not systemic and only desiccates the green foliage that it touches; it kills annual weeds but woody crops and the roots of perennial plants remain unaffected. This causes the characteristic browning of the leaves, which can occur within as little as 30 minutes of treatment under strong light conditions, and complete desiccation within a few days (Brown & *al*., 2004). In Marrakech region of Morocco, *C. rotundus* infestations in olive orchards become a serious constraint for many olive farmers. This study aims to evaluate different doses of glyphosate and paraquat herbicides on *C. rotundus* infestation in olive plantation.

II. MATERIAL AND METHODS

A trial of chemical control of *C. rotundus* was conducted in SAADA INRA research station in Marrakech region of Morocco during 2013-2014 growing season. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Each block contained five elementary plots, four plots of which were treated with the herbicides and the untreated plots serving as control. Treatments were carried out on January 03 April 2014 with Knapsack herbicide sprayer with nozzle delivering a 3 bar jet equipped with a nozzle cover to protect the trees from spray drift. The spray volume per hectare is 200L. Treatments consist on two *glyphosate* rates of application and two *paraquat* rates of application (Table 1).

Table.1: Applied herbicides in experimental site

Herbicide treatments	Herbicide active ingredient	rate of application (g/hectare)
Treatment 1	glyphosate	540 g/ha
Treatment 2	glyphosate	1080 l/ha
Treatment 3	paraquat	400 g/ha
Treatment 4	paraquat	800 g/ha

Observations were made at 30 and 90 days after application of herbicides. Observations concerned Visual rating of efficacy on *C. rotundus* following a scale ranging from 0 to 100% (where 0% is ineffective while 100% is a total destruction of weeds) and biomass reduction. *C. rotundus* dry biomass reduction percentage= [*C. rotundus* dry biomass weight in control plots – *C. rotundus* dry biomass weight in treated plots] x 100 / [*C. rotundus* dry biomass weight in control plots]. Calculation of dry *C. rotundus* biomass were made by collecting *C. rotundus* in each plot using a quadrant of 1m x 1m. Samples were dried in an oven at 75 ° C for 48 hours. Then, dry plant material in each plot was weighed with a precision balance. Statistical analyzes were performed with IBM SPSS Statistics, version 21.0 using the analysis of variance (ANOVA).The difference among treatment means was compared by Tukey's test at $P = 0.05$.

III. RESULTS AND DISCUSSION

1. Effect on visual efficacy rating

Statistical analysis revealed significant differences in the efficacy between treatments. Data in table 2 show that glyphosate at 1080 g/ha and paraquat at 400 g/ha recorded 100% of *C. rotundus* control at 30 days after treatments. Glyphosate at 540 g/ha and paraquat at 400 g/ha recorded 85% and 80% respectively at 30 days after treatment. At 90 days after treatment, glyphosate at 1080 g/ha showed the best efficacy recording 80% of *C. rotundus.* Conversely, paraquat at 400 g/ha, paraquat at 800 g/ha and glyphosate at 540 g/ha recorded weak efficacy varying from 20% to 50% of *C. rotundus* control at 90 days after treatment.

Table.2: Effect of herbicides on visual efficacy rating of C. rotundus

Treatments	Visual efficacy rating (%) (30 DAT*)	Visual efficacy rating (%) (90 DAT*)
Glyphosate at 540 g/ha	85^{b}	40^{b}
Glyphosate at 1080 g/ha	100^{a}	80^{a}
Paraquat at 400 g/ha	80^{b}	20^{c}
Paraquat at 800 g/ha	100^{a}	50^{b}
Pα = 0.05	*< 0.001*	*< 0.001*

**DAT: Days After Treatment. Significant differences within the same column and means followed by the same letter do not differ at p= .05 according to Tukey's test.*

2. Effect on weed dry biomass reduction

Statistical analysis revealed significant differences in the efficacy between treatments. Result in table 3 show that glyphosate at 1080 g/ha showed the best control of *C. rotundus* recording 78.6% of *C. rotundus* dry biomass reduction 90 days after treatments. Paraquat at 400 g/ha, paraquat at 800 g/ha and Glyphosate at 540 g/ha recorded weak efficacy varying from 34.7% to 53.5 % of *C. rotundus* dry biomass reduction at 90 days after treatment. Some authors reported that glyphosate applied at 1080 g/hectare in Gharb region in Morocco showed only 48.23% on monocotyledonous weed dry biomass reduction including *C. rotundus* (Bensellam & Bouhache, 2007). In our trial, glyphosate at 1080 g/ha recorded 78.6% of *C. rotundus* dry biomass reduction. This difference registered between regions could be explained by climatic conditions that differ from one region to another.

Table 3: Effect of herbicides on weed dry biomass reduction of C. rotundus

Treatments	C. rotundus dry biomass reduction (%) (90 DAT*)
Glyphosate at 540 g/ha	45.4^{b}
Glyphosate at 1080 g/ha	78.6^{a}
Paraquat at 400 g/ha	34.7^{c}
Paraquat at 800 g/ha	53.5^{b}
Pα = 0.05	*< 0.001*

**DAT: Days After Treatment. Significant differences within the same column and means followed by the same letter do not differ at p= .05 according to Tukey's test.*

IV. CONCLUSION

This study has shown that glyphosate at 1080 g/ha gave the best control of *C. rotundus*. Paraquat achieved excellent efficacy on *C. rotundus* at 30 days after treatment but not at 90 days after treatment. Glyphosate at 1080 g/ha could be recommended to olive farmers in Marrakech region of Morocco when *C. rotundus* infestation is dominant. This study should be repeated on other perennial weed species to evaluate their response to herbicides application.

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Influence of tillage and straw mulching on purple nutsedge (*Cyperus rotundus* L.) in Olive Orchards

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***Abstract*— *A weed control trial was conducted in order to investigate the effect of tillage and straw mulching on Cyperus rotundus infestation in olive orchards. Tillage was applied on six olive trees and straw mulching was applied on six olive trees. Observations on C. rotundus were carried out 30 and 90 days after treatments after application of tillage and straw mulching. Results showed that the average tillage efficacy recorded 80.3±3.5% of visual efficacy rating at 30 DAT, which is higher than the efficacy of straw mulching that recorded an efficacy of 57.8±3.4%. The average tillage efficacy recorded 71.8±3.4% of visual efficacy rating at 90 DAT, which is higher than the efficacy of straw mulching that recorded an efficacy of 43.8±4.8%. C. rotundus infestation increased at 90 days after tillage compared to 30 days after tillage. The average tillage efficacy recorded 62.3±3.5% on C. rotundus dry biomass reduction at 90 DAT, which is higher than the efficacy of straw mulching that recorded an efficacy of 34.8±5.3%. Thus, frequent tillage could be recommended to prevent C. rotundus re-infestation in Olive Orchards.*

***Keywords*— Tillage, straw mulching, purple nutsedge, Cyperus rotundus, Olive Orchards, Morocco.**

I. INTRODUCTION

The olive tree plays an important socio-economic role in the Mediterranean basin. Olive tree has multiple functions of controlling erosion, reclaiming agricultural land and increasing the income of populations in mountain areas. It is the main tree fruit cultivated in Morocco covering an area of 784 000 ha with a total production of 1 500 000 tons of olives (Berrichi, 2002; MADRPMEF, 2019). It is present in different agricultural areas of Morocco except the coast. Weeds are among the constraints of olive tree production are. They compete with olive trees for water, nutrients, and sunlight. Orchard productivity is affected and young orchards may take longer to come into production (G. Steven Sibbett & Louise Ferguson). Weeds can also enhance the activities of other insects and diseases, and cause a fire hazard in the summer. Perennials are common weeds in olive orchards in Morocco. Their mode of multiplication is mainly vegetative by rhizomes, stolons, bulbs, suckers or tubers (Baudry, 2001). These methods of multiplication make their control difficult and limit the effectiveness of control methods (Bensellam, 1997). The purple nutsedge (*Cyperus rotundus* L.) is among the most harmful weed of olive orchards in Morocco. In Marrakech region of Morocco, *C. rotundus* infestations in olive orchards become a serious constraint for many olive farmers. Herbicides are a very effective tool against *C. rotundus* infestation in olive orchards. However, they should be combined with preventive methods in order to achieve the best weed control. Tillage and straw mulching is among the preventive methods applied in Marrakech region. This study aims to evaluate Tillage and straw mulching in their effect on *C. rotundus* infestation in olive plantation.

II. MATERIAL AND METHODS

A trial of weed control using preventive methods on *C. rotundus* was conducted in SAADA INRA research station in Marrakech region of Morocco during 2013-2014 growing season. Preventive methods consist on tillage and straw mulching. Tillage was applied on six olive trees and straw mulching was applied on six olive trees. Tillage and straw mulching were applied on January 10 April 2014. Observations were carried out at 30 and 90 after application of tillage and straw mulching. Observations concerned visual rating of efficacy at 30 DAT (days after treatment) on *C. rotundus* following a scale ranging from 0 to 100% (where 0% is ineffective while 100% is a total destruction of weeds) and biomass reduction. *C. rotundus* dry biomass reduction percentage= [*C. rotundus* dry biomass weight in control plots – *C. rotundus* dry biomass weight in treated plots] x 100 / [*C. rotundus* dry biomass weight in control plots]. Calculation of dry *C. rotundus* biomass were made by collecting *C. rotundus* in each plot using a quadrant of 1m x 1m. Samples were dried in an oven at 75 ° C for 48 hours. Then, dry plant material in each plot was weighed with a precision balance. Statistical analyzes were performed with IBM SPSS Statistics, version 21.0. To find out the differences in *C. rotundus* efficacy, we applied the Student's independent *t*-test at $P = 0.05$.

III. RESULTS AND DISCUSSION

1. Effect on visual efficacy rating at 30 DAT

Data in Table 1 show that the average tillage efficacy recorded 80.3±3.5% of visual efficacy rating at 30 DAT, which is higher than the efficacy of straw mulching that recorded an efficacy of 57.8±3.4%. Indeed, the value of *t*-statistic was 11.15 with a *P*-value lower than significance level of 0.05. Thus, we conclude that there are statistical differences at significance level of 0.05 between the averages of tillage efficacy and the averages of straw mulch efficacy in favor of tillage, which has shown the best efficacy. Figure 1 shows the obtained results.

*Table 1: Effect of tillage and straw mulching on Visual efficacy rating of C. rotundus at 30 DAT**

	Visual efficacy rating (%) 30 DAT*	*t*-statistic**	*P*-value
Tillage	80.3±3.5		
Straw mulching	57.8±3.4	11.150	<0.001

*Data represented are mean ± standard deviation for (n=6). * DAT: Days after treatments. ** Independent t-test.*

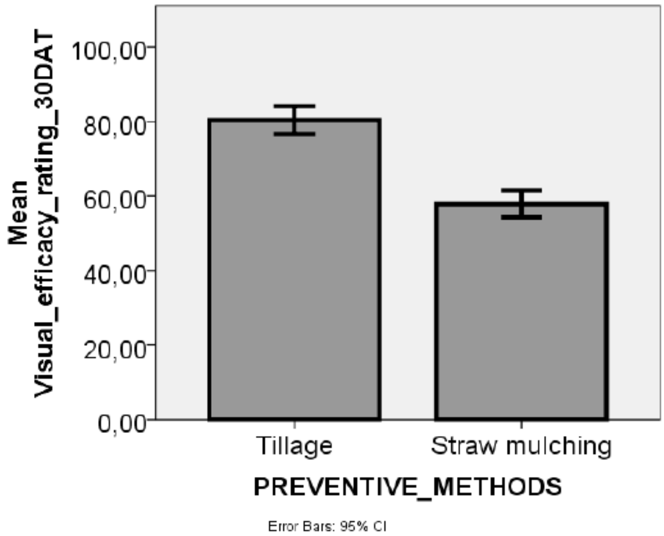

*Fig.1: Effect of tillage and straw mulching on Visual efficacy rating of C. rotundus at 30 DAT**
** DAT: Days after treatment*

2. Effect on visual efficacy rating at 90 DAT

Data in Table 2 show that the average tillage efficacy recorded 71.8±3.4% of visual efficacy rating at 90 DAT, which is higher than the efficacy of straw mulching that recorded an efficacy of 43.8±4.8%. Indeed the value of *t*-statistic was 11.4 with a *P*-value lower than significance level of 0.05. Thus, we conclude that there are statistical differences at significance level of 0.05 between the averages of tillage efficacy and the averages of straw mulch efficacy in favor of tillage, which has shown the best efficacy. Figure 2 shows the obtained results.

*Table 2: Effect of tillage and straw mulching on Visual efficacy rating of C. rotundus at 90 DAT**

	Visual efficacy rating (%) 90 DAT*	*t*-statistic**	*P*-value
Tillage	71,8±3,4		
Straw mulching	43,8±4,8	11.4	<0.001

*Data represented are mean ± standard deviation for (n=6). * DAT: Days after treatment. ** Independent t-test.*

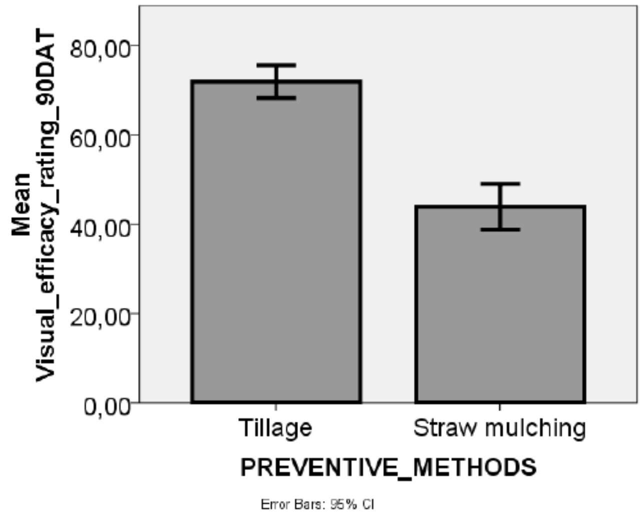

*Fig. 2: Effect of tillage and straw mulching on Visual efficacy rating of C. rotundus at 90 DAT**
** DAT: Days after treatment*

3. Effect on *C. rotundus* dry biomass reduction at 90 DAT

Data in Table 2 show that the average tillage efficacy recorded 62.3±3.5% on *C. rotundus* dry biomass reduction at 90 DAT, which is higher than the efficacy of straw mulching that recorded an efficacy of 34.8±5.3%. Indeed the value of t-statistic was 10.4 with a *P*-value lower than significance level of 0.05. Thus, we conclude that there are statistical differences at significance level of 0.05 between the averages of tillage efficacy and the averages of straw mulching efficacy in favor of tillage, which has shown the best efficacy(Fig. 2). Some authors reported that frequent

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tillage could be effective in reducing *C. rotundus.* However season-long management was essential to prevent *C. rotundus* proliferation over time (Sanjeev K. Bangarwa & *al*., 2008). In fact, Tillage led to a breakup of tubers from aerial shoots, roots, and other tubers in chains. In addition, it brings them close to the soil surface where they are exposed to carbohydrate starvation, desiccation and cold injury (Glaze 1987). However, it is important to mention that tillage in olives can damage shallow roots and may increase erosion in the mountain areas where there is many olive plantations in Morocco.

*Table 3: Effect of tillage and straw mulching on C. rotundus dry biomass reduction at 90 DAT**

	Biomass (%) 90 DAT	*t*-statistic	*P*-value
Tillage	62.3±3.5		
Straw mulch	34.8±5.3	10.4	<0.001

*Data represented are mean ± standard deviation for (n=6). * DAT: Days after treatment. ** Independent t-test.*

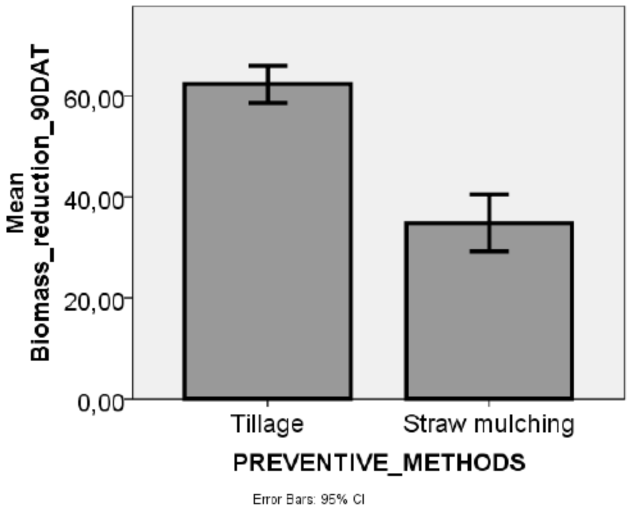

*Fig.3: Effect of tillage and straw mulching on C. rotundus dry biomass reduction at 90 DAT**
** DAT: Days after treatment*

IV. CONCLUSION

This study has shown that tillage gave the best control of *C. rotundus*. Straw mulching showed weak to medium efficacy on *C. rotundus*. In fact, *C. rotundus* infestation increased at 90 days after tillage compared to 30 days after tillage. Thus, frequent tillage is necessary to prevent *C. rotundus* re-infestation. This study should be repeated in different sites on different perennial weeds to evaluate the effect of tillage on weed infestation.

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Effect of persistence in soil of Sulfosulfuron on fababean, chickpea and lentil grown in rotation in semi-arid area

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***Abstract*— *A trial was conducted in greenhouse during 2009-2010 growing season in Chaouia semi-arid area of Morocco in order to study effect of Sulfosulfuron persistence in soil on fababean, chickpea and lentil grown in rotation. Three food legumes were grown in greenhouse pots: fababean, chickpea and lentil. Food legumes seeds were sown in five filled with soil taken from a plot that had been treated the previous year with Sulfosulfuron. Results showed that the highest Sulfosulfuron phytotoxicity was observed on lentil recording 60.8% of phytotoxicity. Phytotoxicity observed on chickpea and fababean pots registered 41.6% and 37.6%, respectively. Thus, intensive tillage is recommended to farmers of Chaouia semi-arid region of Morocco when fababean, chickpea and lentil crops are grown in rotation after cereals, which had been treated with Sulfosulfuron.*

Keywords— ***persistence, soil, sulfosulfuron, chickpea, fababean, lentil, chickpea.***

I. INTRODUCTION

Cereal and food legumes crops play an important role in the arid and semi-arid areas of Morocco. They are food basis for the majority of the population. Weeds remain a major problem to cereals and food legumes production in Morocco. The problem of herbicide residues in soil hinders farmers, limits their strategic choice of crop rotation, and eventually decrease their economic benefits. Many authors reported cases of phytotoxicity due to sulfosulfuron (Abdennadher & Tanji, 2000; El Ghazi, 2003; Hamal, 2005). Sulfosulfuron is new herbicides used in cereal to control weed infestation (Ezzahiri, 2017). It belongs to sulfonylurea chemical family. Sulfosulfuron inhibits the plant enzyme acetolactate synthase (ALS), which is essential for the synthesis of branched-chain amino acids valine, leucine, and isoleucine. Inhibition of amino acid production leds to inhibition of cell division and causes plant death. Sulfosulfuron is a systemic, phloem and xylem mobile herbicide that is absorbed through leaves, and roots (Ezzahiri, 2017). Herbicides residues in soil could injure in some cases crops grown crops grown in rotations. Residual fate and persistence behavior of herbicide in soil is very complex involving several factors (soil, rain off…etc.). This study aims the assessment of the phytotoxicity of Sulfosulfuron residues in soil on fababean, chickpea and lentil in semi-arid area of Morocco.

II. MATERIAL AND METHODS

A trial was conducted in greenhouse of INRA Settat of Morocco during 2009-2010 growing season in order to study the effect of Sulfosulfuron persistence in soil on fababean, chickpea and lentil grown in rotation. The experimental design was completely randomized designs with five replications. Three food legumes tested in greenhouse pots: fababean, chickpea and lentil. Food legumes seeds were sown in five pots filled with soil taken from a plot that had been treated the previous year with Sulfosulfuron herbicide at the rate of application of 19.95 g/ha. Soil physicochemical properties of the experimental site are shown in table 1. Pots size was (30 cm x 30 cm x 40 cm). Observations concerned Visual rating of phytotoxicity on fababean, chickpea and lentil following a scale ranging from 0 to 100% (where 0% is no symptoms of phytotoxicity while 100% is a necrosis on the whole plant). Statistical analyzes were performed with IBM SPSS Statistics, version 21.0 using the analysis of variance (ANOVA).The differences among phytotoxicity means was compared by Tukey's test at *P*= 0.05.

Table 1: Soil physicochemical properties of the experimental site

Clay%	Loam%	Sand %	pH	CaCO3 %	Organic Matter % (0.2 mm)
52.1	25.3	22.6	7.9	13.6	2.54

III. RESULTS AND DISCUSSION

Statistical analysis revealed significant differences between legume species (Table 1). Results in Table 1 showed that the highest Sulfosulfuron phytotoxicity was observed on lentil recording 60.8% of phytotoxicity. Phytotoxicity observed on chickpea registered 41.6%. Phytotoxicity observed on fababean registered 37.6%. There were no significant statistical differences between phytotoxicity registered on fababean and chickpea. Thus, Sulfosulfuron residues on soil were more phytotoxic to lentil than fababean and chickpea.

Table.2 : Observed phytotoxicity of Sulfosulfuron residues in the soil on fababean, chickpea and lentil

Food legume	Phytotoxicity de Sulfosulfuron
Fababean	37.4[a]
Chickpea	41.6[a]
Lentil	60.8[b]
*P*α = 0.05	<0.001

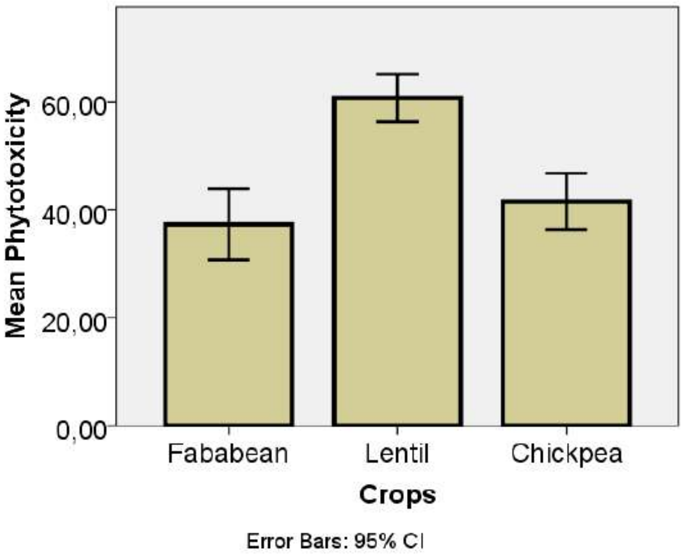

Fig.1: Observed phytotoxicity of Sulfosulfuron residues in the soil on fababean, chickpea and lentil

Several factors influence impact of herbicide residues in soil on subsequent crops. Hens, same herbicide could behave differently from agro-climatic regions and soils. Some authors reported that dissipation of pesticide applied to the soil is a function of dispersion and degradation processes (Grébil, 2001). Dispersion is influenced by an association of transfer processes such volatilization, leaching, water runoff, and plant absorption. Whereas degradation is described by chemical reactions (photodecomposition, hydrolysis...etc.) or biochemical reactions involving soil microorganisms (Grébil, 2001). Bouhache (2018) had listed some measures to avoid the risk of phytotoxicity caused by herbicide residues. In fact, weed management in accordance with the principles of integrated management is an approach to achieve this aim. In addition, the choice of low-half-life herbicides and no risk of herbicide persistence on crops in rotation, the reduction of the recommended dose with equal efficacy, the mixing of two or more herbicides and the earliest possible application are measures that reduce the time of herbicides dissipation in the soil and save the subsequent crops from any adverse side effects. Frequent small irrigations is another possibility to increase chemical degradation of applied herbicides (Bouhache, 2018). In addition, respect of the the time required between the application of herbicides and next crops grown in rotation, indicated on the prospectuses of certain herbicides, is an element to be considered in this management. Thus, it is recommended to read the herbicide prospectus to be informed of the time interval between the application of herbicides and subsequent crops. Finally, it is important to mention that the use of tillage after harvesting is recommended to mix the treated and untreated soil layers and thus disperse the herbicide residues (Zaragoza, 2005, Bouhache, 2018).

IV. CONCLUSION

This study has shown that the highest Sulfosulfuron phytotoxicity was observed on lentil recording 60.8% of phytotoxicity. Phytotoxicity observed on chickpea registered 41.6%. Phytotoxicity observed on fababean registered 37.6%. Intensive tillage is recommended to farmers of Chaouia region of Morocco when fababean, chickpea and lentil crops are grown in rotation after cereals, which had been treated with Sulfosulfuron. This study should be repeated in different sites and different soils for further assessment of Sulfosulfuron residues in soil and its phytotoxicity should be evaluated on other crops grown in rotations.

ACKNOWLEDGMENT

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Sour Apple Concentrate: processing essentials and organoleptic properties

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Abstract—*The aim of this study is to have a new apple-based product with relatively long shelf life. Sans Pareille, Malus domestica, 'mouwachah' was harvested prematurely and sour-apple-concentrate was produced following a traditional Lebanese recipe used to produce pome-granate sauce (PS). Part of the prepared SAC had Salt added (SACSa), another citric acid (SACCA) and both Salt and Citric Acid (SACSaCA). The higher the Brix the significantly higher the density and total caloric content and significantly lower water-acitivity. There was no significant difference in caloric value on Dry-Matter basis. Furthermore, SAC and SACSa had significantly lower pH only at 75ºBrix. Within SACCA and SACSaCA pH at the 75ºBrix is significantly the lowest compared to pH values at 55 and 65ºBrix. The higher the Brix-level the significantly higher the Titratable Acidity (TA) in all products except in SACSaCA where it was significantly higher only at 75ºBrix. Within the same Brix level, TA of SAC and SACSa did not differ significantly, while TA was significantly lower than TA of SACCA and SACSaCA. These products were compared to traditional products PS and grape-verjuice. In terms of aroma, appearance, texture, taste, sourness, sweetness, overall-acceptability and average score of the Sour Apple concentrate products, except for SACSaCA, scored significantly higher than PS and grape verjuice. Within the Sour-Apple-Products the 75ºBrix score significantly the lowest. These results showed that Sour apple concentrate with 65ºBrix with addition of salt or citric acid but not both.*

Keywords— ***Apple, Sans Pareille, Apple juice, Sour Apple Concentrate, mouwashah, Citric Acid, Salt, water activity.***

I. INTRODUCTION

Lebanese "traditional" varieties of apples mainly Red Delicious, Golden Delicious and Sans Pareille (mouwachah) apple, are planted and relatively newer varieties have been introduced such as Gala, Fuji, Scarlett, Super Chief, and Granny Smith. All of these apples had little access to the regional market in last few years due to the regional situation. FAO stats 2013 states that the total Area planted with apples is of 13,604 hectares (ha) producing of 153,151 tons of apples annually constituting 23% of total Lebanese fruits production [1].

Apple juice has pleasant taste and high concentration of phenolic compounds, vitamins, minerals, dietary fiber and antioxidant capacity [2-4]. Some intrinsic and extrinsic factors affect the properties of Fruit-based products that are assessed by different chemical, physical, and sensory parameters. Intrinsic factors include maturity, cultivar, root-stock and pest resistance. Extrinsic factors include growing region, cultivation practices, climate, water stress, storage conditions and the processing technology, packing and transportation [5] . Industrial products from apples include: apple juice, dried apples, apple vinegar and sweet apple molasses [6].

Sans Pareil is woody perennial and Deciduous Fruit. It is an old apple variety which can be eaten fresh or used for cooking. It blooms in March and is harvested in September. This variety is very sweet and juicy is has a large fruit with orange and yellow skin. It is also one of the highly susceptible varieties to fire-blight. This in addition to the problem of marketing all Lebanese apples made this variety suitable to study its development into a shelf stable product resembling two traditional products in the Lebanese market, mainly grape-verjuice and pomegranate sauce. Noting in fruits the harvest time affected pH, organic acids, sugars, taste, but not aroma [7].

The grape-verjuice is simply the juice of unripe grapes, which is boiled and bottled with a 13ºBrix total soluble solid. The low pH (2.6-2.9) and the optional addition of salt makes this product shelf stable grape-verjuice. As for the pomegranate molasses it is done from pomegranate juice which is thickened by boiling to reach around 65-73ºBrix of total soluble solids and a pH value ranging from 1.73 to 3 [8]. Unripe apple juice made from apples harvested in July/august was studied and compared to those sauces mentioned above.

Unripe Sour apple concentrate might be a food ingredient that provides acidity and flavor and as an alternative to vinegar in salad dressings and other applications where the sensory character of acetic acid is unacceptable [9]. Because it can be produced from unripe apple with fire-blight, it might be of great interest to apple growers and to the food industry.

II. MATERIALS AND METHODS

2.1. Sour apple concentrate preparation

The materials used in this study are unripe Sans Pareille, Malus domestica, (mouwachah) which were collected in end of July, washed and pressed to produce Sour Apple juice of 13ºBrix total soluble solids. This juice was thickened by boiling to have Sour Apple Juice, Sour Apple Concentrate plus salt (1%), Sour apple concentrate plus citric acid (1%) and Sour apple concentrate plus both salt and citric acid all with 55, 65 and 75ºBrix total soluble solids (Fig. 1).

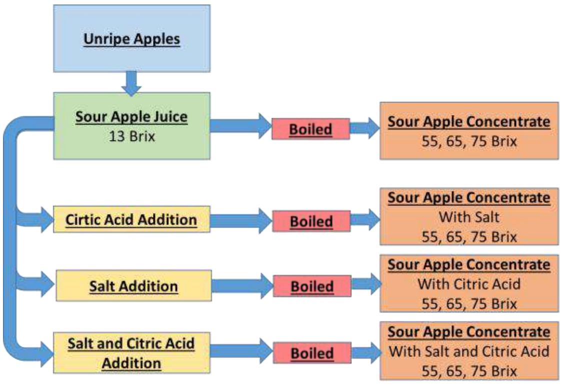

Fig.1: Sour Apple Concentrate production flow

2.1.1. Sour Apple Juice, Grape-Verjuice and Pomegranate sauce

Homemade verjuice and pomegranate concentrate were collected and the physico-chemical properties were recorded.

2.2. Equipment used to measure Physico-chemical properties

Brix Value: Brix Value was measured using Portable hand held RFM700 refractometer (Bellingham and Stanley LTD. United Kingdom).

Weight determination: Weight was measured using Portable electronic balance Model 727 was used to measure the weight with an accuracy of ±1 gr (Jata Hogar).

pH: Microcomputer based pH /conductivity /TDS /salinity and temperature pocket meter Model pH/EC80 was used to measure the pH (Jenco VisionP).

Ash content: Ash was determined using the AOAC 942.05 method.

Volume Determination: 10mL glass graduated cylinder, with sub gradations of 0.1mL (Graduated cylinder, tall form, BLAUBRAND®, class A, Boro 3.3, DE-M).

Caloric Value: Bomb calorimeter IKA C200 was used (KA®-Werke GmbH & Co. KG)

2.3. Sensory Analysis

The sensory attributes attained from 35 taste panelists repeated 3 times include: aroma with 1 having no aroma and 9 having best aroma, appearance with 1 having worst appearance and 9 having the best appearance, texture with 1 being the worst and 9 being the best one, sourness with 1 having no sourness and 9 having the highest sourness, sweetness with 1 having no sweetness and 9 having highest sweetness and overall acceptability with 1 having lowest acceptability and 9 having the highest acceptability. In addition to that, the sensory score of each Sour apple concentrate was calculated by taking the mean of the different sensory attributes.

2.4. Statistical analysis

All tests and analysis were run in triplicates and averaged. General linear model performed via SPSS (statistical Package for the Social Sciences, version 17.0) was used to study the difference between Sour Apple Juice products in terms of density, caloric content on product basis and on dry matter basis, ash content, pH, titratable acidity (gr/100ml) and total soluble solids per titratable acidity among and within the different Brix.

Furthermore, General linear model performed via SPSS was used to assess the difference between the Sour apple concentrate products, with and without salt, with and without citric acid and with both salt and citric acid taking Brix as a covariate.

To study the effect of Brix on the average score General linear model performed via SPSS was used taking salt and citric acid addition as covariate.

III. RESULTS

3.1 Sour Apple Juice, verjuice, pomegranate

The Juice extracted from the unripe apples had a brix value of 13ºBrix which comparable to that of verjuice but much lower than that of pomegranate (Table 1). They did differ significantly in terms of pH where Verjuice showed the lowest and consequently the highest TA and the lowest TSS/TA.

Concerning the juice to concentrate conversion value it was noted that100gr of premature apple was needed to produce 61.3gr Apple Juice with 13ºBrix., 14.5gr SAC with 55ºBrix, 12.25gr SAC with 65ºBrix and 10.63gr SAC with 75ºBrix.

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Table.1 Sour unripe Apple Juice, Verjuice and pomgranate Physical properties

	Apple juice	Verjuice	Pomegranate
	Mean ± SE	Mean ± SE	Mean ± SE
TSS (Brix)	13.0±0.002	13.1±0.002	65.0±0.002
pH	$3.67^{a}\pm0.04$	$2.84^{b}\pm0.04$	$4.04^{c}\pm0.04$
TA (gr/100ml)	$4.53^{a}\pm0.96$	$17.73^{b}\pm1.00$	$10.47^{c}\pm0.95$
TSS/TA	$2.87^{a}\pm0.031$	$0.73^{b}\pm0.03$	$6.21^{c}\pm0.03$

TSS: Total Soluble Solids; TA: Titratable Acidity;
Among rows means with different letters are significantly different

3.2 Physicochemical properties

3.2.1 Density

Comparing the density of the different Sour Apple Concentrate (SAC) [10] products within the same brix, showed that the density of the SAC within 55ºBrix was significantly the lowest while there was no difference when comparing densities of SAC with Salt addition (SACSa), SAC with citric acid addition (SACCA), SAC with Sa and CA addition (SACSaCA). This difference, however, difference disappeared within 65 and 75ºBrixes (Table 2).

Table.2: Density of different sour apple concentrate products

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$1.257^{a\,1}\pm0.002$	$1.320^{b\,1}\pm0.002$	$1.362^{c\,1}\pm0.002$
SACSa	$1.264^{a\,2}\pm0.002$	$1.319^{b\,1}\pm0.002$	$1.359^{c\,1}\pm0.002$
SACCA	$1.264^{a\,2}\pm0.002$	$1.310^{b\,2}\pm0.002$	$1.361^{c\,1}\pm0.002$
SACSaCA	$1.270^{a\,2}\pm0.002$	$1.323^{b\,1}\pm0.002$	$1.363^{c\,1}\pm0.002$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

Within the same product the density of SAC, SACSa and SACA as expected was significantly higher the higher the brix value. The density of SACSaCA was significantly the highest at 75ºBrix, this significance did not show between the 55 and 65ºBrix (Table 2).

3.2.2 Caloric content product basis and dry matter basis

The total caloric content within the product type is as expected where the higher the brix value the significantly higher the caloric content. (Table 3). Furthermore, within the same Brix, 55, 65 and 75, there was no significant difference in the caloric value (Calorie/100gr Product) between SAC, SACSa, SACCA and SACSaCA

Table.3:Caloric content of different sour apple concentrate products per 100 gr product

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$172.13^{a\,1}\pm2.75$	$207.84^{b\,1}\pm2.75$	$233.72^{c\,1}\pm2.75$
SACSa	$177.06^{a\,1}\pm2.75$	$206.04^{b\,1}\pm2.75$	$237.27^{c\,1}\pm2.75$
SACCA	$174.36^{a\,1}\pm2.75$	$206.37^{b\,1}\pm2.75$	$233.40_{c\,1}\pm2.75$
SACSaCA	$176.75^{a\,1}\pm2.75$	$207.10^{b\,1}\pm2.75$	$234.77^{c\,1}\pm2.75$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

The caloric content per dry matter within SAC, SACSa, SACCA and SACSaCA among the different brixes did not differ significantly. The same is true concerning the caloric content of the different SAC products, with and without salt and citric acid, where it did not differ significantly (Table 4).

Table.4 Caloric content of different sour apple concentrate products per 1 gr Dry Matter

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$3.107^{a\,1}\pm0.044$	$3.163^{a\,1}\pm0.041$	$3.127^{a\,1}\pm0.04$
SACSa	$3.144^{a\,1}\pm0.044$	$3.136^{a\,1}\pm0.041$	$3.176^{a\,1}\pm0.04$
SACCA	$3.092^{a\,1}\pm0.045$	$3.185^{a\,1}\pm0,041$	$3.120^{a\,1}\pm0.04$
SACSaCA	$3.134^{a\,1}\pm0.046$	$3.134^{a\,1}\pm0.058$	$3.140^{a\,1}\pm0.04$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

3.2.3 Water Activity

As expected the higher the brix value the significantly lower the water activity within the different SAC products (Table 5).

Table.5 Water activity of the different SAC products

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$0.976^{a\,1}\pm0.116$	$0.84^{b\,1}\pm0.046$	$0.715^{c\,1}\pm0.047$
SACSa	$0.97^{a\,1}\pm0.112$	$0.809^{b\,2}\pm0.046$	$0.701^{c\,1}\pm0.044$
SACCA	$0.971^{a\,1}\pm0.133$	$0.868^{b\,3}\pm0.046$	$0.714^{c\,1}\pm0.045$
SACSaCA	$0.97^{a\,1}\pm0.135$	$0.795^{b\,2}\pm0.044$	$0.671^{c\,2}\pm0.044$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

Within the 55ºBrix the water activity did not differ significantly between the water activity values of SAC, SACSa, SACCA and SACSaCA. Within 75ºBrix the water activity value of SACSaCA was significantly the lowest with the difference in water activity values of SAC, SACSa and SACCA was not significant (Table 5).

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3.2.4 Ash content

As for the Ash-content within SAC showed no significant difference among the different Brixes. Within SACSa and SACCA the ash content at 55 and 65ºBrix did not show any significant difference both being significantly lower than the ash content at 75ºBrix. Within SACSaCA the ash content at 55ºBrix was significantly lower from those 65 and 75ºBrix whose ash content did not differ significantly from each other (Table 6).

Table.6 Ash content per product (gr/gr)

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$0.004^{a\,1}\pm0.002$	$0.005^{a\,1}\pm0.001$	$0.006^{a\,1}\pm0.04$
SACSa	$0.011^{a\,1}\pm0.002$	$0.014^{a\,2}\pm0.001$	$0.016^{b\,2}\pm0.04$
SACCA	$0.011^{a\,2}\pm0.002$	$0.014^{a\,2}\pm0.001$	$0.016^{b\,2}\pm0.04$
SACSaCA	$0.020^{a\,3}\pm0.002$	$0.025^{b\,3}\pm0.002$	$0.028^{b\,3}\pm0.04$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

3.2.5 pH

The pH within the SAC and SACSa showed no significant difference between 55 and 65ºBrix with pH at 75ºBrix being significantly the lowest (Table 7).

Within 55ºBrix, the pH of SAC was significantly the highest compared to pH of SACSa and SACCA, which did not differ significantly from each other. The pH of SACSaCA was significantly the lowest (Table 7).

Table.7 pH of the different Sour apple concentrate

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$3.069^{a\,1}\pm0.04$	$2.983^{a\,1}\pm0.038$	$1.877^{b\,1}\pm0.037$
SACSa	$2.942^{a\,2}\pm0.04$	$2.840^{a\,2}\pm0.037$	$1.923^{b\,1}\pm0.037$
SACCA	$2.870^{a\,2}\pm0.041$	$2.727^{b\,3}\pm0.037$	$1.780^{c\,1}\pm0.037$
SACSaCA	$2.760^{a\,3}\pm0.042$	$2.592^{b\,4}\pm0.053$	$1.930^{c\,1}\pm0.037$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

Within 65ºBrix the pH of all SAC products differ significantly from each other with SAC being significantly the highest, followed by the pH of SACSa and by the pH of SACCA and SACSaCA being significantly the lowest (Table 7).

Within the 75ºBrix the pH of SAC, SACSa, SACCA and SACSaCA did not differ significantly from each other (Table 7).

3.2.6 Titratable Acidity and Total Soluble Solids per Titratable acidity

Titratable Acidity within the SAC products showed that the higher the Brix the significantly higher the Titratable Acidity (Table 8).

Within the same Brix the SACCA had the significantly highest Titratable Acidity, followed by the Titratable Acidity of the SACSaCA which is significantly higher than the SACSa and SAC which were significantly the lowest (Table 8).

Table.8 Titratable Acidity (gr/100ml) of the different Sour Apple products

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$37.18^{a\,1}\pm1.06$	$40.69^{b\,1}\pm0.97$	$49.43^{c\,1}\pm0.96$
SACSa	$35.77^{a\,1}\pm1.05$	$43.35^{b\,1}\pm0.98$	$49.40^{c\,1}\pm0.96$
SACCA	$50.49^{a\,2}\pm1.08$	$62.75^{b\,2}\pm0.98$	$95.19^{c\,2}\pm0.96$
SACSaCA	$46.47^{a\,3}\pm1.09$	$47.57^{a\,3}\pm1.39$	$53.65^{b\,3}\pm0.96$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

Within SAC and SACSa the brix had no clear significant effect on the Total Soluble Solids per Titratable Acidity (TSS/TA). Within SACCA where the TSS/TA at 55 and 65ºBrix were significantly higher than TSS/TA at 75ºBrix. Within SACSaCA the TSS/TA at 55ºBrix was significantly the lowest where the TSS/TA at 65 and 75ºBrix did not differ significantly (Table 9).

Table.9 Total Soluble Solids (Brix) per Titratable Acidity (gr/100ml) of the different Sour Apple products

Brix	55	65	75
	Mean ± SE	Mean ± SE	Mean ± SE
SAC	$1.495^{a\,1}\pm0.031$	$1.613^{b\,1}\pm0.031$	$1.512^{a\,1}\pm0.031$
SACSa	$1.583^{a\,1}\pm0.031$	$1.515^{a\,2}\pm0.031$	$1.513^{a\,1}\pm0.031$
SACCA	$1.119^{a\,2}\pm0.031$	$1.032^{a\,3}\pm0.031$	$0.787^{b\,2}\pm0.031$
SACSaCA	$1.217^{a\,3}\pm0.031$	$1.391^{b\,4}\pm0.031$	$1.394^{b\,3}\pm0.031$

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid;
Among rows: means with different letters are significantly different;
Among Columns: means with different numbers are significantly different

Within the same Brix the SACCA had the significantly lowest TSS/TA, followed by the Titratable Acidity of the SACSaCA which is significantly lower than the SACSa and SAC which were significantly the highest (Table 9).

3.3 Sensory Attributes

3.3.1 Aroma

The aroma score of pomegranate concentrate was significantly the lowest followed by verjuice and SACSaCA which did not differ significantly. Furthermore, verjuice did not differ significantly from SACSa, SAC and SACSa possessed the highest aroma scores (Fig.2).

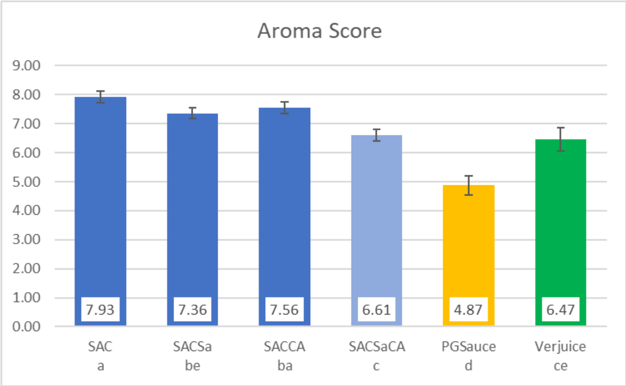

Fig.2 Aroma Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate;
Product means with different letters are significantly different

3.3.2 Appearance

The pomegranate concentrate score significantly the lowest score in terms of appearance followed by SACSaCA and Verjuice with SAC, SACCA and SACSa being the highest (Fig. 3).

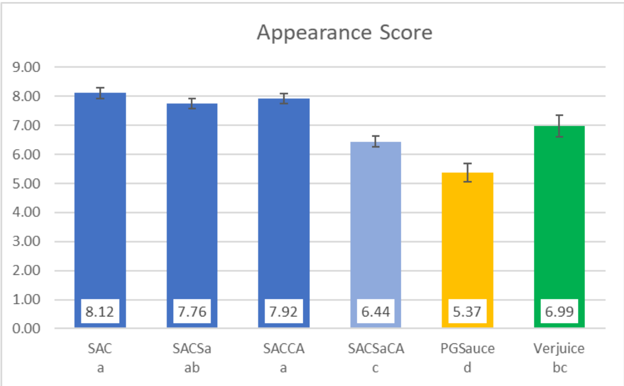

Fig.3 Appearance Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate;
Product means with different letters are significantly different

3.3.3 Texture

The Texture score of pomegranate is significantly the lowest followed by SACSaCA and Verjuice with SAC, SACCA and SACSa being the highest (Fig. 4).

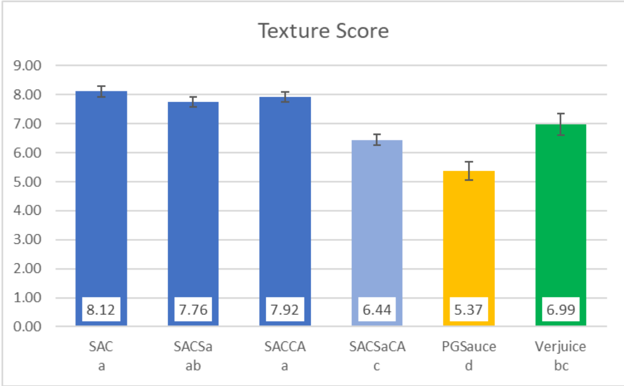

Fig.4 Texture Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate;
Product means with different letters are significantly different

3.3.4 Sourness

The score of sourness was significantly the lowest for pomegranate sauce and SACSaCA followed by the verjuice which did not differ significantly from SAC, SACSa and SACCA which tended to have the highest score (Fig. 5).

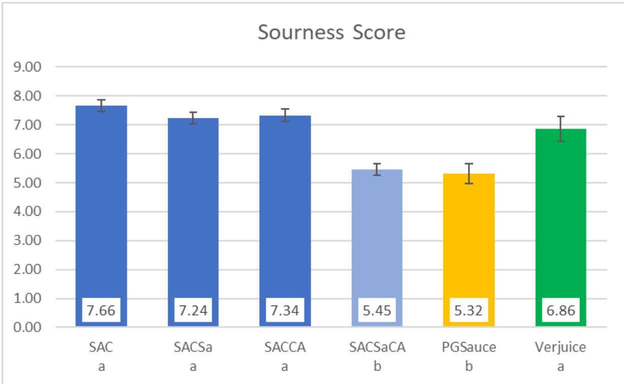

Fig.5 Sourness Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate;
Product means with different letters are significantly different

3.3.5 Sweetness

The pomegranate have the significantly lowest score although it did not differ significantly from the verjuice. The SACSa sweetness score had the significantly highest score. The SACSaCA, SAC and SACA score were in between and in descending order (Fig. 6).

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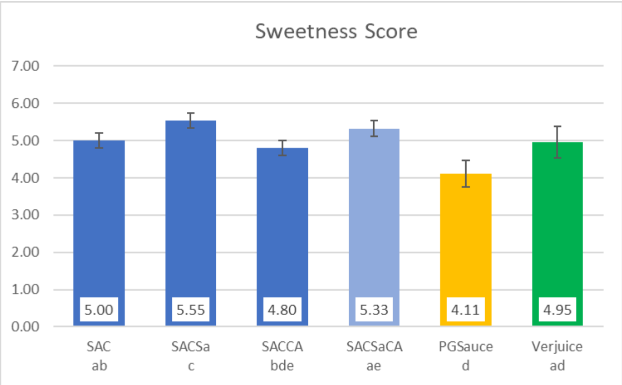

Fig.6 Sweetness Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate; Product means with different letters are significantly different

3.3.6 Taste

The taste scores of the pomegranate followed by SACSaCA are significantly lower than SACSa, SACCA and SAC possessing the highest score. Concerning grape verjuice is not significantly different from pomegranate, SACSaCA and SACSa (Fig. 7).

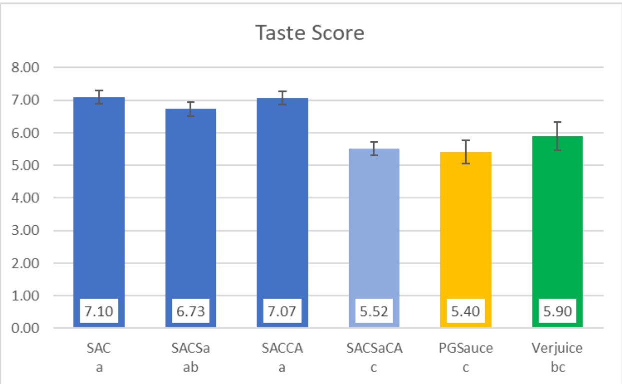

Fig.7 Taste Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate; Product means with different letters are significantly different

3.3.7 Average Score

The average scores of the pomegranate is significantly lower than all SAC products and verjuice and not from SACSaCA. There is no significant difference between the average scores of SAC, SACSa and SACCA and all are significantly than the SACSaCA, pomegranate and grape verjuice (Fig. 8).

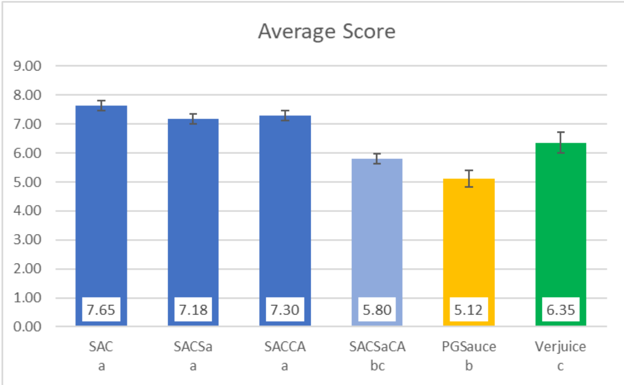

Fig.8 Average Score of different Sour Apple concentrate products, pomegranate sauce and verjuice

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate; Product means with different letters are significantly different

There was no significant difference of the sour apple concentrate of 55 and 65ºBrix. The average score of the sour apple concentrate of 75ºBrix scored significantly the lowest (Fig. 9).

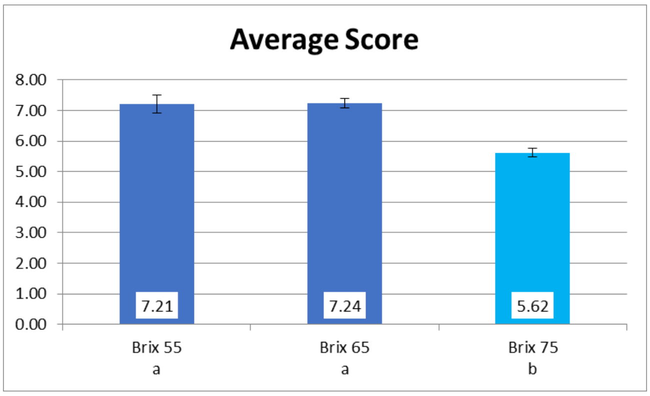

Fig.9 Average Score of different Sour Apple concentrate Brix

SAC: Sour Apple Concentrate, Sa: Salt, CA: Citric Acid; PG: Pomegranate; Product means with different letters are significantly different

IV. DISCUSSION

The level of efficiency is calculated by the gr of juice per 100 gr of apples. As for the character of the sour apple juice in terms of pH which is lower than 4.2 thus starting as a high acid food and the higher the brix the lower the pH. This was reflected in titratable acidity which is higher the higher the Brix and with citric acid addition the much higher the titratable acidity. Furthermore, the ripening index [11] of apples, total soluble solids (Brix) per titratable acidity (gr/100ml) for this study was recorded and sets a benchmark for future studies.

As for the density of sour apple concentrate, it is an important factor to calculate the weight and size of bottle in terms one portion. In addition to that based on this one can study the efficiency of transportation. Taking into consideration the higher the Brix, the higher the density. The caloric content as expected was higher with increasing brix. This is an important information since the new trend in the 21^{st} century is wellness.

Concerning the water activity it is a tool to know the type of the product and to be the basis for future shelf life studies in combination with the pH, titratable acidity and Total Soluble Solids per Titratable Acidity.

After setting the physicochemical properties, the sensory analysis study the perception of people for the apple based new product namely the Sour Apple concentrate in comparison with two traditional products: pomegranate and grape verjuice.

The aroma of the verjuice is scored higher than that of pomegranate which might be due to processing method. The verjuice might be boiled but that of pomegranate it is not only boiled but condensed through evaporation [12]. The aroma of the condensed sour apple concentrate scored higher than pomegranate sauce although it possessed similar production method [13]. This is true in all the other sensory attributes except for the sweetness where those having salt addition, including verjuice [14], had higher scores than those without. This might be because a pinch of salt would increase the sweetness perception. The average score showed that the sour apple concentrate products except the one with both citric acid and salt was significantly higher than the pomegranate sauce and verjuice. In addition to that, the average score of 75ºBrix was lowest thus 65ºBrix should be considered as the highest concentration.

V. CONCLUSION

The sour apple concentrate 75ºBrix showed lower pH and water activity, but in terms of sensory score, the 55 and 65ºBrix scored higher thus should be considered. Unripe apple juice made from apples harvested in July/august can be used to produce Sour apple concentrate a food ingredient that provides acidity and flavor and as an alternative to verjuice and pomegranate sauce in salad dressings and other applications where it scored higher than both [15]. Citric acid addition or salt addition can be used to add some protection to the product. But it is not advised to use both salt and citric acid together. This is a product of interest to apple growers and to the food industry since it increase the potential of having a product with relatively long shelf.

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Variability and Heritability of *Sigah* Brown Rice Mutants (M3) in Vegetative Phase

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Abstract**—** *This research aims to obtain information on genetic diversity, variability, and heritability of Sigah brown rice mutants (M3) in the vegetative phase. This research is conducted from March to September 2018 in the irrigation area of Sungai Sapih, Kuranji, Padang, where the area is 48 meters above sea level. The materials used in this study are M2 rice seeds (13 early maturing mutant lines and 10 tall plant mutant lines + number of tillers), and the original plants of Sigah genotype as a comparison (control). The analysis of genetic diversity, heritability, and variability are tested on plant height, number of productive tillers, and percentage of productive tillers. The selection method used in this study found that there are 8 early maturing mutants and 7 tall plant mutants + number of tillers. The line of early maturing mutants number 89 and 76 have wide diversity range in the term of the character of plant height, number of tillers, and number of productive tillers in the vegetative phase. Further, line 4, 19,134 of tall plant mutants + number of tillers have a wide variety of tillers too.*
Keywords**—Variability, heritability, M3 mutants, brown rice**

I. INTRODUCTION

Indonesia is a wealth paddy country with many variants of rice species in the form of wild species or local cultivars. The Asian continent and Indonesia itself were the centers of rice origin in ancient times. The primary center of rice origin is India, while Indonesia is considered as the secondary center of rice origin. It can also be noted by the existence of many wild species of rice in Indonesia [1].

According to [2] (1993); [3] (2013), local rice cultivars are germplasms that have certain genetic superiority. They have been cultivated continuously making them well-adapted to various specific climatic conditions and land in the planting area. In addition, local rice is naturally tolerant of abiotic stresses, unaffected by pests and diseases, and good in quality. Thus, people who live in a place where they grow, like to eat them.

In general, Indonesian cultivars have superiority in the term of long panicles, round seeds, unfallen seeds, few tillers, photoperiod insensitive, broad leaves, and amylose content. The local varieties, in general, have adapted well to the place or location where the plant is originated. The taste of the rice is also good for the local people and has specific aroma. On the other hand, it has some weaknesses too such as deep and strong rooting but it is not responsive to the provision of fertilizer, deep age, high stems making it easy to fall, and low production. To procure seeds, farmers usually only rely on seeds from their own harvest on an ongoing basis. Thus, the level of seed purity and quality is very low so this affects the harvest. Therefore, the local rice varieties are usually still varied that mainly related to the character of plant height and number of tillers [4].

Plant breeding is an applied science to improve the properties of plants qualitatively and quantitatively. Mendel's law provides a solid basis for the development of plant breeding as a science. A conventional plant breeding is still considered as the main method in improving plant varieties in Indonesia. However, the limited genetic resources used as elders in crossing is an obstacle in conventional plant breeding. The limit of genetic diversity of released rice varieties contributes to the lowering yield potential. Many of the released rice varieties are related to each other, so the diversity is lacking and the yield potential is not different [5].

There are several breeding techniques that can be used to improve the character of plants in a relatively short time period and it can improve one or two characteristics of the plants. One of the techniques is the radiation mutation technique. This mutation technique is used to increase plant genetic diversity. If a character has high genetic diversity, the diversity of characters between individuals in the population will be high too. In this case, the selection will be easier to do to obtain the desired

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traits. The selection method is an effective process for obtaining the desired important traits with a high level of success [6]. This research aims to obtain information on genetic diversity (variability) and heritability of local *Sigah* brown rice M3 mutants in the vegetative phase.

II. MATERIALS AND METHODS

2.1 Implementation Research

This research was conducted for seven months, from March to September 2018, which was located in the irrigation area of Sungai Sapih, Kuranji, Padang, where the area was 48 meters above sea level. The materials used in this study were rice seeds from M2 plant selection (13 early maturing mutant lines and 10 tall plant mutant lines + number of tillers), the original plants from *Sigah* genotype as a comparison (control), urea fertilizer, SP36, KCl, and pesticides for plant care from pest and disease attacks and other materials used during plant care. Tools used were hoes and tractors for land management, waring, spraying pests and plant diseases, weeding, and observation aids.

2.2 Data analysis

Then, the analysis of genetic diversity, heritability, and variability of plant height, number of productive tillers, and the percentage of productive tillers was done after the selection of M3 mutant plants. The phenotype variance score was obtained from observing 15 mutant plants in each line. Next, the variety of environments was obtained by taking *Sigah* samples of 15 control plants. Further, the genetic variance was obtained by subtracting the diversity of phenotypes and environmental variations. Furthermore, the heritability and variability scores in each plant were calculated. The genetic heritability and variability were calculated using the following formula:

$$\sigma^2 = \frac{(\sum x2) - [(\sum X)2/n]}{\text{n-1}}$$

Where :

σ^2 = Variety

n = Total population

σ^2 p= Variety of phenotypes

σ^2 g= Variety of genotypes

σ^2 e= Variety of Environments

The heritability score:

$$H^2{}_{bs} = \frac{\sigma^2{}_g}{\sigma^2{}_p}$$

The heritability convention : low (hbs ≤ 0,2), moderate ($0,2 \le h^2_{bs} \le 0,5$), and height (hbs > 0,5)).

III. RESULTS AND DISCUSSIOND

3.1 The vegetative variables (plant height, the total number of tillers, and number of productive tillers)

Dari hasil seleksi yang telah dilakukan diperoleh (8 galur mutan genjah dan 5 galur mutan tinggi tanaman + jumlah anakan) terdapat variasi antara peubah vegetatif (tinggi tanaman, jumlah anakan total dan jumlah anakan produktif) pada masing-masing galur mutan (Tabel 1). From the selection results, there were variations between vegetative variables (plant height, total number of tillers, and number of productive tillers) in each mutant line (Table 1). They were 8 early maturing mutant lines and 5 mutant lines with tall plant + number of tillers. It could be observed that almost all characters had an average of plant height above 100 cm in the early maturing mutant lines. What was interesting about these mutants was that in addition to their early age, the plant height of the mutants was also ideal. There were three lines that had an average ideal of plant height (below 100 cm). There were also some lines indicated the same, for example, line 47, 76, and 68. The ideal plant height ranged from 90 cm to 100 cm [7]. The plants that were not ideal were more likely to fall than plants that had ideal height.

Table.1. The average of vegetative variables (plant height, total number of tillers, and number of productive tillers) in early maturing mutants and plant height (TT) of mutants + number of tillers (JA) of Sigah brown rice.

Mutant	variabel		
	TT (cm)	JAT (tillers)	JAP (tillers)
A. Early maturing mutants			
47	96.11	13.67	13.11
89	140.80	12.00	11.40
53	142.00	13.18	12.64
58	126.40	20.80	20.20
76	92.89	12.44	12.44
111	163.92	11.23	11.08
4	109.25	15.00	15.00
68	93.80	13.40	13.40
B. Mutant TT + JA			
76	88.93	25.20	25.13
4	97.18	29.55	29.00
19	87.27	25.67	25.33
68	94.00	26.83	26.83
134	90.45	23.42	23.00
209	90.00	24.50	24.00
26	89.00	26.00	25.00
control	143.8	14.33	14.00

Note: TT (plant height), JAT (total number of tillers), JAP (number of productive tillers), JA (number of tillers)

The average total number of tillers in the early maturing mutants was ranged from 10.27 to 15.00 tillers. Meanwhile, the average of productive tillers was ranged from 10.00 to 15.80 tillers. The total number of tillers and productive tillers in line 4 and 68 had the same average score. It could be meant that the total number of tillers in the lines was productive (producing panicles). Whereas, the mutants with tall plant + number of tillers had an ideal plant height ranged from 87.27 cm - 97.18 cm. The plant

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height and number of tillers obtained were the result of genetic influences on plants.

The total number of tillers and the number of productive tillers in tall plant mutants + the number of tillers from all mutant plant lines had a higher average score compared to control plants (*Sigah* genotype). The total number of tillers in this mutant line ranged from 23.42 to 29.55 tillers. On the other hand, the number of productive tillers ranged from 23.00 to 29.00. Of all vegetative variables (plant height, total number of tillers, and number of productive tillers), line 4 was the most attractive mutant line with the ideal plant height and the highest number of tillers. In addition to line 4, line 68 was also attractive with an ideal plant height, the total number of tillers, and the number of productive tillers reached 28.6 points. Thus, all of them were productive tillers. According to [8] (1988), the number of tillers was divided into 3 categories: rich (> 15), moderate (10-15), and low (1-10). The visualization of differences in the appearance of each line with control plants can be seen in Appendix 6.

Moreover, genetic diversity was a quantity that measures the variation in appearance caused by genetic components [9]. The appearance of a plant's phenotype was an interaction between genetic factors and environmental factors. The diversity of phenotypes that appear to be produced by differences in genotype and / or growth environment [10]. But as a result of gamma-ray radiation random mutations that occur in individual plants, cause changes in the appearance of genes in the form of plant traits that can be seen by with bare eyes or not.

On the other side, the estimated score of heritability in a broad sense can be meant as a comparison between total genetic variation and phenotype variety which shows the large proportion of genetic factors in the phenotype of a plant character [11]. The determination of the phenotype diversity, genetic diversity, heritability, variability score, and T-test score on vegetative variables (plant height, total number of tillers, and number of productive tillers) can be seen in Tables 2, 3, and 4.

The results of vegetative variables of early maturing mutant and tall plant mutants + number of tillers in Table 2 showed that the heritability score for both was still in various categories. They were categorized as high, medium and low. The early maturing mutants with high heritability were found in lines 89, 53, 58, 76, and 68. Then, the early maturing mutants with moderate heritability (close to high) were found in line 47, and the early maturing mutants with low heritability score were found in mutants 111 and 4. Whereas, the variability score of all mutants was almost classified to have wide range tiller diversity, yet there were only some lines classified as narrow range tiller diversity, such as lines 111 and 4.

Table 2. The variety of phenotypes, genotypes, heritability, and variability of brown rice (M3) with the tall plant mutants variable in vegetative stage

Line	Average	σ^2_P	σ^2_G	h^2	category	2.Sd	var.	T.hitung
47	96.11 * ± 10.33	106.61	40.44	0.38	Sedang	20.65	Luas	16.93
89	153.60 Tn ± 19.05	362.80	296.63	0.82	Tinggi	38.09	Luas	0.25
53	142.00 Tn ± 17.30	299.20	233.03	0.78	Tinggi	34.59	Luas	0.39
58	126.40 * ± 21.14	447.04	380.87	0.85	Tinggi	42.29	Luas	3.14
76	92.89 * ± 16.03	256.86	190.69	0.74	Tinggi	32.05	Luas	12.02
111	163.92 * ± 7.83	61.24	4.93	0.08	Rendah	15.65	Sempit	9.08
4	109.25 * ± 8.96	80.25	14.08	0.18	Rendah	17.92	Sempit	13.90
68	93.80 * ± 17.33	300.20	234.03	0.78	Tinggi	34.65	Luas	10.95
76	88.93 * ± 9.09	82.64	16.47	0.20	Rendah	18.18	Sempit	21.80
4	97.18 * ± 9.77	95.36	29.19	0.31	Sedang	19.53	Luas	17.39
19	87.27 * ± 4.40	19.35	13.83	0.71	Tinggi	8.80	Luas	38.85
68	94.00 * ± 10.66	113.60	47.43	0.42	Sedang	21.32	Luas	17.18
134	90.45 * ± 4.52	20.47	14.95	0.73	Tinggi	9.05	Luas	36.03
209	90.00 * ± 11.31	128.00	61.83	0.48	Sedang	22.63	Luas	17.58
26	89.00 * ± 1.41	62.24	3.93	0.06	Rendah	2.83	Luas	24.57

Note: *) Significantly different at the 0.05 level according to the T-test; Tn) Not significantly different; σ2P = the variety of phenotypes; σ2G = the variety of genotypes; h2 = heritability; Sd = Standard deviation; ’’ ’’ ’ = Early maturing mutants; ’’ ’’ ’ = tall plant mutants, number of tillers.

Table.3: The variety of phenotypes, genotypes, heritability, and variability of brown rice (M3) with the total number of tillers in the vegetative stage

Line	Average	σ^2_P	σ^2_G	h^2	category	2.Sd	var.
47	13.67 Tn ± 4.44	19.75	4.08	0.21	Sedang	8.89	Sempit
89	12.00 Tn ± 5.10	26.00	10.33	0.40	Sedang	10.20	Luas
53	10.27 Tn ± 3.23	10.42	5.25	0.50	Tinggi	6.46	Sempit
58	16.40 * ± 5.28	27.84	12.17	0.44	Tinggi	10.55	Luas
76	12.11 * ± 2.67	7.11	8.56	1,00	Tinggi	5.33	Luas
111	11.23 * ± 3.96	15.69	0.03	0.00	Rendah	7.92	Sempit
4	15.00 Tn ± 2.94	8.67	7.00	0.81	Tinggi	5.89	Luas
68	13.40 Tn ± 4.28	18.30	2.63	0.14	Rendah	8.56	Sempit
76	25.20 * ± 4.02	16.17	0.50	0.03	Rendah	8.04	Sempit
4	29.55 * ± 5.59	31.27	15.61	0.50	Sedang	11.18	Luas
19	25.67 * ± 7.81	60.95	45.29	0.74	Tinggi	15.61	Luas
68	26.83 * ± 4.22	17.77	2.10	0.12	Rendah	8.43	Sempit
134	23.42 * ± 5.78	33.36	17.69	0.53	Tinggi	11.55	Luas
209	24.50 * ± 3.54	12.50	3.17	0.25	Sedang	7.07	Sempit
26	26.00 * ± 5.66	32.00	16.33	0.51	Tinggi	11.31	Luas

Note: *) Significantly different at the 0.05 level based on the T-test; Tn) Not significantly different; σ2P = the variety of phenotypes; σ2G = the variety of genotypes; h2 = heritability; Sd = Standard deviation; ’’ ’’ = Early maturing mutants; ’’ ’’ ’= tall plant mutants, number of tillers.

The tall plant mutants + number of tillers with high heritability score were found in lines 19 and 134. Whereas, the tall plant mutants + number of tillers with moderate heritability score (almost high) were found in line 47, and with low heritability score were found in line

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76 and 26. The variability score of all mutants was almost classified to have wide range tiller diversity, yet there was only one line classified as narrow range tiller diversity. It was line 76. The moderate heritability score showed that the environment did not contribute in the character's appearance of the mutants line. Meanwhile, the narrow variability indicated that the characters in the line were varied and they were not effective for further selection.

The vegetative variables result of early maturating mutants and tall plant mutants + number of tillers were observed in the total number of tillers. Table 3 showed that the heritability score of both early maturing and tall plant mutants + number of tillers was still categorized as high, medium, and low. The early maturing mutants with high heritability were found in lines 53, 76, and 4. Next, the mutants with moderate heritability score were in lines 47, 89, and 53. Then, the mutants with low heritability score were found in lines 111 and 68. Thus, the tall plant mutants + number of tillers with high heritability were seen in lines 19, 134, and 26. The mutants with moderate heritability were line 4 and 209. The last, the mutants with low heritability were lines 76 and 68.

Table.4: The variety of phenotypes, genotypes, heritability, and variability of brown rice (M3) with the number of productive tillers in the vegetative stage

Line	Average	σ^2_P	σ^2_G	h^2	category	2.Sd	var.
47	13.11 ^{Tn} ± 4.14	17.11	2.25	0.13	Rendah	8.27	Sempit
89	11.40 ^{Tn} ± 5.27	27.80	12.94	0.47	Sedang	10.55	Luas
53	10.00 * ± 3.58	12.8-	2.06	0.16	Rendah	7.16	Sempit
58	15.80 ^{Tn} ± 4.92	24.16	9.30	0.39	Sedang	9.83	Sempit
76	11.78 * ± 2.73	7.44	7.41	1,00	Tinggi	5.46	Luas
111	11.08 * ± 4.05	16.41	1.55	0.09	Rendah	8.10	Sempit
4	15.00 ^{Tn} ± 2.94	8.67	6.19	0.71	Tinggi	5.89	Luas
68	13.40 ^{Tn} ± 4.28	18.30	3.44	0.19	Rendah	8.56	Sempit
76	24.80 * ± 3.88	15.03	0.17	0.01	Rendah	7.75	Sempit
4	29.00 * ± 5.10	26.00	11.14	0.43	Sedang	10.20	Luas
19	25.33 * ± 7.39	54.57	39.71	0.73	Tinggi	14.77	Luas
68	26.83 * ± 4.22	17.77	2.91	0.16	Rendah	8.43	Sempit
134	23.00 * ± 5.82	33.82	18.96	0.56	Tinggi	11.63	Luas
209	24.00 * ± 2.83	8.00	6.86	0.86	Tinggi	5.66	Luas
26	25.00 * ± 4.24	18.00	3.14	0.17	Rendah	8.49	Sempit

Note: *) Significantly different at the 0.05 level according to the T-test; Tn) Not significantly different; σ2P = the variety of phenotypes; σ2G = the variety of genotypes; h2 = heritability; Sd = Standard deviation; '' '' ' = Early maturing mutants; '' '' = tall plant mutants, number of tillers.

The vegetative variables result of early maturing mutants and tall plant mutants + number of tiller were observed in the total number of tillers. Table 4 indicated that the heritability score of both early maturing and tall plant mutants + number of tillers were categorized as high, medium, and low. The early maturing mutant lines with high heritability were line 76 and 4. Then, the moderate heritability were line 89 and 58. Lastly, the low heritability were lines 47, 53, 111, and 68. The tall plant mutants + number of tillers with high heritability were line 19, 134, and 209. The moderate heritability was line 4, and the low heritability lines were 76, 68 and 26.

The vegetative variables result of early maturing mutants and tall plant mutants + number of tillers based on the criteria such as plant height parameters, total number of tillers, and the number of productive tillers showed high heritability score. It was proved that the appearance of the characters was influenced by genetic factors. It affected to the ability of these plants to inherit traits in their offspring becoming high. However, it was different with some lines in vegetative variables (plant height, number of tillers, and number of productive tillers) such as the line l11 of early maturing mutants and line 76 of tall plant mutants + number of tillers had low heritability. These results showed that the characteristics of plant height, the total number of tillers, and the number of productive tillers in the line were highly influenced by the environmental factor. On the contrary, the genetic factor did not significantly affected. There was unusual occurrence in line 76. It had more than one heritability. This matter could be caused by an error when the sample was taken. [12] (2011) stated that more than one heritability was biologically impossible. It occurred due to several things; (1) environment differences, (2) inappropriate statistical methods, (3) errors in sampling, and (4) a small number of samples.

Further, the total number of tillers category can also be concluded in some notes. The line 53 of early maturing mutants and the line 209 of tall plant mutants + the number of tillers had high and medium (almost high) heritability or high similarity levels. In this case, the genetic factors affected the character's appearance. Whereas, there was only one line categorized as productive tiller which was line 58. However, the value of genetic diversity of the strains was categorized as narrow. This result showed that genetic diversity in these tillers was considered to be stable and would not change. In fact, the chances of successful breeding were determined by the presence of genes that carry the desired traits and genetic diversity. On the other hand, the genetic diversity was obtained from the diversity of population-forming genotypes and genes that segregate and interact with other genes [13].

IV. CONCLUSION

It is concluded that based on the selection result there were 8 early maturing mutants and 7 tall plant mutants + number of tillers. The line of early maturing mutants number 89 and 76 had wide diversity range in the term of character of plant height, number of tillers, and number of productive tillers in the vegetative phase. Further, the line 4, 19,134 of tall plant mutants + number of tillers have a wide variety of tillers too.

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Performance and Carcass Characteristics of Layer Chickens Fed Diets Containing *Prosopis Africana* Seed Coat Meal Treated with Polyzyme®

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Abstract— *The study evaluated the effect of prosopis Africana seed coat meal (PASCM) on the performance and carcass characteristics of three hundred (300) Nera brown layer chickens that were fed for a period of 39 weeks. The birds were randomly allotted to 5 experimental diets with 3 replications of 20 birds each. The diets were formulated with the inclusion of PASCM at 0, 15, 20, 25 and 30% levels for treatments T_1, T_2, T_3, T_4 and T_5, respectively and the data collected were subjected to analysis of Variance in a completely randomized design. Results obtained showed that all the productive parameters were affected ($P < 0.05$) by the dietary treatments except mortality that was not affected ($P > 0.05$). Carcass parameters, carcass cut and internal organs were not affected ($P > 0.05$) by the dietary treatments however, GIT parts were affected ($P < 0.05$) by the diets. Performance indicators and carcass characteristics showed that 20% PASCM inclusion level resulted in optimum production and hence recommended for adoption.*

Keywords— ***PASCM, Pullet Layer Chickens, Performance and Carcass Characteristics.***

I. INTRODUCTION

Inadequate supplies of feedstuffs at economic prices continue to limit the production of animal protein in Nigeria. This is because the cost of animal feed accounts for 60% and 70% of the cost of production in poultry enterprises in Nigeria. Nutritionists and other professionals therefore, strive to reduce this cost to maximize profit (Aletor, 2005; *Odeh et al*., 2012).

This high cost of feed ingredients has scared some farmers from investing in poultry business (Musa and Olarinde, 2008). The conventional feed like maize continues to be expensive. Maize constitutes the main component of energy diet in poultry production in Nigeria, suggesting that any increase in the price of maize may increase the price of animal products. Therefore, there is the need to find an alternative feed resource which can replace maize (Eruvbetine *et al*., 2003, Kwari, 2008) in the diets of pullet layer chickens. The use of agricultural by-products and kitchen wastes like maize bran, rice bran and *Prosopis africana* seed coat meal (PASCM) etc. as feed resources can be achieved in poultry diet after careful study. This will help to reduce the competition for maize and increase animal protein at a relatively lower cost and improve net profit (Dafwang and Shwarmen, 1996; Oluyemi and Roberts, 2000; Diarra *et al.*, 2002; Yusuf *et al.*, 2008).

The availability of PASCM and its free acquisition brings it into focus as a replacement for maize in poultry nutrition. PASCM is high in crude fibre and low in energy compared to maize diet but can be used to replace maize as energy source (Sanni, 2015; Abang *et al.*, 2016) in layer chickens diets with some exogenous enzymes (e.g polyzme®) fortification (Chesson, 1993; Bedford and Morgan, 1996; Classen 1996). This study was sought to provide alternative feedstuffs to address the global feed crisis with the use of PASCM without affecting the performance and carcass characteristics in layer chicken nutrition.

II. MATERIALS AND METHODS

Experimental Site

This study was conducted at the poultry unit of Ohagwu farm, Ochodu Ukpa Igede, Oju Local Government Area of Benue State, Nigeria. Oju Local Government Area lies between latitude $6^{0}51^{1}$ north and Longitude $8^{0}25^{1}$ east in the Southern Guinea Zone of Nigeria, with a climate that has two

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distinct seasons. The wet season covers mid-March to mid-November, while dry season starts in late November to early March in which high temperature is experienced between February and April. Oju Local Government Area has an annual rainfall ranging from 1200 mm to1500 mm. The temperatures are generally very high during the day, particularly in March and April with a mean daily temperature of 26^{O}C, and daily minimum temperature of 16^{0}C to 21^{O}C and maximum daily temperature of 31^{O}C to 37^{0}C in dry and wet seasons. The relative humidity ranges from 42% to 75% depending on the time of the day and season of the year (Oju physical Setting Online Nigeria.Com, 2003).

Test ingredient

Prosopis africana seed coat meal (PASCM) was sourced from women in Oju Local Government Area that produced food condiment (Okpehe or Dawadawa) from *prosopis africana* seeds.

Experimental Birds and Management

A total of 300 Nera brown pullet layer chickens were randomly divided into five groups in a complete randomized design with each treatment having three replicates containing twenty birds per replicate. The experimental study which lasted for 39 weeks had five diets that were formulated from a mixture of maize, *Prosopis africana* seed coat meal, soybean meal, rice bran, blood meal, bone meal, palm oil and vitamin/mineral/premix as shown in Table 1. They were intensively managed in deep litter system throughout the experimental period. Feed and water were served*ad libitum*.

Dietary treatment

The PASCM was sundried for 10 days and milled. It was then incorporated into 5 diets at 0, 15, 20, 25 and 30% levels for treatments T_1, T_2, T_3, T_4, and T_5, respectively as replacement for maize. The birds were maintained in deep litter system of five treatments withthree replicates each that were fed on layer mash. Wooden nests were provided for the birds to lay their eggs. Also feeders and drinkers were provided to serve feeds and water respectively. The parameters evaluated were feed intake, feed conversion ratio, percentage hen-day production (%HDP), percentage daily egg production, age at first egg lay, egg laying period which is the length of laying period, percentage of egg laid per day per treatment, percentage hen house production(%HHP) and age at peak of egg laying which were obtained in line with the reports of Oladunjoye *et al.* (2008) and Adeyemi *et al.* (2009). Eggs were collected four times daily between 0700 and 1600 hours to prevent breakages.

Carcass Analysis

At the end of the experiment, three layer chickens from each treatment (i.e. one from each replicate) were randomly selected and slaughtered. The live-weight, plucked weight, dressed weight, cut-up parts and organs were weighed and measured. The cut up parts were individually expressed as percentage of the plucked weight while organ weights were expressed as the percentage of the live weight.

Chemical Analysis

Homogenous samples of *Prosopis africana* seed coat meal, T_1, T_2, T_3, T_4 and T_5 diets were subjected to chemical analysis for proximate composition and gross energy determination in the Kappa Biotechnology Laboratory, Research Support R & D and Analytical Service, Trans Amusement Park, Old Airport, Bodija GPO Box 12033, Ibadan, Oyo State, using the standard methods as indicated by A.O.A.C. (2000) and ballistic bomb calorimeter, respectively. *Prosopis africana* seed coat meals and feed samples were analyzed for crude protein using Kjeldahl technique; other proximate compositions that were analyzed for include ether extract, crude fiber and ash according to A.O.A.C. (2000) procedure. The nitrogen-free extract (NFE) was obtained by subtracting the % moisture, % crude protein (CP), % crude fiber (CF), % ether extract (EE) and % ash from 100 and difference gave NFE (Aduku, 1993; Esonu, 2000). Metabolizable energy (ME) was calculated using the formula of Pauzenga (1985): (Metabolizable energy (ME) (Kcal/kg) =37 x % CP + 81.1 x %EE +35.5 x %NFE.

Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA) in a completely randomized design using the procedure outlined in the Minitab (2014). Where significant difference between treatment means occurred, they were separated using Minitab (2014) software.

Results and discussion

The Performance Indices of Layer Chickens Fed Diets Containing *Prosopis africana* Seed Coat Meal

The effect of dietary PASCM on the performance of laying pullets (Table 2) showed that as the PASCM inclusion levels increased and maize decreased in the diets, percentage hen day production (%HDP) and other indices decreased except the FCR, age at first egg production and mortality. Age at first egg production increased with increased levels of maize substitution with PASCM but mortality for birds on PASCM diets (T_2, T_3, T_4 and T_5) did not followed the same decreasing trend with increased in the level of PASCM. The decrease in the values of these parameters with increased level of PASCM inclusion in the diets implies that the PASCM may have reduced the efficient utilization of protein and energy of the diets due to its phytonutrients content. This result is in line with the result of Kamdoon (2015) had reported the impact of phytonutrients of PASCM which was responsible for decrease in feed intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility in laying quails.

The values of %HDP recorded in this study ranged from 34.48 – 64.90 is lower than the range of values (64.97 – 68.47) reported by Okoeguale and Eruvbetine (2009) when unconventional feed supplemented with feed enzyme was fed to layer chickens. The differences in the values of %HDP obtained in this study and the reported values may be due to differences in the strain of birds used. The feed intake in laying hens (g/bird/day) as recorded in this study averaged between 113.32 – 126.35 which is higher than the values of 76.70 – 80.11 reported by Adeyemi *et al.* (2009) but comparable with the values (120 – 150) reported by Aduku (1993). Feed conversion ratio (feed/dozen egg) obtained ranged from 1.42 – 2.18. Aduku (1993) reported value of 2.65. FCR in 0 %(control diets) showed most superiority over the PASCM based diets and efficiency of FCR decreased with increased levels of PASCM in the diet. This may be due to the PASCM contributory effect of higher fibre content asit replaced energy cereal grain (maize) (Aina, 1990) which necessitates the need for consumption of more feed to meet the energy requirement since birds eat to meet their energy needs (Lesson and Summers, 1997). More so, feed enzyme (polyzyme®) inclusion in PASCM based diets could not result in increased digestibility and therefore led to reduction in nutrient uptake. This finding is in support of the work of Okoeguale and Eruvbetine (2009) that supplemented feed enzyme with unconventional feed high in fibre recorded decrease in nutrient digestibility, reduction in nutrient uptake and poor performance.

The number of egg lay per hen (104.19 – 177.49), dozen egg/hen (8.64 – 14.79), hen-housed production (%) (37.48 – 64.90) and percentage egg production (34.73 – 58.40) showed decrease with increased levels of maize replacement by PASCM. This may be due to the fact that the birds became less efficient in utilizing the protein and energy content of the diets for productive functions due to inherent anti-nutritional factors in PASCM. Njoku and Obi (2009) and Sanni 2015) have reported the anti-nutritional factors in PASCM that affect performance in livestock and poultry. Age at first egg laying period (days) increased with increased levels of maize substituted with PASCM. The 0% (control diet) PASCM inclusion level recorded egg production at the age of 133.00 days earlier than T_2, T_3, T_4 and T_5 (158.33, 155.69, 168.67, 174.33 days, respectively). As the PASCM inclusion levels increased the age at first egg laying production increased. Egg laying period (day) however, decreased with increased levels of maize replaced by PASCM since the age at first egg production occurred earlier with less maize replaced by PASCM in the diets. This result supports the view of Njoku and Obi (2009) and Sanni (2015) who observed that anti-nutritional factors in PASCM reduce performance in livestock and poultry.

Feed cost per dozen eggs decreased with increased levels of maize substituted with PASCM. Treatment T_1 (0% control diet) had the highest feed cost per dozen egg (₦265.72) while T_5 (30% PASCM) recorded the least cost (₦ 158.98). This is because the unit cost of PASCM was cheaper than the same unit cost of maize and more also less feed was consumed in PASCM based diets compared to 0%(control diet). This result agrees with the report of Shamwol (2015) who observed that feed cost and cost of feed per gain decreased with increased levels of PASCM in the diets of laying Japanese quails. Hen-housed egg production (%) and percentage egg produced decreased with increased inclusion levels of PASCM in the diets. This may be due to the PASCM contributory effect of higher fibre content and other anti-nutritional factors of the feed as it replaces energy cereal grains (Aina 1990) which necessitates the need for consumption of more feed to meet the energy requirement since birds eat to meet their energy needs (Lession and Summers, 1997). More so, enzyme inclusion in PASCM based diets (T_2, T_3, T_4 and T_5) could not result in increased digestibility and therefore led to reduction in nutrient uptake.

The egg yolk cholesterol mean values ranged from 226.66 – 263.33mg/100g. The egg yolk cholesterol values were significantly ($P < 0.05$) affected by the dietary treatments. The values decreased linearly across the treatment groups. The highest and lowest values of cholesterol were observed in the groups fed 0% (T_1) and 30% (T_5) PASCM inclusion levels, respectively. The lowest level of egg yolk cholesterol observed in 30% (T_5) PASCM inclusion level could be attributed to high fibre content of PASCM based diets. This result is in line with the report of Idowu *et al. (*2000) who observed that dietary fibre binds with fat and its associates and therefore reduced their assimilation and further deposition in the tissues, organs and products. This result is also in agreement with the hypothesis that increased dietary fibre often result in reduction in the availability of cholesterol for incorporation into lipoprotein (Storey and Furumoto, 1990). This result also shows that there is an inverse

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relationship between the level of fibre in the diet and the cholesterol level of the egg yolk.

The non-significant (P > 0.05) among the treatment means for the mortality observed in this study may imply that the feed was not the cost of the mortality. The diets may have been nutritionally adequate to sustain the hen's health and production despite the high level of crude fibre in the PASCM based diets. This result is in harmony with the finding of Fagbenro and Adebayo (2000) and Akinola and Ekine (2018) who observed that poor quality feed and poor environmental conditions cause high mortality, low productivity, feed condemnation and low rate of return on investment.

Carcass Parameters of Layer Chickens

Result of carcass yield of layer chickens fed on diets containing *Prosopis africana* seed coat meal is presented in Table 3and showed that the live weight, pluck weight, dresses weight, visceral weight and dressing percentage that were not significantly (P > 0.05) affected by the dietary treatments. The dressing percentage varied from 53.59% to 60.63% in the birds fed the experimental diets. The results obtained for dressing percentage in this study were similar across the dietary treatments. These results are in agreement with that of Torres *et al.* (2013) and Lakurbe *et al.* (2018) who reported that there were no significant different (P > 0.05) among the treatments in the whole carcass or weight of carcass parts of broiler chickens fed on sorghum based diets as energy source.

Carcass Cut of Layer Chickens

The evaluation of carcass cut of layer chickens in Table 4 showed that only the breast cut was significantly (P < 0.05) affected by the dietary treatments. This result agrees with the report of Abu (2016) who showed that there were no-significant different in meat yield and meat distribution among carcass cut or the proportional weight of the major visceral organs of broiler birds fed on *Prosopis* pod meal. The non – significance for most of the carcass cut determined in this study, according to Lakurbe *et al.* (2018) is an indication that the dietary treatments have no adverse effect on the parameters under investigation.

Internal organs

The result of the internal organs of layer chickens evaluated is presented in Table 5 and it showed that the liver, lung, heart, spleen, fat and caecum were not affected (P > 0.05) by the dietary treatments however, the kidney, pancreas, Proventriculus, gizzard, empty gizzard, large intestine and small intestine were significantly (P < 0.05) affected by the dietary treatments. The kidney which is an organ of detoxification, showed variation but was not significantly different from treatment 0% (control diet) PASCM inclusion level. This is an indication that PASCM was not toxic enough to cause increase in the size of the kidney. Also some internal organs showed variation among treatment means, there was no consistent trend established. The variation among the treatments according to Yunusa *et al.* (2014) may not be due to different energy sources but probably due to varietal and individual differences among birds in feed consumption.

Gastro-Intestinal Tract (GIT)

The GIT length, small intestine, and large intestine that were expressed as the percentage of GIT length were affected (P < 0.05)by the dietary treatments however caecum that was also expressed as the percentage of GIT length did not show significant different (P > 0.05) among the dietary treatments. The indices of the GIT that showed variation did not follow any consistent trend. Therefore the variation may be due to individual differences in feed consumption rather than the effect of diets on the GIT indices. This result confirms with the result of Yunusa *et al*. (2014) who reported variation in characteristics including the GIT indices of avian species, fed different energy sources suggested that the difference may be due to varietal and individual differences.

III. CONCLUSION

The results of this study showed that most of the productive parameters were affected (P < 0.05) by the dietary treatments. Mortality was not affected (P > 0.05). Carcass yield, carcass cut and internal organs were not affected (P > 0.05) by the dietary treatments but GIT parts were affected (P < 0.05) by the diets. From the results obtained on the effect of PASCM on the layer performance and carcass characteristics 20% PASCM level of inclusion is recommended for optimal productivity since egg production constitutes the main index in layer chicken production.

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Table1: Ingredients and Dietary Composition of Pullet Layer Chicken Diets

	Experimental diets				
Ingredients	0%	15%	20%	25%	30%
Maize	54.00	45.90	43.20	40.50	37.80
PASCM	-	8.10	10.80	13.50	16.20
Sobean meal	20.00	20.00	20.00	20.00	20.00
Rice bran	14.00	14.00	14.00	14.00	14.00
Palm oil	1.00	1.00	1.00	1.00	1.00
Blood meal	2.00	2.00	2.00	2.00	2.00
Bone meal	5.00	5.00	5.00	5.00	5.00
Limestone	3.00	3.00	3.00	3.00	3.00
Vit./Min/permit	0.25	0.25	0.25	0.25	0.25
Salt (Nacl)	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25	0.25
Enzymes	-	+	+	+	+
Total	100.00	100.00	100.00	100.00	100.00
Analyzed nutrients					
Dry matter	88.58	87.29	87.52	89.90	86.64
Crude protein	16.33	16.64	16.92	17.06	16.40
Crude fibre	4.48	5.59	5.12	5.47	5.48
Ether extract	3.41	3.76	4.42	4.33	4.52
Ash	12.19	11.38	11.13	11.74	11.86
Nitrogen-free Extract (NFE)	62.50	62.64	62.40	61.35	61.6
ME (kcal/kg)*	3099.51	3144.34	3199.70	3160.31	3160.17

ME:metabolizable energy

PASCM = *Prosopis africana* seed coat meal

- ❖ Vitamin/mineral premix supplied the following additional nutrients per kg of feed.

Table 2: Effect of Experimental Diet on Productive Parameters of Layer Chickens

	Experimental Diets					
Parameter	T_1	T_2	T_3	T_4	T_5	SEM
% HDP	64.90^a	53.27^b	50.02^b	47.72^b	37.47^c	2.10
No of egg laid/hen	177.49^a	145.44^b	136.55^b	130.00^b	104.18^c	0.00
Dozen egg/hen	14.79^a	12.12^b	11.38^b	10.84^b	8.68^c	0.70
% Mortality	10.00	11.66	10.00	10.00	10.00	0.00
% egg production	58.40^a	46.97^b	44.93^{bc}	42.88^c	34.93^d	0.00
Average feed intake(g/bird/day)	119.34	110.56	107.52	107.70	106.25	0.43
FCR 1.8kg feed/dozen egg	1.42^a	1.71^a	1.75^a	1.83^{ab}	2.18^b	0.04
% hen day house production	58.40^a	48.97^b	44.93^{bc}	42.88^c	34.93^d	0.06
Feed cost/dozen egg(₦)	265.72^a	215.88^b	204.44^{bc}	195.14^c	158.94^d	0.20
Date of first egg lay (days)	133.00^c	158.33^b	155.66^b	168.66^a	174.33^a	0.50
Egg laying period	278.33^a	252.66^b	255.00^b	244.66^{bc}	237.00^c	0.09

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Age at peak of laying	240.00^{b}	251.33^{ab}	254.33^{ab}	264.66^{a}	259.66^{a}	0.04
Yolk cholesterol (mg/100g)	263.33^{a}	256.66^{b}	250.64^{bc}	247.21^{c}	237.12^{c}	0.01

abcd means with different superscripts in the row are significantly different (p<0.05)

% HDP = percentage hen production

AFI = Average feed intake

Number (No.) of egg laid/hen

FCR = Feed conversion ratio

HHEP = Hen-Housed egg production (%)

Table 3: Effect of Prosopis africana on Carcass Parameters of Layer Chickens

Carcass yield	Experimental Diets					
	T_1	T_2	T_3	T_4	T_5	SEM
Live wt (g)	1900.00	1933.30	1833.30	1733.30	1866.70	36.34
Pluck wt (g)	1535.00	1571.30	1506.70	1489.30	1551.70	43.10
Dressed wt (g)	1150.67	1125.33	1075.67	1051.33	998.33	30.10
Visceral wt (g)	1205.00	1215.30	1161.00	1135.70	1076.30	30.85
Dressing %	60.46	58.21	58.54	60.63	53.59	1.12

Wt = weight

SEM = Standard error of mean

Table 4: Effect of Prosopis africana on Carcass Cut of Layer Chickens

Carcass cut (% DW)	Experimental Diets					
	T_1	T_2	T_3	T_4	T_5	SEM
Breast	24.43^{b}	29.42^{a}	24.72^{b}	26.39^{ab}	25.51^{ab}	0.69
Back	16.12	16.86	16.00	16.41	16.69	0.20
Thigh	12.77	12.63	12.87	12.92	13.07	0.20
Drum stick	11.86	12.44	12.40	12.69	12.64	0.15
Neck	8.27	8.19	8.58	8.18	8.17	0.17
Shank	3.33	3.46	3.49	3.67	3.41	0.06
Head	4.18	4.45	4.55	4.29	4.35	0.11
Wing	12.21	11.81	11.43	11.24	10.95	0.24

^{a,b} Means with different superscript in the same row are significantly different (P<0.05)

SEM = Standard error of mean

DW = Dressed weight

Table 5: Effect of Prosopis africana on Internal Organs of Layer Chickens

Internal Organs (% LW)	Experimental Diets					
	T_1	T_2	T_3	T_4	T_5	SEM
Liver	2.06	2.26	1.85	2.33	2.56	0.15
Lungs	0.51	0.43	0.50	0.52	0.47	0.01
Heart	0.72	0.59	0.61	0.60	0.57	0.02
Kidney	0.73^{a}	0.67^{ab}	0.75^{a}	0.78^{a}	0.52^{b}	0.03
Spleen	0.08	0.06	0.05	0.05	0.09	0.01
Pancreas	0.21^{ab}	0.17^{ab}	0.23^{a}	0.21^{ab}	0.12^{b}	0.01
Proventriculus	0.43^{ab}	0.44^{ab}	0.50^{a}	0.34^{b}	0.35^{b}	0.01
Gizzard	3.65^{ab}	3.89^{ab}	3.86^{ab}	4.26^{a}	3.30^{b}	0.12
Empty gizzard	2.24^{ab}	2.10^{b}	2.35^{ab}	2.67^{a}	1.94^{b}	0.08

Fat	1.47	0.68	0.45	0.68	0.75	0.20
Large intestine (g)	0.76^{ab}	0.82^{ab}	0.91^{ab}	1.02^{a}	0.60^{b}	0.05
Small intestine (g)	3.42^{ab}	4.51^{ab}	4.66^{a}	4.39^{ab}	2.96^{b}	0.25
Caecum (g)	0.82	0.65	1.39	0.95	0.87	0.11

^{a,b} Means with different superscript in the same row are significantly different (P<0.05)

SEM = Standard error of mean

LW = Live weight

Table 6: Effect of Prosopis africana on GIT Parts of Layer Chickens

GIT parts	Experimental Diets					
	T_1	T_2	T_3	T_4	T_5	SEM
GIT length (cm)	203.67^{b}	228.67^{a}	249.00^{a}	242.33^{a}	201.67^{b}	5.94
Small intestine (%GIT)	70.28^{b}	79.07^{a}	77.10^{a}	75.75^{ab}	76.78^{a}	0.19
Large intestine (% GIT)	7.02^{a}	5.98^{ab}	5.48^{b}	6.59^{ab}	6.29^{ab}	1.03
Caecum (% GIT)	19.50	17.19	16.68	16.65	18.65	0.63

^{a,b} Means with different superscript in the same row are significantly different (P<0.05)

SEM = Standard error of mean

GIT = Gastro intestinal tract

T_1 = Control diet

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Microplastic in *Cymodocea rotundata* Seagrass Blades

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***Abstract*—*Microplastic known to contaminate all ecosystem in the ocean , including seagrass bed. Leaf is one of the important parts of seagrass. The presence of ephibiont that attached to the surface of the seagrass leaves, causes the leaves' surface texture becoming rough, enabling microplastic entrapped in it. To assess the extent of microplastic accumulation, seagrass samples were collected from Barrang Caddi Island. Of the 15 seagrass blades harvestedin Barrang Caddi Island, there are 23 total items of microplastic observed, comprises91% of microfibers and 9% were microfragments.*

***Keywords*— Seagrass, blades, ephibiont, microplastic.**

I. INTRODUCTION

Plastic waste pollution in aquatic ecosystem is now a global concern (Andrandy,2011; UNEP, 2015). 60-80% of total marine waste is estimated to consist of plastic waste, there is no consensus about the total amount of plastic in the ocean or how much is added / stored annually, although the model predicts that around 10 million tons of plastic are added each year, with the total amount accumulated in 2025 estimated at least 155 million tons(Iñiguez *et al*., 2016). Jambeck *et al*, (2015) described the distribution of plastic waste in several countries, one of which is Indonesia. In that survey, Indonesia is estimated to produce 0.48 - 0.29 million tons/year of plastic waste. The large amount of plastic that collected and accumulated in marine waters is a problem that might disturb the stability of the ecosystem in sea waters(Gray, 2017). Although plastic is persistent, along with the time it can be degraded into smaller particles by waves, sunlight (photo-degradation), oxidation, and mechanical abrasion (UNEP, 2015). Plastic fragment which degraded, often called microplastic (herein after referred to as MPs), have particle sizes of less than 5mm (Tankovic, 2015). Degraded plastics are widely distributed in ocean waters. Size and density affect the presence of this MPs in the aquatic environment. Plastics with high density, that exceeds of seawater (1.02 g cm^{-3}) will sink and accumulate in the sediment (Woodall *et al*., 2015), while low-density particles tend to float on the sea surface (Suaria and Aliani, 2014). With a small size will ease for MPs to be carried by currents and waves that are easily trapped in the ecosystem in the sea, one of which is the seagrass ecosystem.Recent findings on MPs contamination on different seagrass percent coverage was conducted by Tahir *et al*. (2019) clearly shown the potential of MPs transfer pathways to the food chain. Research conducted by Gross (2018) found the presence of MPs on the leaves of*Thalassia testudinum*. Potential mechanisms to explain how plastic can be found in seagrasses, the first is that MPs suspended in the water column are trapped in epiphytes found in seagrass leaves.Second, MPs may stick to seagrass blades via adhesive biofilms (Rummel, 2017). Each type of seagrass has a different morphological form, ranging from cylindrical leaves on *Syringodium*, to the ribbon leaves in *Enhalus*, *Cymodocea*, *Posidonia*, *Thalassia*, and *Zostera*. These various forms of morphology have different effects on the epiphytic community in seagrass beds (Reynold, 2018). This is related to the difference in surface area provided by each of the seagrass plants for epiphytic attachment. Seagrass with a larger surface area allows more epiphytes to stick, which in turn allows more MPs trapped as well. The presence of MPs in seagrasses will certainly have an impact on seagrasses themselves and the organisms that make seagrasses as their food stuff. Seagrass is well known as one of the primary producers in the seawhich plays an important role in the food chain network in marine waters. The presence of MPs in seagrass beds has a potential to enter the body of herbivore organisms, especially those eating seagrass leaves and organisms that eat epiphytes on the surface of the seagrass leaves. In seagrass, plastic can act as inhibitor in the process of photosynthesis so that it can cause disruption of seagrass growth itself (Mandasari, 2014). Consideringthe dangers and impacts that can be generated, it is important to see the extent to which MPs can enter and reside in seagrasses ecosystem. This study aims to look at the presence of MPsin medium-sized seagrassplant such as *Cymodocea rotundata*

II. RESEARCH METHOD

2.1 Field collections

Sample of *Cymodocea rotundata* were collected on August 3th 2019, at Barrang Caddi Island, nearby Makassar City. A total 15 *seagrass* blade were hand-harvestedalong the seagrass beds.Samples were put into sample bag and transported toLaboratory of Marine Ecotoxicology, Hasanuddin University for later laboratory analysis, including sample preparation, microscope observation and Fourier transformed infrared spectroscopy (FTIR).

2.2 Measurement of Area and Percentage of Epiphytic Cover in Seagrass Leaves.

Prior to the observation of MPs in seagrass, the length and width of the seagrass leaves were measured using caliper. To calculate the percentage of epiphytic cover on a seagrass blade, each seagrass leaf was measured by leaf area covered by epiphytes.

2.3 Microplastic Analysis

To prevent any contamination of the samples, the microscope, glass surfaces and all the tools for MPs analysis, were cleaned with tissue paper and sterile distilled waterbetween each imaging session on each sample analysis. Blades of *Cymodocea rotundata* were examined, photographed and analyzed using a Euromex stereo microscope SB 1903. The presence of MPs on the blades was recorded along with the color and shapes as either micro-fibers or micro-fragments.To find out the types of polymers in observed MPs, an analysis was conducted using *Fourier Transform Infrared Spectrophotometer* (FTIR).

III. RESULTS AND DISCUSSION

3.1. Microplastic Found in *Cymodocea rotundata*

Of the 15 seagrass blades collected, 13 blades (87%) were found containing microplastic (MPs), 2 blades (13%) did not appear to contain MPs. The number of MPss found in all blades are 23 items. Where 4 leaf blades with epiphytic percentage <25% found (2 MPs). 7 seagrass leaf blades with 25-50% epiphytes, found (13 MPs). 3 seagrass leaf blades with 50-70% epiphytes, found (6 MPs) and 1 seagrass leaf blade with epiphytes> 70%, present (2MsP) (Fig. 1). For MPs abundance on the leaves of *Cymodocea rotundata*were ranged from 0.271- 1.139 MP/cm^2.

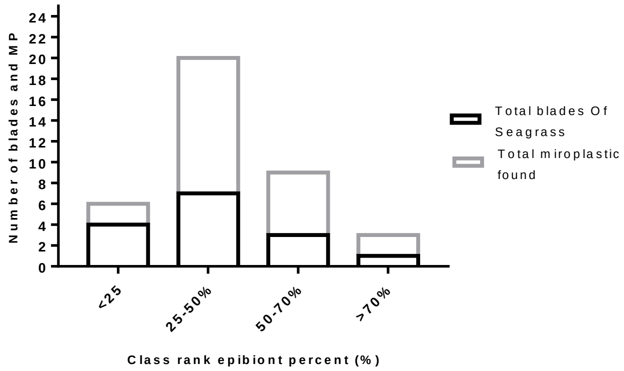

Fig.1: Abundance of MPs observed in seagrass blade of Cymodocea rotundata.

Other research also found the presence of MPs reigns on epiphytes attached to different types of seagrass leaves, *Thalassia hemprichii*, on 15 seagrass blades, a total 71 MPs were found consisting of 12 microbeads and 59 in the form of microfilaments (Goss, 2018). In addition, 199 MPs on *Enhalus acoroides* leaves and 126 MPs on *Thalassia hemprichii* leaves have also been found (Sawalman, 2018). And there are several mechanisms that can explain how MPs can be trapped in seagrass leaves, first through epiphytic species attached to the surface of the seagrass leaves which may be able to capture MPs suspended in the water column, seagrass surfaces overgrown with epiphytes will be rough so that MPs can be trapped in them, secondly MPs can stick to adhesive biofilms (Gross, 2018). In addition, the large number of epiphytes found on the surface of seagrass blades can also allow more MPs to be trapped. The number of epiphytes on the surface of seagrass blade depend on the size of the blades, where total abundance of *Enhalus acoroides* epiphytic meiofauna composition, comprises of 145 individuals from 10 classes (leaf tips: 114 individuals, leaf base: 31 individuals), also in *Thalassia hemprichii*comprises of 64 individuals from 7 classes (leaf tips: 35 individuals, leaf base: 29 individuals), and *Cymodocea rotundata*, consist of 42 individuals from 7 classes (leaf tips: 20 individuals, leaf base: 22 individuals) (Lestari,2010). Statistical analysis using linear regression has revealed no significant difference in the abundance of epiphytes and the amount of MPs observed (*P* value = 0.566). However, by comparing the results of research from previous studies (Gross, 2018) and (Sawalman, 2018), it can be seen that larger-sized seagrasses such as *Enhalusacoroides* and medium-sized *Thalassiahemprichii* have a greater amount of MPs abundance compared to small-sized seagrass *Cymodocearotundata*.

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3.2 Shape and color of microplastic found

Overall, the MPs forms found from 15 seagrass blades (Fig. 2) there are 91% were microfibers and 9% were micro-fragments. Of the 15 seagrass blades comprehensively examined. Furthermore, a total of 21 micro-fibers were found ranging from 0 to 3 item/blade, while for the micro-fragments were ranging from 0 - 1 per single blade.Previous research has also found MPs, and the microfiber is the most dominant found in seagrass blades (Sawalman, 2018; Goss, 2018).Other studies have also shown the presence of MPs fibers and fragments in the seaweed epiphyte *Fucus vesiculosus* (Gutow, 2016). The large number of microfibers found may have been caused by the sampling location in the fishing activity area and the fishing area parked the boats, where most of the fishing gear used by fishermen came from ropes or plastic sacks that had degraded.In line with previous studies that this type of fiber MPs is widely used in the manufacture of clothing, rigging, various forms of fishing gears such as fishing rods and fishing nets (Nor and Obbard, 2014).

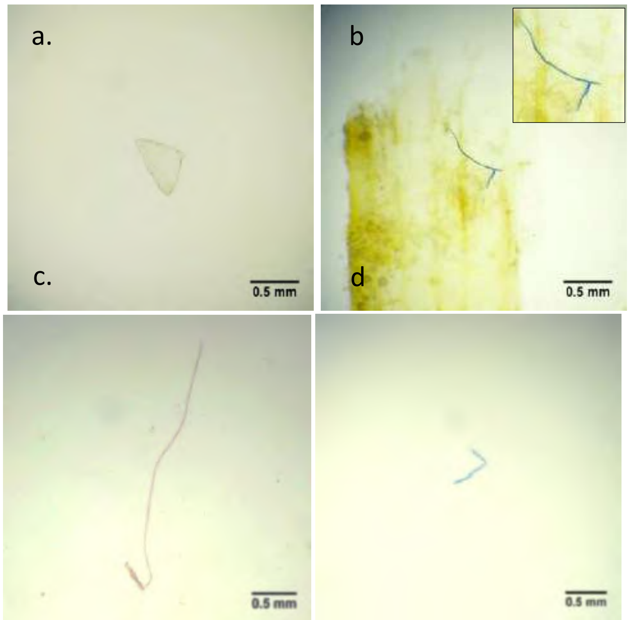

Fig.2: Microplastic (MP) found in seagrass blades Cymodocea rotundata; (a.) Fragment, (b-d) Line

From a total of 15 samples of seagrass leaves, MPsfound in 3 colors predominated by blue (82%), clear and red (9%) each. For seagrass leaf size, from 23 MPs obtained, the size ranges from 1.053 mm – 4.081mm.With the small size of the MPs that is in the seagrass blades can be incorporated into the body of the herbivore through the process of the food chain. One example of a case involving the entry of MPs when consuming seaweed contaminated with MPs is the laboratory experiments conducted by Gutow(2016),found seaweed *Fucus vesiculosus* which was contaminated with MPs, given to be consumed by *Littorinalittorea* resulted that the MPswere found in the stomach and in the gut. The impact of the presence of MPs that enter and digested in organisms has been noted in research by Nobre *et al.*(2015) where toxins carried by MPs can cause anomalies during embryonic development of invertebrates urchin species *Lytechinus variegatus*. The impact of MPs is not only on organisms that eat seagrass leaves that are contaminated with MPs, but as one of the primary producers the presence of MPs can also threaten the growth of the seagrass itself. According to Yokota (2018), one of the primary producers in the algae is Cyanobacteria, showing its interaction with MPs can change the process of photosynthesis of algae, growth, gene expression, colony size and morphology. The existence of these changes may be caused by adhesion and/ortransfer of pollutants absorbed from MPs.

3.3 *Fourier Transform Infrared Spectrophotometer* (FTIR) Analysis.

FTIR analysis was carried out to determine the polymers ofMPs items found on *Cymodocea rotundata* were resulted as polystyrene (PS) (Figure 3) and Nylon (Figure 4) polymers.

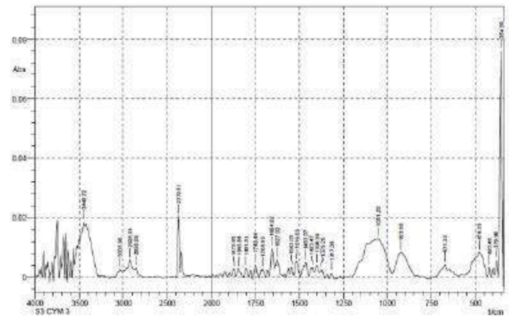

Fig.4: Microplastic FTIR spectrographshowing compatibility with Polystyrene polymers.

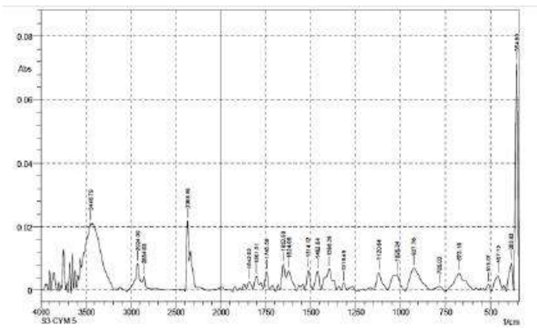

Fig.5: Microplastic FTIR spectrograph showing compatibility with Nylon polymers.

Techniques in comparing the size, shape and type of polymer content may be useful in providing insight to determine the sources of MPs in the ocean. Generally the types of polymers found in the sea consisted by polyethylene, polystyrene, polypropylene (Browne, 2011). Of the microplastic FTIR analysis the polystyrene polymers that are widely used to make food containers and plastic bags, and the nylon polymers are used to make fishing rope, were identified in the present studies and confirmed their wide distribution in the marine environment.

IV. CONCLUSION

Microplastic has been found in seagrass blades of *Cymodocea rotundata*, with the dominant form being microfiber. The presence of MPs in seagrass is thought to be trapped by epiphytes on the surface of seagrass leaves, but it remains uncertain on how long MPs can reside in seagrass and how much the contribution of seagrass as a pathway for MPs entry into organisms, especially herbivores.

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Beach Tourism Development Strategy in Coastal Area District Tete Bone, South Sulawesi, Indonesia

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Abstract— *The aim of this study was to determine the potential for coastal tourism and Tete Beach tourism development strategy.This research was conducted in August 2019. The collection of data such as water quality, the feasibility of coastal tourism, and interviews. Data analysis using Travel Suitability Index (IKW). Zone Capability (DDK) and the Strengths, Weaknesses, Opportunities, Threats (SWOT). Results of the research suggest that the potential of the Tete Beach Tourism relatively accordance withthe number of visitors to ± 128 / day cooperation between local communities, the government and the Indonesian National Armed Forces (TNI) is an author suggested a strategy for the development of nautical tourism in Tete Beach.*
Keywords*— *Beach Tourism, Coastal Area, DDK.

I. INTRODUCTION

Tourist activity has become one of the attractions most in demand by foreign and domestic society. It can be seen from the number of foreign tourists throughout the month of February 2018 amounted to 1.20 million, then increased to 1.27 million people throughout the month of February 2019 and domestic tourists to visit in December 2018 reached 1.100.67 people and increased to 1,158 .162 people in January 2019 (central statistical agency, 2019). This is because the activities have a wide range of tourist attractions appeal, three of them, namely nature such as flora and fauna, has a characteristic of an ecosystem, the unique natureand cultivation of natural resources (fields, plantations, livestock, and fisheries). Social tourism in the form of traditional culture, legacy history, arts and crafts and attractions of special interest.

One of the many attractions of interest in Indonesia in the form of nature, with the type of marine tourism, this is because Indonesia is a country that has a potential range of submarine and terrestrial (beach) which can be utilized, as well as the richness and density of coral reefs located on Pulau Pisang (Lazuardi, et al., 2013), the abundance of fish species in the National Park Publications (Sulisyati, et al., 2016) contained Turtle Conservation in the village Lamanggo (Buangsampuhi, et al.,2019)as well as the beauty of the topography in the form of cliffs dotted coral reefs Bodies Gantarang (Sani and Mahadjir, 2019), in addition to the beauty beneath the sea, land (the beach) Indonesia is quite awesome with the panoramic beauty of the beach, located in Watu Frog (Vitello, 2018), the beauty of the sunset and sunrise at the beach Menganti, Kebumen (Febriansyah and Alfiano, 2018).

With a wealth of potential that is quite interesting, making tourist areas in Indonesia as a field of local revenue due to tourist traffic which continues to grow each year (central statistical agency, 2019) following the tourist sites that are frequently visited by tourists according to Marine and Fisheries Ministry that is, Senggigi Beach, on the island of Lombok offers Attraction destinations white sand, snorkeling, and sunbathing and sunset views. Pangandaran island located in Java Island offers attractions sunrise and sunset. Kuta Beach in Bali, providing attractions such as sunset and waves. Parangitis beach located in Yogyakarta, this island presents attractions such as the myth that embodies trine Parangitis Beach (Volcanos, Kraton, Yogyakarta, and Parangitis). Bunaken in Manado bay beaches offers travel objects offered in the form of waves, Attraction steep cliffs, and diving. Raja Ampat beach Attraction providing diving, with the amount of marine life spread in Indonesia (Marine and Fisheries Ministry, 2018).

Some of the tourist sites of the beach is a tourist destination visited by many tourists with characteristic tourist attraction each, this is because characteristic of a tourist area can become Pull-in itself for tourists, as well as characteristic of Tete be tombolo, a natural phenomenon of

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tombolo this is only found on some beaches only, so this may be the main attraction for a tourist area, not only is it in Tete there are also several attractions other like panorama waves, white sand along the coastline, and expanse of mountains who are on the edge and the middle of the sea (Muh.Yahya, 2015).However, the number of attractions found on the Tete Beach does not make Tete Beach as one of the tourist destinations that are much in demand by tourists, this is likely due to the lack of development of several attractions contained in the Tete Beach, so that researchers are interested in conducting research on development strategies marine tourism area on the Tete Beach

II. RESEARCH METHOD

2.1 Time and Location Research

The study was conducted in Tete, District Tonra Bone regency. Parameter measurements performed at 3 points with a distance of ± 100m station Figure 1)

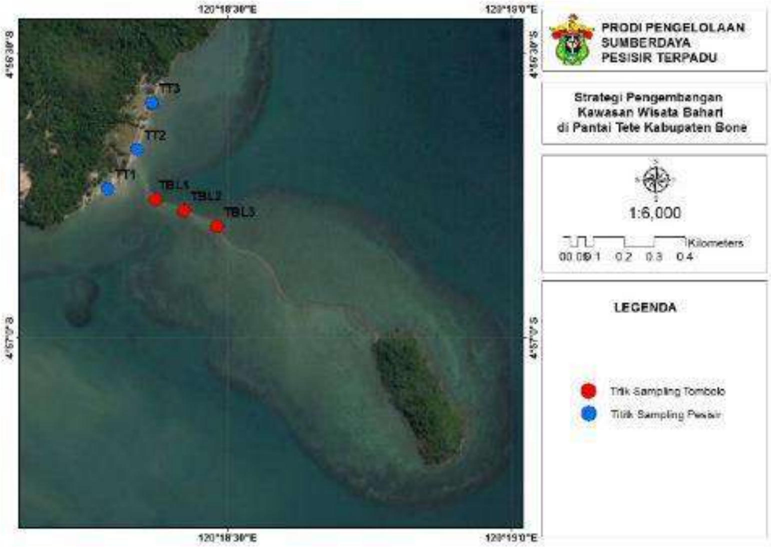

Fig.1: Map Location Research

2.2 Methods and Data Analysis

2.2.1 Data Collection Methods

Data were collected in the form of primary data and secondary data. Primary data includes data obtained directly through field observation techniques, survey and interview techniques. Then the secondary data obtained in agency-related agencies nautical tourism development strategy in Tete Beach. Primary and secondary data will be used to determine the priority of the development of alternative strategies most appropriately carried out, using SWOT analysis.

2.2.2 Data Analysis

2.2.2.1 Analysis of Water Quality Parameters

Analysis of water quality parameters is done in situ and ex-situ (laboratory). Eligibility Criteria valuation based on the Ministry of Environment No.51 of 2004 (Table 2)

Table 2 Criteria Parameter Water Quality Standards

No	Parameter	Unit	BIMutu
Physics			
1	Brightness	m	**>3**
2	Stink	-	**Notsmelling**
4	Waste	-	**Nothing¹(4)**
5	Temperature	°C	**Natural ¹(C)**
Chemical			
1	pHd		**6,5-8,5**
2	Salinitye	%	**Natural³ (ᵉ)**
Biology			
1	**Coliform**	**MPN**	**200 (ᶠ)**

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2.2.2.2 Compatibility Matrix Analysis For Beach Tourism

Matrix analysis of the suitability for use Table suitability travel tour coast beaches as follows:

Table 3 Matrix's compliance To Beach Tourism

No.	Parameter	Quality	Category			
			score 3	score 2	score 1	score 0
1	Beach Type	0200	White sand	White Sand Reef Mixed Fractions	Black Sand, Little Rough	Mud, Gravel, Rough
2	The width of the beach	0200	> 15	10-15	3- <10	<3
3	Base Materials Bodies	0170	Sand	Reefs Sandy	Muddy sand	Mud, mud Sandy
4	Water Depth (m)	0:13	0-3	> 3-6	> 6-10	> 10
5	Bodies Brightness (%)	0:13	> 80	> 50-80	20-50	<20
6	Flow velocity (cm / sec)	0080	0-17	17-34	34-51	> 51
7	The slope of the beach (°)	0080	<10	10-25	> 25-45	> 45
8	Land Cover Beach	0010	Coconut, Land Open	Bushes, thickets, Low Savana	High Thicket	Mangrove, Settlement, Harbor
9	biota Dangerous	0005	There is no	Sea urchins	Pig Fur, Fish Pari	Pig Fur, Fish Pari, lionfish, shark
10	Freshwater availability / Distance Resources Freshwater (km)	0005	<0.5	> 0.5-1	> 1-2	> 2

Then proceed with the following formula (Yulianda, 2019):

Information :

$$\boxed{\sum_{i=1}^{n} (Bi\ x\ Si)}$$

IKW: Tourism Suitability Index

n: Number Parameter

Bi: Parameter Weights All i

Si: Parameter Score All i

Then the class determination using three classification

S1 = is in accordance with IKW> 2.5

S2 = accordance with IKW <2.0 - <2.5

S3 = not in accordance with IKW <1 - <2

S4: Not Available <1

2.2.2.3 Capability Analysis of Tourism Region

Analysis of carrying capacity of tourist areas using the following formula:

$$DDK = K\ X \frac{Lp}{Lt}\ X \frac{Wt}{Wp}$$

Information :

DDK = The carrying capacity of the region DDK

K = Potential ecological visitors per unit area

Lp = Area or length of the area that can be utilized

Lt = Unit areas for certain categories

Wt = time allotted by region for tourism activities in one day

Wp = time spent by a visitor to any Specific activity (Yulianda, 2019)

2.2.2.4 SWOT Analysis

SWOT analysis is done by identifying four things, namely, strengths, weaknesses, opportunities, and threats in Tete by specifying three important points on 4 it and determine strategies that will be run by combining four components SWOT (Rangkuti, 2005)

III. RESULTS AND DISCUSSION

3.1 Water Quality Parameters

Tete beach water quality parameters such as pH, temperature, salinity, brightness, and coliform are presented in Figure 2 as follows:

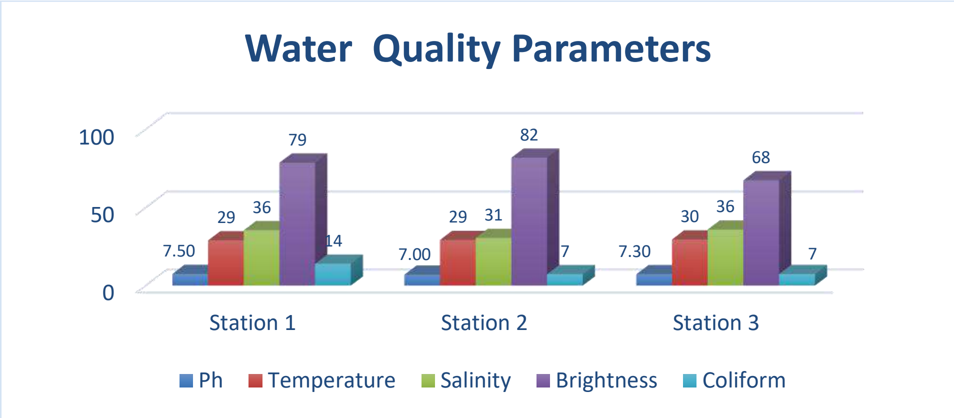

Fig.2: Parameters TeteBeach Water Quality

Based on Figure 2, three stations have the result of significant water parameters and meets the standards set by the Environmental Decree 51 of 2004 to serve as a tourist area, so it is safe for bathing and swimming tourist activity.

3.2 Compliance Matrix Parameter Beach Tourism

The suitability of the parameter measurement Beaches Tete is based on 11 parameters referred to in geology and physics aspects of a beach., Following 11 aspects of the parameters presented in Table 4

Table 4 Parameter Conformity Tete Beach Tourism

Parameter	Weight (Bi)	Station					
		1		2		3	
		Score (Si)	Ni	Score (Si)	Ni	Score (Si)	Ni
Depth (m)	0125	0730	0375	0820	0375	0760	0375
Beach Type	0200	White sand	0600	White sand	0600	white sand mixed rubble	0400
The width of the beach	0200	6,900	0200	6110	0200	4,400	0200
Basic materials	0170	sand	0510	Muddysand	0170	sandy coral	0340
Flow velocity (m / s)	0080	0:03	0240	0:02	0240	0:24	0240
The slope of the beach (°)	0080	4	0240	4	0240	3	0240
Bodies Brightness (%)	0125	78.7	0250	81.89	0375	67.5	0250
Land Cover Beach	0010	open field	0030	open field	0030	open field	0030
biota Dangerous	0005	there is no	0015	there is no	0015	there is no	0015
Freshwater availability (km)	0005	400 m	0015	200 m	0015	300 m	0015
Total number			2,475		2,260		2105
value Compliance			2946		2,690		2,506
Category Suitability		A Great Fit		A Great Fit		Corresponding	

Based on Table 4, each station has a tourist suitability index (IKW) different. This can be seen from Table 4 that where stations 1 and 2 have IKW equal to the weight of 2.9 and 2.6, while the station has a weight IKW reached 2.5, which means that stations 1 and 2 in the category is suitable for conversion to tour, while at station 3 classified accordingly (Yulianda, 2019), although there are differences in the results of IKW,overall a very appropriate location classified coast to serve coastal tourist area.

3.3 Carrying Capacity of Tourism Region

Tete beach has a land area of about ± 888,000 m, with a variety of tourist activities in it, it makes the necessary calculations Tete beach carrying capacity of tourist areas (DDK) to set activates as well as the number of tourists who can visit (Yulianda, 2019). The following table bearing capacity calculations TeteBeach tourist area

Table 5. Carrying TeteBeach Tourism Region

No.	Tourism type	The area of land (m^2)	Carrying Capacity of Region / People
1	Beach	24.500	69.619
2	Pool	19,300	34 276
3	Boat	856. 000	6,081
4	Tombolo	6400.000	18 186

Based on Table 5, tourist activity beach Tete there are 4 types, namely beaches, swimming, boating and tombolo by land area and carrying capacity vary, based on the calculation, the beach Tete can accommodate ± 128 people per day, by division 6 activity boat, beaches 69 people, 18 people tombolo and 34 people swimming travel people.

3.4 Management Strategy Based on SWOT

Management strategies using swot is the final step after conducting various analyzes, the results of this method are used as an alternative to beach tourism management strategies Tete, the following table using the Tete Coastal management strategy swot:

Table 6. TeteBeach Management Strategy (SWOT)

internal / **external**		**Strength (S)**	**Weakness (W)**
		• Travel biology and physic relatively good condition • The relatively strong security because it is managed by Indonesian National Armed Forces • Supporting facilities adequate travel	• Access (road) is not good • The distance to the city farthest travel (61km) • Diversity unfavorable conditions • Within 1 year 2 months are set up as army training schedule
Opportunities (O) • As the land is public economy income • There is uniqueness (tombolo) that became Pull • The existence and the beauty of the beach Tete has been widely known to the public about		• PromotingTete coast as one of the unique tourist areas • Designing service-based activities to improve the local economy • Board held information on the object and tourist facilities as well as schedule a visit within 1 year	• Eduwisata about Tantara training activities in a given month • Conducting marine transportation so that tourists enjoy the ride • Holding a beach rides
Threats (T) • There is a conflict between the government and the military • The presence of sand mines around so disturbing tourist transport lines • There is a conflict between the military and society		• Cooperation between local government and the military in travel management • Give a warning to the perpetrators of coastal miners to be responsible for damages access (roads) • Utilizing the local community in maintaining cleanliness and facilities around the beach Tete	• Having the government as an investor in the form of sea transport, and TNI as a tour manager beach. • Utilizing the public in the development of coastal tourism activates • zForming a group tour guide for tourists visiting during training by utilizing the surrounding communities.

Based on table 6 there are various strategies that need to be done by considering four important aspects, as well as promote the Tete, designing activities based services to the surrounding community, held an information board, education and tour on the training ground Tantara, conduct sea transportation, organized rides beach, giving warning for mining industry, utilizing the services of the public to maintain cleanliness and preservation Tete Beach, as well

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as doing the most important thing is the cooperation between the Indonesian National Armed Forcesas a manager, and calls on the government as an investor and the local community as providers of tourism activities.

IV. CONCLUSION

4.1 Conclusion

TeteBeach has the potential to be a tourist area, which can accommodate about ± 128 people per day with natural beauty and worthiness owned, as well as adequate facilities to support the convenience of tourists. ss

4.2 Recommendations

There should be cooperation between the government, police and the community for the development of management.

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Effect of the gamma Radiation on Vegetative Development of the Native Grapevine (*Vitis vinifera L.*) in morocco

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Abstract*— This study was conducted at the Larache experimental field, CRRA Tangier. For the discrimination of irradiated and control grapevines, 14 characters and 10 replicates for each parameter were used for this study according to the OIV code. The characters were evaluated in different organs of the plant, the twig and the mature leaves. The results showed a differentiation between irradiated and control grapevines. 23% of the irradiated grape varieties had a drop branch while only 3% of the control varieties showed this type of branch. 7% of the irradiated grape varieties showed a copper coloring of the upper face of the leaf whereas no control plant showed this coloration. 40% of the irradiated grape varieties showed a slightly longer petiole compared to the median vein in comparison with 7% of the control varieties having the same character. 43% of irradiated grape varieties showed a tooth shape with a mixture of two straight sides and two convex sides while most control grapes (57%) showed a tooth shape with two straight sides. It was also observed that some irradiated grape varieties (10%) showed the shape of the superior lateral sinus with slightly overlapping lobes and 3% of the irradiated grape varieties showed a form of the lateral sinus superior very widely open while the latter two forms are absent in groups of control grapevines. The irradiated grape varieties showed a higher density of main hairs compared with irradiated grape varieties. For the qualitative parameters it has been observed that the length of the teeth in the irradiated plants is never very short whereas in the control plants the length can be short and very short. The length of the tendrils and the length of the internodes are higher in the irradiated plants than in the control plants.

***Keywords*— Grapevine, Gamma irradiation, qualitative parameters, quantitative parameters, Morocco.**

I. INTRODUCTION

The traditionally grown grapevine belongs to the species *Vitis vinifera L*. It is a remarkable agronomic species, steeped in history and symbols, and offering exceptional diversity [1]. Grapes, *Vitis vinifera L* are widely distributed in the tropics and subtropics with ranges extending into the temperate regions [2]. Grape occupies the first position among the fruits in the world in terms of area and production [3]. The world area occupied by grape is 7.63 million hectares producing 64.29 million tons per annum [4]. The cultivated grapevines differ from each other in the appearance of their foliage and clusters; they make up what grapevine growers call grape varieties and botanists of cultivars. The correct identification of grape varieties or hybrids is based essentially on the morphology of all vegetative organs. The description of the varietals is necessary for the establishment, a description code containing the definition of botanical terms used for a good international understanding. In 1951, the International Office of Vine and Wine published an ampelographic vocabulary, which served as a basis for the drafting of the "International Ampelographic Register". Study of morphology of plants, bud and morphology of grapes is the first way to detect grapevine cultivars especially during in situ collection of plants [5, 6, 7, 8] and establishment of relationships between grapevine cultivars and resolution of different classification problems [9]. Many studies have been used for the identification of grapevine varieties [10, 11, 12]. Like other plants of agronomic interest such as lemon or coffee, clonal diversity in the grapevine is very important [13]. Although conserving the general varietal characteristics, the clones can be distinguished from each other by specific ampelographic characters. In the vineyard, this clonal diversity is of major importance for growers since it allows a varietal improvement without changing grape identity in accordance with the regulations set by the Protected Designations of Origin.

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In Morocco, the grapevine is one of the oldest cultures, it has known in the past important wine areas. Currently the requirement of grape was fulfilled through the import from other countries using hard carned foreign currencies as the demand is increasing with the increase in population. Despite this decline, viticulture occupies a fairly important place in the Moroccan socio-economic level. The varietal spectrum of the national table grapevine is mostly composed of very old local grapes, dominated by Doukkali and Abbou and the rest is occupied by foreign grape varieties strongly represented by Valency and Muscats [14]. The improvement projects of the grapevine in Morocco are very limited; recently the National Institute of Agronomic Research of Morocco launched a program of genetic improvement of the grapevine of table by the use of induced mutagenesis. An adequate dose of gamma rays for mutation induction has recently been defined by [15]. Including this objective a set of table grape mutants installed in the INRA Larache grapevine collection was studied in order to see if there is a phenotypic difference between the irradiated and control grapevines.

II. MATERIEL AND METHODES

The vegetative material consists of irradiated and control grapevines planted at the Larache experimental station. We tried to study a set of qualitative and quantitative parameters of the irradiated grapevines and compared them with the control plants. A total of 15 characters and 10 repeats for each parameter were used for this study. The traits were evaluated in different plant organs, the branch and young leaves and mature leaves (Table 1). The ampelographic characters have been described using the OIV descriptors [16, 17]. The main component analysis was carried out by SPSS software® version 20.0.

Table 1 Studied character

N	Code	characters
1	OIV006	Branch: port
2	OIV051	Young sheet: color of the higher face
3	OIV067	Break into leaf adult: form limb
4	OIV093	Break into leaf adult: length of the petiole compared to the length of the median
5	OIV076	Break into leaf adult: form teeth
6	OIV077	Break into leaf adult: length of the teeth
7	OIV8017	Branch: length of the gimlets
8	OIV353	Branch: length of the entrenoeuds
9	OIV079	Break into leaf adult: general form of the petiolar sine
10	OIV074	Break into leaf adult: profile
11	OIV079	Break into leaf adult: general form of the petiolar sine
12	OIV086	Break into leaf adult: density of the hairs lying of the principal veins
13	OIV084	Break into leaf adult: density of the hairs laid down between the veins
14	OIV087	Break into leaf adult: density of the drawn up hairs of the principal veins

III. RESULTS AND DISCUSSION

1. *Qualitative characteristics of the young shoot*

1.1. Branch port

The following figure represents the distribution of the percentage of the port of the grape varieties of the control and irradiated groups of the grapevine; one notices that 17% of the irradiated grape varieties presented a port of the semi-drooping shoot, whereas only 7% of the control grape varieties showed the same type of port of the branch. Also, 23% of the irradiated grape varieties had a drop branch while only 3% of the control varieties showed this type of shoot. However, most of the 60% control varieties showed a type of erect branch.

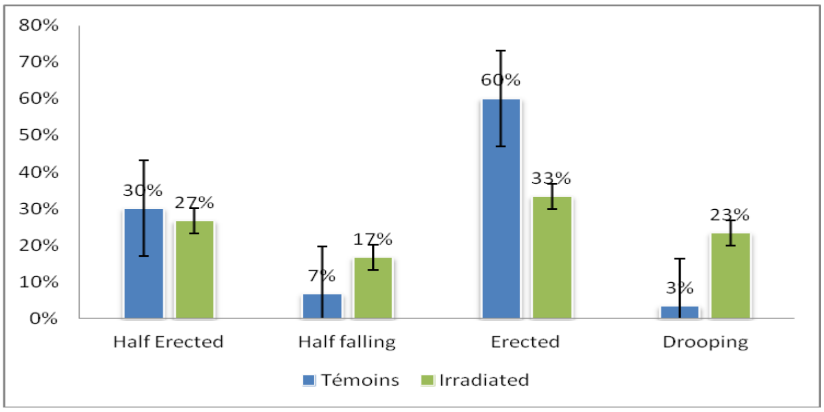

Fig.1. Distribution of branch port in control and irradiated groups

2. *Qualitative characteristics of the young leaf*

2.1. Color of the upper side of the leaf

The leaves have all the terms of passage from pale green to dark green and their color is accentuated by age. If theoretically differences in hues can be sufficient to distinguish certain grape varieties, practically it is impossible to establish a correct scale whose interpretation can vary with the observer. In autumn the

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grapevines are generally yellowish in color. However, the dyes and some varieties become naturally reddish.
The color distribution of the upper face of the leaf is shown in Figure 2. We notice six classes of the color of the upper face of the leaf. The three classes such as the yellow, yellow and red color of the upper face of the leaf are almost homogeneous for both control and irradiated grapevine. Whereas for the green color of the upper surface of the leaf we saw 33% of the control grape varieties against this coloration against 23% of grape varieties irradiated since the same coloration. 30% of irradiated varieties showed a green color with tanned beaches of the upper face of the leaf against 23% these grape varieties are witnesses of the same coloring. We observed 7% of irradiated grape varieties on a copper coloring of the upper surface of the leaf.

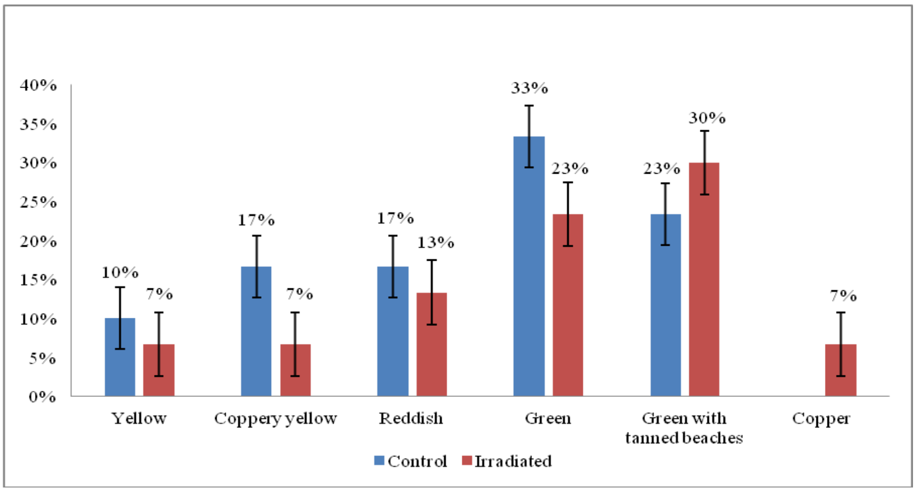

Fig.2. Distribution of upper leaf color in control and irradiated grapevine groups

3. Qualitative characteristics of the mature leaf
3.1. Shape of the limb
The appearance of the surface of the limb is a secondary character, but it can sometimes modify so much the foliage, that the latter then takes a characteristic appearance. The shape of the limb varies in five groups according to Figure 3. We note that most irradiated and control grape varieties showed a wedge-shaped shape. An almost homogeneous distribution of the other classes of the limb shape was observed in both control groups and irradiated.

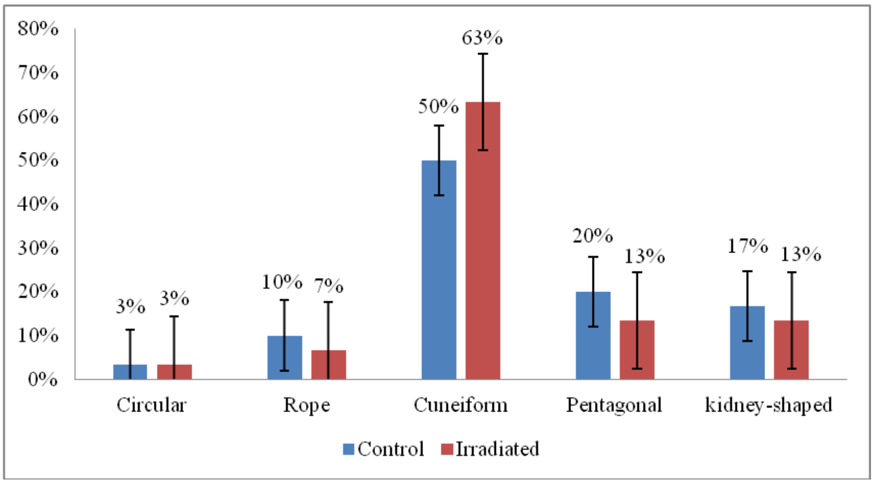

Fig.3. Distribution of the limb shape in the group of irradiated and control grapevines.

3.2. Length of the petiole compared to the length of the midrib
The distribution of the petiole length in relation to the midrib is shown in Figure 4. From the figure we observed that 40% of irradiated grape varieties showed a slightly longer petiole compared to the midrib in comparison with 7% of the control varieties that showed a longer petiole. 33% of the control and irradiated varieties have the length of the petiole is equal to the length of the midrib.

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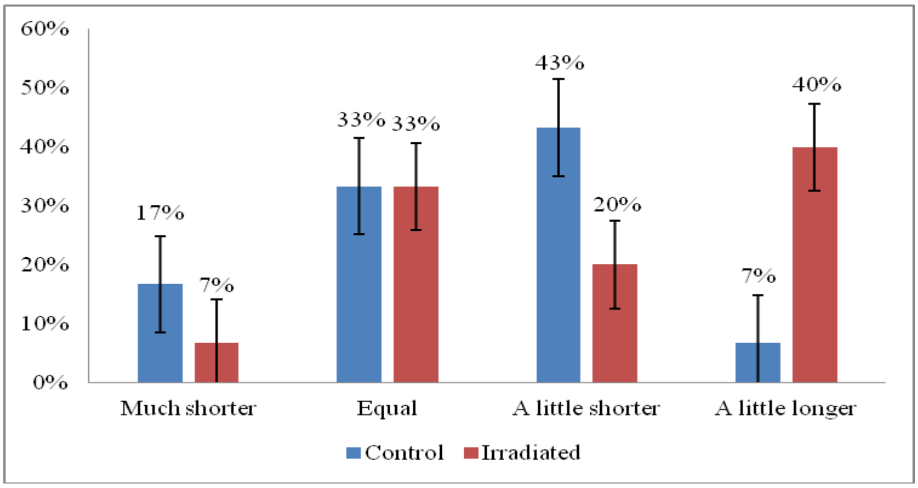

Fig.4. Distribution of the petiole length compared to the median vein

3.3. Tooth shape

The serration is a very good visual ampelographic character, unfortunately very difficult to code, because the same leaf is found with different shaped teeth and unequal depth. By analyzing Figure 5 we note that 43% of irradiated grape varieties showed a tooth shape with a mixture of two straight sides and two convex sides. Also, 23% of the irradiated grape varieties showed a tooth shape with a concave side and a convex side compared to 57% of the control grape varieties showed a tooth shape with two straight sides.

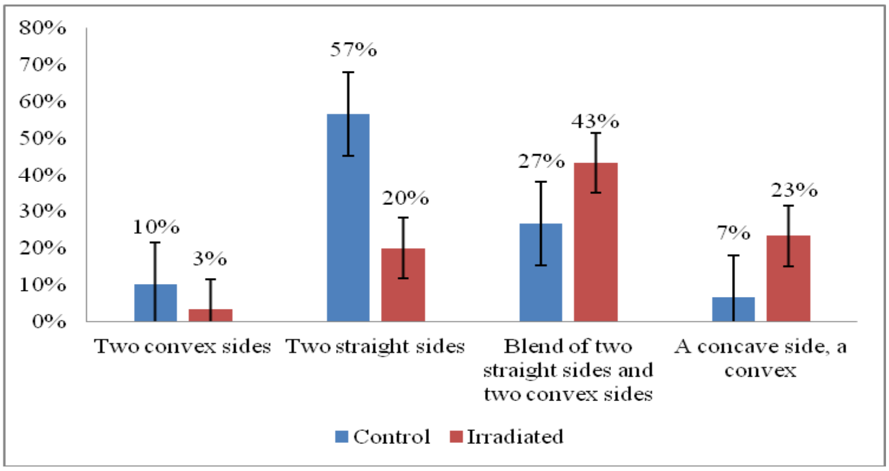

Fig.5. Shape of the teeth in the grapevines of the control and irradiated groups

3.4. Degrees of opening or sinus overlap

The petiolary sinus often makes an important contribution in the ampelographic distinction. Its shape is at the sum of the angles of the ribs, that is to say Σ. The shape of the sinus is variable. When they are shallow or almost void, they are often in the form of a deep V. They affect particular forms according to whether the bottom of the sinus is concave or acute. Similarly both sides of the sinus can remain substantially parallel (in glove finger). The shape of the upper lateral sinuses influences the aspect of the medial lobe of the leaf. While in whole or weakly cut leaves, it is broad, compact not detaching from the limb on the contrary in the grape varieties with deep upper lateral sinuses, the median lobe is more or less narrowed, sometimes giving a pace "wasp size" .

The following figure shows the general shape of the petiolar sinus. We note that 82% of irradiated grape varieties have a wide open petiole sinus. Also 3% of irradiated grape varieties showed a petiole sinus very widely open.

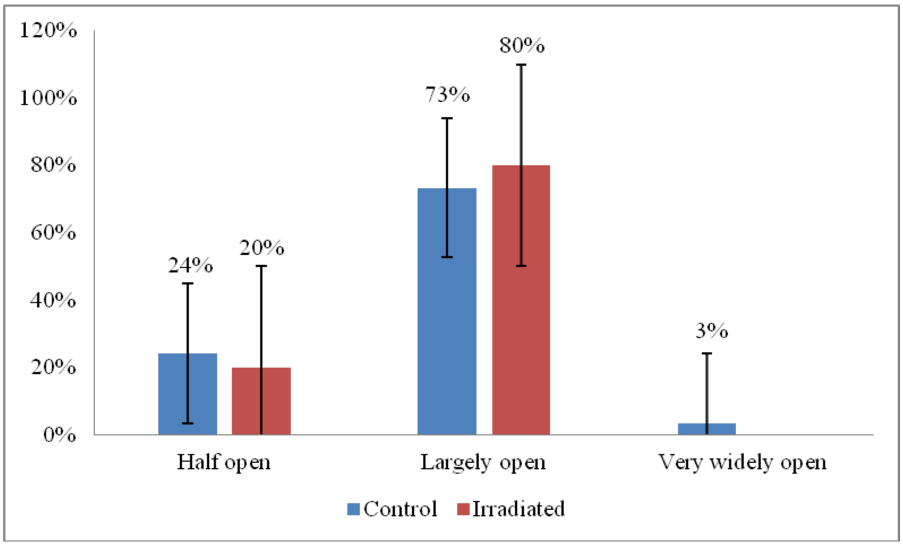

Fig.6. General shape of the petiolar sinus

3.5. Leaf profile

The grapevine leaf alone is an excellent organ for determining and classifying species or grape varieties. The following figure shows the leaf profile in irradiated and control grapevine plants. We note a homogeneous distribution of the different forms of leaves in the two groups of control and irradiated grapevines. Most of the 47% irradiated grapes have the shape of flat leaves. Only 10% of the irradiated and control grape varieties showed a profile of the corrugated sheet.

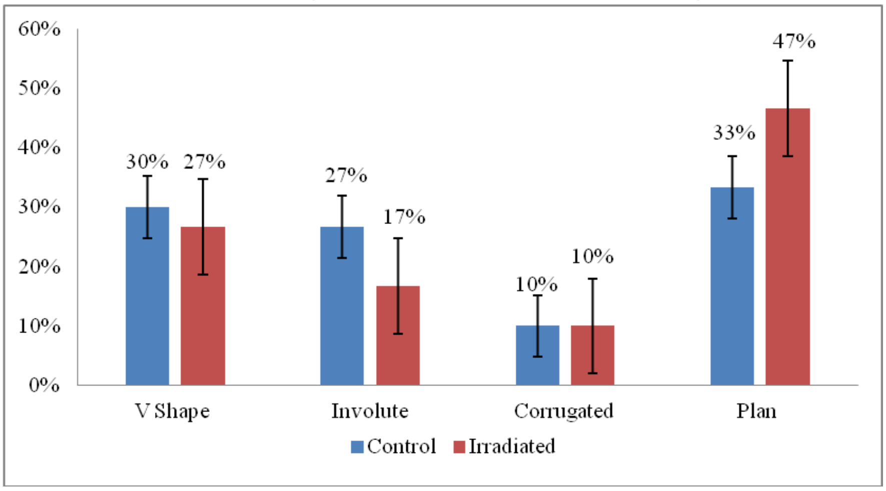

Fig.7. Profile of the sheet at the groups of grapevines irradiated and pilot

3.6. Upper lateral sinus shape

The shape of the superior lateral sinus varies according to the group of vines studied. From Figure 9 it is noted that the shape of the upper lateral sinus is open in most varieties and in both control groups and irradiated, also the closed form of the petiole sinus is present in both control forms and irradiated. It was observed that some irradiated grapes (10%) showed that the shape of the upper lateral sinus with slightly overlapping lobes. And 3% of the irradiated varieties showed a form of the lateral sinus superior widely open. These last two forms are absent from the control grapevine groups.

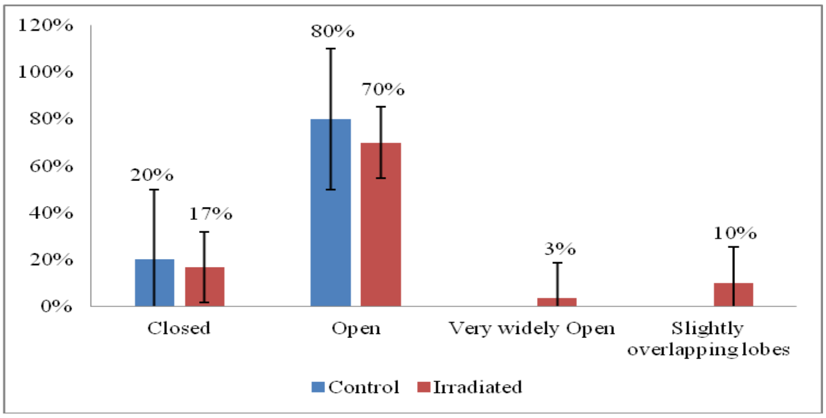

Fig.8. Upper lateral sinus form in control and irradiated groups of grapevines

4. Villus leaf

The grapevine is often covered by hairs of different shapes with varying intensity. The villosity of budding corresponds to that of the adult leaf. In fact, everything happens as if the quantity of woolly hairs remained constant during the successive increase of the limb to its final size. Thus the varieties with cottony budding will have leaves whose blade below will be cottony, downy, araneous, according to the initial density of the hairs and the final size of the limb.

4.1. Density of the coated hairs of the main veins

A homogeneous distribution of the density of the coated hairs of the main veins in irradiated and control grapevine groups was observed according to Figure 10

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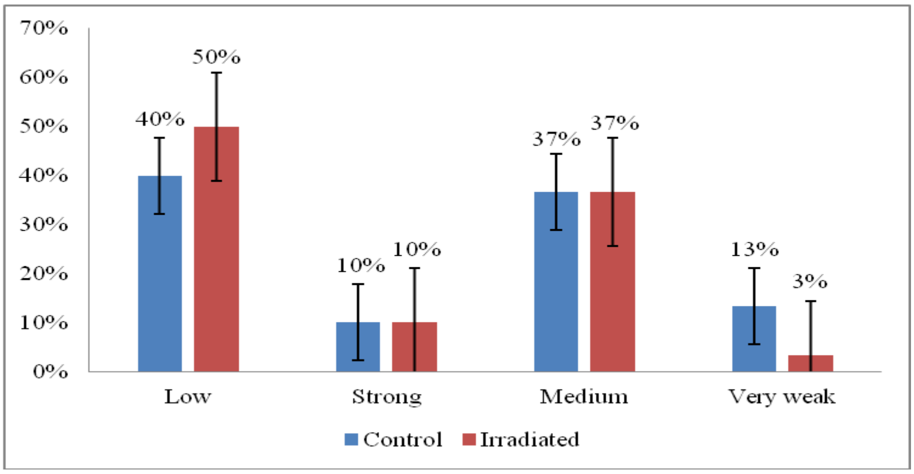

Figure 9: shows a homogeneous distribution of the density of the lungs

4.2. Density of the hairs lying between the veins

The following figure shows the distribution of the density of the hairs lying between the veins in irradiated and control vine groups. We observe a homogeneous distribution of the density of the hairs lying between the veins in the two groups. Most irradiated grape varieties showed a dense pile density between the veins, whereas most control grapes showed a low density of hairs lying between the veins.

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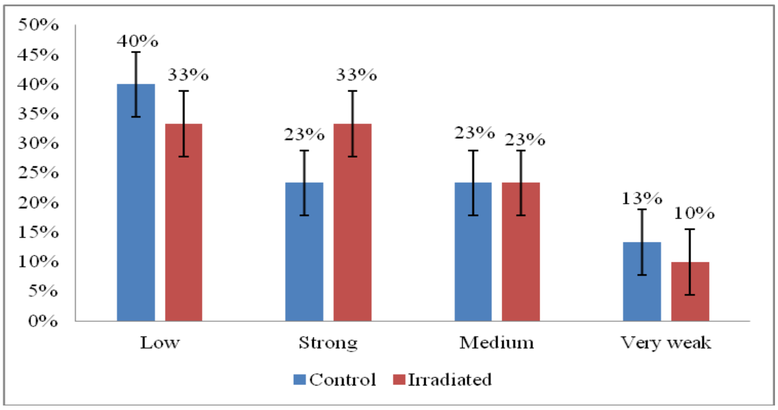

Fig.10. Density of the hairs lying between the veins

4.3. Density of upright hairs of the main veins

The following figure shows the density of the upright hairs of the main veins. Most of the irradiated grape varieties 47% showed a low density of hairs erected from the main veins. The control grape varieties showed a higher density of the primary veins than the irradiated grape varieties.

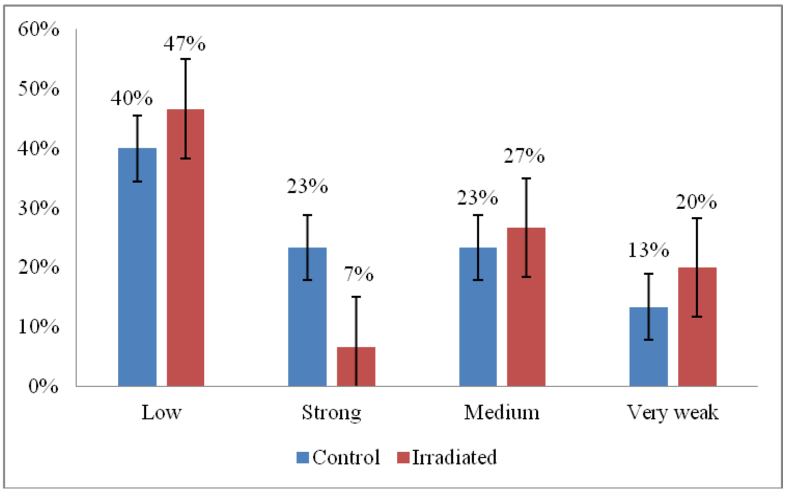

Fig.11. Density of upright hairs of the main veins

5. Quantitative characteristics

5.1. Length of teeth

The serration is a very good visual ampelographic character, unfortunately very difficult to code, because on the same sheet we find teeth of different shape and unequal depth. Figure 1 shows the variation in tooth lengths in irradiated and control plants. It is noted that the length of teeth in irradiated plants varies between long to medium and never very short, whereas in control plants this length is short and very short.

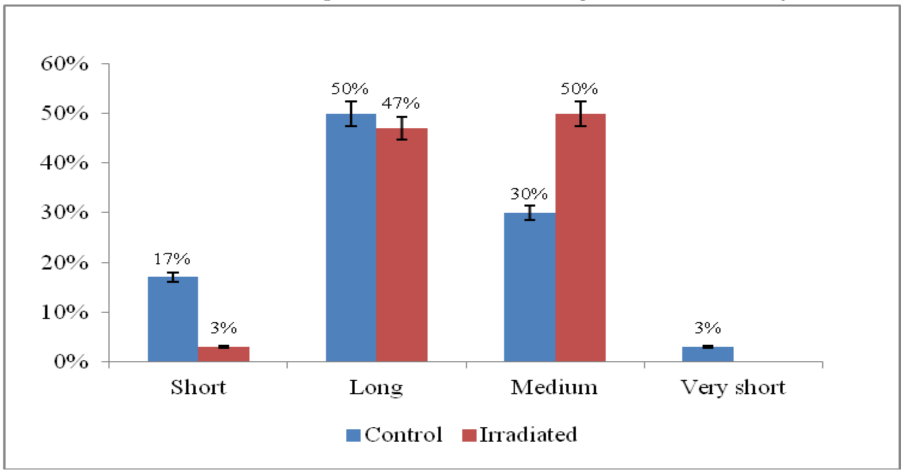

Fig. 12: Length of teeth in irradiated and control grapevines

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5.2. Length of the tendrils

The length of the tendrils in control plants varies from 1.5 cm to 18 cm, whereas in irradiated plants it varies from 2.3 cm to 15 cm. Most control plants showed a length of tendrils of 6 cm, whereas in irradiated plants most showed a length of tendrils of 9 cm.

5.3. Length of internodes

The length of internodes in control plants varies from 2.5 cm to 8 cm, whereas in irradiated grapevine plants this length varies from 2.5 cm to 10.5 cm.

6. Projection the groups in the plan

Using different qualitative and quantitative parameters we performed a principal component analysis of two groups of grapevine. The result is shown in the following figure. We note that the irradiated and control grapevines form two well-distinguished groups.

Some vines of the irradiated group are found in the group of grapevines control the same thing for some grapevines of the control group that are in the group of irradiated grapevines. From the figure that there is a clear differentiation between the two groups of irradiated and control grapevines. This differentiation deserves to be further clarified by studying these individuals in more detail and by using other approaches to differentiate mutants from irradiated ones.

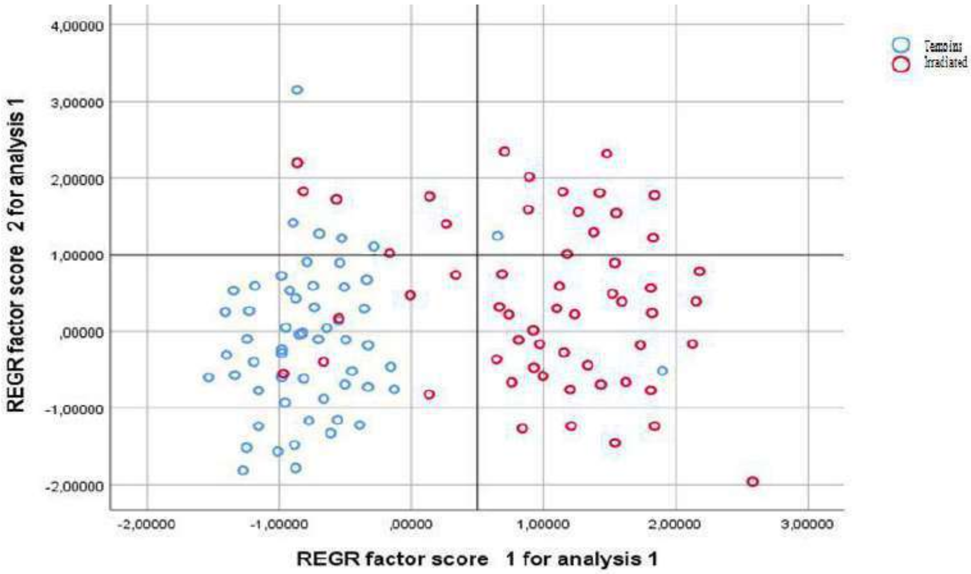

Fig.13: Projection of the groups in the plan (1,2)

IV. CONCLUSION

The different methods used proved very useful for the characterization of mutants and controls of grapevines grown at the Larache experimental area, CRRA Tangier. Classical ampelography is still the method most used in a practical way by a large number of people. The descriptions made on the various organs of the plant remain stable over time and have proved to be very typical and characteristic for each grape variety. Our results motivate the existence of a differentiation between the irradiated and control grapevines and which deserves to be further enlightened with the aid of molecular markers.

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Use the biometric parameters to characterize the local fig (*Ficus carica L*) in Tafza area of Morocco

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***Abstract*— *In objective to identify and characterize the richness of fig tree in limited area of northwest of morocco, abroad prospection was carried out in northwest Morocco. It included zones located in Tafza region as well as in the provinces of chefchaouen. A total of 130 accessions were sampled, studied and identified by used the biometric analyses include in European program GEN LMBO 029. As a result of the study a total of 13 different fig varieties were identified. Several synonymies and homonymies were detected. Comparison of the ecotype shows the high significatif difference, the conservation of the local cultivars is highly recommended.*
***Keywords*— Ficus carica L, genetic resource, biometric analysis, Tafza area, Morocco.**

I. INTRODUCTION

In Morocco, the fig tree is a tree of great importance for the Moroccan population and which fulfills several functions: social, economic and environment [1]. Among the fruit crops, the fig tree occupies the fourth place after the olive, the rosacea with pips and rosacea with. However, in the Northern region (Rif), the fig tree is one of the main agricultural resources and occupies the second place after the almond tree from the point of view of fruit plantations. The fig tree is considered in Morocco as a fruit species of secondary importance [2], [3]. However, at the regional scale and from the point of view of agro-diversity, its varietal diversity and its omnipresence designate it as one of the characteristic elements of the agro-ecosystems of the Rif Mountains [4], [5]. The study of the shape of the fruit is of paramount importance because it is according to this characteristic that one decides on the treatment to be subjected to it. The fruits are of variable shape within the same tree and during the same season [6]. To avoid this hazard, we often rely on the presence or absence of neck. Other authors rely on three dimensions of the fruit to differentiate the varieties: the length C, the diameter D, and the distance A separating the base from the center of the circle of diameter D. The first work concerning the pomological description of fig varieties has been done by Tayou in 1985 but from limited surveys to the Chefchaouen region. The evaluation of the morphological and pomological diversity of the fig tree in northern Morocco [7] showed that fig cultivars are highly diverse and provide a large collection of genotypes. However, because of many possible cases of synonymy and homonymy, the pomological characterization is insufficient for the establishment of reference genotypes of figs in Morocco. The objective of this work is the study of diversity of fig tree in the located area of the northwest of Morocco, by using the biometric analysis.

II. MATERIAL AND METHODS

Plant material

The plant material used in this study was constituted by the fig fruits, a total of 13 fig fruit prospected at different localities in Tafza region in Northwestern of Morocco. In total 130 accessions were gathered. In many cases, either isolated plants or plants located at old fig plantations areas were sampled. Cultivars were selected for their large distribution and their commercial value in the three regions. Samples of 130 homogenous fruits (three replicates of 10 fruits each) were chosen for each ecotype. Fruits were selected ripe and free from diseases.

Approach Biometric

A sample of ten fruits was randomly collected from different branches of the tree. The weight of the fruit was measured fruit by fruit with a precision scale in the laboratory. For these same samples, weight, of fruit, the dimensions of the fruit (length Width Height) and ostiole width were measured using a caliper (Fig. 2). The descriptors used were adapted list drawn up by European program GEN LMBO 029 [8]. The general appearance of the fruit corresponds to its external form. In this aspect, we are interested in the shape and size of the fruit.

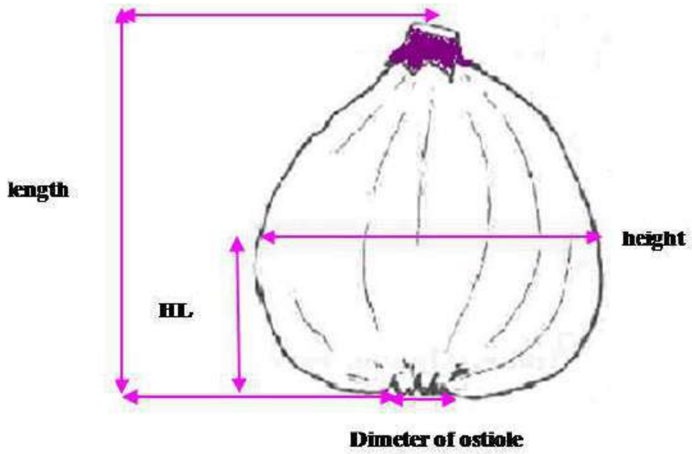

Fig.1: Representative schema of measurements made on fig fruit

Statistical analysis

Comparison between the ecotype was made possible by statistical analysis of collected data. Data were subject to one-way analysis of variance (ANOVA) and the results are given as means ± standard deviations (SD). Statistics were performed using Statistical Package for the Social Sciences (SPSS version 13.0; SPSS *Inc*.).

III. RESULTS AND DISCUSSION

13 varieties were identified in Tafza region and 130 accessions were collected either as unidentified or with local names. As result of the biometric approach the prospected plant material was characterized.

The average weight of the varieties studied varied from 11.4 g to 44.22 g (Table 1). The Al chitoui variety shows the highest weight while the Hafri variety shows the lowest weight. The analysis of variance showed nine homogeneous groups from the weight fruit (Table 1).

The average length of the fruit varied between 3 cm and 4.7 cm (Table 1), the variety Al Rounbiz showed the height value of the length of the fruit whereas the varieties Al qouti and Mouslikh showed the lowest value of the length of the fruit. We distinguished eight groups significantly different from the Length of fruit (Table 1). The varieties Hafri, Al gaouzi and ounq hmam are not significantly different. The varieties Al chatoui, Kouhli and Al rounbiz each one formed one group significantly different to the other group (Table 1).

The width of the fruit varied between 2.8 cm and 4.4 cm (Table 1), the Hafri variety shows the weaker value of width fruit while the varieties Bakour and Lndbar shows the greatest value of the width of the fruit (Fgi.2). The analysis of variance shows five groups significantly different from the width of fruit. The varieties Ounq hmam, Assal, Al qouti, Al aroui, Larchan, Mouslikh are significantly not different for the parameter Width of fruit (Table 1).

The value of HL varied between 1.5 cm and 2.3 cm (Table 1), the varieties Al chatoui and Bakour shows the greatest value while the variety Hafri shows the lowest value of HL. The analysis of variance shows six groups significantly different from the parameter HL (Table 1).

The diameter of the ostiole varied between 0.1 cm and 0.87 cm (Table 1) the variety Hafri shows the smallest value of the diameter of the ostiole whereas the variety Larchan shows the greatest value of the diameter of the ostiole. The analysis of variance shows that the varieties Assal, Bakour, Al aroui and Lndbar are not significantly different (Table 1).

The length/ Width ratio of the fruit varies between 0.78 and 1.16 (Table 1), the greatest value of the length / width ratio of the fruit was observed in the Hafri variety, while the lowest value was observed in the Al qouti variety. The analysis of variance shows seven groups significantly different. The varieties Hafri, Al chitoui, Al aroui and Al rounbiz are not significantly different from the ratio Length/width (Table 1).

The length / HL of the fruit varied between 1.4 and 3.9 (Table 1), it is found that the variety Mouslikh showed the lowest value of the ratio length / HL while the variety Hafri showed the most great value. Table 1 shows three groups significantly different (Table 1).

In these results, we find that the variety Al chitoui (Fig.3) showed the greatest value of the weight of the fruit and the value of HL of the fruit. However the Bakour and Lndbar varieties (Fig.2) showed the greatest value of the width of the fruit; when the variety Larchan shows the greatest value of the diameter of the ostiole.

Table 1: Biometric characteristics of fig cultivars harvested from the region

Variety	Weight (g)	length (cm)	width (cm)	HI (cm)	Diameter of ostiole (cm)	Length/Width	Length/HL
Hafri	11,4 a	3,2ab	2,8a	1,5a	0,1a	1,16ef	3,9b
Al chitoui	44,22 f	4,91f	4,24d	2,3e	0,43bcd	1,15ef	2,10ab
Al gaouizi	13,9 ab	3,1ab	3,1ab	1,6ab	0,2ab	0,9cd	1,9ab
Ounq hmam	36,33 def	3,21ab	3,7c	1,9cd	0,38abc	0,8ab	1,6a
Assal	28,71 cde	4,5de	3,7c	1,98d	0,51cd	1,20f	2,2ab
Bakour	43 f	4c	4,4d	2,3e	0,5cd	0,9bc	1,7ab
Al qouti	21,7 abc	3,04a	3,8c	1,9cd	0,63d	0,78a	1,5a
Al aroui	26,7 bcd	4,1cd	3,6c	1,9cd	0,5cd	1,13ef	2,1ab
Larchan	24 abcd	3,9c	3,8c	1,8cd	0,87e	1,0cd	2,1ab
Mouslikh	20 abc	3,03a	3,6c	1,8cd	0,61d	0,8ab	1,4a
Lndbar	34,8 def	4,1cd	4,4d	2,1e	0,5cd	0,9bc	1,8ab
kouhli	16,8 abc	3,49b	3,3bc	1,7b	0,2a	1,05de	1,97ab
Al rounbiz	40,25 ef	4,72ef	4,2d	2,17e	0,47bcd	1,12ef	2,1ab
Pα = 0.05	0	0	0	0	0	0	0

Significant differences within the same column and means followed by the same letter do not differ at Pα ≤0.05 according to Duncan test

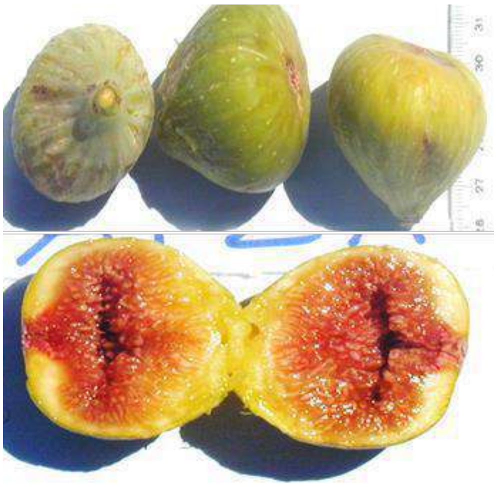

Fig.2: Fruit of variety Landbar from Tafza area

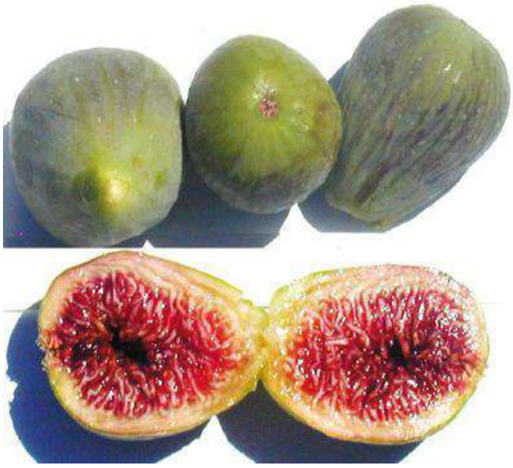

Fig 3. Fruit of variety Chitoui from Tafza area

IV. CONCLUSIONS

In this work we have characterized and identified figs in the region of Tafza located north of Morocco. This study shows the existence of a great morphological diversity of figs in this region. Thus 13 varieties were identified and showed a great biometric diversity of the fruits. Considered as genetic resources, these varieties must be preserved in a germplasm bank for preservation.

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Access to Agricultural Information among Rural Farmers –A Case of Ido Local Government Area Ibadan, Oyo State, Nigeria

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***Abstract*— The study examined the level of access to agricultural technology information among rural farmers in Ido Local Government, Ibadan, Nigeria. Primary data were collected with the aid of pre-tested questionnaires and 100 respondents were selected through a two stage sampling procedure. The data was analyzed using descriptive statistics and chi-square was used to test the hypothesis.The result of the analyses indicated that (29.1%) falls within the active age bracket of 40-60years, more than half (61.6%) had no formal education, 70.9% were male and 68.6% were indigene of the study area. Majority(58.1%) of the respondents had a little information about weather and climate, 48.8% had no information about tillage while (50%) had a lot of information about weed control and fertilizer. Most of the farmers had little information about government related information (4.7%), market related (7%) and harvesting techniques(8.1%). 50% of the respondents in the study area strongly agreed that source of information is constraint in agricultural production while 3.5% strongly disagreed about the source of information being a constraint. The hypothesis test revealed that there is significant relationship between marital status (< 0.05), education level (< 0.05) and the level of access to agricultural information in the study area. Based on the result, the study recommends that information should be disseminated to the farmers in the language that they understand and also, adult education should be encouraged in the study area to keep farmers informed towards on agricultural production practices.*
***Keywords*— Access, Agricultural information, Climate, rural farmers, Production.**

I. INTRODUCTION

Information is considered a vital resource alongside land, labour, capital, skills. It is facts or knowledge provided or learned as a result of research or study (Smith, 2001). Information is knowledge needed to answer some questions faced by people in their day to day activities.

The concept of information was coined by an American scientist called Robert Taylor. He stated that information is the process of asking questions. No one can categorically claim to know all the information needs especially in information relied sector like agriculture where there are new and rather complex problem facing farmers every day. It is safe to assert that the information need of farmers revolve around the resolution of problems such as pest hazard, weed control, soil fertility, farm credit, labour shortage soil erosion among others.

Obidike (2011), Petros *et al.,* (2018) maintains that the greatest challenge facing agricultural sector is the delivery of useful information for rural communities. In most developing countries agriculture is the most important economic activity providing food, employment, foreign exchange and raw materials to industries. A significant proportion especially in the developing world has been suffering from hunger and malnutrition.

Rural farmers account for the greater part of the population of any developing country such as Nigeria. The government of a developing country have a major responsibility of ensuring that there is adequate development in their various communities and local government which could lead to effective and efficient agricultural systems that will not only supply food and animal protein but also foster the utilization of natural resources in a sustainable manner (CGIAR,1995).

When the rural farmers lack access to knowledge and information that would help them achieve maximum agricultural yield; they are not only in the grope of the dark but are driven to the urban centres in search of formal

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employment, as the only option for survival (Munyua, 2000).Blait (1996) pointed out that the less expensive input for improved rural agriculture development is adequate access to knowledge and information in areas of new agricultural technologies, early warning system (drought, pest, diseases) improved seedlings, fertilizer, credit, market prices.

The general lack of awareness among farmers can be attributed to their high illiteracy (Mgbenka and Mbah, 2016),this contribute to the low level of agricultural findings. Farmers need information on production technology that involves cultivating, fertilizing, pest control, weeding and harvesting, they also need information relating to loan such as names of lenders, location and types of existing credit to reap greater profit. Grass root organs such as village heads and local.officials are used to diffuse information because of their personal touch with the small scale farmers.

The gap between theory and practice can only be reduced if correct methods of communication are implemented. These methods includes both direct and indirect communication. By directcommunication reference is made to situation where feedback can be provided instantly. Methods such as meetings and farmers' day are some examples of this mode of communication. Access to information is necessary for improving rural people livelihood (Mbagwu*et al.,*2018). Despite its key role in socio economic development, very few people in developing countries have access to adequate information. The information environment of the rural areas is distinct from that of the urban environment due to obvious differences. The rural areas are mostly inhabited by people with low economic potentials, illiterates, semi- illiterates, social amenities including agencies for the information dissemination. The factor of illiteracy or low level of illiteracy, school dropouts among others who have limited or no access to social amenities including agencies for the information dissemination. The factor of illiteracy or low level of illiteracy acts as a great inhibitor to information access and assimilation in rural communities in Nigeria.

Information that can help solve a problem in a development process has been a topic of extensive debate. According to Gaal (2017), the lack of adequate and relevant information as impacted negatively on any development process although academics and researchers are aware of the value of information in development, there are some concern that information is still not perceived as being as important as other resources.Meanwhile, the choice of information sources varies in individual traits, among agricultural information seekers variables such as farm size, years in farming, age, level of education, and level of income influence the choice of information sources (Riesenberg et al., 1999). If this factors can be noted it would be easier to improve access of information in rural areas. Most information services are focused in the urban areas rather than in the rural areas where help is really needed and there is a large population that lives there. Limited infrastructures, low level of illiteracy, lack of suitable information services and lack of technical competence as among the barriers to delivery of information services in rural areas in developing countries (Kamba 2009).The messages carried are not tailored to the information needs of rural populations. Even when the information is relevant, it is seldom aimed at the proper time and so does not get to the targeted audience. Another major constraint is the use of print media, leaflet and newsletters as message carries are of limited use in reaching illiterates farmers technical language used in communicating information is incomprehensible to farmers.

The objective of the study is to determine the access of information among rural farmers, identify the type of information available to them as well as the constraints encountered towards their access to agricultural information by rural farmer.

II. METHODOLOGY

Study area

Oyo state is one of the South Western zone of Nigeria. Oyo state is covered by Oyo-State Agricultural Development Programme (ADP) with four zones namely; Saki, Ogbomosho, Oyo and Ibadan/Ibarapa zones. Ido local government falls within the Ibadan/ Ibarapa agricultural zone.The annual rainfall ranges from 1,200 – 1,300 mm. The area lies within the rainforest region of Nigeria and has two distinct seasons, the raining season from April to October with an August break and dry season from November to March. The temperatures vary from a minimum of 21ºC in July to a maximum of 39ºC in February. A good percentage of the populace are engaged in agriculture; producing staple crops. The state is divided into three agro ecological zones which are: the rainforest, the savannah and the derived savannah. The vegetation of the zone is evergreen forest found in the southern part. Ido local Government is located between 3'39E and 3'45E and latitude of 7'47N rainforest.

Sampling procedure

A two stage sampling technique was used for this study. The first stage involve a purposive selection of Ido local government because it is an agrarian community**.** The second stage involve a**r**andom selection of eleven villages. The third

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stage involve a random selection of 10 respondents from each village except Apesan and Gedegbe where we have few farmers and just 5 respondents were selected from each village making it a total of one hundredin the study area. The selected villages are ;Apesan, Gedegbe, FenwaAdelakun, AkindeleEgbarin, Ojuloge, Bako, AbaTesan, Alagbede, Fafunwon and Gedegbe;

Method of data Analysis

The objectives wereanalyzed using descriptive statistics such as tables, frequencies and percentages and chi-square as appropriate.

III. RESULT AND DISCUSSION

Table 1: Socio Economic Characteristics of respondents

Variable	Frequency	Percentage
Gender		
Male	61	70
Female	25	29.1
Total	86	100
Age		
Below 20	15	17.4
21-40	22	25.6
41-60	25	29.1
Above 62	24	27.9
Total	86	100
Education		
No formal education	53	61.6
Primary education		
Secondary education	22	25.6
Diploma		
Bsc	8	9.3
Marital status	2	2.3
Single	1	1.2
Married	44	51.2
Divorced	4	4.7
Widowed	20	23.3
Total	86	100
Household size		
1-5	49	57
5-10	36	42
Above 10	1.0	1.0
Total	86	100
Religion		
Christianity	28	32.6
Islamic	54	62.8
Traditional	4	4.7
Total	86	100

Source: Field Survey

Table 1 shows that 70.9% of the respondents are male and 20.9% are females. This implies that more males engage in farming than females. Also 17.4% of the respondents were aged below 20 years, 25.6% were aged between 21-40 years, 29.1% were aged between 41-60 years while 27.9% are above 62 years. This result is similar to that of Hoping (2004) who reported that farmers are of an average of 40 years. Majority (61.9%) had no formal education, this is line

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with the findings of Sawio(1999) that majority of farmers had no formal education, 25.6% had primary education, 9.3% had secondary education,2.3% had diploma while just 1.2% are degree holders.

The table also revealed that 62.8 % practice Islam , 32.6% are Christians while 4.75 were of the traditional belief . Also, 51.2% were married, 23.3% were widowed, 20.9% are single while just 4.7% were divorced. The findings also shows that 57% of the respondents have a household size of 0-5 members, 42% had household members of between 5-10, just 1% had above 10 members.

Table 2: Access to information among sampled respondents

Agric information	Very much(frequency)	%	A little	Percentage	Unsure	Percentage	Not at all	Percentage
Weed control	43	50	28	32.6	2	2.3	13	15.1
Farm Mechanisation	20	23	32	37.2	2	2.3	32	37.2
Pest control	46	53.5	10	11.6	8	9.3	22	25.6
Weather and Climate	12	14.0	21	24.4	3	3.5	50	58.1
fertilizer	61	70.9	25	29.1	0	0	0	0
Tillage	10	11.6	27	31.4	7	8.1	42	48.8
Seed varieties	16	18.6	22	25.6	5	5.8	43	50
Land preparation	8	9.3	19	22.1	1	1.2	58	67.4
Planting method	11	12.8	16	12.8	1	1.2	58	67.4

Source: Field survey

Table 2 shows that 50% of the respondents had “very much” information on weed control, 32.6% had a little information on weed control, 2.3% were unsure and 15.1% did not get any information at all. 23% of the respondents had very much information about mechanization while 37.2% had a little information on farm mechanization, 2.3% were unsure and 37.2 did not get information. Also 53.5% of the respondents had very much information about pest control, 11.6% had a little information, 9.35 % were unsure if they had information or not while 25.6% did not have information pertaining to pest control, this result shows that more of the respondents had information about pest control. Also 14% of the respondents in the study area had very much information about weather and climate, 24.4% had little information, 3.5% were unsure, majority (58.1%) did not have access to any informationat all. 70% of the respondents had very much information about fertilizers, 29.1% had a little information, none of the respondents were unsure and none of the respondents

Table 3: Production Related Information Level of Access

Production related	Very much		A little		Unsure		Not at all	
	frequency	percentage	frequency	percentage	Frequency	percentage	Frequency	percentage
Storage	23	26.7	55	64.0	3	3.5	5	5.8
Processing	29	33.7	49	57.0	4	4.7	4	4.7
Tools and equipment	17	19.8	54	62.8	3	3.5	12	14.0

Source: field survey

NOTE: All figures in parenthesis are measured percentage

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Table 3 shows the level of access of respondents to production related information. 23.3 % of the respondents attested that they had very much information about storage of their products 64.0% had a little; 3.5% said they were unsure and just 5.8% of them did not have access to any information at all. Also, 33.7% of the respondents in the study area had very much information about processing of their produce, 57.0% had a little information, 4.7% were unsure and 4.7% did not have access to any information at all. However, 19.8% of the respondents had very much information about tools and equipment for processing and storing their goods, 62.8% had a little information, 3.5% were unsure and 14% of them did not have access to any information at all.

Table 4: Level of Access to Government Related Information

Government Related	**Very much**		**A little**		**Unsure**		**Not at all**	
	Frequency	Percentage	frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Government Policy	4	4.7	36	41.9	6	7.0	40	46.5
Infrastructure	10	11.6	37	43.0	6	7.0	33	38.4
Programme	20	23.3	35	40.7	6	7.0	33	38.4

Source : field survey

NOTE: All figures in parenthesis are measured in percentage.

From table 4 above, 4.7% of the respondents attested that they had very much information about government policy, 41.9% had a little, a while 3.0% were unsure and 46.5% did notb have access to any information at all. Also 11.6% of the respondents had very much information about government infrastructures, 43% had a little information, 7.05% were unsure and 38.4% of them did not have access to any information at all. 23.3% of the respondents in the study area had very much information, 40.7% had a little information, 7.0% were unsure and 29.1% did not have access to any information at all.

Table 5: Level of access to market related information among sample respondents

Market related	**Very much**		**A little**		**unsure**		**Not at all**	
	frequency	Percentage	frequency	Percentage	frequency	percentage	Frequency	Percentage
Market price	6	7.0	50	58.1	1	12	29	33.7
A variable market	6	7.0	59	68.6	3	3.5	18	20.9

Source: field survey

NOTE: All figure in parenthesis are in percentage

Table 5 shows that 7% of the respondents had very much information about market price at which to sell their goods, 58.1% had a little, 1.2% were unsure and 33.7% did not have access to any information which shows that most of the respondents did not have access to any information about market price. Also 8.1% of the respondents got very much information about available market price, 60.5% had a little information about available market, 3.5% were unsure and 27.9% did not have access at any information at all.

Table 6: Level of access to harvesting related information among sampled respondents

Harvesting related	**Very much**		**A little**		**unsure**		**Not at all**	
	Frequency	Percentage	Frequency	percentage	frequency	Percentage	frequency	Percentage
Method	7	8.1	52	60.5	3	3.5	24	27.9
Tools	4	4.7	52	60.5	2	2.3	28	32.6

NOTE: All figures in parenthesis are in percentage

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Table 6 reveals that 8.1% of the farmers had very much information about harvesting methods, 60.5% had little information 3.5% were unsure and 27.9% did not have access to any information at all. Also 4.7% of the respondents had very much information, 60.5% had a little, 3.5% were unsure and 32.6% did not have access to any information at all.

Table 7: Constraints to access of information among respondents

Constraints	**Strongly agree**		**Indifferent**		**disagree**		**Strongly disagree**	
	Frequency	Percentage	Frequency	Percentage	frequency	percentage	frequency	Percentage
Source	43	50.0	4	4.7	36	41.9	3	3.5
Cost of getting information	59	68.6	4	4.7	18	20.9	5	5.8
Availability	56	65.6	7	8.1	12	14.0	11	12.8
Cost of utilization	68	79.1	13	15.1	3	3.5	2	2.3
Government policy	74	86.0	9	10.5	1	1.2	2	2.3
Others	73	84.9	10	11.6	1	1.2	2	2.3

Source: Field Survey.

Table 7 shows the constraints to access and usage of agriculture information. 50% of the respondents in the study area strongly agreed that source of information is constraint, 4.7% were indifferent, and 41.9% disagreed and 3.5% strongly disagreed about the source of information being a constraint. Also 68.6% of the respondents agreed that the cost of getting information is a constraint to access and usage of agricultural information, 4.7% were indifferent, 20.95% disagreed and just 5.8% of the strongly disagreed about it. 65.1% of the respondents agreed that availability of information is a constraint, 8.1% were indifferent, 14 disagreed and 12.8 strongly disagreed, 79.1% of the respondents strongly agreed that cost of utilization is a constraint, 15.1% were indifferent, 3.5% disagreed and 2.3% strongly disagreed. 86% agreed that government policy is a constraint, 10.5% were indifferent, 1.2% disagreed and 2.3% strongly disagreed. Other constraints mentioned by the respondents were, access to loan, collateral problems, language barrier etc. 84.6% of the respondents agreed that the other constraints were greater, 11.6% were indifferent, 1.2% disagreed and 2.0% strongly disagreed.

Hypothesis of the study

Chi-square was used to analyze the hypothesis, since it is measured at a nominal level. H01: There is no significant relationship between the socio economic characteristics of the respondents and access to agricultural information.

VARIABLES	**X2**	**DF**	**P-VALUE**	**REMARK**
Age	2.088	3	0.554	Non significant
Gender	2.229	1	0.135	Non significant
Marital status	11.307	3	0.010	Significant
Household size	1,835	2	0.400	Non significant
Religion	3.103	2	0.212	Non significant
education	9.920	4	0.042	Significant

The result from the table above revealed that there is a relationship between the socio economical characteristics and access to agricultural information. From the table the martial status has a significant relationship with the access to agricultural information (0.010) in the study area and also the level of education also shows a positive significant relationship (0.042), and thus the h01 hypothesis which states that there is no significant relationship between socio economical characteristics and access to agricultural information is rejected and a new hypothesis which states that there is a significant relationship between socio economical characteristics and access to agricultural information is accepted.

IV. CONCLUSION

The study examined the level of access to agricultural information of rural farmers in Ido local government, Nigeria. From the results of the study most of the farmers in the study area had no formal education (61.6), (70.9) the highest percentage of the respondents were male and (29.1) were within the active age bracket of 41-60 year, also 68.6% of the respondent were indigenes of the study area. Most of the respondents in the study area had a little information about weather and climate, machines, tillage seed varieties, land preparation and planting information while a large percentage had a lot of information about weed and fertilizer due to the actions of profit oriented organizatiosn who give the farmers this information so as to be able to sell their goods. Most of the farmers had little information about production of goods, government related information, market related and harvesting techniques. From the result, it can be observed that the respondents in the study area have an inadequate level of information. However there is a significant relationship between educational level, marital status and the access to agricultural information which means that the household size and marital status had a positive effect on the level of access to agricultural information in the study area.

V. RECOMMENDATION

The following are the recommendation made based on the findings of the study.

* Agricultural information should be extended to remote villages.
* Agricultural information should be explained in the simple and understandable language to the rural farmers
* Loans with little or no collateral should be given to farmers.
* Farmers input band machinery should be sold to farmers at subsidized rate
* Installment payment for inputs and machinery should be accepted.
* Adult education should be encouraged

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Benefits of forest legislation enforcement on the Status of Onigambari Forest Reserve

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Abstract— *Forest legislation enforcement is a fundamental strategy needed for the regulation of use and development of forest reserves. Effective law enforcement is necessary for continued production of goods and services. However, gaps have been noticed in the forest legislations' enforcement in the study area. Simple random sampling was used to select 93 forest dwellers from 3 purposively selected adjoining communities in Onigambari forest reserve: Onigambari (28), Busogboro (38) and Onipe (27). Samples were drawn in proportionate to sizes. A set of questionnaire was used to obtain data on factors responsible for weakness in enforcement of forest legislations and various benefits being derived by the host community dwellers from the use of forest reserve irrespective of the existence of forest legislations. Data were analyzed using descriptive statistics and logit regression at* $\alpha_{0.05}$*. Respondents were 42±13.7 years, mostly male (75.9%) and married (81.9%). The identified factors responsible for weakness in enforcement of forest legislations included low activities of extension agents (77%) and lack of effective supervision by the representatives of the State governments (Managers). Benefits being derived by the forest dwellers were achievement of sustainable forest production, employment generation and attention to road network with odd-ratios, 62.18, 2.10 and 1.70 respectively. The study substantiate the existence of weakness in enforcement of forest legislations in Onigambari forest reserve and also revealed an array of benefits being derived by the forest dwellers. Therefore for continuity and improvement in the goods and services provided by the reserve, it is necessary for the government to build institutional capacity so as to fostering better enforcement of forest legislations and compliance.*

Keywords— ***Law enforcement, Weak enforcement, Forest benefits, Forest dwellers.***

I. INTRODUCTION

Policy is the basis for which legislation is needed to regulate the use and development of forest resources, while law enforcement is an integral part of the overall management of the National Forest System. Law enforcement personnel, line officers, and appropriate staff ensure that prevention, investigation, enforcement, and programme management requirements are fully integrated into all National Forest System resource management programmes. Law enforcement personnel operate as full partners within the forest service organization in carrying out the agency's mission, especially in upholding federal laws and regulations that protect natural resources, agency employees, and the public. Accomplishment of the forest service law enforcement mission is a product of trust, co-operation, and collaboration between law enforcement personnel and other agency employees (USDA, 2015).

FAO (2010) reported that every year, a significant proportion of the world's timber is harvested, transported, processed and traded in violation of national laws. Illegal logging and associated timber trade have far reaching environmental, social and economic consequences, including loss of biodiversity and habitants, political instability, increased income disparities and market distortions. While accurate data on the scope of illegal forest activities is not available, the World Bank estimates that losses from illegal logging in terms of global market value are more than US$10 billion annually and lost government revenues total about US$5billion. The magnitude of the problem has prompted governments, with the help of international and non-governmental organizations as well as the private sector, to step up their analysis of the socio-economic causes and consequences of illegal logging. Studies have shown that issues to be resolved include (i) Flawed policy and legal frameworks (ii) Uncertainty surrounding forest tenure (iii)

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Weak law enforcement; insufficient information on the forest resources, coupled with increased demand for forest resources (iv) Corruption and lack of transparency. Several processes are therefore underway at international, regional and national levels to combat forest crime and improve compliance.

Effective policies (legal reform) require an assessment of other capacity and willingness of government and others to implement new legal strategies, a commitment to monitoring the effects of legal strategies, a commitment to monitoring the effects of legal changes over time and a responsiveness to the result of the monitoring.

One of the factors that militate against sustainable forest management is the absence of a National Forestry Act. Apart from using the provision of the Act to regulate forestry practices in Nigeria and to give also a legal backing for the National Forest Policy, it would further enable us to meet the obligations on the treaties and conventions relevant to forestry development to which Nigeria is a signatory. The first ever National Forestry Act has also been evolved to back the policy which has been passed to council for approval though it is yet to see the light of the day.

Conclusively, there are lot of benefits from enforcement of forest legislation for both the host communities and the manager of forest reserves (Government). Some of these benefits are pointer to the existence of economic well-being of the forest dwellers. That is why the importance of community participation cannot be ruled out in effective management of forest reserves, while one of the benefits of community participation in forest management is the improvement in socio-economic well- being of the people (Banjo and Abu, 2014).

Hence, this paper addressed the weakness in enforcement of forest legislation and also identified various benefits derived by the host community dwellers from the use of the forest irrespective of the existence of forest legislations.

II. METHODOLOGY

Study Area

Onigambari forest reserve was declared to be Ibadan forest reserve by a resolution of Ibadan council on 14th September 1899 and take its name from a nearby village called Onigambari. The reserve is located on latitude $7^0 25^1$ and $7^0 55^1$N, and longitude $3^0 53^1$ and $3^0 9^1$E located in the Oluyole local government of Oyo State, Nigeria (Fig. 1). The forest reserve, which used to be part of the lowland rainforest, is now in the Guinea savanna and Derived savanna zones. It covers a total land area of 13932.18 hectares consisting of five zones: Mamu, Onigambari, Busogboro, Odo-Ona, Onipe and Alabata. The Mamu and Onigambari zones were predominantly made up of Gmelina plantations. The topography is generally undulating, lying at altitude between 120-150m above sea level and the area is bounded by two Rivers (Rivers Ona and Awon). Mean annual rainfall ranges between 1200mm to 1300mm with two distinct wet seasons.

Sampling Techniques and Data Analysis

Purposive sampling was used to select three villages among the adjoining communities in Onigambari forest reserve (Within 1-3km radius) and these include: Onigambari, Onipe and Busogboro with the projected population of 1,428, 1271 and 1,018 respectively. Diaw *et al*., (2002) was therefore adapted to sample from the population. Hence, 10% sampling intensity was used to randomly sample respondents in the communities where their population is less than 500, 5% for between 500 and 1000, and 2.5% for over 1000.

Therefore, 28 respondents were reached in Onigambari, 38 in Busogboro and 27 in Onipe, totaling 93 respondents that were sampled for the study. Hence, 93 structured questionnaires were administered for primary data collection while 83 were retrieved. The returns represent 89% of the total number of questionnaire administered to the forest dwellers in the study area. Data were analyzed using descriptive statistics and inferential statistics (Logit regression analysis).

Population Projection

The current sampled communities' population was projected from 1991 communities' census report to 2019 using the formula below.

$P_n = P_o e^{rt}$ ------(1)

Where: P_n= Final population

P_o= Initial population i.e 1991

e= Exponential

r= Growth rate (Average of 3.5%)

t= Time interval (x-1991) yr(s)

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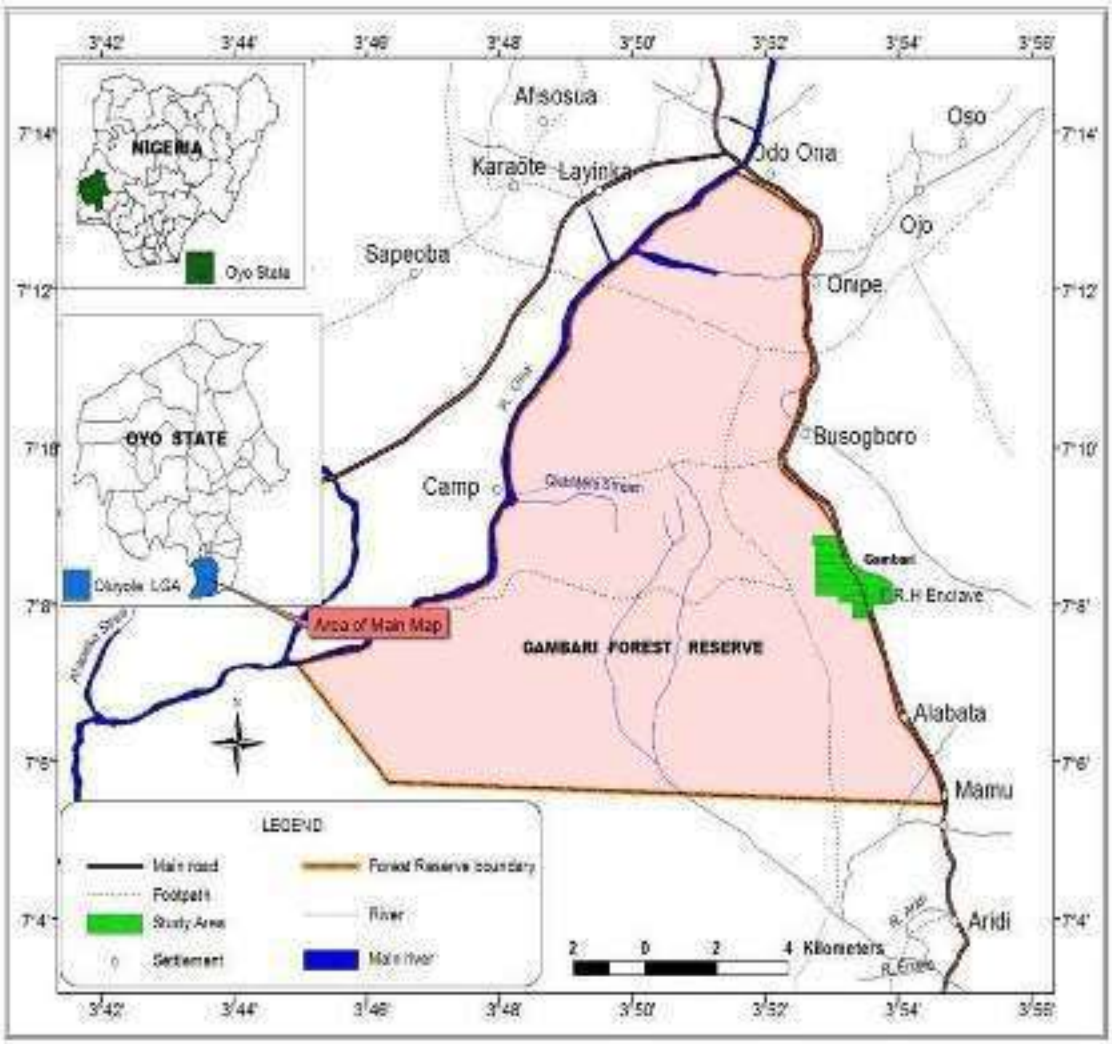

Fig 1: Map of Onigambari Forest Reserve showing the Forest Adjoining Communities

Logistic regression

The binary logistic models are useful in a situation whereby the dependent or response variable is binary in nature. This implies that they can have only two possible values. The models therefore describe the relationship between one or more continuous independent variable (s) to the binary dependent variable.

The two common binary models are the logit and probit. The logistic model is particularly preferred because of the unique information it provides. Distinct information provided by logit is the odds ratio. It is defined as the ratio of the odds of an event occurring in the group to the odds ratio of it occurring in another goup (Deeks, 1996 and Davies, 1998). The logistic model of a response p between 0 and 1 is given as:

Logit (p) = log (p/1-p) = log (p) - log (1-p) ----- (ii)

The simplest form of logistic model is expressed as:

Logit (pi) = a + bx1 + -------- + bx4 --------------- (iii)

Where:

Pi = Probability of significance of benefits derived from Onigambari forest reserve (Dependent variable)

Xi = Vector of predictor or independent variables a and b = regression parameters.

The independent variables are:

X_1 = dummy variable indicating whether Attention to Road Network (ARN) has been a benefit being derived from the existence of the forest reserve.

X_2 = dummy variable indicating whether Establishment of Industry (EIND) has been a benefit being derived from the existence of the forest reserve.

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X_3 = dummy variable indicating whether Rural Electrification (REL) has been a benefit being derived from the existence of the forest reserve.

X_4 = dummy variable indicating whether Efficient Forest Administration (EFA) has been a benefit being derived from existence of the forest reserve.

X_5 = dummy variable indicating whether Recruitment for Regular Inventory by the government (RRI) has been a benefit being derived from existence of the forest reserve.

X_6 = dummy variable indicating whether Stem down of Rural-Urban Migration (SRUM) has been a benefit being derived from existence of the forest reserve.

X_7 = dummy variable indicating whether Enhancement of Economic Growth (EEG) has been a benefit being derived from existence of the forest reserve.

X_8 = dummy variable indicating whether Employment Generation (EG) has been a benefit being derived from existence of the forest reserve.

X_9 = dummy variable indicating whether More Land Under Cover (MLUC) has been a benefit being derived from existence of the forest reserve.

X_{10} = dummy variable indicating whether Achievement of Sustainable Forest Production (ASFP) has been a benefit derived from existence of the forest reserve.

X_{11} = dummy variable indicating whether Increase in Income Propensity of the dwellers (IIP) has been a benefit derived from existence of the forest reserve.

X_{12} = dummy variable indicating whether access to Raw Materials for Industries (RMI) has been a benefit derived from existence of the forest reserve.

III. DISCUSSION

Table 1 showed the socio-economic characteristics of the respondents in the study area. Information on gender revealed that 75.9% of the respondents were male while 24.1% were female. The average age of the respondents was 42±13.7 years. It could be inferred that the size of the economically active population engaged in forest and other related activities is presently high and this agrees with the findings of (Faleyimu, 2010). The implication for this could probably be tied to the fact that forestry sector serves as easier way to satisfy the basic needs of life (Food, Shelter and Clothing). Poor or weak forest legislation enforcement may therefore pose a lot of danger to the world's clamour for effective conservation to ensure sustainability of forest resources.

Among the 83 respondents, the result revealed that 81.93% were married. This implies that majority of the forest dwellers are married and thereby, involving in the forest livelihood activities which cater for their household needs. This finding concurs with the research work of Akinbile (2007) that marriage confers responsibility.

In terms of educational status of the respondents, majority of them had secondary (31.3%) and primary (34.9%) education. This could be attributed to the influence of the environment on the forest dwellers. Only few who are determined to study further leaves such environment. Many often do not care to study further inasmuch they start making money at a very tender age. This agrees with (Adejumo, 2016).

As touching the occupation of the respondents, majority of them were farmers (47.0%) and artisans (32.5%) and these are the predominant occupation in such environment since they are majorly concerned about how to cater for their families and not in pursuit of living a luxury life.

Also, the study revealed that most of the respondents (73.5%) are native of the study area while only few of them (20.5%) must have settled in the area many years ago in search of opportunity for subsistence farming. This uphold the report of Calibre and SCC (2000) which reported that the people living in and around the forests include smallholders practicing subsistence farming and settlers who have come in the areas to search for new opportunity in agriculture.

Table 1: Socio-economic Characteristics of the Respondents in the Study Area

Socio-economic Characteristics	Frequency	Percentage (%)
Gender		
Male	63	75.9
Female	20	24.1
Total	83	100
Age (Years)		
21-30	18	21.7
31-40	26	31.3
41-50	22	26.5
51-60	-	-
61-70	17	20.5
Total	83	100
Marital Status		
Single	68	81.9
Married	12	14.5
Widow	3	3.61
Educational Status		
No formal education	26	31.3
Primary	26	31.3

Secondary	29	34.9
Tertiary	2	2.4
Total	83	100
Occupation		
Artisan	27	32.5
Farming	39	47.0
Trading	17	20.5
Total	83	100
Nativity		
Within the Study Area	61	73.5
Outside the Study Area	22	20.5
Total	83	100

Source: Field Survey, 2019.

Weakness in Enforcement of Forest Legislation

Table 2 showed the respondents' responses to the reasons for weakness in enforcement of forest legislation in the study area. It was evident that low activities of extension agents (77.1%) and lack of effective supervision by the representatives of the State government (77.1%) were the major factors responsible for weakness in enforcement of forest legislation in the area. Truly, it is so obvious that forestry extension agents are not sufficient as it ought to have been compared with what is obtainable in the agricultural sector. One could easily identify agricultural extension agents who monitor agricultural activities and at the same time provide useful information on new ideas. Through this medium, farmers are more sensitized and are also advised to do the right thing that would boost their yield.

Either consciously or unconsciously, agricultural extension agents participate in enforcement of valuable agricultural legislations, which would improve the performance of the sector. As a matter of fact, diverse agricultural research institutes have been established by the Federal government to research into different areas of agriculture. The case is different in forestry. Forestry has only one research institute in Nigeria (Forestry Research Institute of Nigeria), so the task of forest extension has been left for them. It is necessary to stress that Forestry Research Institute of Nigeria has been doing wonderfully well in the dissemination of forestry innovations to farmers and environmentalists. Precisely, her Department of Forest Economics and Extension has been going out on fortnight basis to educate farmers on the new ideas in forestry and how agroforestry would help them to maximizing the use of land.

However, the role of forestry extension agents in enforcement of forest legislation cannot be over-emphasized as they could also assist in educating the farmers or forest dwellers on the importance of forest policies and its benefits.

As responses by the respondents also showed that lack of effective supervision is another factor responsible for weakness of forest legislation's enforcement, it is important to stress that success in any project evaluation is highly depended on the manager's capability of supervision, co-ordination and control. Meanwhile, the long-term fortunes of forestry depend largely on the institutions put in place as well as the performance of the managers (Adeyoju, 2001). This is also supported by Faleyimu (2010) which stated that irrespective of the sustainability strategy and the enabling environment, the human role is indispensable. Also accountability and transparency are important components of effective supervision and co-ordination.

Other prominent factors responsible for weakness in enforcement of forest legislation were low income earning of the rural dwellers (67.5%) and characterized illiteracy level of the forest dwellers (65.1%) There is an adage which says "A hungry man is an angry man", therefore forest dwellers with low income capability tends to violate government laws so as to meet his or her urgent immediate need. For instance, various policies placed on firewood extraction may not be perfectly enforced while huge success in this regards may be extremely difficult. Prevalent illiteracy level of the forest dwellers required periodical sensitization by the forestry extension agents so as to ensure strict compliance.

Table 2: Weakness in Enforcement of Forest Legislation

Weakness	Agree (%)	Disagree (%)	Undecided (%)
1. Low activities of extension agents	64(77.1)	15(18.1)	4(4.8)
2. Lack of effective supervision by the government	64(77.1)	19(22.9)	-
3. Illiteracy level of the dwellers around forest reserve	54(65.1)	24(28.9)	5(6.0)
4. Lack of Capital/ low income power among	56(67.5)	24(28.9)	3(3.6)

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	the rural dwellers			
5.	Lack of co-operation and co-ordination among the rural dwellers	46(55.4)	32(38.6)	5(6.0)
6.	Structure and belief of the environment	48(57.8)	30(36.1)	5(6.0)
7.	Too much access to forest reserve due to the satisfaction that government want to do for his people around forest reserve.	46(55.4)	28(33.7)	9(10.8)
8.	Problem of finance needed and monitoring of the implementation.	45(54.2)	31(37.3)	7(8.4)
9.	Inadequate information from the policy maker.	50(60.2)	28(33.7)	5(6.0)

Source: Field Survey, 2019.

Logit Regression Model for Forest Dwellers' Benefits from Onigambari Forest Reserve

The Binary Models

Binary Regression Models

Binary regression models obtained for forest dwellers' benefits from Onigambari Forest Reserve (Table 3).

$$BDFF = 28.68 + 0.57\,ARN - 6.90EIND - 0.25EFA - 0.88RRI + 0.14SRUM + 0.63EEG + 3.16EG - 5.89MLUC + 8.35ASFP - 1.78IIP - 0.04RMI \text{ ------------------------- (iv)}$$

N = 83, Final loss = 0.00, Chi-square (df ; 12) = 95.99. Odd-ratio (Unit change). Constant (28.68); ARN (1.70), EIND (0.001), REL (O.14), EFA (0.78), RRI (0.41), SRUM (1.15), EEG (2.10), EG (23.50), MLUC (0.002), ASFP (62.18), IIP (0.17), RMI (0.960).

Where,

BDFF - Forest dwellers' Benefits from Onigambari Forest Reserve

ARN - Attention to Road Network

EIND – Establishment of Industries

REL – Rural Electrification

EFA – Efficient Forest Administration

RRI – Recruitment for Regular Inventory by the Government

SRUM – Stem down of Rural-Urban Migration

EEG – Enhancement of Economic Growth.

EG – Employment Generation

MLUC – More Land Under Cover

ASFP – Achieving of Sustainable Forest Production

IIP – Increase in Income Propensity of the dwellers

RMI – Raw Materials for Industries

Model presented above for Forest dwellers' benefits from Onigambari forest reserve gave overall significant fit to the data judging from $_{\chi}{}^{2}$Value that was significant at $p<0.05$. Achievement of Sustainable Forest Production (ASFP) had the highest odd-ratio of 62.18 followed by Employment generation (EG) with the odd-ratio of 2.10 and lastly, Attention to Road Network (ARN) with the odd-ratio of 1.70 respectively.

Therefore, the significant benefits identified to be enjoyed by the forest dwellers in and around Onigambari forest reserve were Achievement of Sustainable Forest Production (ASFP) by the management of the forest which paved way for the continuous production of forest products and services which in turn affects the livelihood of the dwellers positively. Others are Employment Generation (EG), Enhancement of Economic Growth (EEG) in the area and Attention to Road Network (ARN) in the study area. There was sufficient evidence that the model were statistically significant. In other words, the higher the value of odds-ratio, the higher the level of significance of these benefits enjoyed by the forest dwellers in Onigambari forest reserve. Hence, it clearly indicated the variable (s) i.e benefits that mostly derived from the study area. The implication was corroborated by Deeks (1996); Bland and Altman (2000) that the logit model provides information on the consequence of one variable on the other. Therefore, identifying these benefits indicated that the existence of Onigambari forest reserve has impacted the livelihood of forest dwellers in the area.

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Table 3: Logit Binary Nature for Forest Dwellers' Benefits from Onigambari Forest Reserve
Dependent Variable (BDFF) = Forest Dwellers' Benefits from Onigambari Forest Reserve (Yes =1), (No =0)

Independent Variables	Coefficient	Odd-ratio
Whether (ARN) has been a benefit being derived from the existence of the forest reserve.	0.57	1.70*
Whether (EIND) has been a benefit derived from the existence of the forest reserve.	-6.9	0.001ns
Whether (REL) has been a benefit being derived from the existence of the forest reserve.	-1.94	0.14ns
Whether (EFA) has been a benefit being derived from the existence of the forest reserve.	-0.25	0.78ns
Whether (RRI) has been a benefit being derived from the existence of the forest reserve.	-0.88	0.41ns
Whether (SRUM) has been a benefit being derived from the existence of the forest reserve.	0.14	1.15ns
Whether (EEG) has been a benefit being derived from the existence of the forest reserve.	0.63	2.10*
Whether (EG) has been a benefit being derived from the existence of the forest reserve.	3.16	23.50*
Whether (MLUC) has been a benefit being derived from the existence of the forest reserve.	-5.89	0.002ns
Whether (ASFP) has been a benefit being derived from the existence of the forest reserve.	8.35	62.18*
Whether (IIP) has been a benefit being derived from the existence of the forest reserve.	-1.78	0.17ns
Whether (RMI) has been a benefit being derived from the existence of the forest reserve.	-0.04	0.96ns
Model χ^2 (df, 12) = 95.99, Final loss = 0.00; p<0.05		

***=Significant at p<0.05; ns = Not significant**

IV. CONCLUSION AND RECOMMENDATION

This study uphold the existence of weakness in enforcement of forest legislation in Onigambari forest reserve and found low activities of extension agents and lack of effective supervision by government (Managers) to be significantly responsible for weakness in enforcement of forest legislation in the area. The study also identified the various benefits being derived by the forest dwellers from the forest reserve due to the existence of forest legislation in the area to be achievement of sustainable forest production, employment generation, enhancement of economic growth in the area and attraction of attention to the road network in the study area.

Therefore, building of institutional capacity to foster better enforcement of forest legislation and compliance is of paramount importance to achieving sustainable forest management in the reserve, such that more benefits can be enjoyed by the management of the reserve, forest dwellers and country at large.

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Extraction of Catechins from *Areca catechu* L.Peel with different Solvent Type for Feed Additive of Broiler

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***Abstract*—*Catechins are secondary metabolites compound of flavonoids group that is naturally produced by plants and have health benefits as cholesterol-lowering, antioxidant, antimicrobial in mice.Rapid growth in broilers is often followed by high-fat growth as well, thus causing a high cholesterol content in the broiler's meat especially in thigh meatand wings.Feeding extract catechin in broiler as feed additive will reduce cholesterol or fat in broiler's meat. The catechins could be extracted by maceration method, so far there is no information about the type of solvent and extraction method for extraction of catechin from Areca catechu peel. The aims of this study to obtain the best combination of solvent type and maceration duration in extracting of catechins from betel nut peel. Areca catechu peel used in this study is a green-colored fruit peel, which washarvested from betel nut seeds in Batu Balang village, Lima Puluh Kota District, West Sumatra Province, Indonesia. This study was performed by using Factorial Experimental in Completely Randomized Design withtwo factors. The first factors was solvent types (water with an initial temperature at 80°C, acetone distillate, and ethyl acetate distillate) and the second factors wasmaceration duration (6 hours, 12 hours, and 18 hours), each combination treatment was replicated 3 times.The variables observed were percentage of water content, percentage of yield, and percentage of total catechins. The results showed there was a significant interaction (P <0.05) between type of solvent and maceration duration on yield percentage andtotal catechins percentage, while there was no interaction betweentype of solvent and maceration duration(P> 0.05) on percentage ofwater content. Both type of solvent and maceration duration were significantly (P <0.05) affectedyield percentage andtotal catechins percentage, whilepercentage ofwater content did not affect. It concluded the combination of distillate acetone solvent and maceration duration for 6 hours was the best combination to obtain catechin extract from Areca catechu peel. In this condition the percentage of water content was 10.53%, yield percentage was 7.13%, and percentage of catechin extract was 25.53%.*

***Keywords*—Areca catechu L. peel, catechin extract, feed additive, maceration duration, solvent type.**

I. INTRODUCTION

Areca catechu L. is classified as a monocot plant of the Palmae family which is spread almost in all regions in Indonesia. According to the Central Statistics Agency (2017), Areca nut fruit production in Indonesia reached 314.51 tons with an area of 143,927 hectares. Areca nut seed is one of the potential non-oil export commodities in the international market. In 2016 the export volume of betel nuts reached 219,127 tons with a value of US $ 277.78 million or around Rp. 3.9 billion(Yudha, 2017).

Areca nut fruits are exported in the form of seeds, so that after harvesting the Areca nut seeds will leave the fruit peel which is a waste and has not been widely used. Areca nut peel waste reaches 76% of the weight of fresh Areca nuts fruits (Mahata *et al*., 2018).Areca nut peel contains 65.41% water, 34.59% dry matter, 2.22% protein, 0.15% fat, 47.02-53.96% crude fiber, 0.28% Ca, 0.36% P, and energy metabolism 1116 kcal / kg and analysis of betel nut peel with van Soest method showed betel nut peel contains 59.07% Neutral Detergen Fiber (NDF), 44.74% Acid Detergen Fiber (ADF), 27.44% cellulose, 14.32% hemicellulose, and 17, 30% lignin (Mahata *et al*., 2018).Furthermore, Mahata *et al.*(2018) explained the peel of betel nuts contained phytochemical compounds such as catechins of 1.466%, total polyphenols 1.693%, total alkaloids 1.382%, and contained fatty acids consisting of 1.83% myristate, palmitate 16.386%, stearate 2.751%, oleic 34.130%, linoleic 2.918%, and linolenic 0.171%.

Catechins was a popular phytochemical compound in Areca catechu plant. Catechins is a metabolic secondary from tannin which produce by plant naturally (Gruenwald*et al*., 2000). Previous research showed catechins are beneficial for health, as cholesterol-lowering, antioxidants, antimicrobials. Some researcher reported that there was a decrease in the activity of pancreatic cholesterol esterase (pCEase) in vitro in mice given areca seed extract supplements with a reduction in plasma cholesterol by 25%, and did not change triglyceride concentrations (Park *et al*., 2002). Furthermore, Ikeda (2008) reported that giving catechins from green tea was able to lower total cholesterol levels in rat blood plasmabecause catechins were able to effectively inhibit the absorption of cholesterol in the intestine.Yunarto *et al*. (2015) also reported, ethyl acetate fraction of gambier leaf extract containing catechins could inhibit the action of HMG-CoA reductase in mevalonate synthesis of HMG-CoA in cells, to reduce total cholesterol, triglycerides, LDL and not increase HDL in rat blood plasma.Raederstorff *et al.* (2003) also reported that the administration of epigallocatechin-gallate from green tea to a dose of 0.7 g/day/kg body wight (BW) was able to reduce LDL and blood plasma of mice. Mechanism of LDL reduction by catechins is reduced by the production of apolipoprotein B which is the main constituent component of LDL and as a radar for LDL receptors in cells (Babu and Liu, 2008).

Broiler is chicken that has high productivity, as meat producer. Broilers have relatively short growth and meat production compared to other livestock.Broiler chicken meat is one of the sources of animal protein that is widely consumed by the community because it is relatively inexpensive, easy to obtain, and has soft fibrous meat so that it is favored by all ages. Muliani (2015) stated the rapid growth of broilers is often followed by high-fat growth as well, thus causing a high cholesterol content in the broiler's body especially in thigh meat and wings. Along with public awareness of health, cholesterol content becomes the public's consideration in consuming broiler meat.The alternative offered is the use of catechins form betel nut peel as additive feeds on broiler rations to lowering cholesterol and fat in broiler meat.

The use of catechins in Areca catechupeel as a feed additive for broiler rations is limited because of high crude fiber content andit still bound in plant cells, so that it is necessary to proces by extracting of catechin from Areca catechu peel.Extraction is the process of separating the material from the mixture using an appropriate solvent. The extraction method is divided into 2 ways, by cold proces namedmaceration method, and by heat proces named soxhlet extraction. Maceration is the simplest method of extraction by immersing the sample powder in a suitable solvent at room temperature.Soxhletation is a method of extraction carried out in a device called soxhlet with a polar solvent based on its boiling point. Catechin extraction can be done by the maceration method. The choice of the maceration method in this study because the catechin compound was susceptible to heat, so it was not good to use the soxhlet method (Damanik *et al*., 2014).This is supported by Cheong *et al*. (2005) that the concentration of catechin compounds decreased in the Soxhlet method compared with the maceration method.

The choice of solvent type is very important when the extractionprocess. The solvent in maceration extraction process must be in accordance with the material to be extracted, and the solvent must be able to separate quickly after shaking or maceration extraction (Mamonto *et al*., 2014). Catechins are not soluble in cold water but dissolve in hot water, in alcohol, ethyl acetate, and almost insoluble in chloroform, benzene, and ether (Hidayatullah, 2008).The most efficient solvent for extraction of catechins from green tea is in hot water with temperature at 80°C with maceration duration at 60 minutes and obtained catechin at range 64-97% (Uzunalic *et al*., 2006).According to Damanik *et al*. (2014) stated the best solvent for extracting catechins from gambier is ethyl acetate concentration 95% with temperature at60°C, and maceration timeat 6 hours with catechin extraced is 87.14%. According to Satriadi (2011) tannin extract on Areca catechu seeds from Pelaihari area in South Kalimantan can be obtained from both water solventat temperature 80°C with 12 hour maceration duration and acetone solvent with maceration duration for 12 hour, and tannin extracted obtained from both of solvent was 17.97%, and 19.04% respectively.

This study aims was to obtain the best treatment combination between the type of solvent and the best maceration time, to get the optimal catechin extract from the peel of betel nuts as a feed additive broiler ration for lowering broiler blood serum cholesterol.

II. MATERIAL AND METHOD

2.1. Materials Research

The materials used in this study are green betel nut peel collected from Nagari Batu Balang, Lima Puluh Kota District, West Sumatra Province, Indonesia. In addition, aquades, water solvents (with an initial temperature at

80ºC), acetone distillate, and ethyl acetate distillate. To measure the catechin content of areca nut peel extract, ethyl acetate pro analysis (pa) 99,5%and catechin standard were used.

The tools used in this research to produce Areca catechu peel flour are oven, grinding machine (ADR MPJ 200), 10 mesh sieve (ABM brand). Areca catechu peel extraction tools used were Erlenmeyer 250 ml volume tubes, aluminum foil, rotary evaporator brand (RV 10 digital V), stirring rods, analytical scales, Whatman 42 filter paper diameter 125 mm, thermometer, incubator shaker (New Brunswick brand). Catechin levels were measured with a UV spectrophotometer, with quartz cuvette equipment, ultrasonic bath, analytical balance, blender, excavator, 50 mm measuring flask, watch glass, petri dish, oven, ordinary filter funnel, 2 ml pipette, Erlenmeyer with a 100 mm sharpener, and qualitative filter paper No.42.

2.2. Research Implementation

This study was performed by using Factorial Experimental in Completely Randomized Design with two factors. The first factors was solvent types (water with an initial temperature at 80ºC, acetone distillate, and ethyl acetate distillate) and the second factors was maceration duration (6 hours, 12 hours, and 18 hours), each combination treatment was replicated three (3) times.

2.2.1. Sample Preparation

Areca catechu peel waschopped and dried using an oven at 60ºC until the water content reaches 14%. Then the dried Areca catechu peel is milled by using grinding machine, then sieved with 10 mesh size sieve. Areca catechu peel flour which has been obtained then used for the extraction of catechins by the maceration method with different solvents.

2.2.2. Catechin extraction

Catechinwas extracted from Areca catechu peel flour by maceration method using different solvent (water with an initial temperature of 80ºC, acetone distillate, ethyl acetate distillate) by Uzunalic *et al.*(2006) method.The maceration extraction of Areca catechu peel flour is carried out by weighing 20g of Areca catechu peel flour, and placed into a 250 ml Erlenmeyer, then add with 180 ml of different solvent (water solvent with an initial temperature of 80ºC, acetone, and ethyl acetate) in different Erlenmeyer for each solvent. Furthermore, Erlenmeyer was wrap with aluminum foil to avoid direct sunlight (avoiding light-catalyzed reactions) and they were shaked at incubator shaker for 10 minutes at 60ºC, and stirred for all parts of Areca catechu peel flour particles wereevenly mixed with solvent, so that extraction can be carried out perfectly. After 10 minutes, the Erlenmeyer was removed from the shaker incubator and left at room temperature according to different maceration duration treatment (6, 12, and 18 hours), then filtered by using Whatman paper filter number 42. The filtrate obtained was collected in a bottle and wrapped with aluminum foil, then put into a rotary evaporator to vaporize its solvent, so a dry extract of catechins is obtained from each solvent.

2.3. Observed variables

2.3.1. Percentage of Water Content

The measurement of water content was obtained based on the AOAC method (Association of Official Analytical Chemists, 1990).

2.3.2. Percentage of Yield

Extract yield was calculated by ratio of extracted weight produced with the weight of the extracted Areca catechu peel, as below:

% Yield = Extract mass (g) / (Mass Simplicia (g) x 100%

2.3.3. Percentage of Total Catechin

The analysis of the content of the catechin flour of Areca catechu peel is done by SNI method (Indonesian National Standard, 2000).

III. RESULT AND DISCUSSION

3.1. Effect of Solvent Type and Maceration Time on Percentage of Moisture Content of Crude Catechin Extract from *Areca catechu* L.

The average percentage of the water content of crude catechin extract of Areca catechu L. peel in each treatment can be seen in Table 1.

Table 1. Mean percentage (%) of the water content of crude catechin extract of Areca catechu L. peel based on the solvent type and maceration time.

Type of Solvent	Maceration Time			Average
	M1 (6 hours)	M2 (12 hours)	M3 (18 hours)	
P1 (water with an initial temperature of 80ºC)	10,95	10,91	10,85	**10,90**
P2 (acetone distillate)	10,53	10,47	10,96	**10,65**
P3 (ethyl acetate distillate)	9,93	10,17	10,93	**10,35**
Average	**10,47**	**10,52**	**10,91**	

Note: ns: not significantly different (P> 0.05)

SE: Standard error (0.019)

Based on the results of an analysis of variance showed that there was no interaction (P> 0.05) between the type of solvent (factor A) and maceration time (factor B) to the percentage of water content from crude catechin extract of Areca catechu L.Table 1 shows the percentage of the water content of crude catechin extract of Areca catechu L. peel is 9.93 to 10.96%.

Extract water content is the weight of the extract after drying at a temperature of 105 ° C for 30 minutes or after obtaining a constant weight and expressed in percent (Ratnani *et al*., 2015). Determination of water content aims to provide a minimum limit or the range of the amount of water content in areca nut peel extract. There is no interaction between the type of solvent and the maceration time allegedly because there is no limitation of the time of evaporation of the solvent so that the solvent used at the time of extraction evaporates completely and produces a thick extract with almost the same water content. Prasetyo and Inoriah (2013) reported the process of drying or evaporation of solvents in an extract that is less than optimal can affect the high water content of the extract.This result is different from Damanik (2014) who reported that the highest water content of gambier leaf extract was found in water solvent which was 10.225% and the lowest water content was found in ethyl acetate 95% solvent which was equal to 0.225% with limitation of solvent evaporation time for 60 minutes. The high water content in water is due to the boiling point of the water solvent being higher than ethyl acetate. According to Susanti (2012) states that a solvent must have a boiling point low enough so that the solvent is easily evaporated without using high temperatures in the purification process, ethyl acetate is a type of solvent that has a relatively low boiling point of 77oC so that it is easily evaporated.

DepKes (2008) states that the water content standard of plant extracts in general is <10%, and the water content of catechin extracts from gambir is <14%. Zulharmita et al., (2012) stated the water content of traditional medicine preparations and extracts should not be more than 10%. The water content of EKKBP obtained in this study has met the specified extract water content standard. EKKBP water content in this study ranged from 9.93 to 10.96%, higher than the results of research Ozdemir et al. (2018) the water content of catechin extracts in black tea ranged from 4.78 to 5.45%. Yunarto et al. (2015) reported the moisture content of the ethyl acetate fraction of gambir leaf extract was 2.23%. However, lower than the results of research Ratnani et al (2015) states that the water content of the bitter leaf extract is 13% exceeds the allowed standard (<10%), causing a high rate of microbial contamination of 3.1 x 107 CFU / g with a standard that is no more than 104 CFU / g. According to Yunarto et al. (2017) stated that good storage of catechin extracts <10% because gambier plant catechin compounds are hygroscopic (can draw air water) causing microbial activity is unstable and easily oxidized. The same thing was also reported by Utami (2017) that moisture content exceeding 10% can cause the extract to be easily overgrown with microbes which will reduce the stability of the extract.

3.2. Effect of Solvent Type and Maceration Time on Percentage of Total Yield of Crude Catechin Extract from *Areca catechu* L.

The average percentage of total yield of crude catechin extract of *Areca catechu* L. peel in each treatment can be seen in Table 2.

Table 2. Average percentage (%) yield of crude catechin extract of Areca catechu L. peel with different types of solvents and maceration times.

Type of Solvent	Maceration Time			Average
	M1 (6 hours)	M2 (12 hours)	M3 (18 hours)	
P1 (water with an initial temperature of 80°C)	8,20^{a}	8,14^{a}	7,94^{b}	**8,09^{a}**
P2 (acetone distillate)	7,13^{c}	6,91^{d}	6,78^{e}	**6,94^{b}**
P3 (ethyl acetate distillate)	1,44^{f}	1,38^{f}	1,37^{f}	**1,40^{c}**
Average	**5,59^{a}**	**5,48^{b}**	**5,36^{c}**	

Note: Superscripts with different letters show significantly different effect (P <0.05)

Based on the results of the analysis of variance showed that there was an interaction that significantly different effect (P <0.05) between factor A (a type of solvent) and factor B (maceration time) to the percentage of the total yield of crude catechin extract of Areca catechu L. peel.

DMRT test results showed that the treatment of P1M1 (water solvent with an initial temperature of 80oC and maceration time 6 hours), and P1M2 (water solvent with an initial temperature of 80oC and maceration time 12 hours) gave a significant effect on the treatment other.

The yield of crude catechin extract of Areca catechu L. peel obtained in this study ranged from 1.37% to 8.20%. The highest total yield of crude catechin extract of Areca catechu L. peel was obtained from the treatment of water with an initial temperature of 80 ° C with maceration periods of 6 and 12 hours with values of 8.20% and 8.14%, respectively. The total yield of crude catechin extract of Areca catechu L. peel with the highest acetone solvent was obtained at 6 hours maceration time which was 7.13%. While the lowest yield was found in the treatment of ethyl acetate solvents with different maceration times (6, 12, and 18 hours), namely: 1.44%, 1.38%, and 1.37%.

The high yield of EKKBP in the treatment of P1M1, and P1M2 because catechin compounds found in the skin of betel nuts are polar and will dissolve with polar solvents. According to Sayuti (2017), the suitability of the nature of the solvent and the dissolved substance will affect the percentage of yield. Catechin compounds are polyphenol compounds (Yeni *et al*., 2017), polyphenol compounds are polar (Evans, 2000). According to Tiwari *et al*. (2011), water is a universal polar solvent, and acetone is also classified as a polar solvent, so the yield of EKKBP becomes high in the combination of treatments P1M1, P1M2, followed P1M3, and P2M1. Ethyl acetate is a semi-polar solvent, and is not suitable for dissolving polar catechin compounds, so the total yield of EKKBP is low in the treatment of P3M1, P3M2, and P3M3. Firdiyani *et al*. (2015) stated that ethyl acetate is a solvent with semipolar characteristics.

Types of solvents different in this study showed significant differences in the yield content of EKKBP. The yield content obtained from the water solvent with an initial temperature of 80oC (8.09%) higher than acetone (6.94%) and ethyl acetate (1.40%), and the yield of acetone is higher than ethyl acetate. The high yield of water solvents is due to its nature as a universal solvent and the level of polarity is higher than that of acetone so that more polar substances or compounds from areca nut peels can dissolve, and accumulate into more yields than acetone solvents and ethyl acetate. According to Burdick & Jackson (2012) air is a polar solvent with a polarity index of 10.2, acetone is a polar solvent with a polarity index of 5.1, while ethyl acetate is a semi-polar solvent with a polarity index value of 4.4.Ethyl acetate solvents are classified as semi-polar solvents so they are less able to dissolve polar substances or compounds.

In this study, the longer maceration time (6, 12, and 18 hours) the reduced yield of EKKBP obtained for all types of solvents. The highest yield was obtained at 6 hours maceration (5.59%), then the extension of maceration time was 12 hours, the yield was reduced at 5.48%, and at 18 hours the yield was 5.36%. The low yield due to prolongation of maceration is caused by the process of withdrawal and the amount of compounds from betel nut peel that can dissolve in each solvent has reached a maximum so that the extension time is not much more compounds left in the peel of betel nut. The results of this study differ from those reported by Kamaluddin (2014), that the longer the extraction time will increase the extract yield. According to Kristian *et al*. (2016) the longer the extraction time, the chance for the material to contact the solvent will be greater, so that the total yield obtained will be high up to the saturation point of the solution, but the number of certain compounds will decrease after reaching the optimal time.

3.3. Effect of Solvent Type and Maceration Time on the Percentage of Total Catechins from Betel Nut Peel

The average percentage of total catechins from the peel of betel nut in each treatment can be seen in Table 3.

Table 3. Average percentage (%) of catechins from the extraction of betel nut (Areca catechu L.) based on the type of solvent and maceration time

Type of Solvent	Maceration Time			Average
	M1 (6 hours)	**M2 (12 hours)**	**M3 (18 hours)**	
P1 (water with an initial temperature of 80°C)	5,60^{d}	4,65^{d}	4,46^{d}	**4,90^{b}**
P2 (acetone distillate)	25,53^{a}	16,89^{c}	17,17^{c}	**19,87^{a}**
P3 (ethyl acetate distillate)	23,68^{b}	17,68^{c}	17,90^{c}	**19,75^{a}**
Average	**18,27^{a}**	**13,07^{b}**	**13,18^{b}**	

Note: Superscripts with different letters show significantly different effect ($P < 0.05$)

Based on the results of an analysis of variance showed that there was an interaction that gave a significantly different effect ($P < 0.05$) between factor A (a type of solvent) and factor B (maceration time) to the percentage of total EKKBP catechins.

DMRT test results showed that P2M1 treatment (acetone solvent and maceration time 6 hours) had a significantly different effect on other treatments.

The percentage of total catechins obtained in this study ranged from 4.13% to 25.53%. The highest percentage of total catechins was obtained from the treatment of acetone with maceration of 6 hours, 25.53%, followed by an ethyl acetate solvent with a 6-hour maceration time of 23.68%. While the lowest catechin compounds were found in the treatment of water solvents at a temperature of 80 ° C with different maceration periods (6.12, and 18 hours), respectively: 5.60%, 4.17%, and 4.13%.

In this study, it was found that the high yield did not reflect the high catechin value, this was because when extracted with polar or semi-polar solvents, other phytochemical compounds that matched the solvent also dissolved, causing a high total yield. According to (Achyadi *et al*., 2018), the size of the yield cannot indicate the quality of the product, because the yield is of small value does not necessarily have a low-quality product, and vice versa, the yield of a large value is not necessarily the product has a low quality value . According to Vuong *et al*. (2010), the catechin extract obtained contained other components besides the catechins which were dissolved in the solvent as well as the residual solvent remaining, thereby affecting the high yield.

Types of solvents different in this study showed significant differences in the total catechin content of betel nut peel extract. The levels of catechins obtained from acetone solvents (19.87%) were not significantly different from those of ethyl acetate (19.75%) higher than those of catechins with water solvents at 80°C (4.90%). This is because acetone is an extraction solution that matches its polarity with the extracted compound, the areca nut peel catechin. This is supported by Sangthong *et al*. (2013) reported that acetone is the best solvent for extracting catechin compounds from areca seed. The choice of the type of solvent must consider the suitability of the solvent with the solute (polarity), toxicity, volatility, availability, and price of the solvent. Acetone is a polar solvent, liquid, colorless, food-grade (harmless if used as a solvent for food analysis) and flammable (Tiwari *et al*., 2011).

In this study the longer the maceration time (6, 12 and 18 hours) the reduced levels of EKKBP catechins obtained for all types of solvents. The highest catechin levels were obtained at 6 hours maceration time (18.27%), then prolongation of maceration time to 12 hours, catechin levels were reduced at 12.91% and at 18 hours the catechin content level was 13.49%. The ability of a solvent to dissolve a compound is also influenced by the length of time of maceration. According to Maulida & Naufal (2014), the longer the extraction process, the longer the contact between the solvent and the solute, so that the solute dissolution process will continue until the solvent is saturated with the solute. However, in this study, an extended period of maceration showed lower levels of total catechins. According to Sintha (2008), the extraction time on each material has an optimum limit, the addition of time beyond its optimum limit has no effect because the compound that has moved to the solvent will experience a saturation point and has been extracted optimally.

IV. CONCLUSION

It concluded the combination of distillate acetone solvent and maceration duration for 6 hours was the best combination to obtain catechin extract from Areca catechu peel. In this condition the percentage of water content was 10.53%, yield percentage was 7.13%, and percentage of catechin extract was 25.53%.

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Ridolfia segetum Moris (false fennel) response to different rates of application of Metsulfuron Methyl

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Abstract— *High infestations of Ridofia segetum can cause wheat crops yield reduction. The aim of this study is to investigate the effect of Metsulfuron Methyl on R. segetum infestation in a soft wheat crop. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Each block contained 4 elementary plots, 3 plots of which were treated with three rates of application of Metsulfuron Methyl and one untreated control plot. Trial were conducted in Ouazzane region of Morocco in January 2018. Treatments were carried out with a knapsack sprayer with the nozzle delivering a 3 bar jet. A quadrant of 1m x 1m was used to calculate percentage of R. segetum density reduction and biomass reduction. R. segetum dry biomass were determined using an oven at 75 ° C for 48 hours. Then, weighed with a precision balance. Results showed that treatments with Metsulfuron Metsulfuron Methyl at 6 g/ha and 9 g/ha gave the best control of R. segetum infestations recording respectively 92.5% and 94.6% of R. segetum density reduction and 92.7% and 94.6% of R. segetum dry biomass reduction. Metsulfuron Methyl at 3 g/ha recorded the lowest efficacies 61.9 and 48.8% respectively on R. segetum density reduction, and R. segetum dry biomass reduction.*

Keywords— ***Ridolfia segetum, Metsulfuron Methyl, wheat, density, biomass.***

I. INTRODUCTION

Weeds are a serious problem in cereal crops in Morocco and severely affect production yields (Zimadahl & El Brahli, 1992; Boutahar, 1994; Taleb, 1996; Bouhache, 2007; Bouhache, 2017). *Ridolfia segetum* Moris (false fennel) belongs to Apiaceae botanical Family. It's an annual plant, hairless, fennel-smelling. Stem upright, usually 30 cm to 1 m high. Leaves divided into very thin segments (Tanji, 2005). Top leaves reduced. Inflorescence in umbels of 10 to 40 rays of 3 to 8 cm. Corolla with 5 yellow petals fringed. Ovoid seeds, 2 to 3 mm long and 0.5 to 1 mm wide, hairless, smooth, brown, 5 thin ribs (Tanji, 2005). Plant present in different region of Morocco usually on clay soils. Plant eaten by livestock especially before flowering (Tanji, 2005). Metsulfuron Methyl is an herbicide that belongs to the Sulfonylureas family. A small amount of active ingredient is used to kill weeds in cereal crops. It is an herbicide used to exterminate many annual or perennial broadleaf weeds (Ezzahiri & *al*., 2017). It is a systemic herbicide absorbed by root and leaves to inhibit cell division in the shoots and roots of the plant. It can be sprayed on leaves or soil and its action has some remanence. *Ridolfia segetum* become a serious problem in cereal fields in Ouazzan region of Morocco by reducing yields and making harvesting difficult. The aim of this study is to compare the effect of three doses of Metsulfuron Methyl on the *Ridolfia segetum* infestation in in a soft wheat crop in the Ouazzan region of Morocco.

II. MATERIAL AND METHODS

A weeding trial was conducted in Ouazzane region of Morocco during 2017-2018 growing season. The experimental design was Randomized Complete Block Design (RCBD) with three replications. The distance between the blocks was 2 meters and the distance between plots was 1 meter. Each block contained 4 elementary plots, 3 plots of which were treated with the post-emergence herbicides tested (Table 1) and one untreated control plot. The size of the elementary plots was 2m x 5m (10 m²). Treatments was carried out on January 5, 2018 with a Knapsack herbicide sprayer with nozzle delivering a 3 bar jet. The spray volume per hectare is 200L. Treatments consist on three rates of application of Metsulfuron Methyl (Table 1). Observations were at 60 days after application of herbicides. Observations concerned Percentage of *R. segetum* density reduction and biomass reduction. *R. segetum* density reduction percentage= [*R. segetum* density in control plots – *R. segetum* density in treated plots] x 100 / [*R. segetum* density in control plots], Calculation of the density at the

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experimental level of the plot was made by a quadrant of 1m x 1m. *R. segetum* dry biomass reduction percentage= [*R. segetum* dry biomass weight in control plots – *R. segetum* dry biomass weight in treated plots] x 100 / [*R. segetum* dry biomass weight in control plots]. Calculation of dry *R. segetum* biomass were made by collecting *R. segetum* in each plot using a quadrant of 1m x 1m. Samples were dried in a drying oven at 75 ° C for 48 hours. Then, dry plant material in each plot were weighed with a precision balance. Statistical analyzes were performed with IBM SPSS Statistics, version 21.0 using the analysis of variance (ANOVA).The differences among treatment means was compared by Tukey's test at *P*= 0.05.

Table 1: Applied herbicides in experimental site

Herbicide treatments	Herbicide active ingredient	rate of application (g/hectare)
Treatment 1	Metsulfuron Methyl	3 g/ha
Treatment 2	Metsulfuron Methyl	6 g/ha
Treatment 3	Metsulfuron Methyl	9 g/ha

III. RESULTS AND DISCUSSION

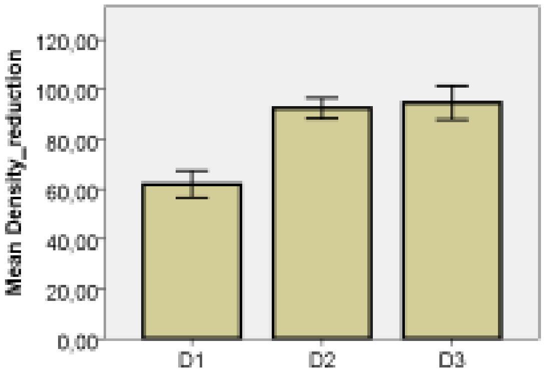
Effect on *R. segetum* density reduction

Statistical analysis revealed significant differences between treatments (Table 2). Results in Table 2 showed that the best *R. segetum* density reduction was obtained by Metsulfuron Methyl at 6 g/ha and 9 g/ha recording respectively 92.5% and 94.6% of *R. segetum* density reduction. Metsulfuron Methyl at 3 g/ha showed medium efficacies that did not exceed 61.9 % of *R. segetum* density reduction.

Table 2: Effect of treatments on R. segetum density reduction (%)

Treatments	*R. segetum* density reduction (%)
Metsulfuron Methyl at 3 g/ha	61.9^{a}
Metsulfuron Methyl at 6 g/ha	92.5^{b}
Metsulfuron Methyl at 9 g/ha	94.6^{b}
Pα = 0.05	<0.001

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1 : Metsulfuron Methyl at 6 g/ha; D2 : Metsulfuron Methyl at 6 g/ha; D3 : Metsulfuron Methyl at 9 g/ha
Error Bars: 95% CI

Fig.1: Effect of treatments on R. segetum density reduction (%)

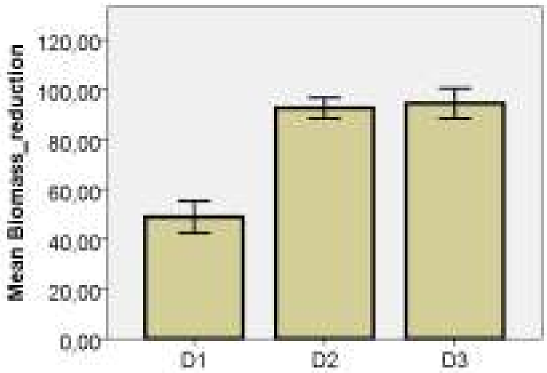
Effect on *R. segetum* dry biomass reduction

Statistical analysis revealed significant differences between treatments (Table 3). Data in Table 3 indicate that the best *R. segetum* dry biomass reduction was achieved by Metsulfuron Methyl at 6 g/ha and 9 g/ha recording respectively 92.7 % and 94.5% of *R. segetum* dry biomass reduction. Concerning the effect of Metsulfuron Methyl at 3 g/ha, results showed weak efficacies that did not exceed 48.8% of *R. segetum* dry biomass reduction. Suresh Kumar & *al*. (2011) reported that Tank mixture of clodinafop 60g/ha + metsulfuron methyl 4 g/ha and clodinafop 120 g/ha + metsulfuron methyl 8 g/ha with and without surfactant 0.2% provided excellent control of weeds in wheat crop.

Table 3: Effect of treatments on R. segetum dry biomass reduction (%)

Treatments	*R. segetum* density reduction (%)
Metsulfuron Methyl at 3 g/ha	48.8^{a}
Metsulfuron Methyl at 6 g/ha	92.7^{b}
Metsulfuron Methyl at 9 g/ha	94.5^{b}
Pα = 0.05	<0.001

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1 : Metsulfuron Methyl at 6 g/ha; D2 : Metsulfuron Methyl at 6 g/ha; D3 : Metsulfuron Methyl at 9 g/ha
Error Bars: 95% CI

Fig.2: Effect of treatments on R. segetum dry biomass reduction (%)

IV. CONCLUSION

This study has shown that the herbicide Metsulfuron Methyl at 6 g/ha and 9 g/ha gave the best control of F. parviflora. Metsulfuron Methyl at 3 g/ha showed weak to medium control of F. parviflora. Thus, Metsulfuron Methyl at 6 g/ha can be recommended to farmers in Ouazzane region when *R. segetum* infestation is dominant. This study should be completed with the assessment of Metsulfuron Methyl residues in soils and its phytotoxicity to crops grown in rotations.

ACKNOWLEDGMENTS

The authors are grateful to all technicians of ONCA Ouazzan for providing necessary facilities for conducting this research work.

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Antibacterial Activities of Lactic Acid Bacteria from Langsat Fruit (*Lansiumdomesticum*) against Phatogenic Bacteria and Spoilage Bacteria

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Abstract— *Langsat fruit (Lansiumdomesticum) sample collected from 4 district in Minahasa, North Celebes. Lactic Acid Bacteria (LAB) isolates were found at that samples. The study aim to examinedthe antibacterial activity of Lactic acid bacteria (LAB) from Langsat fruit sample against pathogenic bacteria Escherichia coli (Gram Negatrive Bacteria) and spoilage bacteria Staphylococcus aureus (Gram Positive Bacteria) using agar well diffusion method.The isolation result obtained 42 isolates of LAB namely 10 isolate were obtained from Southeast Minahasa district, 8 isolates were obtained from the southern district of Minahasa, 12 isolates were obtained from from the Minahasa district, and 12 isolates were obtained from North Minahasa district. Fourty two isolates LAB identified as Lactobacillus genera based on profile matching method were effective against bacteria indicator (pathogenic and spoilage bacteria). The antibacterial activity showed through the formation of a clear zone around well that containing LAB isolates. The diameter inhibition of LAB isolates ranged from 3,0 – 17,0 mm. Overall, the isolated LAB showed the remarkable inhibitory effect against both Gram positive (spoilage bacteria) and Gram negative (pathogenic bacteria). However, the spectrum of inhibition was different for the isolates tested. These results suggest that this potent isolates could be used as a natural biopreservativesagent.*

Keyword*— *Langsat fruit, LAB, Antibacterial, Lactobacillus, well diffusion.

I. INTRODUCTION

Langsat Fruit (*Lansiumdomesticum*) is a tropical fruit that will only grow well in tropical regions such as Indonesia and is an endemic fruit in Minahasa, North Sulawesi Province. Langsat fruit has benefits and uses for the human body because it has a high nutritional content. Fresh fruits have a limited shelf life and are easily damaged. This is caused by spoilage microorganisms found in these fruits [16]. Likewise with Langsat fruit, although it has benefits for the human body, this fruit decomposes more quickly when picked from the tree.

Fruits are a substrate for bacterial growth because they have high carbohydrate content and high organic acids. LAB are found in environments that are rich in nutrients such as meat, vegetables, fish, fruits, fermented products and so on. Some studies have succeeded in obtaining LAB in fruits and vegetables including Markissa Fruit Fermentation [9], Dried Fruit (Raisins and Fig) from Morocco [1], Ackee fruit from Nigeria [6], Cabbage Juice [15],Mango fruit [4];[5], and Cayene pepper fermentation[11].

Lactic acid bacteria also produce metabolites and some of them have antibacterial activity including organic acids (lactic acid, acetic acid, propionic acid, sorbic acid, benzoic acid), hydrogen peroxide, bacteriocin and diacetyl[3], but some strains capable of synthesizebacteriocin antimicrobial compounds [2]. Antimicrobial compounds produced by lactic acid bacteria have the potential as natural preservatives to improve the safety of food products.

This study aims to isolate LAB which has antibacterial activity fromLangsat Fruits from Minahasa, North Sulawesi.

II. MATERIALS AND METHODS

Sampling : Langsat fruit samples were collected from 4 districts in Minahasa, North Sulawesi Province, namely Southeast Minahasa districts (Mitra), South Minahasa districts (Minsel), Minahasa districts (MI), and North Minahasa districts (Minut).

Isolation and Purification Lactic Acid Bacteria

The initial stage of LAB isolation from Langsat fruit was enrichment. 10 g of Langsat fruit sample was mashed and put in a 100 ml erlenmeyer containing MRS broth medium pH (5.5), then grown at room temperature for 2 days.

LAB isolation was carried out using the pour plate method. Take 1 ml of fermented Langsat fruit then put into a test tube containing 9 ml of sterile Aquades (10^{-1} dilution). Dilution is continued until the 10^{-7} dilution series, then from each series of dilutions 10^{-5}, 10^{-6} and 10^{-7}, 1 ml of sample is taken and poured into a petri dish and added with MRSA + 1% CaCO3. Incubation is carried out at 37 ° C for 2-3 days until a clear zone is formed around the bacterial colony. Each colony which has different morphological appearance and clear zone formed is isolated and then purified [7]. LAB colonies were purified on the MRS medium so that by the quadrant streak method until separate colonies were obtained [8].

Cell morphology, Gram staining and catalase test, motility, non-spore forming were performed as a preliminary screening forLAB.The selectedLAB were maintained as stock cultures at -80 °C in 10% skim milk and 20% glycerol.

Antibacterial Activity of Lactic Acid Bacteria

The antibacterial activity test uses the well diffussion assay against test bacteria (*E.coli*ATCC35218 and*S.aureus* ATCC 25923). Test bacteria were taken as much as 15 ml and then inoculated on Nutrien soft agar (0,75% agar) for each bacterium of 50 µl in petridish inoculated by pour plate. The solid medium was perforated using a 6 mm diameter perforator.MRS hard agar poured on petri dish and allow to solidify, then overlaid with nutrient broth were prepared previously and then in place at a temperature of 4 ° C for 1 hour.Culture of LAB was taken as much as 50 µl and inserted into the wellbore. Petridish that have been inoculated with testbacterial were incubated at 37ºC for 24 hours. After that, the inhibitory zone produced and measured based on the diameter of the antimicrobial area [8].

III. RESULT AND DISCUSSION

Isolation of Lactic Acid Bacteria

Samples of LangsatFruit were collect from 4 district of Minahasawere used for isolation of LAB. 50 isolates ofbacteria-acid producing that produce clear zones around the colony. The formation of a clear zone occurs because $CaCO_3$in the MRS medium is to be dissolved by acids produced of bacteria acid production.After being confirmed as LAB, 42 isolates of LABwere obtained(10 isolates were obtained from Southeast Minahasa district, 8 isolates were obtained from the southern district of Minahasa, 12 isolates were obtained from from the Minahasa district, and 12 isolates were obtained from North Minahasa district).All these isolates were gram positive, rods or cocci, appeared singly, in pair, chain, tetrad. Cell were non motile and nonsporing, they gave negative reaction for catalase. These strains were then classified into genus level using profile matching method. Based on the profile matching method that 42 isolates were putatively identified as genus *Lactobacillus*.

Table 1. Screening of lactic acid bacteria isolates into genera level by profile matching method.

Character	Isolate	*Lactobacillus**
Amount of isolates	42	
Gram stain	+	+
Shape	Rod	Rod
Cell arrangement	Single, pair and chain,	Single, pair and chain
Production gas from glucose	-	-
Catalase	-	-
Spore formation	-	-
Motility	-	-
Fermentation type	Homofermentati ve	Homofermentati ve

*) Bergey's Mannual of Systematic Bacteriology

AntibacterialActivity of Lactic Acid Bacteria

The antibacterial activity of LAB isolates (culture) were tested against pathogenic bacteria and spoilage bacteria are summarized in (Table 2) by using agar well diffusion assay, and figure (1) illustrate the zones of inhibition against pathogenic bacteria and spoilage bacteria under study. The diameters of the inhibitionzones were varied it ranged between 2,0 to17,0 mm. All of LAB isolate haveinhibitory activity againts pathogenic bacteria and spoilage bacteria. IsolatesLMT2, LMS8, LMI7, and LMU2 have the highest diameter of inhibition zones 16,5-17,0 mm (S.aureus). Isolates LMT2, LMS8, LMI7, and LMU7 have the highest diameter of inhibition zones 7,0-8,0 mm (E.coli) thisrevealed that the LAB inhibited all the pathogenic bacteria and spoilage bacteria tested.

Previous studies have shown that most of the selected strains of lactic acid bacteria had good antagonistic activity against pathogens including Escherichia coli [12]. According to [13] whose mentioned that inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger.

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Generally the antibacterial components produced by LAB can inhibit the growth of gram-positive bacteria and gram-negative and the same was stated by [14].

Tabel 5. Selection of LAB Isolates based on antagonistic activity (diameter of the clear zonaproduced againstindicatot bacteria)

No	Isolate Code	*E.coli* ATCC 35218 (mm)	*St. aureus* ATTC 25923 (mm)
	Southeast Minahasa		
1	LMT1	3,0	13,2
2	LMT2	**7,0***	**16,5***
3	LMT3	6,0	6,0
4	LMT4	3,0	16,0
5	LMT5	2,0	12,5
6	LMT6	3,0	11,2
7	LMT7	5,0	6,0
8	LMT8	5,0	14,5
	South Minahasa		
9	LMS1	3,0	15,7
10	LMS2	2,0	16,3
11	LMS3	5,0	13,5
12	LMS4	2,0	7,5
13	LMS5	3,0	11,2
14	LMS6	3,0	11,2
15	LMS7	6,0	13,7
16	LMS8	**7,0***	**17,0***
17	LMS9	5,0	15,7
18	LMS10	3,0	11,5
	Minahasa		
19	LMI1	2,0	10,0
20	LMI2	3,0	3,0
21	LMI3	5,0	10,0
22	LMI4	4,0	11,0
23	LMI5	4,0	13,7
24	LMI6	3,0	13,7
25	LMI7	**7,0***	**15,0***
26	LMI8	6,0	11,0
27	LMI9	4,0	12,0
28	LMI10	5,0	14,0
29	LMI11	3,0	6,0
30	LMI12	3,0	6,0
	North Minahasa		
31	LMU1	6,0	16,0
32	LMU2	5,0	**17,0***
33	LMU3	3,0	10,0
34	LMU4	3,0	11,0
35	LMU5	2,0	11,7
36	LMU6	6,0	11,7
37	LMU7	**8,0***	12,0

38	LMU8	6,0	15,0
39	LMU9	4,0	12,0
40	LMU10	3,0	12,0
41	LMU11	4,0	10,0
42	LMU12	3,0	10,0

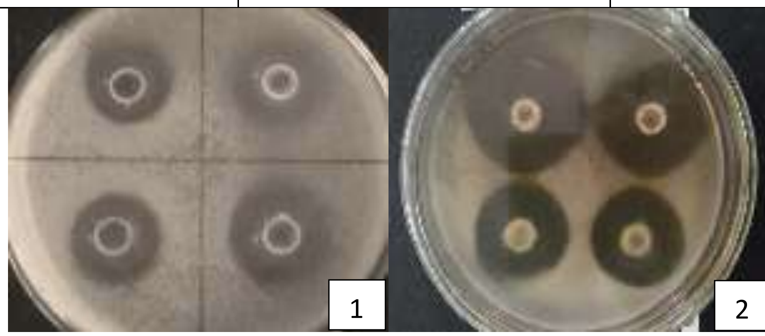

Fig.1: Antibacterial activity of LAB against pathogenicbacteria and spoilage bacteria.
(1). Escherichiacoli, (2). Staphylococcus aureus

The activity on inhibition of variety of bacteria by LAB due toa combination of many factorsproduced by LAB e.g. production of lactic acid whichreduce pH of Langsat fruit and also other inhibitory substances such as bacteriocins, hydrogen peroxide, diasetylwhich are responsible for the mostantimicrobial activity [10].

IV. CONCLUSION

The results were obtained42isolates oflacticacidbacteriawhichcan begroupedinto thegenera*Lactobacillus*. All ofisolates LABhaveantibacterial activityagainstthe growth ofpathogenic bacteriaandspoilage bacteria. LMT2, LMS8, LMI7,LMU2, and LMU7 have the highest antibacterial activity.

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Avena sterilis L. (wild oat) response to Clodinafop-propargyl in wheat crops in Morocco

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***Abstract*— *Avena sterilis is problematic weed that affects wheat production. The aim of this study is to investigate the effect of Clodinafop-propargyl on A. sterilis infestation in a soft wheat crop. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Each block contained 4 elementary plots, 3 plots of which were treated with three rates of application of Clodinafop-propargyl and one untreated control plot. Trial were conducted in Ouazzane region of Morocco in January 2018. Treatments were carried out with a knapsack sprayer with the nozzle delivering a 3 bar jet. A quadrant of 1m x 1m was used to calculate percentage of A. sterilis density reduction and biomass reduction. A. sterilis dry biomass were determined using an oven at 75 ° C for 48 hours. Then, weighed with a precision balance. Results showed that treatments with Clodinafop-propargyl at 60 g/ha and 80 g/ha gave the best control of A. sterilis infestations recording respectively 96.1% and 99.4% of A. sterilis density reduction and 98.2% and 99.7% of A. sterilis dry biomass reduction. Clodinafop-propargyl at 40 g/ha recorded lower efficacies 75.3% and 80.1% respectively on A. sterilis density reduction, and A. sterilis dry biomass reduction.*

Keywords*— *Avena sterilis, Clodinafop-propargyl, wheat, density, biomass.

I. INTRODUCTION

Weeds compete on water, minerals and sunlight and decrease wheat production in Morocco (Zimadahl & El Brahli, 1992; Boutahar, 1994; Taleb, 1996; Bouhache, 2007; Bouhache, 2017). *Avena sterilis* L. (wild oat) belongs to *Poaceae* botanical Family. It is an annual plant. Upright 50 cm to 1.50 m high. Leaves with limbs usually hairy at its base and on the margin. Eroded-acute ligule. No auricles. Panics 30 to 50 cm long. Lower flowers covered with silky hairs and twisted dorsal edges (Tanji, 2005). Upper hairless flowers. Flowers stand out together at maturity. Equal glumes, 3 to 5 cm, Spikelet with 3 to 5 flowers. Spread flower Spikelet forming an open V. Brown or black seeds, 2 to 3 cm long and 3 to 6 mm wide, 5 to 6 cm long (Tanji, 2005). Plant quite abundant throughout Morocco. It grows on different types of soil. It is consumed by animals. Infestations on cereal fields may led to refusal of the harvested product by Moroccan seed companies (Tanji, 2005). Clodinafop-propargyl is a systemic herbicide absorbed by leaves to control grasses. It belongs to the Aryloxyphenoxy-propionate 'FOPs' family. It causes inhibition of acetyl CoA carboxylase (ACCase) (Ezzahiri & *al*., 2017). ACCase enzyme catalyzes the fatty-acid synthesis. This herbicide inhibit the ACCase enzyme activity, thus blocking the production of phospholipids necessary for synthesizing the lipid bilayer, which is indispensable for cell structure and function. *Avena sterilis* become is a serious problem in cereal fields in Ouazzan region of Morocco that causes reduction of cereal production. The aim of this study is to compare the effect of three doses of Clodinafop-propargyl on *Avena sterilis* infestation in a soft wheat crop in the Ouazzan region of Morocco.

II. MATERIAL AND METHODS

A weeding trial was conducted in Ouazzane region of Morocco during 2017-2018 growing season. The experimental design was Randomized Complete Block Design (RCBD) with three replications. The distance between the blocks was 2 meters and the distance between plots was 1 meter. Each block contained 4 elementary plots, 3 plots of which were treated with the post-emergence herbicides tested (Table 1) and one untreated control plot. The size of the elementary plots was 2m x 5m (10 m²). Treatments was carried out on January 2, 2018 with a Knapsack herbicide sprayer with nozzle delivering a 3 bar jet. The spray volume per hectare is 200L. Treatments consist on three rates of application of Clodinafop-propargyl (Table 1). Observations were at 60 days after application of herbicides. Observations concerned

Percentage of *A. sterilis* density reduction and biomass reduction. *A. sterilis* density reduction percentage= [*A. sterilis* density in control plots – *A. sterilis* density in treated plots] x 100 / [*A. sterilis* density in control plots], Calculation of the density at the experimental level of the plot was made by a quadrant of 1m x 1m. *A. sterilis* dry biomass reduction percentage= [*A. sterilis* dry biomass weight in control plots – *A. sterilis* dry biomass weight in treated plots] x 100 / [*A. sterilis* dry biomass weight in control plots]. Calculation of dry *A. sterilis* biomass were made by collecting *A. sterilis* in each plot using a quadrant of 1m x 1m. Samples were dried in a drying oven at 75 ° C for 48 hours. Then, dry plant material in each plot were weighed with a precision balance. Statistical analyzes were performed with IBM SPSS Statistics, version 21.0 using the analysis of variance (ANOVA).The differences among treatment means was compared by Tukey's test at *P*= 0.05.

Table 1: Applied herbicides in experimental site

Herbicide treatments	Herbicide active ingredient	rate of application (g/hectare)
Treatment 1	Clodinafop-propargyl	40 g/ha
Treatment 2	Clodinafop-propargyl	60 g/ha
Treatment 3	Clodinafop-propargyl	80 g/ha

III. RESULTS AND DISCUSSION

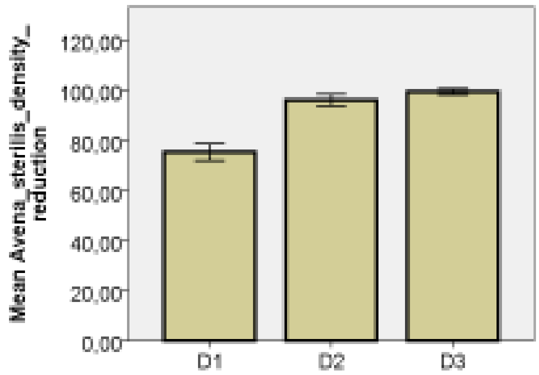
Effect on *A. sterilis* density reduction

Statistical analysis revealed significant differences between treatments (Table 2). Results in Table 2 showed that the best *A. sterilis* density reduction was obtained by Clodinafop-propargyl at 60 g/ha and 80 g/ha recording respectively 96.1% and 99.4% of *A. sterilis* density reduction. Clodinafop-propargyl at 40 g/ha showed lower efficacy 75.3 % of *A. sterilis* density reduction (fig. 1).

Table 2: Effect of treatments on A. sterilis density reduction (%)

Doses	*Avena sterilis* density reduction
Clodinafop-propargyl at 40 g/ha	75.3[a]
Clodinafop-propargyl at 60 g/ha	96.1[b]
Clodinafop-propargyl at 80 g/ha	99.4[b]
Pα = 0.05	<0.001

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



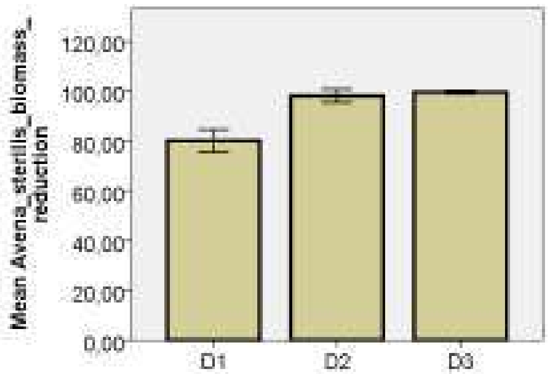
D1: Clodinafop-propargyl at 40 g/ha; D2: Clodinafop-propargyl at 60 g/ha; D3: Clodinafop-propargyl at 80 g/ha
Error Bars: 95% CI

Fig.1: Effect of treatments on A. sterilis density reduction (%)

Table 3: Effect of treatments on A. sterilis dry biomass reduction (%)

Doses	*Avena sterilis* density reduction
Clodinafop-propargyl at 40 g/ha	80.1[a]
Clodinafop-propargyl at 60 g/ha	98.2[b]
Clodinafop-propargyl at 80 g/ha	99.7[b]
Pα = 0.05	<0.001

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1: Clodinafop-propargyl at 40 g/ha; D2: Clodinafop-propargyl at 60 g/ha; D3: Clodinafop-propargyl at 80 g/ha
Error Bars: 95% CI

Fig.2: Effect of treatments on A. sterilis dry biomass reduction (%)

Effect on *A. sterilis* dry biomass reduction

Statistical analysis revealed significant differences between treatments (Table 3). Data in Table 3 indicate that the best *A. sterilis* dry biomass reduction was achieved by

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Clodinafop-propargyl at 60 g/ha and 80 g/ha recording respectively 98.2 % and 99.7% of *A. sterilis* dry biomass reduction. Concerning the effect of Clodinafop-propargyl at 40 g/ha, results showed lower efficacy that did not exceed 80.1% of *A. sterilis* dry biomass reduction (fig. 2).

IV. CONCLUSION

This study has shown that the herbicide Clodinafop-propargyl at 60 g/ha and 80 g/ha gave the best control of *A. sterilis*. Clodinafop-propargyl at 40 g/ha lower control of *A. sterilis*. Thus, Clodinafop-propargyl at 60 g/ha can be recommended to farmers in Ouazzane region when *A. sterilis* infestation is dominant. This study should be completed with the assessment of Clodinafop-propargyl effect on other problematic grasses.

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The authors are grateful to all technicians of ONCA Ouazzan for providing necessary facilities for conducting this research work.

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Increased *Oscillatoria* sp. Population on Integrated Cultivation Ponds of Rice and Tiger Shrimp (*Penaeus monodon*) in Idle Land

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***Abstract*— *Tiger shrimp culture that is maintained integrated with rice on idle land because of sea water intrusion has been tested in 2018 through the Innovative Adaptive Technology of Brackish Fisheries (INTAN-AP) pioneering from the Fisheries Research Center of the Human Resources Research Agency. There were 5 pond of rice fields used in the experiment in July-December 2018 in Oring Countryside, Lawallu Village, Barru Regency, South Sulawesi. Rice seeds of INPARI 34 and 35 varieties were kept in the paddy fields and PL 45 tiger shrimp were cultivated in the caren field, while re-fertilization was carried out using N, P, K fertilizers while shrimp were given commercial feed during cultivation based on weight gain per sampling. Plankton sampling was carried out in tiger shrimp ricecultivated land, every 7 days using plankton net mesh size No. 25, and then analyzed at the plankton laboratory in Research Institute for Coastal Aquaculture and Fisheries Extension in Maros. Then the data is calculated the abundance of the population then discussed descriptively. The results found that the population of Oscillatoria sp phytoplankton increases every time they conduct sampling until it reaches the peak of its population, along with the period of fertilizer application and the increase in the amount of feed given to tiger shrimp. The highest population peak at the 7th sampling in the pond 5 was 25,316 cells / L and in the pond 4 when the 6th sampling was 22,300 cells / L.*
***Keywords*— co-culture, rice, tiger shrimp, phytoplankton, Oscillatoria sp.**

I. INTRODUCTION

Tiger shrimp (*Penaeus monodon*) culture that is maintained integrated with rice plants on idle land due to the influence of sea water intrusion has been tested in 2018, which is a pioneering research program of the Research Center for Fisheries, Human Resources Research Agency of the Ministry of Maritime and Fisheries Affairs of the Republic of Indonesia. The land used is the pond rice fields owned by members of farmer groups that have long been neglected because they cannot be planted optimally because of the presence of sea water intrusion so that they are exposed to brackish conditions. In line with technological developments in the field of genetic engineering, varieties of rice seeds that are tolerant to saline conditions have been recommended by Indonesian Center for Rice Research, Sukamandi in the presence of INPARI 34 and INPARI 35 varieties that have been cultivated in the brackish land (Anonim, 2018).

Co-culturing shrimp/fish with aquatic plants, including natural macrophytes, rice and vegetables, is a recommend measure to re-use the residual nutrients and to reduce the eutrophication in aquaculture ponds (Henry-Silva and Camargo, 2006; LIU-Yaobin, *et al*., 2019). The results from paddy field have showed that rice-fish co-culture could enhance nutrients use efficiency and reduce nutrients loss to environment because of the complementary use of nutrients between fish and rice (Hu et al., 2013; Xie et al., 2011). Therefore, rice-fish co-culture may have the inherent advantage and great potential in the mitigation of gaseous N loss from aquaculture. Research from Fengbo Li (2019), indicated that rice-fish/shrimp co-culture was an efficient measure to mitigate the gaseous N loss from intensive aquaculture ponds.

The land used has been reconstructed into 2 parts, namely the paddy field for rice plants and caren as a location for raising tiger shrimp in a contiguous spread of location. In the process of maintaining the two commodities (rice and tiger shrimp), some of the same cultivation techniques ranging from land preparation in the form of land management, liming, repeated fertilization, eradication of pests, control of growth to harvest (Sahabuddin, 2019).

Specifically in the fertilizing activities on rice paddy fields and caren / tiger shrimp cultivation land using fertilizer made from N, P, and K, it is possible to create stimulation for the emergence of certain types of phytoplankton with a rapid rate of population growth due to high fertility so as to stimulate the high phytoplankton population. Aquatic fertility can be seen from the content of organic materials that exist in water, where the presence of organic materials is the main nutrient that can supply the needs of producer biota (producer) which in the end the producer is a food source for fish chicks, shrimp, and other types of aquatic biota. Phytoplankton is one of the factors that influence water quality fluctuations. Phytoplankton production in intensive aquaculture is influenced by the presence of nutrients in the water, especially nutrients from the nitrogen (N) and phosphate (P) groups. The availability of nutrients in intensive cultivation is determined by the presence of the amount of organic matter and the level of its breakdown by bacteria. The organic material comes from artificial feed that is not consumed (leftover feed) and excretion from shrimp (Budiardi *et al*, 2007). An increase in artificial feeding will increase the content of organic matter and nutrients which in certain limits will increase the primary productivity of waters (Boyd, 1979).

In the area of aquaculture, the presence of phytoplankton is very important because phytoplankton play a role as primary producers and contributors of dissolved oxygen in these waters, so that its existence can be used as an indicator of fertility in waters, in fertile and productive waters the abundance of phytoplankton is found to be more abundant (Yuliana, 2008).

Aquatic environmental conditions such as temperature, salinity, and nutrient content determine the amount and type of plankton that is in waters. If the salinity of pond water has the high amount of plankton, it is not different from the plankton in coastal waters, the salinity is very low then the type of plankton has no different from plankton fresh water (Sachlan, 1972). Abundance of plankton, especially phytoplankton in waters is influenced by the availability of nutrients, especially nitrogen (N) and phosphate (P). The composition and abundance of plankton in waters will change in response due to changes in physical, chemical, and biological environmental conditions (Reinolds et al., 1984).

Oscillatoria sp is a phytoplankton of the *Cyanophyceae* type (Guiry, and Guiry, 2019). Phytoplankton is often found abundant in brackish waters. Therefore, it is necessary to conduct studies and analyzes on the emergence and increase of *Oscillatoria* sp phytoplankton populations in co-culture of rice and tiger shrimp (*P. monodon*) on idle land.

II. METHODS AND MATERIAL

The research was conducted in July-December 2018 in the rice and tiger shrimp co-culture ponds in Lawallu Village, Barru Regency, South Sulawesi. Plankton samples were taken at the location of rice brackish water and tiger shrimp. The rice that grows in the paddy fields of INPARI 34 and 35 varietiesis recommended by Indonesian Center for Rice Research, Sukamandi. After planting rice in the paddy field, fertilizing I, II, and III using urea, SP_{36} and ZA fertilizers is conducted. Fertilization is carried out on caren land to grow phytoplankton before the spreading of the PL 45 tiger shrimp seeds.

Plankton sampling is conductede every 7 days, by filtering water in caren as much as 100 liters, then the results of 100 mL filter are put into a plankton bottle that gives lugol solution for analysis in the laboratory. Plankton abundance is analyzed based on the following formula, modification of the APHA formula (1989) :

$$\boxed{N = \frac{A}{B} X \frac{C}{D} X \frac{E}{1} X \frac{1}{F}}$$

Explanation:

N = Abundance (Ind/L)
A = ∑ SRC box (1000 boxes)
B = ∑ box of field of view (1 box)
C = ∑ seenInd.
D = ∑ observed box
E = water volume in sample bottle (ml)
F = volume of filtered water in the field

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Fig.1: Sampling of plankton at the location of co-culture rice and tiger shrimp in Lawallu Village, Barru Regency, South Sulawesi.

Data from observations and phytoplankton density calculations were analyzed and population growth calculated then discussed descriptively related to the period of fertilizer application in the paddy fields and caren fields.

III. RESULTS AND DISCUSSION

Description of Oscillatoria sp phytoplankton

Oscillatoria sp phytoplankton reproduces by fragmentation, forming long cell filaments which can break into new fragments called hormogonia. Hormogonia can grow into new, longer filaments. Usually the filament breaks into a number of fragments called homogonia. Each hormogonium consists of one or more cells and grows into filaments with cell division in one direction. *Oscillatoria*sp also carry out photosynthesis, each oscillator filament consists of trichomes consisting of cell lines. The tip of the trichome oscillates like a pendulum in reproduction, it occurs only in a vegetative way(https://en.wikipedia.org/wiki/Oscillatoria).

Oscillatoria sp phytoplankton found in the field in shrimp rice brackish water as recorded on a microscope monitor screen is shown in Figure 2, while the pattern of population increase of *Oscillatoria* sp is shown in Figure 3, at the first sampling the lowest population number was in pond 3 which was 102 cells / L , the highest in the freshwater channel (SAT) is 510 cells / L. Freshwater channels are the main source of water that is pumped into each pond, in addition there are reservoirs that are a source of sea water to maintain the stability of brackish conditions with 5-7 ppt salinity.

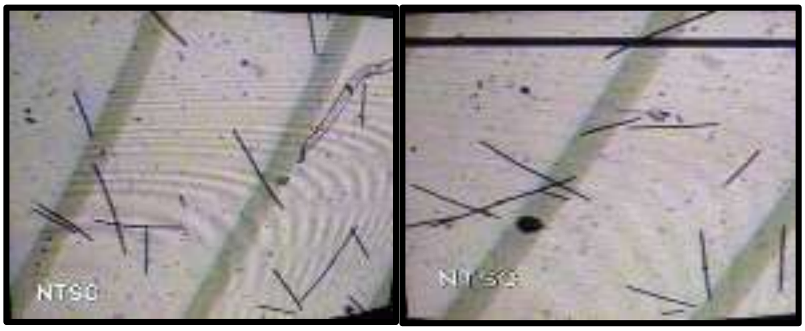

Fig.2: Abundance of Oscillatoria sp. results of observations on a microscope monitor screen

Population growth began to increase during the 2nd sampling, after supplementary fertilizers I was made on the paddy field, as well as the 3rd sampling the population increased especially in the pond 4 of 5,240 cells / L and pond 5 of the 3,500 cells / L population, this condition shows an indication of an increase in population after supplementary fertilizers I and II in the paddy fields. This condition can occur because the fertilizers used are N and P fertilizers, after soaking into the paddy fields can seep into the caren land, which can spur theincreasing nutrient content which is the main element that can increase the fertility of the waters so as to spur an increase in the population of *Oscillatoria* sp phytoplankton, as Ariadi *et al* (2019) that the high density of *Oscillatoria* sp as a type of *Blue Green Algae* plankton is caused by pond conditions that are eutrophic or hyper-eutrophic. Furthermore, *Oscillatoria* sp phytoplankton can capture nitrogen diffusion well so that the population is easy to bloom (Aliviyanti *et al*, 2017).

At 5th sampling, population surge after the 3rd supplementation fertilization, the presence of nutrient seepage (nutrient) can stimulate fertility. Pond environmental conditions will affect the composition, abundance and distribution of plankton physical, chemical and biological contained therein. Reinolds *et al*. (1984), argues that plankton abundance in waters is influenced by several environmental parameters and physiological characteristics.

According to Pirzan and Utojo (2010), pond water quality that is well managed in a range that is in accordance with the growth of plankton as a natural feed of aquaculture organisms can increase pond productivity. The highest abundance of *Oscillatoria* sp. in pond 5 is at the 7th sampling valued at 25.316 ind / L. When the abundant population of *Oscillatoria* sp can interfere with the growth of tiger shrimps because it will be a competitor in oxygen absorption, then it causes obstructions of sunlight to penetrate the water surface due to the dense population of the plankton so that the water color changes to dark green. This can happen due to the condition of pond waters that are green and the presence of excessive nutrient input such as nitrates and phosphates resulting in nutrient enrichment (blooming). The high population of toxic phytoplankton in a waters can cause various negative consequences for aquatic ecosystems, such as the reduction of oxygen in the water which can cause the death of various other aquatic creatures (Damar, 2006).

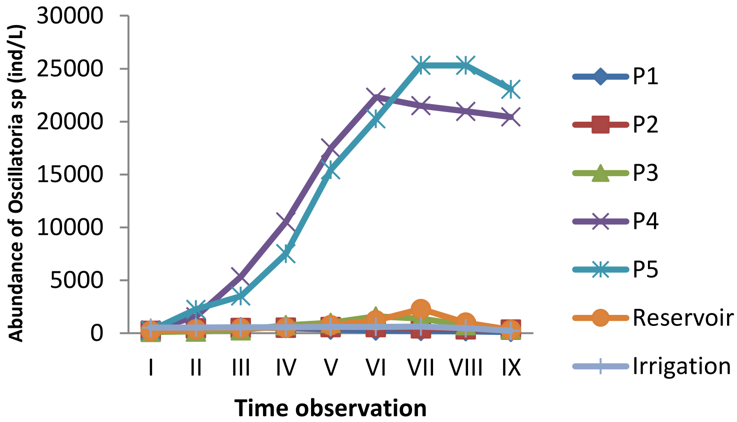

Fig.3: Increased of Oscillatoria sp phytoplankton population during the period of rearing co-culture tiger shrimp and rice

In other conditions the lowest abundance in the first pond when the last sampling with a population of *Oscillatoria* sp phytoplankton was only 110 Ind / L. The existence of plankton in ponds besides functioning as fish and shrimp feed can also act as one of the ecological parameters that can describe the fertility of a pond area. The presence of plankton, especially phytoplankton, plays a very important role in aquatic ecosystems, including pond waters. Phytoplankton act as primary producers and contributors of dissolved oxygen in the waters, so that their existence can be used as an indicator of the fertility of water. The higher the abundance of phytoplankton, the more fertile the waters (Yuliana, 2008).

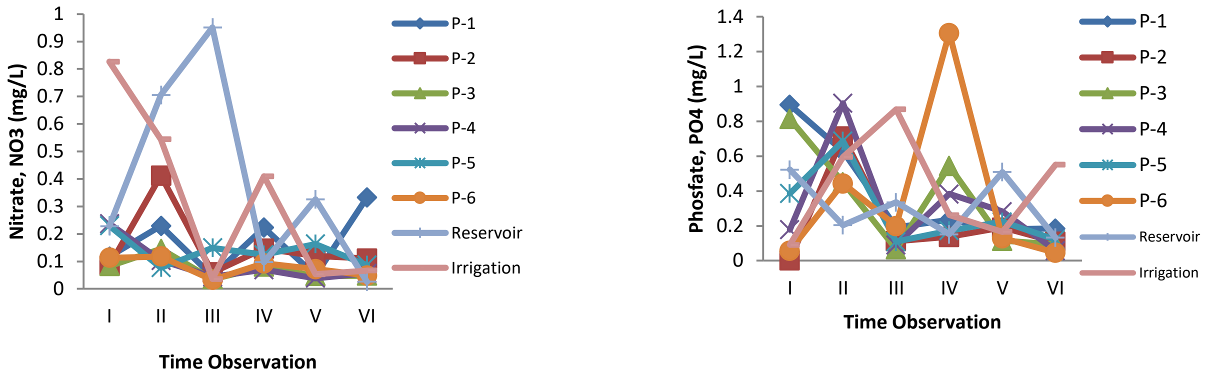

Fig.4: Phosphate and nitrate content in the rearing pond of co-culture tiger shrimp and rice during the experiment

The highest concentration of nitrate content in pond 2 when 8th sampling was 0.562 mg / L and the highest phosphate in 10th sampling pond 4 was 2.6474 mg / L, after the 3rd suplementation fertilizing, the high concentration of nutrient content spurred the increase *Oscilatoria* sp. population, but during the observation the content of nitrate and phosphate was still within the limits tolerated by *Oscillatoria* sp. phytoplankton. Usman et al (1995) stated that the nitrate optimum for algae growth ranged from 0,900-3,500 mg/L. According to Aziz et al (2015) the total value of P in brackish water ponds ranged from 0.62-5.88 mg/L. Phosphorus content in waters is influenced by the presence of phosphorus-containing materials in the waters, such as phosphorus fertilization (SP fertilizer). The lowest limit for phosphate concentration for optimal algae growth ranges from 0.018-0.090 mg/L P-PO_4 and the highest limit ranges from 8.90-17.8 mg/L P-PO_4, if nitrogen is in the form of nitrate. If N is in the form of ammonium, the highest limit is 1.78 mg /L P-PO_4 (Andarias 1991). According to Rudiyanti *et al*. (2010), in order to be able to grow algae optimally, suitable environmental elements are required, such as nitrate and phosphate content, temperature, water depth, brightness, salinity, acidity and soil texture.

IV. CONCLUSIONS

The increase of *Oscillatoria* sp phytoplankton population occurred during the 5th sampling in pond 5, occurring after suplemenatation I, II and III fertilizing in paddy field. Fertilization contributed to the increase in peak population of *Oscillatoria* sp in caren.

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Effect of Clodinafop-propargyl on *Phalaris paradoxa* L. (awned canary-grass) in wheat crop

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***Abstract*— *Phalaris paradoxa is problematic weed that decrease considerably wheat crop yields. The aim of this study is to investigate the effect of Clodinafop-propargyl on Phalaris paradoxa infestation in a soft wheat crop. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Each block contained 4 elementary plots, 3 plots of which were treated with three rates of application of Clodinafop-propargyl and one untreated control plot. Observations concerned Percentage of Phalaris paradoxa density reduction and biomass reduction. Results showed that treatments with Clodinafop-propargyl at 60 g/ha and 80 g/ha gave the best control of Phalaris paradoxa infestations recording respectively 96.2% and 98.5% of Phalaris paradoxa density reduction and 95.5% and 99.3% of Phalaris paradoxa dry biomass reduction. Clodinafop-propargyl at 40 g/ha recorded lower efficacies 77.1% and 82.3% respectively on Phalaris paradoxa density reduction, and Phalaris paradoxa dry biomass reduction.*

***Keywords*— Phalaris paradoxa, Clodinafop-propargyl, wheat, density, biomass.**

I. INTRODUCTION

Weeds are a major problem on wheat production in Morocco as they compete on water, minerals and sunlight and make harvest operation more difficult (Zimadahl & El Brahli, 1992; Boutahar, 1994; Taleb, 1996; Bouhache, 2007; Bouhache, 2017). *Phalaris paradoxa* L. (awned canary-grass) belongs to *Poaceae* botanical Family. It is an annual plant. Upright 20 cm to 1.20 m high (Tanji, 2005). Leaves 10 to 20 cm long and 5 to 10 mm wide. Membrane ligules, 3 to 4 mm long. No auricles. Inflorescence is a compact and rough panicle, sometimes wrapped by the upper leaf, cylindrical, narrowed at the base, 3 to 10 cm long and 1 to 2 cm wide. Palms of the panicle made up of beams of 5 to 7 spikelets (Tanji, 2005). Central spikelets are fertiles, the others steriles. Oval seeds, hairless, shiny, 3 to 4 mm long and 1 to 2 mm wide without chip at the base, usually having 3 long lines on each side. Seedling is hairless, coiled prefoliation. First leaves 5 to 10 cm long and 1 to 2 mm wide. Membrane ligule, 1 to 4 mm. Seed determination makes it easier to recognize the seedling (Tanji, 2005). Plant lying on different types of soil and consumed by animals. Seeds are usually consumed by birds. Clodinafop-propargyl is an herbicide that belongs to Aryloxyphenoxy-propionate 'FOPs' family. It is a systemic herbicide absorbed by leaves to control grasses. It causes inhibition of acetyl CoA carboxylase (ACCase) which is an enzyme that catalyzes the fatty-acid synthesis (Ezzahiri & *al*., 2017). Clodinafop-propargyl inhibits the ACCase enzyme activity, thus blocking the production of phospholipids necessary for synthesizing the lipid bilayer, which is indispensable for cell structure and function. *Phalaris paradoxa* decrease considerably cereal yields in Ouazzan region of Morocco. The aim of this study is to compare the effect of three doses of Clodinafop-propargyl on *Phalaris paradoxa* infestation in a soft wheat crop in the Ouazzan region of Morocco.

II. MATERIAL AND METHODS

A weed control trial was conducted in Ouazzane region of Morocco during 2017-2018 growing season. The experimental design was Randomized Complete Block Design (RCBD) with three replications. The distance between the blocks was 2 meters and the distance between plots was 1 meter. Each block contained 4 elementary plots, 3 plots of which were treated with the post-emergence herbicides tested (Table 1) and one untreated control plot. The size of the elementary plots was 2m x 5m (10 m²). Treatments was carried out on January 2, 2018 with a Knapsack herbicide sprayer with nozzle delivering a 3 bar jet. The spray volume per hectare is 200L. Treatments consist on three rates of application of Clodinafop-propargyl (Table 1). Observations were at 60 days after application of herbicides. Observations concerned Percentage of *Phalaris paradoxa* density reduction and biomass reduction. *Phalaris paradoxa* density reduction percentage= [*Phalaris paradoxa* density in control plots – *Phalaris paradoxa* density in treated plots] x 100 / [*Phalaris paradoxa* density in control plots],

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Calculation of the density at the experimental level of the plot was made by a quadrant of 1m x 1m. *Phalaris paradoxa* dry biomass reduction percentage= [*Phalaris paradoxa* dry biomass weight in control plots – *Phalaris paradoxa* dry biomass weight in treated plots] x 100 / [*Phalaris paradoxa* dry biomass weight in control plots]. Calculation of dry *Phalaris paradoxa* biomass were made by collecting *Phalaris paradoxa* in each plot using a quadrant of 1m x 1m. Samples were dried in a drying oven at 75 ° C for 48 hours. Then, dry plant material in each plot were weighed with a precision balance. Statistical analyzes were performed with IBM SPSS Statistics, version 21.0 using the analysis of variance (ANOVA).The differences among treatment means was compared by Tukey's test at *P*= 0.05.

Table 1: Applied herbicides in experimental site

Herbicide treatments	Herbicide active ingredient	rate of application (g/hectare)
Treatment 1	Clodinafop-propargyl	40 g/ha
Treatment 2	Clodinafop-propargyl	60 g/ha
Treatment 3	Clodinafop-propargyl	80 g/ha

III. RESULTS AND DISCUSSION

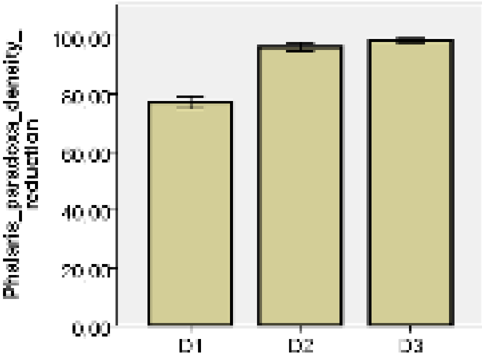
Effect on *Phalaris paradoxa* density reduction

Statistical analysis revealed significant differences between treatments (Table 2). Results in Table 2 showed that the best *Phalaris paradoxa* density reduction was obtained by Clodinafop-propargyl at 60 g/ha and 80 g/ha recording respectively 96.2% and 98.5% of *Phalaris paradoxa* density reduction. Clodinafop-propargyl at 40 g/ha showed lower efficacy recoding 77.1 % of *Phalaris paradoxa* density reduction (fig. 1).

Table 2: Effect of treatments on Phalaris paradoxa density reduction (%)

Doses	*Phalaris paradoxa* density reduction
Clodinafop-propargyl at 40 g/ha	77.1[a]
Clodinafop-propargyl at 60 g/ha	96.2[b]
Clodinafop-propargyl at 80 g/ha	98.5[b]
Pα = 0.05	*<0.001*

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1: Clodinafop-propargyl at 40 g/ha; D2: Clodinafop-propargyl at 60 g/ha; D3: Clodinafop-propargyl at 80 g/ha
Error Bars: 95% CI

Fig.1: Effect of treatments on Phalaris paradoxa density reduction (%)

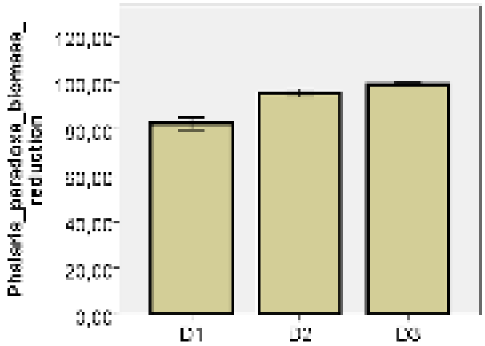
Effect on *Phalaris paradoxa* dry biomass reduction

Statistical analysis revealed significant differences between treatments (Table 3). Data in Table 3 indicate that the best *Phalaris paradoxa* dry biomass reduction was achieved by Clodinafop-propargyl at 60 g/ha and 80 g/ha recording respectively 95.5 % and 99.3% of *Phalaris paradoxa* dry biomass reduction. Concerning the effect of Clodinafop-propargyl at 40 g/ha, results showed lower efficacy recording 82.3% of *Phalaris paradoxa* dry biomass reduction (fig. 2).

Table 3: Effect of treatments on Phalaris paradoxa dry biomass reduction (%)

Doses	*Phalaris paradoxa* density reduction
Clodinafop-propargyl at 40 g/ha	82.3[a]
Clodinafop-propargyl at 60 g/ha	95.5[b]
Clodinafop-propargyl at 80 g/ha	99.3[b]
Pα = 0.05	*<0.001*

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1: Clodinafop-propargyl at 40 g/ha; D2: Clodinafop-propargyl at 60 g/ha; D3: Clodinafop-propargyl at 80 g/ha
Error Bars: 95% CI

Fig.2: Effect of treatments on Phalaris paradoxa dry biomass reduction (%)

IV. CONCLUSION

This study has shown that the herbicide Clodinafop-propargyl at 60 g/ha and 80 g/ha gave the best control of *Phalaris paradoxa*. Clodinafop-propargyl at 40 g/ha lower control of *Phalaris paradoxa*. Thus, Clodinafop-propargyl at 60 g/ha can be recommended to farmers in Ouazzane region when *Phalaris paradoxa* infestation is dominant.

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Lolium rigidum Gaudin (annual ryegrass) response to different doses of Clodinafop-propargyl in wheat crops

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Abstract— *Lolium rigidum is a harmful weed specie in wheat crops. The aim of this study is to investigate the effect of Clodinafop-propargyl on Lolium rigidum infestation in a soft wheat crop. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Each block contained 4 elementary plots, 3 plots of which were treated with three rates of application of Clodinafop-propargyl and one untreated control plot. Observations concerned Percentage of Lolium rigidum density reduction and biomass reduction. Results showed that treatments with Clodinafop-propargyl at 60 g/ha and 80 g/ha gave the best control of Lolium rigidum infestations recording respectively 95.1% and 96.8% of Lolium rigidum density reduction and 92.5% and 64.6% of Lolium rigidum dry biomass reduction. Clodinafop-propargyl at 40 g/ha recorded lower efficacies 65.7% and 61.9% respectively on Lolium rigidum density reduction, and Lolium rigidum dry biomass reduction.*
Keywords— ***Lolium rigidum, Clodinafop-propargyl, wheat, density, biomass.***

I. INTRODUCTION

In Morocco, weeds reduce considerably cereal crop yields by competing with the crop for resources such water, minerals and sunlight (Zimadahl & El Brahli, 1992; Boutahar, 1994; Taleb, 1996; Bouhache, 2007; Bouhache, 2017). *Lolium rigidum* Gaudin (annual ryegrass) belongs to *Poaceae* botanical Family. It is an annual plant. Hairless. Upright, 20 to 70 cm high (Tanji, 2005). The underside of leaves is shiny and smooth, with a width of 2 to 6 mm. White and translucent ligules. uncrossed auricles. Spikes, 10 to 50 cm. Spiklets, 1 to 2 cm long, inserted into excavations of the rachis (stem) and equipped with 2 to 12 fertile flowers (Tanji, 2005). Glumes orally equal to or greater than the spelts. Lower glumes usually without awns. Lanceolate oval, brown, hairless seeds seeds. Ventral surface is concave and the dorsal surface is convex. Seeds size are 4 to 7 mm long and 1 to 1.5 mm wide. Hairless seedling. First leaves of 4 to 7 cm x 1-2 mm, with shiny and smooth leaves on the underside, with a ligule of less than 1 mm. Auricles are absent or small, becoming visible from the third leaf (Tanji, 2005). Seed detection makes it easier to recognize the seedling. *Lolium rigidum* is a weed which is quite abundant throughout Morocco. It is existing on different soils and environments and consumed by animals. Some hybrids or varieties are used in Morocco to improve the production of rangelands and fallows (Tanji, 2005). Clodinafop-propargyl is a post emergent herbicide which belongs to Aryloxyphenoxy-propionate 'FOPs' family. It is a systemic herbicide absorbed by leaves to control grasses. It causes inhibition of acetyl CoA carboxylase (ACCase) which is an enzyme that catalyzes the fatty-acid synthesis (Ezzahiri & *al.*, 2017). Clodinafop-propargyl leds to the inhibition of ACCase enzyme activity, thus blocking the production of phospholipids necessary for synthesizing the lipid bilayer, which is indispensable for cell structure and function. *Lolium rigidum* is a harmful weed specie in cereal crops in Ouazzan region of Morocco. The aim of this study is to compare the effect of three doses of Clodinafop-propargyl on *Lolium rigidum* infestation in a soft wheat crop in the Ouazzan region of Morocco.

II. MATERIAL AND METHODS

A weed control trial was conducted in Ouazzane region of Morocco during 2017-2018 growing season. The experimental design was Randomized Complete Block Design (RCBD) with three replications. The distance between the blocks was 2 meters and the distance between plots was 1 meter. Each block contained 4 elementary plots, 3 plots of which were treated with the post-emergence herbicides tested (Table 1) and one untreated control plot. The size of the elementary plots was 2m x 5m (10 m²). Treatments was carried out on January 2, 2018 with a Knapsack herbicide sprayer with nozzle delivering a 3 bar jet. The spray volume per hectare is 200L. Treatments consist on three rates of application of

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Clodinafop-propargyl (Table 1). Observations were at 60 days after application of herbicides. Observations concerned Percentage of *Lolium rigidum* density reduction and biomass reduction. *Lolium rigidum* density reduction percentage= [*Lolium rigidum* density in control plots – *Lolium rigidum* density in treated plots] x 100 / [*Lolium rigidum* density in control plots], Calculation of the density at the experimental level of the plot was made by a quadrant of 1m x 1m. *Lolium rigidum* dry biomass reduction percentage= [*Lolium rigidum* dry biomass weight in control plots – *Lolium rigidum* dry biomass weight in treated plots] x 100 / [*Lolium rigidum* dry biomass weight in control plots]. Calculation of dry *Lolium rigidum* biomass were made by collecting *Lolium rigidum* in each plot using a quadrant of 1m x 1m. Samples were dried in a drying oven at 75 ° C for 48 hours. Then, dry plant material in each plot were weighed with a precision balance. Statistical analyzes were performed with IBM SPSS Statistics, version 21.0 using the analysis of variance (ANOVA).The differences among treatment means was compared by Tukey's test at $P= 0.05$.

Table 1: Applied herbicides in experimental site

Herbicide treatments	Herbicide active ingredient	rate of application (g/hectare)
Treatment 1	Clodinafop-propargyl	40 g/ha
Treatment 2	Clodinafop-propargyl	60 g/ha
Treatment 3	Clodinafop-propargyl	80 g/ha

III. RESULTS AND DISCUSSION

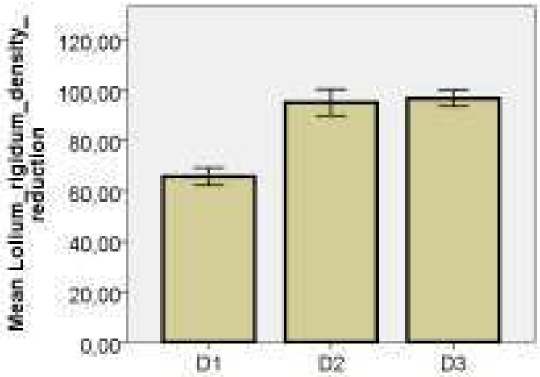
Effect on *Lolium rigidum* density reduction

Statistical analysis revealed significant differences between treatments (Table 2). Results in Table 2 showed that the best *Lolium rigidum* density reduction was obtained by Clodinafop-propargyl at 60 g/ha and 80 g/ha recording respectively 95.1% and 96.8% of *Lolium rigidum* density reduction. Clodinafop-propargyl at 40 g/ha showed lower efficacy recoding 65.7 % of *Lolium rigidum* density reduction (fig. 1).

Table 2: Effect of treatments on Lolium rigidum density reduction (%)

Doses	*Lolium rigidum* density reduction
Clodinafop-propargyl at 40 g/ha	65.7^{a}
Clodinafop-propargyl at 60 g/ha	95.1^{b}
Clodinafop-propargyl at 80 g/ha	96.8^{b}
Pα = 0.05	*<0.001*

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1: Clodinafop-propargyl at 40 g/ha; D2: Clodinafop-propargyl at 60 g/ha; D3: Clodinafop-propargyl at 80 g/ha
Error Bars: 95% CI

Fig.1: Effect of treatments on Lolium rigidum density reduction (%)

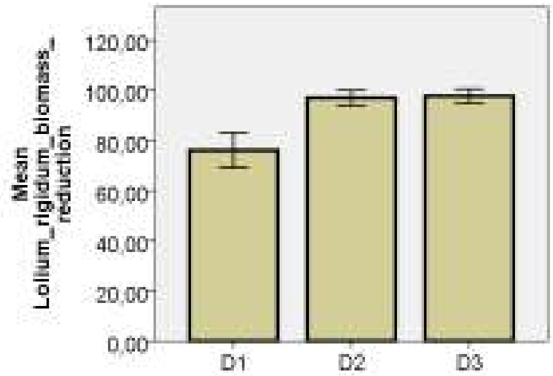
Effect on *Lolium rigidum* dry biomass reduction

Statistical analysis revealed significant differences between treatments (Table 3). Data in Table 3 indicate that the best *Lolium rigidum* dry biomass reduction was achieved by Clodinafop-propargyl at 60 g/ha and 80 g/ha recording respectively 92.5% and 94.6% of *Lolium rigidum* dry biomass reduction. Concerning the effect of Clodinafop-propargyl at 40 g/ha, results showed lower efficacy recording 61.9% of *Lolium rigidum* dry biomass reduction (fig. 2).

Table 3: Effect of treatments on Lolium rigidum dry biomass reduction (%)

Doses	*Lolium rigidum* density reduction
Clodinafop-propargyl at 40 g/ha	61.9^{a}
Clodinafop-propargyl at 60 g/ha	92.5^{b}
Clodinafop-propargyl at 80 g/ha	94.6^{b}
Pα = 0.05	*<0.001*

Significant differences within the same column and means followed by the same letter do not differ at P= 0.05 according to Tukey's test



D1: Clodinafop-propargyl at 40 g/ha; D2: Clodinafop-propargyl at 60 g/ha; D3: Clodinafop-propargyl at 80 g/ha
Error Bars: 95% CI

Fig.2: Effect of treatments on Lolium rigidum dry biomass reduction (%)

IV. CONCLUSION

This study has shown that the herbicide Clodinafop-propargyl at 60 g/ha and 80 g/ha gave the best control of *Lolium rigidum*. Clodinafop-propargyl at 40 g/ha lower control of *Lolium rigidum*. Thus, Clodinafop-propargyl at 60 g/ha can be recommended to farmers in Ouazzane region when *Lolium rigidum* infestation is dominant.

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Composition of *Vernoniaamygdalina* and its Potential Health Benefits

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***Abstract*—** *Vernoniaamygdalina is a perennial shrub. It is a vegetable, commonly used in foods and traditional medicine in tropical Africa. Traditional users report that this plant possesses observable health benefits. It contains many nutrients and phytochemicals such as iodine, alkaloids, anthraquinones, edotides, sesquiterpene lactones and steroid glycosides. These bioactive compounds are associated with the plant's health benefits. This review is a comprehensive update on the composition of V. amygdalina, linked to its food and medicinal uses. Traditional medicinal uses not yet supported by scientific evidence have been indicated. Clinical studies to substantiate the health-promoting effects of this plant product are lacking. More animal models experiments are required to prove the potency of this plant product. Grey areas where research is required to promote effective use of this plant product are indicated. Metadata for the health benefits of this plant product is based on inferences from compositional analysis and animal bioassays. There is a need to establish these claims in humans. Sources of information for this review were obtained from online search engines. The searches were restricted to information available as primary or secondary sources and effort was made to keep to only recent literature. Very important literature before that was not recent was also included. There is only very little information on the food uses of V. amygdalina which has been related to potential health benefits. There are a lot of claims on this plant-based on ethnomedicine and traditional uses. Vernoniaamydgalina is rich in nutrients and phytochemicals and this invariable account for its positive influence on health. Compounds associated with its anticancer effects, antioxidant properties, antimalarial properties, anti-inflammatory properties, antimicrobial properties and hypolipidaemic effects have been identified but most mechanisms of action have not been elucidated in humans.*

Keywords*—** ***Antioxidant properties, bioactive compounds, chemical composition, health benefits, Vernoniaamygdalina*.*

I. INTRODUCTION

Vernoniaamygdalina is a perennial shrub with leaves that have a bitter taste popularly known as bitter leaf. Some species are bitterer than others **(Kokwaro, 1993).** Consumers usually wash the leaves with water (sometimes with water and salt) to reduce bitterness. The leaves may also be boiled before washing, (or boiled after washing) in a bid to make it more palatable. Washing of bitter leaf involves intermittent squeezing. The leaves may be exposed to sunlight for short periods to ensure that it does not get too small during size-reduction involved in the squeeze-washing process. Local Nigerian names for *V. amgdalina* are: "Ewuro" (Yoruba) "Onugbu" (Igbo) "Oriwo" (Bini language), "Ityuna" (Tiv language), "Chusardoki or fatefate" (Hausa language) and "Etidot" (Ibiobio) **(Kokwaro, 2009).**

Traditionally, *V. Amgdalina's* is believed to be effective in preventive health care and for the management of several disease conditions in humans **(Burkill, 1985).** Several research studies suggest that its effective use is largely dose-dependent **(Ibrahim et al., 2011).** It may demonstrate toxic effects in some cases such as when it is being used to promote fertility **(Saalu et al., 2013)** or as an antimalarial **(Omoregie et al., 2011).** Hydrodistillation extracts of this plant product are rich in essential fatty acids (like oleic acid and linoleic acid) **(Amaechi et al., 2018);** essential oils with insecticidal properties such as eucalyptus; linalool; α-pinene; and some natural preservatives such as benzene are found in *V. amydalina* **(Asawalam et al., 2006).** Acetone and methanol extracts of *V. amygdalina* contain steroid glycosides, sesquiterpene lactones and edotides **(Oluwaseun et al., 2017).** The leaves have been most extensively investigated and used, while the stem and roots have received limited research attention. The stem and roots have been identified to have anti-bacterial properties against periodontal bacteria, explaining its use as a chewing stick in some traditional settings. The distribution

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of bioactive compounds in plant parts (leaves, stem and roots) has not been fully established.

1.1. *Vernoniaamygdalina*plant

Vernoniaamgdalina belongs to the Asteraceae (Compositae) family. This plant family is made up of herbs, shrubs, or trees. It is the largest family of flowering plants. It has up to 1,620 genera and more than 23,600 species **(Kokwaro, 1993).**Vernonia is a genus of about 1,000 species of herbs and shrubs and*V. amygdalina* as the most important species **(Kokwaro, 2009).** In Nigeria, Zimbabwe and South Africa, it is usually domesticated(**Toyang and Verporte, 2013).** The shrub is about 13m high. The full-blown mature leaf is elliptical, petiolate and has a diameter of about 6mm **(Toyang and Verporte, 2013).** The leaves are dark green with a characteristic odour and a bitter taste. The tree is commonly propagated through cutting **(Anonymous, 1999).** *Vernoniaamygdalina* grows in many parts of Africa. It regenerates easily, producing a large mass of forage and is drought resistant **(Bonsi et al., 1995a).** Figure 1 shows the leaves of *Vernoniaamygdalina.*

Fig.1. Vernoniaamygdalina leaves.

II. NUTRIENT COMPOSITION AND HEALTH BENEFITS OF VERNONIAAMYGDALINA

V amygdalina is rich in nutrients. The protein content of the leaves is quite high (sometimes up to 20%). The lipid content is also relatively high (4.7%) when compared with other leaves. There is the need to carry out further research on the protein and lipid profile of *V. amygdalina* to have better understanding of the nutrient benefits of the plant product. The stem and the root contain significant amounts of fat (34.03 and 30.15%) respectively **(Bonsi et al., 1995b).** The leaf contains higher amounts of the mineral elements iron and copper than the stem and the roots (see Table 1a). It contains significant amounts of ash (8-17%).The leaf is particularly rich in iodine (35.8µg), iron (5-14%) and copper (6-10mg/100g). It is also rich in thiamine, vitamin A and vitamin E (Table 1a). Other nutrients, found in *V. amygdalina* leaves that have been studied are presented in Table 1b. *V. amygdalina* leaves are also rich in reducing sugars (14.3%) especially sucrose (13.2%). It is a good source of arabinose, maltose, galactose, glucose and fructose. It also contains significant amounts of the flatulent sugar: raffinose(see Table 1b).

Table 1a: Nutrient composition of Vernoniaamygdalina

Parameter	Leaves	Stem	Roots
Protein (%)	19.23-45.1	6.71	7.30
Moisture (%)	7.92-91.4	18.50	12.0
Total lipids %)	4.70		
Fat (%)	0.4	34.03	30.15
Ash (%)	7.72-15.2	17.99	11.01
Total carbohydrate(g)	68.35	-	-
Crude fiber (%)	1.5-9.75	-	-
Dietary fibre	25.47	-	-
Reducing sugar (g)	14.31	-	-
Energy	392.67	-	-
Carbohydrate (CHO) (%)	10.0	-	-
Non detergent fiber (NDF)	23.7-50.2	-	-
Acid detergent fiber (ADF)	13.9-39.5	-	-
Hemicellulose	3.5-11.5	-	-
Dry matter (%)	20.08	-	-

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Minerals			
Magnesium	88.1-261.93	-	-
Sodium (mg/100g)	8.5-136.36	-	-
Calcium (mg/100g)	67-278	-	-
Phosphorus (µg·g^{-1})	61.6-67	-	-
Potassium (mg/100g)	21.1-60.9	-	-
Zinc (mg/100g)	8.05-51.08	0.14	0.26
Manganese (mg/100g)	5.56		
Iron (mg/100g)	5.0-14.2	0.12	0.09
Copper (mg/100g)	6.01-10.2	0.021	0.022
Iodine (µg/100 g)	35.82	-	-
Vitamins			
Ascorbic acid (mg/100g)	20.4-228.4	49.00	10.30
Thiamine (mg/100g)	100-170	0.50	0.37
Riboflavin (mg/100g)	3.10	0.13	0.15
Nicotinamide (mg/100g)	0.41-1.65	0.03	0.15
Pyridoxine (mg/100g)	2.6	-	-
Vitamin A (IU/100g)	30.90-34.6	21.5	30.90
Carotenoids (mg/100g)	30.0	-	-
Vitamin E (IU/100g)	37.3	106.20	35.83

(Bonsi et al., 1995a; Bonsi et al., 1995b; Areghore et al., 1997; Akinyele et al., 2014; Ilondu, 2010; Georgewill and Georgewill, 2010)

Table 1b:Carbohydrates and proteins found in Vernoniaamygdalina Leaves

Sugar Content mg/100g)	Value	Amino acid (mg/100g)	Value
Glucose	7.20	Glycine	4.63
Sucrose	13.20	Cysteine	1.84
Fructose	6.00	Casein hydrolysate	96.99
Lactose	2.61	-	-
Galactose	6.56	-	-
Arabinose	9.25	-	-
Raffinose	5.10	-	-
Maltose	7.24	-	-
Reducing sugar (g)	14.31	-	-

(Georgewill and Georgewill, 2010)

2.1. Highlights of Important Nutrients found in *V. amygdalina*and their potentials for promoting health

2.1.1. Minerals

V amygdalina is rich in zinc (8.05-51.08mg/100g), copper (6.01-10.2mg/100g) and iodine(35.82 µg/g). The wide range of values for copper and zinc are reports from different authors. World health Organizations recommendation for zinc (2.27and 2.89mg/daily, respectively for pregnant and lactating women) is much lower than the observed values for *V. amygdalina*. The normal range of copper in the serum is 80-155µg/dl for women and 70-140µg/dlfor men). The FDA recommends 1mg iodine per day, while some experts have recommended up to 14mg/day. These values are much lower than the observed values for this plant product, indicating that regular consumption of *V. amygdalina* will ensure adequate amounts of the mineral elements for normal body functions. Iodine is normally found in sea products and lack of iodine and its health consequences has compelled national fortification programmes for public health improvement. Lack of iodine may lead to metabolic diseases such as goiter, cretinism, and impaired intellectual ability. It is a global public health issue that affects fertility and pregnancy. Symptoms of copper deficiency include inability to concentrate, fatigue and a poor mood. Low amounts of copper are associated with low dopamine levels(**Lei et al., 2019).**Zinc plays an important role in wound repair, immune response and tissue regeneration. Zinc deficiency affects the skin, brain cells, skeletal system, reproductive system and the gastrointestinal tract(**Shivi et al., 2017).** Iodine is required for the formation of thyroid hormones and proper functioning of the thyroid gland.In the human body, iodine banks are found in the breast and ovaries, suggesting that these two organs may require substantial amounts of iodine for their proper development and functions **(Ahat et al., 2010).**

2.1.2. Vitamins

Data from Table 1a shows that *V. amygdalina* is a rich source of vitamin A, vitamin E and thiamine. The stem is exceptionally rich in vitamin E. Vitamin A is required for cell differentiation and reproduction in addition to its role in vision. It also boosts the immune system. Vitamin E has very strong antioxidant properties and is beneficial to lipid tissues. Thiamine is intricately involved in energy and carbohydrate metabolism. The content of these vitamins in *V. amygdalina* is higher than in many other green leafy vegetables. The daily requirement of vitamin A for adult women is 700mcg per day. Vitamin B_1 is important for energy production through the breakdown of carbohydrates; activation of the immune system; communication between the brain and nerve cells and communication between cells and tissues. Thus the high content of vitamin B_1 in *V amygdalina* implies that this plant product will promote the overall health and well-being of humans. Alcohol impairs the transport of vitamin B_1 to tissues where it can be utilized. This can result in cognitive impairment **(Jadeja and Deuka, 2014).**

2.1.3. Protein, carbohydrate and Lipid content

The protein content of *V. amygdalina* is comparatively high for green leafy vegetables (19.23-45.10 % for leaves; 6.71% for the stem and 7.30% for the root)and should be further investigated for its detailed amino acid profile. The lipid content is also high (4.7%) and requires further research input to decipher the detailed lipid profile. V. amygdalina carbohydrate is a rich source of sucrose, arabinaose, maltose, galactose glucose and fructose. This leafy vegetable also contains significant amounts of raffinose which is a flatulent sugar **(Georgewill and Georgewill, 2010)**

III. PHYTOCHEMICAL COMPOSITION OF VERNONIAAMYGDALINA

Phytochemical screening shows that *V. amygdalina* contains alkaloids, steroids, flavonoids, phenols, saponins, terpenes, cyanogenic glucoside, tannin, anthraquinone, phytate, oxalate, lignans(**Areghore et al., 1997**). The phytochemical constituents of *V. amygdalina*are shown in Table 1c.The phytochemical constituents of the stem and roots have received limited research attention.

Table 1c:Phytochemical constituents of V. amygdalina

Parameter	*V. amygdalina* leaf	Stem	Roots
Total phenolics (mg/100 g DW)	156.40	-	-
Total flavonoids (mg/100 g DW)	6.25	-	-
Saponin (%)	1.425-5.97	13.21	28.52
Flavonoid (%)	4.89	1.02	0.51
Tannin	9.62	-	-
Phytate	3.95	-	-
Oxalate	0.62-5.36	-	-
Cyanogenic glycoside	1.11	-	-
Alkaloids (%)	2.16	7.02	6.11
Anthraquinone	0.14	-	-
Steroid	0.38	-	-
Phenol	3.24	-	-
Polyphenols (mg/100g)	9.75	-	-

(Udochukwu et al., 2015)

3.1. Phytochemical compounds from*Vernoniaamygdalinawith scientifically established medicinal properties*

Bioactive compounds identified in *V. amygdalina* are many.

3.1.1. Terpenoids:

The terpenoids include several sesquiterpene lactones such as: vernolide, vernodalol, vernolepin, vernodalin, vernomygdin, hydroxyvernolide, vernodalinol, vernomenin, vernolic, 11, 13-dihydrovernodalin, 11, 13-dihydrovernorodeline, 4, 15-dihydrovernodalin, 1, 2, 3, 15, 11, 13, 2', 3'-octahydrovernodalin and epivemodalol(**Njan et al., 2008**). Some of these compounds may be associated with the ability of V. *amygdalina* to regulate blood sugar **(Njan et al., 2008; Nwaoguikpe, 2010; Amaechi et al., 2018).**

3.1.2. Flavonoids

The flavonoids found in this plant are luteolin (a flavone); luteolin 7- 0-β- glucoside,myricetin (3,5,7,3',4',5'-hexahydroxyflavone) and luteoin 7-0- glucoronoside. The flavonoid content of the leaf is much higher than that of the stem and the roots. The nature of the flavonoids found in the stem and roots need to be studied in details. In higher plants, flavonoids are involved in the filtration of ultraviolet light, nitrogen fixation, cell cycle inhibition; as chemical messengers and for the protection of plants against certain diseases. Flavonoids are known to demonstrate two major mechanisms of action (either through direct scavenging of free radicals ;or interfering some enzyme activities such as with nitric oxide synthase activity or xanthine oxidase activity) **(Nijveldt et al., 2001).**

3.1.3. Steroid glucosides:

The steroid alcohol found in *V. amygdalina* is 7, 24 (28)-stigmastadien-3-β-ol. The steroid glucosides isolated from *V. amygdalina* includevernoniosides A_1, A_2, A_3, A_4 B_1 B_2 B_3 D_1 D_2 and vernoniosides E. Several bioactive compounds have been isolated from *V. amygdalina* including Vernodalin, Vernomygdin, Vernoniosides (A_1, A_2, A_3, A_4, B_1, B_2, B_3, D& E), Vernodalol and Epivernodalol(**Owen et al., 2011).**

IV. MODE OF ACTION OF BIOLOGICALLY ACTIVE COMPOUNDS IN VERNONIAAMYGDALINA

Some bioactive compounds from *V. amygdalina* have been associated with specific medicinal properties. The sesquiterpene lactones are associated with antimicrobial properties; antiprotozoal actions, and anti-tumour activities **(Kupchan et al., 1969; Owoeye et al., 2010; Alara et al., 2017).** The steroid glycosides possess antihelminthic and anti-inflammatory properties. They are also beneficial in handling gastrointestinal disorders. The edotides (peptides) have anticancer properties and relieve oxidative stress. The saponins lower cholesterol levels by interacting with bile acids causing accelerated metabolism of cholesterol in the liver, thereby making serum cholesterol levels to be reduced (Potter et al., 2008). Saponins may also suppress the production of mediators of inflammation such as histamine, serotonin and prostaglandin (Potter et al. 2008). They are also known to exhibit hypoglycaemic activity possibly by suppressing the transfer of glucose from the stomach to the small intestine and retarding glucose transport in the small intestine (Rao et al., 2004). The flavonoids and terpenoids all have anticancer, antioxidant, antimicrobial and hepatoprotective properties **(Owoeye et al., 2010; Atangwho et al., 2012).** Also, the terpenoids possess antileukemia, analgesic and anti-nociceptive properties **(Khalafalla et al., 2009).** Myricetin has been associated with hypolipidaemic and antidiabetic effects (Ref).Table 2 summarizes the specific actions of bioactive components of *V. amygdalina*. Mode of action of the bioactive compounds includes: causing a rise in the level of phase 11 enzymes; slowing down of the proliferation of cells; suppressing pro-inflammatory mediators.

Table 2:Phytochemical compounds in Vernoniaamygdalina leaves and their mode of action

Type of Compound	Bioactive compound	Medicinal property	References
sesquiterpene lactones	vernodalin, vernomigdin,vernodalol, epivernodalolvernolepin,vernolide, vernolepin, vernodalin, hydroxyvernolide, vernodalol, vernomygdin, vernomenin, 4,15dihydrovernodalin, 1,2,11,12′,3′ hexahydrovernodalin,	Antibacterial, anaesthetic, antifungal, anti-inflammatory, antiprotozoal, and antimicrobial. Inhibition of breast cancer cell growth; antitumor activity;	**(Burkill, 1985; Kupchan et al., 1969; Erasto et al., 2006;Luo et al., 2017;Owoeye et al., 2010; Atangwho et al.,**

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	1,2,4,15,11,13,2′,3′ octahydrovernodalin, epivernodalol, and vernonioside A1, A2, A3, A4, B1, B2, B3, and B4		**2012;2013)**
Steroid glycosides	Vernonoisides A_1, A_2, A_3, A_4, B_1, B_2, B_3, D and E	Antihelminthic. Anti-inflammatory properties; Gastrointestinal disorders	**(Leung et al., 1968; Atangwho et al., 2014;Quasie, 2016;Yeap et al., 2014).**
Edotides (peptides)	Arabinogalactan; 1, 8-dihydroxy-3, 7-dimethoxy-xanthone; 4,8-dihydroxy-2,7-dimethoxy-xanthone;1,2-dihydroxy-6, 8-dimethoxy-xanthone; 3,7,8-trimethoxy-1-hydroxy-xanthone;Andrographidoid A,B,C,D and E	Oxidative stress; anticancer	**(Jisaka et al., 1992; 1993)**
	vernolide and vernodalol	Bactericidal	
Flavonoids, tannins, saponins,	luteolin, luteolin 7-O-β-glucuronoside and lutelin7-O-β-glucoside;	antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral	**(Adama et al., 2011); (Kambizi and Afolayan, 2001); (Erasto et al., 2006; Dua et al., 2009; Xu et al., 2015;Erasto et al., 2007;Igile et al., 1994; Nwaoguikpe, 2004**
Triterpenoids	thiamine, ascorbic acid, pyridoxine, glycine, cysteine, casein hydrolysate, eucalyptol, beta pinene, myrtenal, and alpha-murolol	anticancer, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, antileukaemia, analgesic, anti-nociceptive	**(Erasto et al., 2007);(Farombi, 2011)**

Food uses of *Vernoniaamygdalina*

The *Vernoniaamygdalina* leaves are mainly used for human consumption. Washing is often necessary to reduce the bitter taste caused by water-soluble saponins. The bitterness in *V. amygdalina* is caused by sesquiterpene lactones (e.g. vernodalin, vernolepin and vernomygdin) and steroid glucosides (vernoniosides). The fresh leaf is usually washed before it is added to food during preparation. *V. amygdalina* may be used to prepare dishes such as soups (Ogbono, Egusi, Okro and the popular bitter leaf soup), African breadfruit, yam porridge and the like. The leaves are used as a vegetable and stimulate the digestive system. The leaves have been introduced in the beer brewing industry as an alternative to hops **(Areghore, 1998).** The stems are eaten by domestic animals. They are also used by humans as a chewing stick. The leaves may be used as a hop substitute and an antioxidant **(Adama et al., 2011).** The stem and roots of *V. amygdalina* are used as a medicinal chewing stick and in traditional health care.

V. ETHNOBOTANICAL USES OF V. AMYGDALINA

Vernoniaamygdalina is widely used traditionally for the treatment and management of many ailments. In some settings, it is used to treat and manage diabetes, malaria and stomach disorder. They may be eaten to reduce fever. Furthermore, they are used as local medicine against leech, which feeds on blood and which may act as a potential reservoir for pathogenic viruses. The leaf extract may be applied to wounds, especially when fresh for quick healing. It is not usually applied to open stale wounds. Water extract of the leaves or the raw leaves may be chewed to reduce worm infestation, constipation, hiccups and kidney problems. A decoction of the leaves or the roots is also locally used for the treatment of schistosomiasis, cough, hepatitis, sexually transmitted diseases and microbial infections **(Goergewill and Georgewill, 2010; Udochukwu et al., 2015).** While the roots and the leaves of *V. amygdalina* are used in traditional medicine to treat fever, hiccups and kidney problems **(Igile et al., 1995);** the wood, from the root, may be used to clean the tooth **(Okunlola et al., 2018).** Fresh leaves of *V. amygdalina* have been reported to be very good as a purgative **(Atangwho et al., 2017).** It is used in some parts of Africa to prepare cough remedy **(Egharevba et al., 2014).**

VI. MEDICINAL USES OF VERNONIAAMYGDALINA

Several research reports have shown that *V. amygdalina* possesses the following medicinal properties; it protects the liver; it is an antioxidant; it is an antimicrobial agent, and it prevents the agglutination of red blood cells. It is also chemoprotective, cytotoxic, anthelmintic, hypo-lipidaemic and abortifacient **(Akinyele et al., 2014; Ilondu et al., 2010).** Bitter leaf is also said to cure anorexia; Ascaris infestation, typhoid and breast cancer **(Areghore et al., 1998).**

VII. MANAGEMENT OF TYPE 2 DIABETES

Vernoniaamygdalina has been observed to assist the regeneration of beta cells of the pancreas after they have been destroyed artificially by streptozotocin or alloxan (substances which are used to artificially induce diabetes in experimental animals. V. amygdalina promotes the uptake and utilization of glucose by muscle and liver cells of the human body. **(Erasto et al., 2006; Luo et al., 2017; Amaechi et al., 2018**). However, none of the isolated compounds (in this plant product) has been shown through research to be directly responsible for the anti-diabetic properties of *V. amygdalina*. Only limited studies have been carried out on the mechanism of anti-diabetic action of V. amygdalina. The reports on mechanism of action in diabetes are scanty, since the derangements soon after the onset of the disease go beyond the β-cell sequestration, such that regeneration of β-cell alone may not entirely address the complications. It is necessary to study the impact of *V. amygdalina* on carbohydrate metabolism - the biological molecule whose metabolism is grossly affected in diabetic condition. Animal experiments showed a lot of accumulation of vacuoles in the pancreas of diabetic rats (Figure 3-negative control). The streptozotocin-induced diabetic pancreas showed apoptotic beta-cell alterations. The endoplasmic reticulum was more readily visible in the pancreas of diabetic rats. Ingestion of *V. amygdalina* reduced the accumulation of vacuoles; reduced the visibility of the endoplasmic reticulum and showed docked insulin granules **(Amaechi et al., 2018).**

7.2. Antimicrobial properties: Aqueous, ethanol and methanol extracts of *V. amygdalina* inhibit bacteria and fungi associated with the spoilage and pathogenicity of foods. Organisms such as *Candida albicans, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and Escherichia coli were found to be susceptible to extracts of *V. amygdalina* leaves. Chewing sticks made from *Vernoniaamygdalina* wood prevent the proliferation of periodontal bacteria. Leaves showed activities against various pathogenic bacteria and viruses. **(Alo, 2012; Adetunji, 2013; Akinyele, 2014).** Saponins, flavonoids, and alkaloids are responsible for the antimicrobial properties of V. amygdalina. Specific compounds which have been associated with the antimicrobial effects of this plant product include Dihydrovernodalin, vernodalol and vernolide (sesquiterpene lactones) **(Akinyele, 2014).**

7.3. Cancer prevention and management

Aqueous extracts of *Vernoniaamygdalina* leaves inhibit cell growth and multiplication. This biological activity enables it to retard the growth of cancer cells. In tests with rats, a sesquiterpene extract from the leaves of *V. amygdalina* was found to prevent liver damage. Vernoniaamygdalina extract is toxic to cancerous cells. It suppresses metastasis (the spreading of cancerous cells) **(Izevbigie et al., 2014).** It also suppresses the production of an enzyme which is responsible for the production of high levels of estrogen-a hormone associated with the development of breast cancer when it is produced in very high levels. The phytochemicals which are responsible for the anticancer effects of V. amygdalina include: vernomygdin, vernodalin, vernomenin, and vernolepin **(Izevbigie et al., 2014).**

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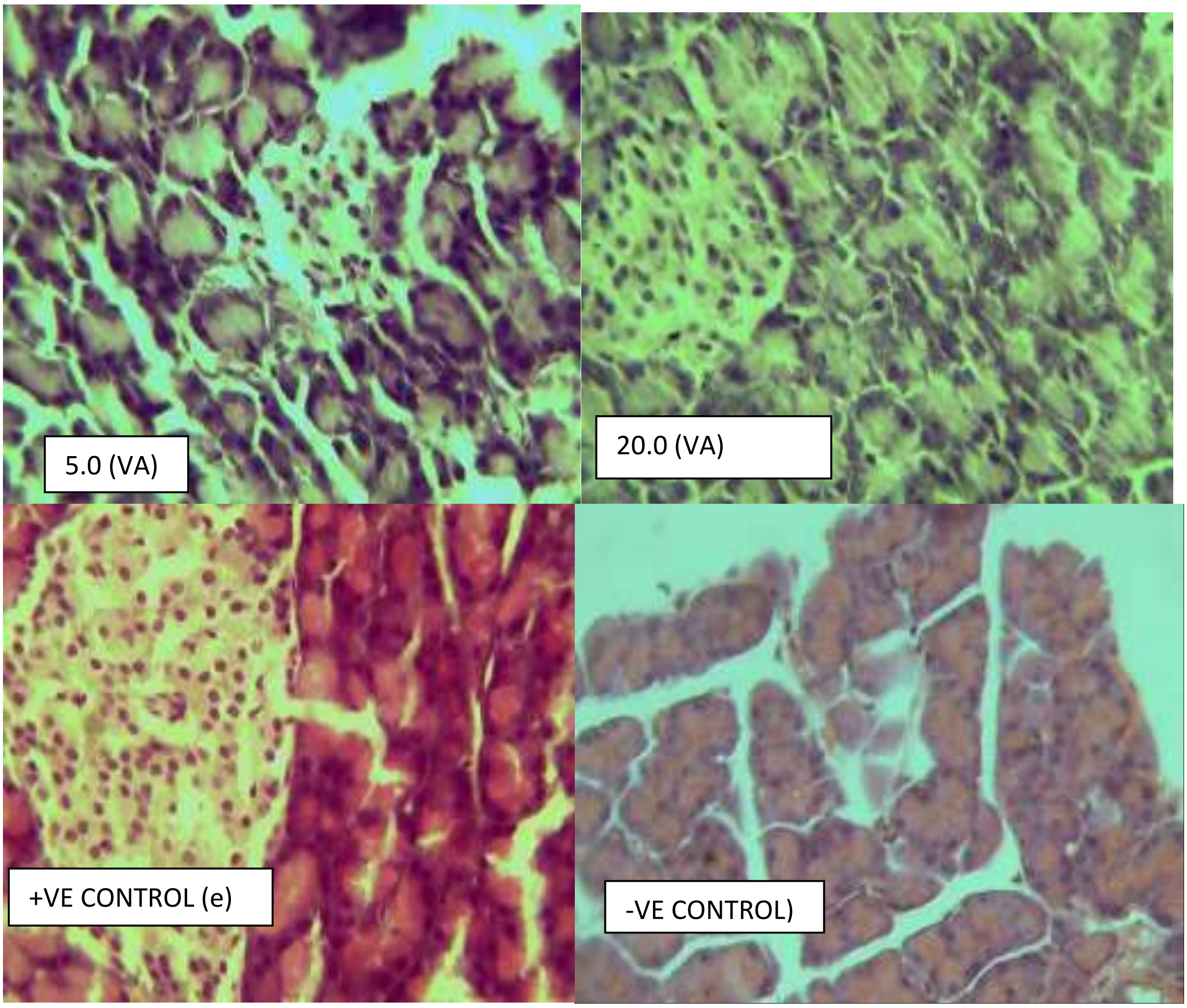
7.4. Antimalaria

Extracts of leaves and root bark showed antimalarial activity against *Plasmodium berghei* which causes malaria when tested in vivo in mice and against *Plasmodium falciparum* in vitro (**Kambizi and Afolayan, 2001**). These extracts suppress the growth of malaria parasites. The leaf extract has also been observed to restore the efficiency of drugs like chloroquine after the malaria parasites had developed resistance towards them (**Iwalokun, 2008; Njan et al., 2008; Egharevba et al., 2014).**



Source: [Amaechi et al., 2018]

Fig.3 Photomicrograph of sections of the pancreas from experimental rats fed Vernoniaamygdalinaat various concentrations.

Legend to Figure 3 Negative control refers to untreated diabetic animals,
Positive control refers to non-diabetic animals fed standard diet without plant product (*Vernoniaamygdalina).*

7.5. Antioxidant properties

Some chemical compounds isolated from the leaves of *Vernoniaamygdalina* (edotides, and sesquiterpene lactones) elicit remarkable antioxidant and chemopreventive properties in cell cultures and rodent models (**Yeap et al., 2014; Wong et al., 2013; Abosi and Raseroka, 2003).** The biochemical and molecular are mechanisms are fundamental to cancer prevention and boosting of the immune system. Inflammation may be caused by oxidative stress and will lead to the development of many chronic diseases. Antioxidants will ameliorate damages caused by radiation, pathogens or stress before more complicated disease situations develop(**Hussain et al., 2016**).

Table 3: Potential health benefits of components of Vernoniaamygdaina

Potential health benefit	Nature of nutrients and phytochemicals involved	Possible mode of action	References
Anti-malaria	Vernodalin, vernodalol,vernoleptin , vernolin, hydroxyvernodalin	It suppresses the growth of the malaria parasite	Kraft *et al*., 2003;Njan*et al*., 2003; Tona*et al.,* 2004; Masaba, 2000; Challad and Willcox, 2009; Egharevba et al., 2015
Diabetes prevention and management	Not yet known	V. amygdalina enhances glucose uptake and utilization in muscle and liver cells; it improves glucose tolerance and postprandial blood glcoselevels in normal subjects. Regeneration of beta cells of the pancreas.	Erasto*et al.,* 2009; Abraham, 2007;Ebong *et al*., 2008; Eteng*et al.,* 2008; Erasto et al., 2009; Uchenna et al., 2008; Akpaso et al., 2011; Ong et al., 2011; Ojimelukwe*et al*., 2012; Amaechi et al., 2018
Antioxidant activity	Polyphenols, tannins, saponins, flavonoids	They ameliorate damages caused by radiation, pathogens And stress.	Roginsky et al. 2003; Oboh et al., 2008; Fasakin and Aluko, 2011; Adesanonye et al., 2010; Adesanoye and Farombi, 2010; Farombi et al., 2011; Nwabjo, 2005; Erasto et al., 2005; Haliwell et al., 2005;
Antimicrobial property	Sesquiterpene lactones (Vernodalol, vernolide)		Udochukwu et al., 2015; Omoregie *et a*l., 2011; Adetunji et al., 2013; Oyedeji et al., 2013; Alo et al., 2012
Cancer prevention and management	Sesquiterpene lactones	Vernodalinol inhibits the growth of cancer cells	Izevbigie et al., 2004; Sweeny et al., 2005; Oputa and Izevbigie, 2006; Gresham, 2008; Oyugi et al., 2009; Yadjou et al., 2013
Hypolipidaemic effect		Vernodalinol reduces the cholesterol/lipid ratio	Adaramoye et al., 2007; Adaramoye et al., 2008; Adaramoye et al., 2009
Prevention and control of inflammation	Tannins, flavonoids, saponins, glycosides, mineral elements		Kim et al., 2004

VIII. CONCLUSION

This review collates information on the nutrient and phytochemical composition of *V. amygdalina* and relates it to the health benefits associated with the plant. *V. amygdalina* is a protective food and should be consumed to promote health. Grey areas that need empirical research input are highlighted. Detailed chemical and nutrient composition especially of the stem and roots of *V. amygdalina*(phytochemicals, vitamins, minerals, and constituent sugars) should be determined in order to present a complete nutrient and chemical composition profile of this plant. In vitro and in vivo studies, cell culture assays; rodent model experiments and animal bioassays have been carried out to decipher the pharmacological effects of *V. amygdalina*, but more in-depth research is needed as well as clinical studies with humans.

Areas that need more in-depth research include the mechanism of action of this plant product in cancer prevention, diabetes management and anti-inflammatory diseases management. Further research is also required to establish whether *V. amydalina* promotes fertility or not. There is need to understand whether the relationship between this plant product and fecundity is dependent on

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the plant part or on the dosage level or both. More animal experiments are required to ascertain traditional claims. More clinical trials are required to ascertain therapeutic effects.*V. amygdalina* is a potent food and medicinal plant that may be used in the dietary management of cancer and other tropical diseases. The full potential of this plant has not been fully exploited. This review stimulates further scientific research into the biological activities, with the view to discovering novel or lead pharmaceutical agents in *Vernoniaamygdalina.*

DECLARATION OF INTEREST

The author declares that there was no conflict of interest.

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Effects of Composting on Growth and Uptake of Plant Nutrients and Soil Chemical Properties After Composting with Various Comparison of POME

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Abstract— *Effects of Composting on Growth and Uptake of Plant Nutrients and Soil Chemical Properties After Composting with Various Comparison of POME aims to find out the provision of compost with various comparisons of POME in improving soil chemical properties and to know the treatment of compost types with various comparisons of POME to Plant Nutrient Growth and Uptake research this will be carried out in the Research will be carried out in the Bangun Bandar PT. Socfindo, Serdang Bedagai district, Dolok Masihul sub-district with a height of ± 25 m above sea level. The research will be carried out in January - August 2018. As for the method used The mixture of soil and compost samples that have been incubated for 1 month is taken from each sample as much as 1 kg and analyzed the nutrients in the laboratory by the wet decstructive method pH H2O 1: 2.5 , N-Total (Kjeldahl destilation), C-Organic (Walky and Black titration), P-Bray II (spectrophometer) and CEC (Ammonium acetate pH 7). Plant Height (cm), Plant Bump Diameter (cm), Weight Measurement , Wet and Dry Weights, Weights, Dry Roots, Nisbah, Roots Results of the study Compost giving with various comparisons of POME 1: 1.3, 1: 1.9 and 1: 2.6 improves the soil chemical properties: total N incubated for 1 month. The compost treatment with various POME 1: 2.6 comparisons gave the highest results. The compost treatment with various POME comparisons significantly affected plant height, plant tuber diameter, canopy wet weight, canopy dry weight and root canopy ratio, leaf P nutrient content, Ca levels leaf and plant nutrient uptake (N, P, K, Ca, Mg). In general the treatment of compost with various POME 1: 1.9 comparisons showed the highest results.*

Keywords*— *Composting, Soil Chemical Properties, Plant Nutrient Uptake, Comparison of POME

I. INTRODUCTION

The emergence of palm oil mills accompanied by the results of waste in the form of solids and large amounts of liquid [7]. Liquid waste from palm oil mills still has the potential as environmental pollution because it smells, has color, contains COD, BOD values and high suspended solids. If the waste is discharged directly into the environment, then some will settle, decompose slowly, consume dissolved oxygen, cause turbidity, emit a sharp odor and can damage the environmental ecosystem [3]. Waste produced by oil palm plants can provide great benefits for life, including as organic fertilizer and as charcoal. Utilization of these wastes is processed into compost. Composting is one way to increase nutrient value and reduce the volume of oil palm empty fruit bunches, the most widely used method is aerobic composting because it is cheap and easy to do and does not require too difficult process control. While composting anaerobicly utilizes microorganisms that do not need air in degrading organic material [5]

Palm oil mill waste management is by utilizing the waste to be processed into compost with a bunker system (aerated bunker composting system), because this system can reduce methane gas emissions. In the process of making compost, it is expected that all waste will be used up (zero waste), but in reality until now only all of the liquid waste has not been used (still +50% of the total liquid waste generated from the factory). have been us ing GIS mapping of actual and potential land suitability for oil palm[11].

.The results showed that with the addition of PKS liquid waste, the average temperature of the TKS heap was higher, the nutrient content of the compost formed had increased. Only the disadvantage with aerobic composting produced unpleasant odors in the first and second weeks of composting, in the treatment given urea the foul odor occurred until the 5th week. However, if liquid waste is given in fresh form, then no rotting odor is formed [10]. According to [5] compost from oil palm bunches has been made simply by adding palm oil mill wastewater.

The results of this composting contain 3.45% potassium, 0.022% phosphorus, organic carbon (C) 29.76% and 54.39% water content. Showed that the composting method used the Bunker system, the ratio between the yield of empty bunches to POME was 1: 5 producing compost with the highest nutrient / nutrient content of Nitrogen, Phosphorus and Potassium over a period of 25 days [14]. The treatment of compost significantly affected plant height, plant tuber diameter, canopy wet weight, canopy dry weight and root canopy ratio was 25% POME [15]..

Utilization of compost in the main nurseries is one of the most important materials applied in the nursery media. From the results of experiments on the main nurseries of oil palm showed that the application of compost in planting media, can increase plant height and the number of midribs on oil palm seedlings [4]. According to [13], that compost can increase soil CEC from 4 to 6 cmol / kg and also soil pH. Fertilization of oil palm seeds can be done by adding inorganic and organic fertilizers [2].

II. MATERIALS AND METHODS

Place and time of research

The research will be carried out in the Bangun Bandar PT. Socfindo, Serdang Bedagai district, Dolok Masihul sub-district with altitude± 25 m above sea level. The research will be conducted in January - August 2018

Materials and methods

This study uses oil palm seedlings from DxP Deli Lame variety from PT. Socfindo who is 3 months old after the seedlings. The materials used include: NPK 15-15-6-4 (nutrient content was analyzed using the wet decstructive method), top soil (ultisol), oil palm empty fruit bunches that have been "pressed", solid ex-decanter, ashes cyclon boiler dust, liquid waste from fat pit (POME - Palm Oil Mill Effluent), fungicides with active ingredients mankozeb and insecticides.The tools used in this study are bunker buildings, polybags measuring 42.5 cm x 50 cm x 0.2 mm, wire sieves, wooden boxes measuring 1.5 mx 1.5 mx 0.6 m, hoes, tools ground drill, scales, ZE 30D sprinkles, gauges (roll, calipers), ovens, and stationery.

2.3.Parameters Observed

Soil Chemical Characteristics Soil mixtures and compost samples that have been incubated for 1 month are taken from each treatment as much as 1 kg and analyzed for nutrients in the laboratory with wet H2O 1: 2.5, N-Total (Kjeldahldestilation), C- Organic (Walky and Black titration), P-Bray II (spectrophometer) and CEC (Ammonium acetate pH 7). Plant Height (cm), Diameter of Crop Bump (cm), Measurement of Weights, Wet and Dry .Weights, Weights, Dry Roots, Nisbah, Rootbill

III. RESULTS AND DISCUSSION

3.1.Chemical Properties of Soil After Composting with Various Comparison of POME

Changes in soil chemical properties: pH, C-organic, total N, C-organic, P-Bray II and CEC in the provision of compost with various comparisons can be seen in Table 1.

Table 1. Chemical Properties of Soil 1 month after Incubation Type of Compost with Various Comparison of POME

Compost Treatment (TKKS: POME)	pH-H_2O	C-Org.	N total	P-Bray II	KTK
		%	%	mg/kg	cmol/kg
Control	6.79	1.22	0.25	635.68	12.41
	Very high	*Low*	*High*	*Very high*	*Low*
1 : 0.6	6.56	1.55	0.25	871.59	13.22
	Very high	*Low*	*High*	*Very high*	*Low*
1 : 1.3	6.33	2.08	0.26	629.19	13.13
	Very high	*Low*	*Very high*	*Very high*	*Low*
1 : 1.9	7.03	1.46	0.27	684.38	14.81
	Very high	*Low*	*Very high*	*Very high*	*Low*
1 : 2.6	7.02	1.36	0.40	640.13	12.79
	Very high	*Low*	*Very high*	*Very high*	*Low*

Description: Criteria for assessment of soil chemical properties according to Fairhurst and Hardter (2010).

From Table 1 above shows that the treatment of compost with various comparisons of POME did not change the status of the chemical properties of pH, P-Bray II and soil CEC. However, it showed a change in nutrient status of C-organic in the type treatment with various POME 1: 1.3 comparisons compared to other treatments that was 2.08%. Likewise, the change in the total N status (very high criteria) in compost type treatment with various comparisons of POME 1: 1.3, 1: 1.9 and 1: 2.6 where the higher the POME ratio the higher the total N content.

3.2Effects of Composting with Various POME Comparisons on Plant Nutrient Growth and Uptake

Based on the results of variance shows that compost treatment with various

comparisons of POME has a very significant effect on plant height, tuber diameter, canopy wet weight, canopy dry weight and has a significant effect on root canopy ratio, but does not significantly affect root wet weight and seed root dry weight Palm oil. Growth of oil palm seedlings in compost treatment with various POME comparisons can be seen in Table 2.From Table 2 above it can be seen that all compost treatments with various comparisons of POME 1: 0.6, 1: 1.3, 1: 1.9, 1: 2.6give a better response than the control of increasing plant height, tuber diameter, canopy wet weight, weight dry crown and root canopy ratio. The compost treatment with various POME 1: 1.9 comparisons gave the highest yields to the growth of oil palm seedlings.

3.2.Discussions

The treatment of compost with various POME comparisons did not significantly affect the chemical properties of the soil, namely pH, organic C, total N, available P and CEC. It is suspected that in this study, the seedling planting media used were previously mixed with rock phosphate fertilizer as a base fertilizer of 75 g / 20 kg of soil, which can be seen that the pH, total N and P-content were available with the criteria: high-very high in the treatment control (Table 1).

Table 2. Growth of Oil Palm Seedlings by Providing Compost Types with Various Comparison of POME

Compost Treatment (TKKS: POME)	Pertumbuhan						
	High Plant	Diameter Cob	Weight Wet Header	Weight Wet Root	Weight Dry Heading	Weight Dry Root	Ratio Heading Root
	Cm	cm	G	g	g	g	
Kontrol	83.48b	4,19d	310.53c	118.33	63.69c	35.34	1.81d
1 : 0.6	91.17a	4.79ab	439.00ab	128.60	95.27ab	39.35	2.54ab
1 : 1.3	89.60a	4.64bc	390.67b	114.80	84.33b	36.00	2.33bc
1 : 1.9	93.40a	5.09a	500.13a	137.60	105.67a	41.64	2.53ab
1 : 2.6	91.85a	4.87ab	452.53ab	128.40	105.51a	39.43	2.72a

Note: The numbers on the line followed by the same letters show no significant difference with the DuncanMultipe Range Test (DMRT) at the α level of 5%

The results of the study by explain that the application of oil palm empty fruit bunches compost and various doses of compound fertilizer in soil mixture with OPEFB compost incubated for 14 days has no significant effect on pH, C-organic, N-total and Mg. But statistically, the treatment of compost with various comparisons of POME 1: 1.3, 1: 1.9 and 1: 2.6 can increase the total N content of the soil with very high criteria, the more volume of POME given in the composting process the more the total N content increases. This is in line with the results of compost quality enriched by various POME comparisons, where the more volume of POME used the higher the nutrient content of C-organic, N, P, K and Mg. [15] The treatment of NPK fertilizer dosage has a very significant effect on plant tuber diameter, canopy wet weight and canopy dry weight that is 25-50% the NPK fertilizer dose. and According to POME is rich in organic compounds and carbon dioxide. POME contains large amounts of nitrogen, phosphate, calcium, magnesium, and potassium so that it can be used as fertilizer[6].

The treatment of compost enriched with various comparisons of POME 1: 0.6, 1: 1.3, 1: 1.9, 1: 2.6 has a very significant effect and gives the same response to leaf P levels compared to control. This shows that the provision of compost to the planting medium can increase nutrient absorption which is transplanted to the plant canopy. The results of the study of [12], the treatment of compost application of empty fruit bunches in planting media in the main seedlings of oil palm plants showed a very significant effect on leaf P nutrient content, leaf K, height and diameter of plant tuber. According to [13] the provision of POME is able to increase the CEC so that the

P derived from fertilizers and P which are not available can become available. [9] states that phosphorus is an essential macro nutrient that plays an important role in various processes, such as photosynthesis, assimilation and respiration. According to [16]and [1] phosphorus is a structural component of a number of energy transfer molecules, ADP, ATP, NAD, NADH, as well as DNA and RNA genetic information system compounds. Phosphate is needed by plants for cell formation in the growing root and shoot tissues and to strengthen the stem, so it does not easily collapse in natural ecosystems.

IV. CONCLUSION

1. Giving compost with various comparisons of POME 1: 1.3, 1: 1.9 and 1: 2.6 improves the chemical properties of the soil: the total N incubated for 1 month. Compost treatment with various POME 1: 2.6 comparisons gives the highest results.
2. The treatment of compost with various comparisons of POME significantly affected plant height, plant tuber diameter, canopy wet weight, canopy dry weight and root canopy ratio, leaf P nutrient content, leaf Ca content and plant nutrient uptake (N, P, K, Ca, Mg). In general the treatment of compost with various POME 1: 1.9 comparisons showed the highest results.

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Solid waste management in Kulhudhuffushi, Maldives; Most suitable solution for the Crisis

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***Abstract*— *This research is to find the most suitable, applicable and practical solution for the collection, transfer, transport and disposal of municipal solid waste in Hdh.Kulhudhuffushi of Republic of Maldives. This research was aimed to find the most suitable solid waste disposal method, along with the proper collection system, transfer and transport mechanism. The research covers waste generation sources as, households, businesses, restaurant and institutions. The annual maximum waste generation was from households, with 4,107 tonnes of waste per year. Average generation rate was 1.13 – 1.55kg per capita per day. The total waste generation rate was 5,643.89 tonnes per year. The highest portion of solid waste was kitchen/food waste with 2,181.93 tonnes per year. The total composition of biodegradable and non-biodegradable waste was 68% and 32% respectively. Hence, method of composting was selected over incineration, due to the huge (98%) amount of moisture and low volatility of biodegradable waste. Since, the land ok Kulhudhuffushi dump site is smaller in size mechanical composting was preferred. The non-biodegradable waste would be stored in bunkers and transferred to the incineration plant quarterly each year. The collection facility would be separate collection with rear loading dump trucks as well as multi compartment dump trucks scheduled to collect waste twice a week. For the efficiency of the collection process the town was divided in to three zones with equal number of households, and thereby the collection crew can complete one round of collection within three days' time.*
***Keywords*— biodegradable, collection, generation, highest portion, transferred**

I. INTRODUCTION

Waste management (more specifically solid waste management) is a field with a large scope. This research is completely focused on to population in addition to municipal solid waste types. Therefore, this research would purely focus on solid waste management of Kulhudhuffushi. Though Kulhudhuffushi is one of the largest islands of Maldives, it has a land area of roughly 3.5km2. In the early ages there is no specific areas in the town marked as land fill zones or dump sites for the citizens, to collect dispose the waste. As a result, from the earliest of the times people used to dump the waste near the sea shore and to the ocean. Kulhudhuffushi has a small land mass and with a growing population the area considered as the dump site in the year 2000 was 17,010 square meters. Now this small area of land is over filled and in-need of renovation.

Solid Waste Management one of the greatest challenges confronted by the developed as well as developing countries. Improper techniques used for the handling and lack of waste handling facilities have adverse effect on the environment in addition to the people around. Similarly, the issue of Solid Waste Management is one of the major concerns in the town of Kulhudhuffushi with no proper facilities and techniques to deal with solid waste. Therefore, what would be the most suitable solution for solid waste issue in Kulhudhuffushi?

The aim of this research is to find the most suitable, applicable and practical solution for the collection, transfer, transportation and disposal of solid waste in the town of Kulhudhuffushi of Republic of Maldives.

1. To identify the problems related to Solid Waste Management in Kulhudhuffushi
2. To propose possible solid waste management solutions which could be implemented in Kulhudhuffushi
3. To propose the most suitable collection system for Kulhudhuffushi
4. To propose most convenient waste transportation method for Kulhudhuffushi

II. LITERATURE REVIEW

Any material which is thrown away or discarded as useless and unwanted could be considered as solid waste. When solid waste disposal process is not maintained or managed, it would lead to air, water and land pollution resulting in various unhealthy conditions. Solid waste can be classified to Municipal, Industrial, Hazardous, Agricultural and Biomedical Solid Waste. Each of these types of solid waste has to be treated differently as the composition of the waste changes. [1]

2.1 Solid Waste Terminology

The term Municipal Solid Waste (MSW) is generally used to describe most of the non-hazardous solid waste. [2] Refuse and Trash are the main two components of Municipal Solid Waste. Refuse consists of garbage and rubbish. Garbage contains putrescible or highly decomposable food waste. Hence, garbage cause attracts flies and other insect trouble and also ferment readily resulting in the production of unpleasant odours, hence needing special attention. [3] Rubbish is mostly dry, non-putrescible materials. Rubbish is frequently responsible for the creation of nuisance when it is scattered by careless handling or by wind. [3] Trash is the bulky waste materials that generally requires special handling and is therefore, not collected on a routine basis. [3]

2.2 Quantity and Composition of Waste

Quantity of solid waste the amount of waste by weight or volume generated per capita per year. The composition of waste is the amount of different types of waste present in the municipal solid waste. Information regarding the weight, volume and composition of municipal solid waste is necessary for the proper planning, design and operation of collection and disposal facilities. Weight and volume play a crucial role in determining the size of collection vehicles as well as the sizes of the disposal plants. Moreover, the composition is vital to determine the method of disposal. [4] In the context of the Maldives, waste generation was estimated between 2kg – 5kg per capita per day. Municipal solid waste contains a wide variety of materials, some which could be burnt while others cannot. A portion of MSW can also be recovered and a percentage of it been recyclable.

2.3 Moisture Content and The Density of Solid Waste

Knowledge of the moisture content of the waste is important when the waste is used for energy recovery. The knowledge of moisture contact would provide the amount of heat the waste can produce. [5] Moisture content also plays a key role when the waste is subjected to anaerobic decomposition in sanitary landfills. To subject the organic matter to composting and anaerobic digestion, the water content must be put at the optimum. [5] The moisture content is expressed wet and dry (Pw and Pd respectively) percentage.

$$P_w = \frac{W}{S_w} \times 100 \tag{1}$$

$$P_w = \frac{W}{S_w} \times 100 \tag{2}$$

Where, W is the mass of the moisture, Sd the mass of the dry solid waste and Sw the mass of the wet solid waste.

The most important use of knowledge of the density of solid waste is the determination of its compacted volume. By the knowledge of the compacted density, the volume of landfill space or the storage space can (a compacted volume) can be calculated easily. Compacted volume is also used to determine the size of the collection trucks. [5] Compactor machines compaction rations can vary from 2 to 4. In landfills the compaction ration can vary from 297 to 891 kg/m3. Generally, 475 to 594 kg/m3 can be achieved in landfills with a moderate compaction effort, while a density of 297kg/m3 can be achieved from a poor compacted landfill. [5]

2.4 Solid Waste Collection

The total process of Municipal Solid waste management is divided in Collection, Transfer, Transport and Disposal. About two third of the total cost of solid waste management is covered for waste collection. Collection system of solid waste can be divided in primary collection and secondary collection. Primary collection involves the collection of solid waste from house to house, the point of waste generation to a suitable nearly common location known as the transfer station. From the transfer station the waste the waste would be further collected and transported to the point of disposal. The transfer of solid waste from the transfer station to the disposal site is known as the secondary collection. [3] The process of primary collection is subcategorized into four categories, communal storage, block collection, Kerbside collection and door-to-door collection. [4]

2.5 Transport of Solid Waste

Transportation of solid waste involves the transfer of waste form the transfer station to the disposal site. The process involves heavy motor vehicles and is necessary when the source of the waste is extremely far from the final disposal site. [3]

2.6 Chemical and Energy Properties of MSW

The energy content increases with the amount of volatile matter present in the waste. The energy content of solid

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waste is the heat of combustion released when the waste is burned. Hence, there are two types of heat due to combustion. They are known as higher heat of combustion and lower heat of combustion. [2] The higher heat of combustion includes the heat of vaporization of water. Lower heat of combustion does not include the heat of vaporization of water.

2.7 Composting of Municipal Solid Waste

Composting is the process by which the organic portion of the solid waste is allowed to decompose under carefully controlled conditions in a rather biological process than a chemical process. With proper control of temperature, moisture and aeration, a composting plant is capable of reducing the volume of the raw organic matter by as much as 50 percent. [6] Process of composting stabilizes the waste and produce an end product that can be recycled for beneficial purposes. A complete municipal solid waste composting operation includes sorting and separating, shredding and pulverizing, digestion, product upgrading, and finally marketing.

2.8 Incineration of Municipal Solid Waste

Incineration is expensive, primarily because extensive air pollution control equipment is required and it requires high-level technicians and skilled employees for proper operation and maintenance. However, the advantages of the incinerator often outweigh these disadvantages. [4] Incineration is a chemical process in which the combustion potion of the waste is mixed with oxygen, forming carbon dioxide and water. For the complete combustion the waste must be mixed with appropriate volume of air, and a proper temperature must be maintained for a suitable length of time. [3]

Sanitary landfills are simple, cheap, and effective method of waste disposal. Yet, this method may not be suitable for Kulhudhuffushi due to the lack of land shallow water table. Incineration of solid waste suited for towns where land filling is not available. The method of incineration can reduce the garbage volume by 90%. This method is a highly suitable option however, setbacks like high construction and operation costs would be problem to initiate the project. Composting is similar to sanitary land fill but yields a stable end product which is a good soil conditioner and may be used as a base for fertilizers. Therefore, if this system is to be used along with a fertilization or cultivation island it could be beneficial. However, other costs such as transportation of the solid wastes arrives with this method.

III. METHODOLOGY

3.1 Site Selection

The Journal is published in standard A4 size with a two-column layout for text. Diagrams and tables should be in portrait orientation with either one or two column width. As per the Kulhudhuffushi Island Council, there were 2160 households in Kulhudhuffushi, while 1485 households were contributing to the municipal solid waste. The remaining 531 were under construction. In addition to the household, business establishments were key sources contributing to solid waste. There were 205 retail and whole sale business establishments in Kulhudhuffushi.

3.2 Sample Selection

The target area for the research was the town of Kulhudhuffushi and the people you were using the municipal solid waste management system of Kulhudhuffushi. Hence, citizens from other island were also given change to participate in the research as the target samples were selected on the basis of households, businesses, restaurants and institutions, rather than individuals. The sample size was selected with following equations, put forward by Professor Othman Alsalloum, of King Saud University, Saud Arabia. The sample size was calculated as by considering the total population as the sum of all the household, businesses, restaurants and the institutions. The total sample received from this figure was then further divided among the different categories (household, businesses, restaurants and the institutions) depending on their ration.

The Minimum Sample Size for Infinite Population (Population Larger Than 10,000)

$$n = \frac{Z^2\, P\,(1-P)}{d^2} \tag{3}$$

Where;

n = Minimum Sample Size for a Large Population
Z = Standard Normal Distribution
P = Expected Probability
d = Maximum Allowable Deviation

<u>The Minimum Sample Size for a Small Population (Population Smaller Than 10,000)</u>

$$nspt = \frac{n}{1 + (\frac{n}{spz})} \tag{4}$$

Where;

Nspt = Minimum Sample Size for a Small Population
n = Minimum Sample Size for a Large Population
spz = Size of the Population

A total of 264 samples of data was collected from Households, while 27 samples were collected from the business sector. From restaurants and institutions, a total number of 5 and 12 samples were collected respectively. Therefore, the total number of samples collected was well beyond the required minimum sample size. For the collection of data through questionnaires the household were selected on a random basis to get a generalized idea from the whole town.

The method of interviews was be focused on the authorities' such as Kulhudhuffushi Island Council, Kulhudhuffushi Regional Hospital, Waste Management Corporation of Maldives, Fenaka Corporation, Waste collecting personals and Ministry of Environment and Energy. The data collected from the above-mentioned authorities was an added reliability to the data collected from the target groups with the method of questionnaire.

IV. RESULTS AND DISCUSSION

4.1 Average Composition of Municipal Solid Waste from Households

The maximum composition of municipal solid waste from the households were kitchen / food waste. Only a small fraction 2 percent and 3 percent of the household waste was metal and wood respectively. Moreover, 9 percent of the waste was paper and 4 percent of waste being glass. 5 percent and 9 percent of the waste was clothing and plastic materials respectively. The second highest composition of household waste was considered as the other types of waste, consisting of 21 percent of the total solid waste from household. From the figure below, it was clear that the 84 percent of the waste mentioned as others were leaves. In addition to that, diapers, fish waste, rotten fruits and vegetables adds up to the biodegradable potion of the household solid waste. Hence, 97 percent of the waste mentioned as others were biodegradable waste. A total of 78.4 percent of house hold solid waste was biodegradable. That leaves 21.6 percent, roughly one third of the household waste to be non-biodegradable.

4.2 Average Waste Generation Rate per Households

The average daily rate of waste generation per house hold on a daily basis was a total average of 7.58kg of waste is generated per household. Among which kitchen waste was 3.54kg (as it was 47 percent of the total solid waste). Kitchen waste was followed by others category with 1.57 kilograms, which implies 1.32 kilograms of other waste was leaves. Plastic, metal, wood, paper, glass and clothing was 0.72kg, 0.18kg, 0.19kg, 0.66kg, 0.33kg and 0.39kg respectively. Therefore, 5.91kg out of 7.58kg of waste generated on a daily basis was biodegradable waste.

4.3 Average Composition of Municipal Solid Waste from Businesses

The maximum composition of municipal solid waste from the businesses were paper waste with 34 percent of the total waste composition. Plastic products were the second largest type of waste contributing to municipal solid waste from the restaurants with 22 percent of the total waste. Furthermore, food waste was also quite high in composition with 13 percent. Clothing and wood were with the smallest contribution to municipal solid waste with only 5 percent each. Metal and glass were found to be 10 and 6 percent respectively. Moreover, 5 percent of waste from the businesses were classified as other, and only damaged fruits, vegetable and other food items were included in the category. Therefore, 57 percent of waste from the businesses were biodegradable while 43 percent of the waste generated from the businesses were non-biodegradable.

4.4 Average Waste Generation Rate per Business

The average daily rate of waste generation per business establishments was on a daily basis is a total average of 13.78kg of waste is generated per business outlets. Among which 4.67kg was paper waste, as it was occupying 34 percent of the total solid waste from business outlets. With the second highest composition, plastic waste generation was 3.01kg per day. Kitchen waste was 1.74kg. Kitchen waste was followed by metal, glass, clothing, wood and others with a weightage of 1.38Kg, 0.79Kg, 0.73Kg, 0.72kg and 0.71Kg respectively. Therefore, 7.84kg of the waste was biodegradable per single business establishment. Which result in only 5.91kg of non-biodegradable waste from each business outlet per day.

4.5 Average Composition of Municipal Solid Waste from Restaurants

The maximum composition of municipal solid waste from the restaurants were kitchen and food waste. Plastic products were the second largest type of waste contributing to municipal solid waste from businesses with 19 percent of the total waste. Furthermore, paper was also quite high in composition with 14 percent due to high number of paper serviettes and paper receipts. wood and clothing were having no contribution to the municipal solid waste stream. Glass was with 5 percent contribution to municipal solid, while metal gives a 9 percent contribution due to the tins and cans used in food packaging. Only 1 percent of the total waste generated from the restaurants were considered as others, consisting of dry leaves and garden waste from the tress and small gardens with in the restaurants. Therefore, 67 percent of

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total waste from the restaurants were biodegradable while 33 percent of the waste generated from the businesses were non-biodegradable.

4.6 Average Waste Generation Rate per Restaurant

On a daily basis a total average municipal solid waste generated per restaurant was found to be 48 kilograms. Among this 24.90Kg of waste was kitchen and food waste. Second highest waste type was plastic items with 9.10kg followed by paper items with 6.80kg. Metal waste and glass waste are 4.10kg and 2.60kg per day respectively. As mentioned before, wood and cloth waste were not generated in the restaurants. Therefore, 32.2kg of waste on a daily basis was biodegradable waste from a single restaurant. 15.8Kg of waste from the restaurants were non-biodegradable.

4.7 Average Composition of Municipal Solid Waste from Institution

The above figure represents the percentage composition of municipal solid waste generated on an average basis per single institutions Kulhudhuffushi per day. The largest three categories of solid waste generated from institution includes, paper waste, other types of waste and plastic waste with a waste composition of 36 percent, 27 percent and 23 percent respectively. Other categories of waste were in a small composition. Wood waste was 6 percent of the total waste. Kitchen and food waste along with glass was makes up a waste of only 2 percent each. Metaling waste was observed to be 3 percent and cloth material to be just 1 percent of the total waste. The 27 percent of waste indicated in the other waste category contains 4.5 percent of cardboard and 22.5 percent of dry leaves and garden waste. As a result, only 29 percent of the waste from institutions were non-biodegradable while a whopping 71 percent of waste was biodegradable.

4.8 Average Waste Generation Rate per Institution

The average waste generation rate of an institution was 38.58 kilograms per institution per day. Among the 38.58kg of daily waste 14.07kg was paper waste. Category of others are second in line with 10.24kg. Others category consists of cardboard, dry leaves and garden waste. Plastic was the third most contributing to the solid waste with 8.84 kilograms. Wood, metal, kitchen food, glass and cloth materials were the lowest by weight with only 2.25kg, 1.08kg, 0.94kg, 0.85kg and 0.32kg respectively.

4.9 Comparison of the composition and Quantity of Municipal Solid Waste from Different Sources

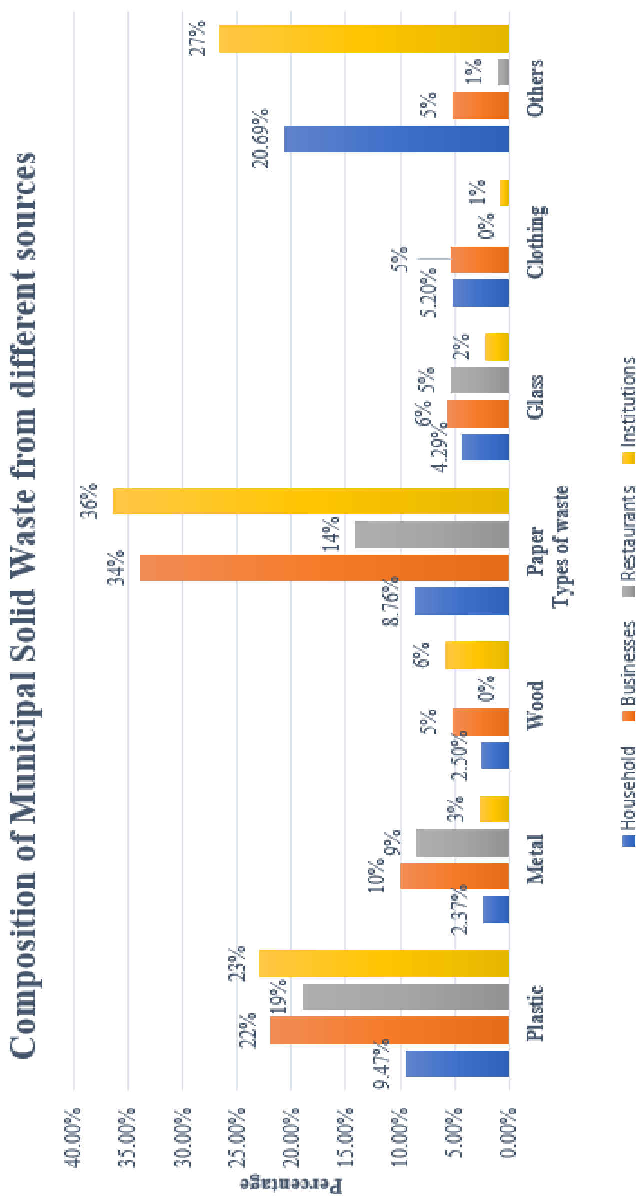

Fig.4.9.1 Comparison of the composition and Quantity of Municipal Solid Waste from Different Sources.

As per the figure it was clear that highest contribution to waste was paper and kitchen waste or food waste. Kitchen waste was highest from household and restaurants while, paper waste was the highest from businesses and institutions.

Although kitchen waste and paper were having highest composition per source, from institutions and businesses, when considering the all the sources the waste of "others" was the second most common type of waste by weight. The kitchen waste from the houses was recorded as 5,256.94 kg/day, while kitchen waste was recorded as 2,328kg. The third highest category of waste was plastic wastes from house hold with 1,065.92kg. This category was followed by paper waste from households and businesses with 985.80kg and 952.43kg respectively.

The total municipal solid waste was found out to be consisting of these eight major categories, Plastic, Metal, Wood, Paper, Glass, Kitchen / Food waste, clothing and others. 39 percentage of the total was kitchen waste, while others waste second highest with 17percentage of the total waste. About 64.71 percent of the others category was biodegradable waste containing dry leaves, fish wastes, garden waste, diapers. The remaining 35.29 percentage of the others contains tires and ash which was non-biodegradable. 68 percent of the total municipal solid waste generated in Kulhudhuffushi was biodegradable was. Only 32 percent of waste was non-biodegradable. Therefore, from the 15,462.70kg (15.46 tonnes) of municipal solid was generated in Kulhudhuffushi 10,514.64kg of waste was biodegradable waste. Only 4948.06kg (4.95 tonnes of waste were non-biodegradable. Kulhudhuffushi generates a total of 5,643,885.5kg (5,643.89 tonnes) of municipal solid waste on a yearly basis. With a population of just under 10 thousand people, the municipal solid waste generation of the town was about 1.13 – 1.55kg per capita per day. A study conducted in New York was proven the municipal solid waste generation per capita was 1.5kg per capita per day. [5] When a yearlong management plan was to be considered 3,837,842.14kg (3,837.84 tonnes) of waste was to be biodegraded and the remaining 1,806,043.36kg (1,806.04 tonnes) of waste was non-biodegraded.

4.10 Current Waste Collection

The current waste collection system of the town of Kulhudhuffushi is one key aspect to be upgraded in order to upgrade the whole waste management system of the town. Furthermore, collection sums up to cost two-third of the total cost of waste disposal. [3] 93 percent of the surveyed population was not satisfied with the current method of waste collection. There were few concerns which was highlighted by a number of households. As a primary concern, 38.08 percent of the people wants to increase the frequency of the waste collection. From the figure below, it is clear that 68.2 percent of the population dispose waste on weekly basis as the collection service scheduled only once a week.

A small percentage of 28.3 disposes the waste on a regular basis. As a secondary concern, a large group of the surveyed population believes that waste categorization was one of the main areas to be made better in order to provide a better waste collection. Source infect can reduce the cost of separation by manual or mechanical sorting, hence reducing the expenses on some aspects to focus on more needed aspects. [5] Furthermore, 16.15 percent of the surveyed house hold was recommending to upgrade the current collection vehicles. The general public was concerned as the types of vehicles currently used for was collection were just normal lorries and pickups. In addition, the general public recommends dustbins in public areas such as market places, parks and beaches so that waste generated on those area would not pollute the environment. These public dustbins could be handled by the collection crew as part of their routine.

4.11 Current Waste Management System

The current method of waste disposal was open burning. [7] Which intern caused a great deal of risk to the general public living near close vicinity of the dump site. Furthermore, the current handler of dumpsite, was concerned that the open burning incidents was not generated by the handler but the trespassing public. Fenaka corporation further added that open burning of local towns was prohibited under the waste management regulation of Maldives and thereby, was not taking part in any such activities. [8] No matter the origination of open burning, improper burning was highly dangerous to both people and to the environment. [9] Due to such concerns the 94.6% of the general public was not satisfied with the current method of waste management in Kulhudhuffushi.

4.12 Existing Trends and Future Contribution of General Public to Improve the Solid Waste Management

In this research it was proven that 93.8% of the people was disposing the waste to the dumpsite although 6.2% of the people chose other areas of the town to get rid of the waste. Disposing the waste to areas with in the town except the areas specifically allocated for was an offence punishable by law as per the waste management regulation. However, illegal dumping of waste was observed by 75.6 percent of the sample being surveyed in this research. Moreover, the locations of most illegal waste disposal include the lake area and beach with 37.31 percent and 33.85 percent respectively of the total illegal dumping. The main reason for illegal dumping was not having a proper penalty to the parties who go illegal dumping. Penalties were mentioned in the waste management regulation regarding illegal dumping of waste. However, the enforcement of these rules and regulations were not practice.

78.3% of the people was paying up to 100 Maldivian Rufiyaa per month on waste collection. Also, it was observed that 54.3% of the people were willing to pay a contribution between 101 – 200 Maldivian Rufiyaa.

Further 28.4% was even willing to contribute up to 300 Maldivian Rufiyaa for the better waste management system.

4.13 Volume of The Solid Waste

Component	Typical Density (kg/m3)	Mass of solid waste per day (Kg)	Volume of Solid Waste per day (m^3)	Volume of Solid Waste per day (m^3)
Plastic	60	1975.78	32.93	12,019.45
Metal	350	624.72	1.78	649.7
Wood	230	470.41	2.05	748.25
Paper	90	2,300.69	25.56	9,329.4
Glass	200	697.65	3.49	1,273.85
Kitchen Waste / Food Waste	300	5,977.89	19.93	7,274.45
Clothing	60	740.62	12.3	4,489.5
Garden Waste	100	2,674.94	26.75	9,763.75
TOTAL			**124.83**	**45,548.35**

Fig.4.13.1 Volume of Slid Waste

4.14 Number of Trips and Number of Trucks

There should be 4 rear loader trucks to collected all the bio degradable was and four multi compartment trucks to collect all the non-bio-degradable waste. With the multi compartment collection trucks multiple types of waste can be separately collected. Further 2 Trucks should be kept to collect waste from public dustbins and on call waste collection orders. Hence, the total number of was collection vehicles.

4.15 Storage Facility for Non-Biodegradable Waste

Non-biodegradable waste could be compacted and stored in the dumpsite and transferred to an incineration facility managed my Waste Management Corporation (WAMCO) on a specific time schedule. Total volume of the non-biodegradable waste coming to the dumpsite was (50.5m3 daily) 18,432.5m3. On the yearly waste generation of 5,643 tonnes of waste 1,805.76 tonnes were non-biodegradable. Hence, the 450Tonne landing craft can remove the non-biodegradable waste from Kulhudhuffushi on a quarterly basis per year (four times per year). Therefore, at a given time the maximum amount of non-biodegradable waste present in the Kulhudhuffushi dump site would be 451.44 tonnes (3,646.75m3). Therefore, 4m x 4m x 150m, two storage bunkers can store the waste until it can be transported from the town.

For biodegradable waste, composting plant would be the most suitable method. Due to the high amount of moisture content incineration would not be a suitable option for biodegradable waste. 98% of the moisture of the total municipal solid waste of Kulhudhuffushi was from biodegradable waste. Non-biodegradable waste only contains 2% of the total moisture, hence making them ideal for incineration. Mechanical composting was the preferred choice for the town of Kulhudhuffushi as the land area does not give provision to establish an open composting facility. Furthermore, mechanical composting can reduce the stabilization time of compost from 5 weeks to 1 week increasing the rate of production and hence the time period waste has to be handled. [4]

V. FUTURE DIRECTIONS

With the development and technological advancement, as well as the population increase could alter the current composition of municipal solid waste in Kulhudhuffushi. For the purpose of this research only collection, transfer, transport and disposal were focused. However, the approach of energy recovery can also be would also be beneficial as the level of energy consumption was forecasted to increase with time due to development.

Furthermore, with the construction and demolition waste was not much of a concern. With the advancement in construction industry and introduction of multi-storey buildings such waste types might not be disposable through the current method of disposal.

In addition, the journey of the non-biodegradable waste from Kulhudhuffushi to the incineration plant was a long journey. Hence, a closer, centralized waste management town could be a future approach, which would not only include waste solutions for Kulhudhuffushi but to the whole “Thiladhunmathi” atoll.

VI. CONCLUSION

The research conducted on the topic Solid waste management in Kulhudhuffushi; most suitable solutions for the crisis was specifically focus on the context of the town of Haa Dhaal Atoll Kulhudhuffushi. For a proper decision of a waste management solution the composition and quantities of the waste generation on a daily basis was a necessity. However, the dump site handler of Kulhudhuffushi was not maintaining any record or data.

Therefore, the daily waste generation from four main sources was measured through questionnaire survey. The four main waste generation sources in Kulhudhuffushi includes, household, businesses, restaurants and institutions. For each type of source, the daily weight of waste generation was measured along with the composition of different types of waste materials. On average a single household generates 7.58kg of waste, while a business outlet was generating 13.75kg per day. Restaurants and institutions generate 48kg and 38.58kg of waste on a daily basis. It was found that the highest composition of municipal solids waste was kitchen/food waste from household and restaurants with 47.72% and 52% respectively. The second highest composition of solid waste was paper waste from businesses and institutions with 34% and 36% respectively.

The weight of solid waste generation from all the sources reaches to 5,643 tonnes per year. Among which the highest contribution was from the households with 4,107 tonnes per year. Business establishments generates 1,023 tonnes of waste on a yearly basis. The proportion of restaurants and institutions was quiet less as the number of units were less in those sources. However, restaurants and institutions were measured to generate 245 and 267 tonnes of waste every year. The largest amount of municipal solid waste was kitchen waste with a total of 2,181 tonnes per year from all sources combine.

Furthermore, 68% of the total municipal solid waste generated on the Kulhudhuffushi is Biodegradable while only 32% of the waste was non-biodegradable. Due to the high portion of biodegradable waste 98% of total moisture content was in biodegradable waste (moisture content of 1,979 tonnes). As a result, the process of incineration was not the most suitable solution to dispose the solid waste is Kulhudhuffushi. With 68% of biodegradable waste disposal by composting was the preferred option for Kulhudhuffushi. However, due to the limitation of land area, open composting was ruled out and mechanical composting is the most suitable solution for the solid waste issue of Kulhudhuffushi. Mechanical composting further reduces the compost settlement type by five times, hence was an added benefit. Hence, the objective of proposing the most suitable waste disposal solution was achieved.

The collection of waste from Kulhudhuffushi was to be conducted by zoning the town in to three areas. Each area was be collected twice a week (the survey suggested that the people of Kulhudhuffushi preferred to increase the collection frequency, hence it could be achieved) on door-to-door collection process. A total 10 dump trucks with 2m3 was preferred for smooth operations. Rear loader type 6 trucks were to be involved in collection process. 4 trucks in the door-to-door collection of biodegradable waste and 2 trucks in the on-call collection. The remaining 4 trucks would be on multiple compartment so collection of non-biodegradable waste. Hence, the objective of proposing a suitable collection method was achieved. In addition to the dump trucks Front end loaders, motor grader, earth movers and steel wheeled compactors were also requirement to maintain the site. The non-biodegradable portion of the waste (1805.76 tonnes per year) would be stored in 4m x 4m x 150m two storage bunkers which would be transferred to the WAMCO incineration plant on quarterly basis per year. Thereby, achieving the third objective of this research project.

Furthermore, the smooth functioning of this method would be achieved when waste was categorized from the sources (source separation). 38.08% of the surveyed population was found to be forwarding the opinion. Moreover, 54.3% was found to be willing to contribute to a better waste management system. As a result, the proper payment scheme should be implemented to maintain a source of income for the maintenance and proper functioning of the facility. 55.8% of the population was engaged in waste reduction from the sources by means of reducing and recycling. Also, the public concerns for the illegal dumping believed the penalties of such offenses should be improved. In addition, the open burning issues were found to be arising not only due to the handler of dumpsite, but due to trespasser as well. Therefore, a proper boundary should be made around the premises of the dumpsite and the accesses control should be fully maintained.

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Assessment of Two-*spotted Spider Mite* (*Tetranychus urticae*) on Potato (*Solanum tuberosum* L.) in Eastern Hararghe, Ethiopia

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***Abstract*— *Potato is an important tuberous vegetable crop in Ethiopia. However, its production is constrained by arthropod pests, nutrient depletion of soil and diseases. Among arthropod pests, two-spotted spider mite (TSSM, Tetranychus urticae) is one of the major arthropod pests affecting potato production and contributing to low yield in eastern Hararghe, Ethiopia. To tackle this problem, the survey was undertaken to assess the distribution, farmers' management practices and their perception of TSSM on the irrigated potato. The data were collected from ninety-six potato producers during the dry season in Haramaya, Kombolcha, Kersa, and Metta districts of Eastern Hararghe Zone by interviewing, observing and recording from October to March in 2018/2019. The meteorological data were obtained from the National meteorological agency, Jijiga station. The number of TSSM/potato plants, prevalence, incidence and severity levels of potato were analyzed using three-stage nested design. Farmers' perceptions and practices were analyzed by using descriptive statistics, while the relationship of weather factors and altitude with the status of TSSM was analyzed using SPSS software program version 16.0 and determination of Pearson correlation was done using SPSS statistical software. This assessment confirmed that TSSM was found in all the districts with variation in number per potato leaves, incidence and severity levels. The highest incidence (37.88%), severity (3), number of TSSM/potato (39) per plant was scored from the Haramaya district and the lowest was recorded from Kombolcha district. Based on the observation and response of the respondents, the TSSM was mostly prevailed in autumn (44.79%) but was the lowest in spring seasons (6.25%). It severely affected potato production at flowering stages (44.2%). But the lowest damage was at the seedling stage (7.4%). The number, prevalence, incidence and severity of TSSM was the highest in Haramaya and the lowest in Kombolcha districts. Data on farmer's perception also showed that 60% of them recognized TSSM on potato plants. The linear correlation coefficient indicated that the incidence (r=-0.546, Y=-0.548X+38.7), number of TSSM/potato plant (r=-0.847, Y=-0.06.232X+38.24) and severity level (r=-0.431, Y=-0.0221X+2.805) was decreased as rainfall intensity increased. The studies suggested that the provision of a resistant variety of potato, chemical pesticides, water supply will help the farmers to increase the production and productivity of potato by decreasing the yield losses due to TSSM. Further research on the seasonal abundance, effect of microclimate, natural enemies and management practices on the population dynamics of TSSM in the major potato growing areas of the eastern part of Ethiopia was suggested from the study.*

***Keywords*— Assessment, Correlation, Farmers Perceptions, Incidence, Prevalence, TSSM.**

I. INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family of Solanaceae; an important tuberous vegetable crops in the world that ranked fourth in the volume of production after rice, wheat and maize. It ranked first among the root and tuber crops, followed by cassava, sweet potato and yam (FAO, 2010).

In Ethiopia potato is important for securing the food demand of the country within a short period of time, cash income, jobs for potato grower and provides high energy, quality protein, substantial vitamins and minerals (Kuarabachew *et al*., 2007; FAO, 2008).

The low potato yield could be attributed to a number of factors including arthropod pests, nutrient depletion of soil and diseases. Arthropod pests and pathogens caused yield losses of potato up to 17.2% (8.1–21.0%) globally (Savary *et al*., 2019). Insect pests account for 16% of yield losses of potato worldwide. It reduced potato tuber yield and quality that could be estimated between 30-70% (Giordanengo *et al*., 2013). In tropical countries, the potato yield loss caused by arthropod pest was higher and approaching 60–70% (Nwilene *et al.*, 2008).

In Ethiopia, the most serious and commonly reported arthropod pests that limit potato production were two-spotted spider mites (*Tetranchus urticae*), cutworm (*Agrotis ipsilon, A. segetum*), white grub (*Brahmina coriacea*), potato tuber moth (*Phthorimaea operculella*) alone caused yield loss of potato up to 42% and 8.7 % in-store and in the field, respectively (Tekalign *et al*., 2015). Two-spotted spider mites are the most common pests found in potato growing areas of eastern Hararghe, Ethiopia (Muluken *et al*., 2016). The adults, nymphs and larvae of TSSM feed mostly on green parts of potato leaves leading to its complete destruction and yield during its heavy infestations, as well as potato tuber sprouts and shoots. As a result, farmers continue to bear huge losses due to these pests. Since potato is an important food security crop and its low production brings an impact on national food security.

Understanding potato production constraints, particularly arthropod pests and farmers 'methods of managing TSSM could be useful in designing an effective integrated pest management (IPM) strategy in a sustainable manner. Smit and Matengo (1995) and Obopile *et al*. (2008) reported that assessing farmers' perceptions of crop production constraints has been used as a tool for documenting pest status and designing suitable pest management options for a particular community. Furthermore, integrating farmers' perspectives of insect pest management by crop entomologists helps the community for the intervention of the local control measure. For example, understanding of farmer's selection criteria of potato varieties might differ from that research scientists had normally assumed. Nonetheless in eastern Hararghe, there is inadequate information on farmers' knowledge, perceptions, and practices in the management of TSSM. Thus, this hindered the development of effective pest management approaches for smallholder farmers. In addition, there is also insufficient information on TSSM status in eastern Hararghe, Ethiopia. Therefore, this research was initiated with the objective of assessing the status of two-spotted spider mite on potato production in selected districts of eastern Hararghe, Ethiopia

II. MATERIALS AND METHODS

Description of the study Area

The survey was conducted in Eastern Hararghe Zone, Oromia National Regional State of Ethiopia on irrigated potato from October to March in the 2018-2019 cropping season. The study area included four major potato producing districts purposely. The selected districts were Haramaya, Metta, Kersa and Kombolcha. From each district, four major potato producing farmer associations were selected. In each farmer association, six respondents, who own potato plant and live along with their family member, were considered. The respondents' farm site was 2-4 kilometers far apart. To avoid any potential bias, the purpose of the survey was made clear for the respondents to enable them to provide the required information. The assessment was made on farmers' perceptions, stage of TSSM prevalence, infestation levels, the season of occurrence, factors favoring the pest development, management practices and severity at farmer's levels on the potato plants. Each site of the district was geo-referenced. Coordinates were mapped using Arc View 10.4 software to show the site spot of the TSSM distribution of TSSM. The meteorological data were obtained from the national meteorological agency, Jijiga stations (Table 1). During the study, potato plants were examined visually aided with magnifying glasses and the sampled leaves of the potato plant was transported to the plant protection research laboratory of Haramaya University to count the number of TSSM by stereomicroscope.

Assessment of Two Spotted Spider Mites (TSSM)

Twenty-four potato farms were selected per district to assess the distributions, infestations, incidence and damage level of two-spotted spider mites. In each potato farm, three potato leaves from ten potato plants were taken randomly to identify whether TSSM was present or absent across a farm in a zigzag manner. The specific potato plant was located by thrown quadrants. The data were recorded based on visual observations by using a simple 10x hand lens, face-to-face structured interview and observations. In a potato farm where TSSM was incident, the number of adults per leaf was counted to determine the number of TSSM per plant. The average of TSSM per potato-sampled leaves was recorded. The prevalence percentage of TSSM per district was calculated as follows

$$\text{Prevalence (\%)} = \frac{\text{Number of fields in FA in which TSSM was found}}{\text{Total number of FA (n=6)}} * 100.$$

Where: FA-Farmers association

$$\text{Incidence (PI)} = \frac{\text{Numberofinfestedplants}}{\text{Totalplantobserved}} \times 100,$$ followed by the work of Kataria and Kumar (2012).

The counted TSSM adult was used to determining the infestation, prevalence, severity and incidence levels on potato plants. The mite infestation level was classified according to the following criteria: none: no spider mites present; light: spider mites found on occasional plants, one to 10 per leaf, some leaf damage (russeting, bronzing, speckling) present; medium: 11 to 50 spider mites per leaf present on numerous plants, leaves speckled, mottled yellow or red; and heavy: more than 50 spider mites per potato plant leaves on most plants, many leaves reddish-brown in color followed the work of Steinkraus *et al*.

(2003), while the severity index was calculated followed by the work of Kataria and Kumar (2012).

Severity index (SI) = Sum of total grade points (1-5 infestation grade G-I to G-V, respectively) of the infested plants / Total number of infested plants observed. It was scored by using 1 to 5-grade level (1= no severity; 2= up to 25% severity; 3= 25-50% severity; 4= 51-75% severity and 5= higher than 75% severity).

Table 1 Means of Agro-meteorological features of districts that includes rainfall, temperature, relative humidity and altitudinal ranges in 2018/ 2019 cropping year

Parameter	Kersa	Metta	Haramaya	Kombolcha
Average Rainfall(RF)(mm)	71.72	85.58	66.58	104
Temperature (˚C)	9-26.2	8.5-25	10.8-25	10.5-23
Relative humidity (%)	15.38-41.27	14.4-44.1	14.9-42.8	13.38-44.27

Obtained from: Ethiopian Meteorological Agency, Jigjiga branch, 2018/2019

Data analysis

The number of TSSM/potato plants, prevalence, and incidence and damage levels of potato were analyzed using a three-stage nested design following the work of Kumlachew *et al*. (2016). Data were subjected to analysis of variance (ANOVA) using SAS version 9.0 software package (SAS, 2002). Means were separated using Tukey's test. Farmers' perceptions and practices were analyzed and compared by using descriptive statistics, while the relationship of weather factors with the status of TSSM was analyzed using SPSS software program version 16.0 and determination of Pearson correlation was done using SPSS statistical software.

III. RESULTS AND DISCUSSIONS

Assessment of TSSM on Potato plants

The two-spotted spider mite distribution and intensity varied across the assessed potato growing districts of eastern Hararghe Zone, Ethiopia. In all the surveyed districts, the TSSM infested the irrigated potato farms (Figure 1). The recording of TSSM on potato plants indicated various degrees of severity. All the surveyed areas showed low to high TSSM infestation levels. During the survey of TSSM, there were also free potato farms recorded in each district (Figure 2). It was highly distributed on the young opened leaves compared with the old leaves of potato-based on the observations. Additionally, two-spotted spider mite densities were higher in potato fields adjacent to dusty roads than potato fields adjacent to asphalt roads. The TSSM also thrived on a wide number of other alternate cultivated and wild host plants, mostly Solanaceae (*Datura stramonium*, *Solanum indicum*, *S. nigrum*, and *S. lycopersicum*), Malvaceae, Fabaceae, Cucurbitaceae and Asteraceae families where the agro-ecological conditions are suitable for potato growing. As far as the current distribution of the TSSM is concerned, all the potato growing kebeles in the surveyed areas were already infested. The discussants in Haramaya disclosed that they observed the infestation of their potato for the first time in 2014 and 2015. They also said that they had never ever seen such kind of problem in their potato farm before and commonly called it "*Machure*" which means pests causing diseases to potato plants. As per the discussant farmers, the TSSM was less in number during the rainy period.

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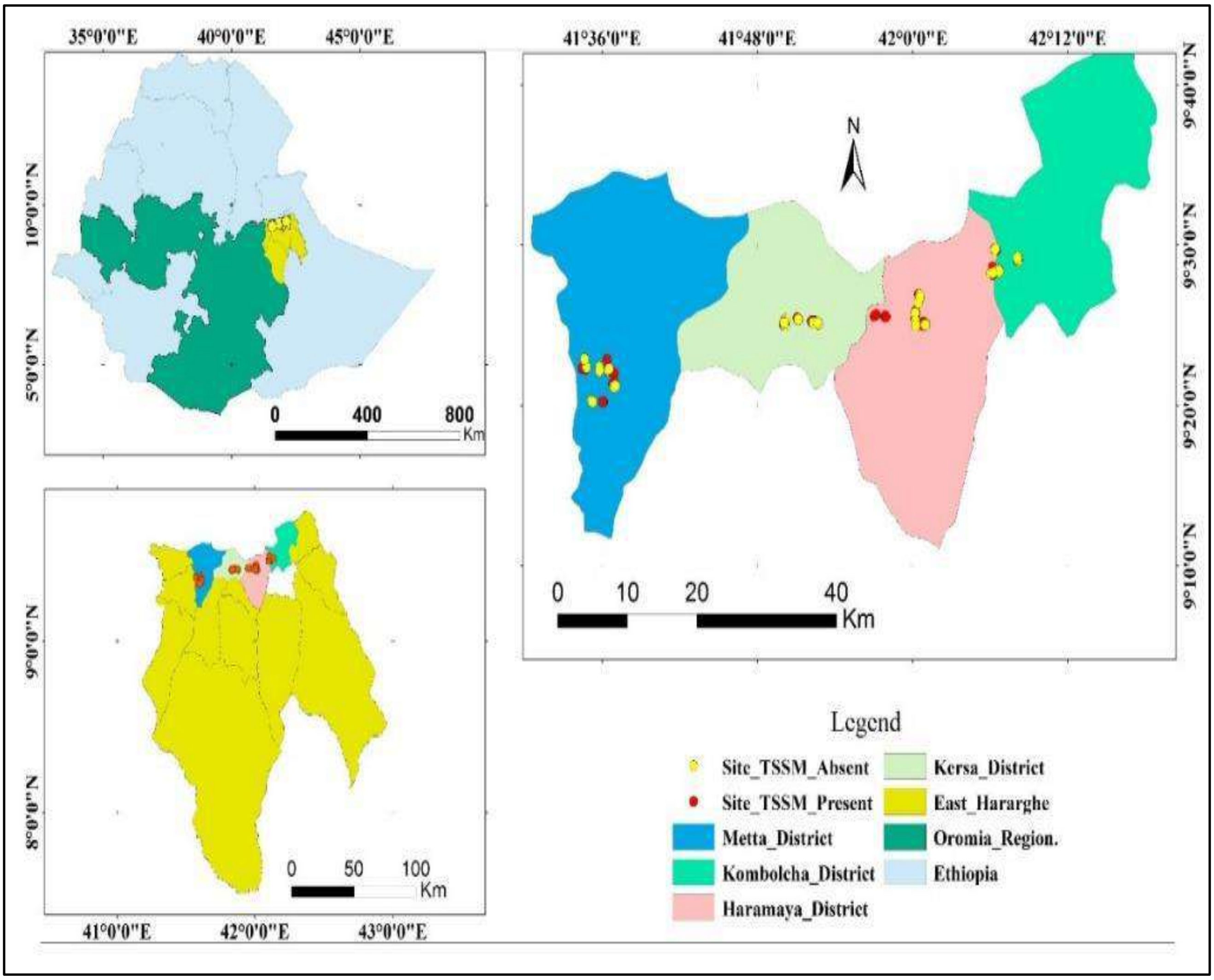

Fig.1: Map of distribution of TSSM on potato producing areas in Eastern Hararghe Zone, Ethiopia during 2018/2019 cropping season

Table 2 Severity status of TSSM in eastern part of the selected districts of Eastern Hararghe Zone, Ethiopia during 2018/2019 cropping seasons

Districts	Kebele	Severity index	Severity status
Haramaya	Ifa Oromia	3	high infestation
	Tuji Gebissa	3	high infestation
	Finkle	3	high infestation
	Damota	3	high infestation
Kersa	Ifa jelala	1	Low infestation
	Handura kosim	3	high infestation
	Meda oda	2	Medium infestation
	Burka weter	2	Medium infestation
Metta	Haro kutir-1	3	high infestation
	Haro kutir-2	2	Medium infestation
	Chelenko lola	3	high infestation
	Dursitu bilisumma	1	Low infestation
Kombolcha	Kekalli	1	Low infestation
	Egu	1	Low infestation
	Bilisuma	1	Low infestation
	Kerensa	1	Low infestation

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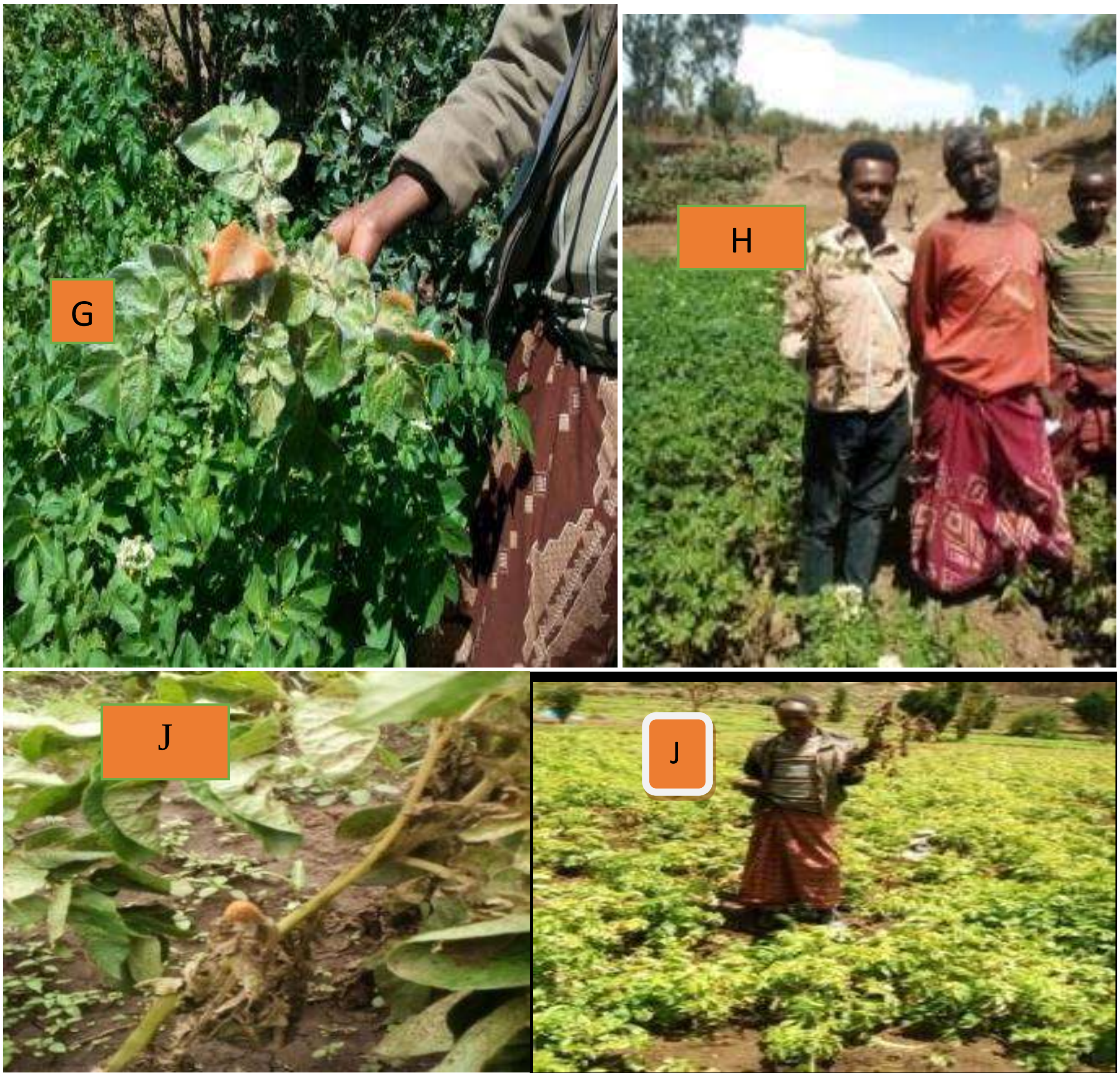

Fig.2 (A-J) Potato farms infested with TSSM in Eastern part of Hararghe Zone, Ethiopia

TSSM was highly infested and severe on potato plant in Haramaya and lower in Kombolcha districts (Table 2). Initially, the TSSM caused yellow mottling but progressively caused scarring, bronzing, and drying out of potato leaves by sucking up the cell contents (Figure 2). The feeding on potato plants is not limited to leaf but also extended to sap contents of green shoots. Thus, the plant photosynthetic ability was reduced that resulted in a decline of overall growth and its yield (Surendra, 2015).

Number of TSSM/potato plant, Prevalence, Incidence and Severity Levels

The incidence of TSSM on potato plant was significantly different across the studied districts (Tukey<0.05). The highest mean incidence level was recorded from Haramaya (37.88%/potato plant), followed by Kersa (29.6%/potato plant) and Metta (25.4%/potato plants). The least incidence level was recorded from Kombolcha districts (7.2%/potato plants) (Table 3). Similarly, the potato plant severity was significantly different among the districts. The highest mean severity of potato plant was scored from Haramaya (3%) followed by Kersa (2.4%) and Metta (2.21%). The lowest potato plant severity was observed from Kombolcha (1.4%). The highest number of TSSM/potato plants was counted from Haramaya (39%), followed by Kersa (27.8%) and Metta (24.4%). The lowest TSSM was recorded from Kombolcha (8.8%) (Table 6). The present finding was lower than the previous report of Muluken *et al*. (2016), which indicated that 80% of potato plant leaves were severely attacked by TSSM. This might be due to the environmental factors such as temperature, rainfall, and relative humidity that was not suitable for reproduction, growth, and development for TSSM in 2018/2019 cropping

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season. As the vegetative or leaf parts of the potato plants were damaged severely, the ability of its biological activity such as photosynthetic activities, uptake of nutrients and water from the soil was reduced. The severely damaged leaves became dry, drop, and covered with webbing, which was produced by protoimmatures, deutoimmatures, and adults. This resulted in a reduction of crop yield as well as aesthetic injuries (Dutta *et al*., 2012). The highest TSSM prevailed in Haramaya district, while Kombolcha district was the lowest (Table 3).

Table.3: Means of percentage of levels of TSSM per potato plants, its prevalence, incidence, severity and their number per leaves in 2018/2019 cropping season in the selected districts of eastern Hararghe, Ethiopia

Districts	Number of TSSM/potato plant	Prevalence	Incidence	Severity
Haramaya	39^{a}	9.733^{a}	37.88^{a}	3^{a}
Kersa	27.8^{b}	6.960^{ab}	29.6^{ab}	2^{ab}
Metta	24.4^{c}	6.284^{b}	25.4^{b}	2.25^{ab}
Kombolcha	8.8^{d}	2.586^{c}	7.2^{c}	1^{b}
CV	20.1	24.5	25.95	30.5
F	12.4	14.11	16.62	6.89
P	0.006	0.0003	0.0001	0.0059
Tukey`s 5%	10.1	5.23	9.45	0.52

Means with the same letter are not significantly different

Farmers Perceptions and Management Practices on Potato TSSM

Farmers perceived that TSSM prevalence followed seasonal changes in environmental factors such as drought and high temperature in potato growing areas. Most of the farmers (60%) identified and understood the TSSMs on potato plants. The majority of the farmers (70%) stated that the TSSM was first noticed in the year 2014 on wild Solanaceous plants and highly problematic and became a pest in 2015 on potato, since then it remained to be a problem on horticultural crops. Of the interviewed farmers, more than half of them were not able to manage TSSMs. Most of them also suggested many problems among which lack of resistant improved varieties and lack of chemical pesticides were the most prominent ones.

The main methods used by farmers in managing potato TSSM pests on their farms were cultural practices. Some of them also applied chemical insecticide and fungicides (figure 4). Roughing of infested plants, irrigating weekly and early planting was the cultural management strategies applied by farmers to minimize the potato yield losses by TSSM. Profit, Mitac and Dimethoate are among chemical insecticides mainly used by farmers. This application was varying across the districts that ranged by 3.5- 14% of the households. There was no combination of insecticide, other pesticides and biological management practices implemented at farmers' level in eastern Hararghe, Ethiopia (Figure 4). Early planting was the most common method used to limit the damage caused by TSSM, however, crop rotation and insecticide applications were being used but on a very low scale. On average, 41-87.5% of the potato grower farmers did not manage TSSM. The use of chemical insecticides was relatively low; being highest in Haramaya followed by Kersa districts and very low in the Metta district (figure. 4). Insecticide application was highest in districts which ranked TSSM as the main arthropod pests damaging potato severely. It was evident that some farmers implemented more than one management strategy to reduce the field infestation of potato by insect pests. Aiming at improving the effectiveness of available management methods is, therefore, desired. The low use of insecticides in potato could partly be due to inefficient inaccessibility, high cost of insecticides which subsistence farmers cannot afford, lack of knowledge about pest biology (of especially TSSM). Many farmers did not know how to manage TSSM. However, farmers who applied insecticides to manage TSSM observed reduced damage. This, therefore, encouraged farmers to apply insecticides three weeks after planting within 10 days in the presence of TSSM. Due to the severity of pest infestation during outbreaks, farmers usually received insecticides from agriculture extension workers or local authorities to spray against TSSM because farmers took the pest seriously. It should be noted, however, that the use of chemical insecticide is not a permanent solution as it can be disastrous to human health due to poor handling and elimination of natural enemies of the pest.

Nearly forty percent of the interviewed farmers also considered TSSM as potato diseases and used Ridomil and

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Mancozeb fungicides to manage it. However, economically it was not feasible, since both pests are different in response to the agricultural pesticides. Sixty percent of the interviewed farmers suggested that climate change including rainfall irregularity and increase in temperature heats caused the excessive infestation of TSSMs on potato. In addition, more than 70% of the farmers mentioned that the shortage of moisture in the soil due to the shortage of water for irrigation and increased temperature increased the rate of infestation levels. The interviewed farmers who recognized TSSM on potato plants indicated that the main cause of its occurrence was shortage of rainfall and moisture in soil.

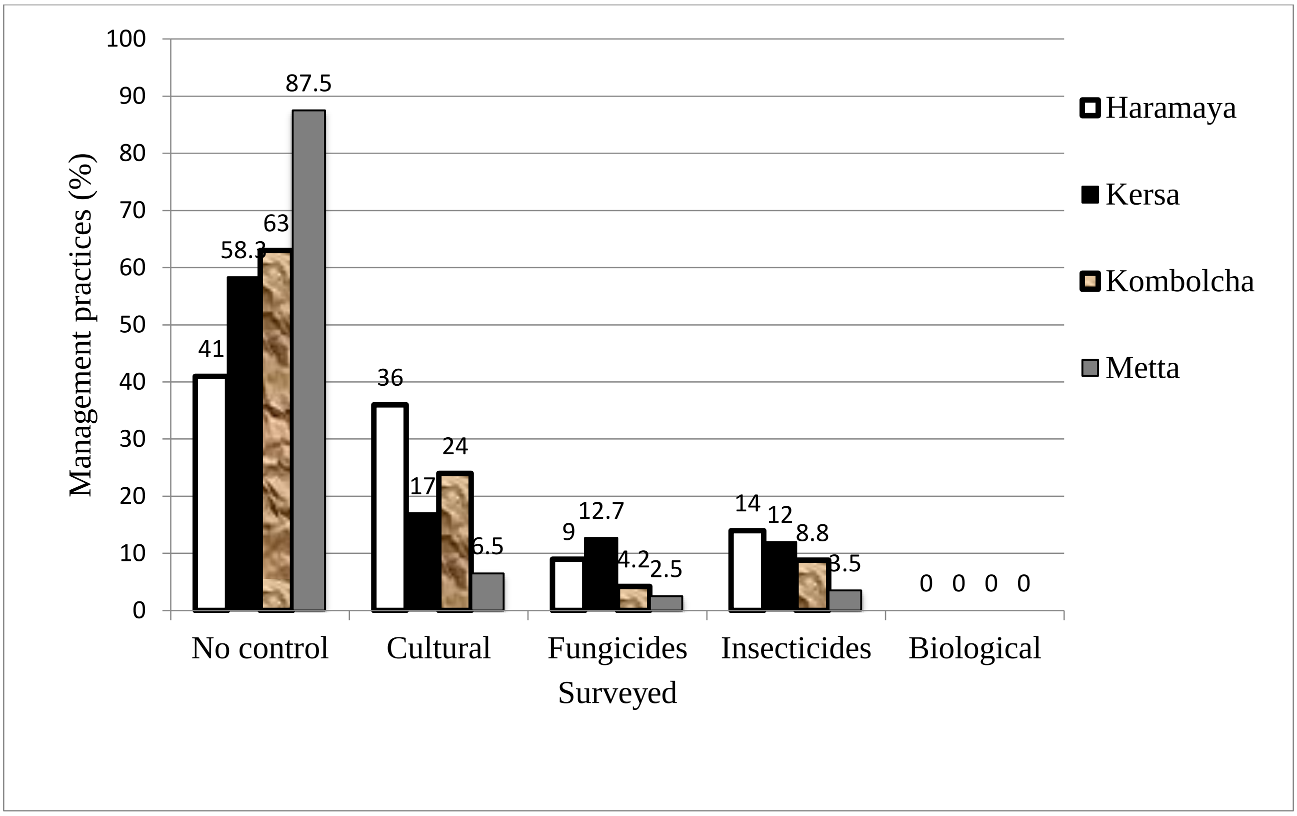

Fig.4: Farmers management practices of TSSMs in potato farm in selected districts of Eastern Hararghe

Based on the observation and response of the respondents, the TSSM mostly prevailed and affected the potato plant during flowering (44.2%) stages. The highest prevalence of TSSM on potato plant was during flowering stages, followed by mid (32.8%) and maturity (15.6%) growth stages, while the least was during seedling (7.4%) stage (Figure 5). The result was contrary to the finding of Wudil *et al*. (2016) who reported that TSSM was more attracted and preferred to the young, tender and succulent parts of the potato plants that provide more nitrogen-rich nutrition. The potatoes at the middle to flowering stages are the most vulnerable to mites' infestation. This finding agreed with Wudil *et al*. (2017) who reported that the older and the younger plant leaves had inferior nutritive value. This might be why TSSM severity was low at these stages comparatively. The middle leaves of potato hosted more mites than the upper and lower leaves when the nutrient is insufficient. Woods *et al*. (2007) also reported that the youngest, fully expanded leaves always had more spider mites than young or old leaves. The TSSMs concentrated on the younger leaves than the older ones. These are an indication of the intra and interplant distribution of TSSM to search a more concentrated nutritive young leaves (Woods *et al*., 2007).

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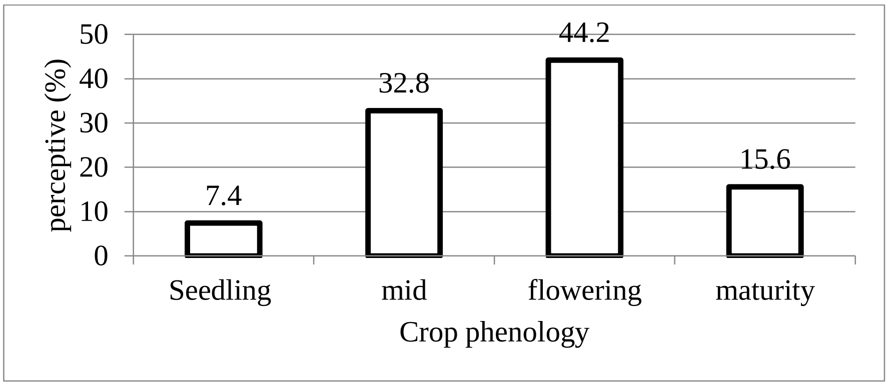

Fig.5: Stages at two spotted spider mites prevalence on potato

Based on the potato producers' perception, the TSSM intensity varied across the season of the year. The highest incident was in autumn (44.79%), followed by winter (36.46%), summer (12.5%) and spring (6.25%) (Figure 6). Its infestation level was higher on irrigated potato in winter and autumn.

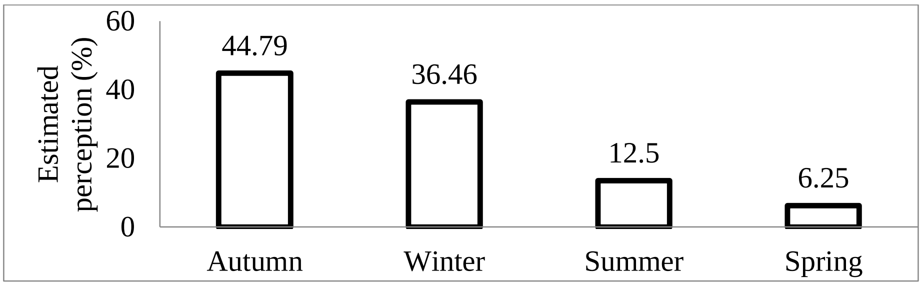

Fig.6: Proportion of farmers' perception on seasonal occurrence of T. urticae on potato plants

These findings agreed with the finding of Ismail *et al*. (2007) who reported that the TSSMs were lowest in summer than autumn and winter. Wilson and Morton (1993) also reported that populations of TSSM declined through November/December, and then increased progressively thereafter at varying times and rates. Understanding the seasonal abundance and intra-crop distribution of *T. urticae* on plant helps to develop sampling strategies for pest management. In line with this study, Shanbaky *et al*. (2016) reported that understanding the seasonal prevalence of *T. urticae*, which varied between seasons helps strongly to influence TSSM. The survival strategy of *T. urticae* appears crucial in determining the extent to which this potential is realized. *T. urticae* was more abundant on the edges of fields than in the interior in its early occurrence, which indicates colonization from an external source. The edge effect diminished with time, suggesting a lack of continuous colonization. The only exceptions to this pattern occurred when *T. urticae* migrated from other alternate sources including *D. stramonium* and *S. indicum* from nearby potato fields.

Influence of weather factors on TSSM

More than 60% of the respondents indicated that TSSMs was more abundant in February-May on the irrigated potato. The linear correlation coefficient indicated that the incidence (r=-0.546, Y=-0.548X+38.7), number of TSSM/potato plant (r=-0.847, Y=-0.06.232X+38.24) and severity level (r=-0.431, Y=-0.0221X+2.805) considerably decreased as rainfall intensity increased. This result indicated that the population of TSSM changed as the environmental conditions across seasonal factors changed including the availability of suitable host plants, high temperature, low rainfall and relative humidity in months and years (Table 4). The TSSM prevalence was intensified across eastern Ethiopia resulting in heavy potato yield losses particularly in 2014 and 2015. It was speculated that changing climate conditions increased the outbreak of TSSM, especially under drought conditions. The data demonstrated that the relationship between climate variables (temperature, relative humidity and precipitation/rainfall) and the status of TSSM during 2018/2019 was inseparable.

The development and incidence of the insect pest were much dependent upon the prevailing environmental conditions such as temperature, relative humidity and rainfall (Woiwod, 1997). The timing of pest insect attacks can vary greatly from region to region and from year to year (Collier and Finch, 2001). The data indicated that the decreasing rainfall intensity, relative humidity and increasing temperature might have contributed to increase in the level of incidence, severity, and number of TSSM on potato growing areas of eastern Hararghe (Table 4). The density of the incidence and severity levels of TSSMs on potato plant varied across the districts, which was relatively lower in Kombolcha. The TSSM population increased in drought season because of accelerated movement to adjacent plants.

Orellana (2014) reported that TSSM density increased during warmer temperatures under dryer weather conditions. If there were no favorable environmental conditions, the potato can tolerate higher spider mite densities without suffering yield loss, especially when weather conditions do not induce any stress on potato plants. This is because mites prefer to thrive in hot and dry environmental conditions. Temperature is influencing the temporal and spatial distribution of mites in the field because it strongly affects its population growth (Gotoh *et al*., 2010). It was confirmed that the TSSM population density, decreased as rainfall and relative humidity increased. The TSSM prefers, low relative humidity, high temperature and low rainfall for distribution to cause severe incidence.

Table 4 Correlation co-efficient between the TSSM and weather parameters

Environmental parameter		Correlation co-efficient	Coefficient determination	Regression equation
Average Temperature (°C)	Maximum	0.576	0.332	Y=0.475x+18.22
	Minimum	-0.671	0.451	Y=-0.001x+21.53
Average relative humidity (%)	Maximum	-0.849	0.721	Y=-0.251x+52.65
	Minimum	0.669	0.448	Y=5.353x+52.65
Average monthly rainfall (mm)	Maximum	-0.6	0.360	Y=-7.784x+62.51

The TSSM the population was higher on water-stressed than well-irrigated plants on the farmer's field based on observations. This finding indicated that environmental factors and pest populations were inseparable. This finding also agreed with previous reports that indicated increasing temperature had significant positive correlation, where relative humidity and rainfall had a significant negative correlation with two-spotted spider mite population in relativity (Manian *et al*., 2014).

The feeding and reproduction capacity of the TSSM increased under high temperature with low relative humidity (Pakyari and Enkegaard, 2012). In addition, the incubation period of *T. urticae* increased with decreasing temperature and total time taken for completing the life cycle decreased with increasing temperature (Paramjit *et al*., 2017). The current study also confirmed that the TSSM increased following the drought conditions, including increasing temperature, decreasing rainfall and relative humidity in eastern Ethiopia (Table 4). Eastern Hararghe zone is characterized by a lower amount of rainfall, low relative humidity and maximum temperature followed by the Elino in the 2014-2015 cropping year. These assumptions might lead us to imagine them as the cause of *T. urticae* outbreak in 2014 on the potato. The population density reached a peak in 2014 and 2015 and caused significant yield losses of potatoes (Muluken *et al*., 2016). The outbreak and yield loss were inseparably linked to changes in weather conditions of the zone. Drier conditions might have encouraged the survival, perpetuation and distribution of potato TSSMs. Conversely, after 2016 the population and its incidence level decreased.

Rainfall exerted its kinetic energy to strike the TSSM washing off from plant shoots and even killed them. The high relative humidity and low temperatures resulted in the delaying of the growth and development of the mite population in the rainy season (Wudil *et al*., 2016). In line with this, Wudil *et al. (*2017) and Barbar *et al*. (2006) also reported that the altitudinal differences of the agro-ecologies influence the rainfall distribution, temperature and relative humidity. The temperature, relative humidity and rainfall are important environmental factors that affect the population dynamics of arthropods in agro-ecosystems (Wudil *et al*., 2016; Prischman *et al*., 2005). Overall, when the rainfall intensity, temperature and relative humidity increased the incidence, severity levels and the number of two-spotted spider mites was negatively affected (Table 4). In line with this study, TSSMs were positively influenced by increasing temperature and negatively affected by increasing rainfall intensity (Wudil *et al*., 2017).

IV. SUMMARY AND CONCLUSIONS

T. urticae is the potato production constraint under favorable dry weather conditions. It causes devastating yield losses unless proper management techniques are developed to mitigate the problem during favorable weather conditions. This study, therefore, aimed to assess the distribution, farmers' perceptions and management practices of farmers against *T. urticae* on irrigated potato in four districts of eastern Hararghe zone (Haramaya, Kombolcha, Kersa and Metta). The attempt was also made to relate, the TSSM distribution, incidence, severity and their numbers with weather factors in the eastern Hararghe zone. The parameters decreased with the increment of rainfall, but increase as temperature increased.

Potato plant was more severely damaged during mid and flowering stages than at seedling and maturity stages. To mitigate this problem, some of the farmers practiced cultural techniques and also applied synthetic chemical pesticides, but nearly 40% of the respondents were not familiar to the pest as it is new to the environment and considered as plant diseases. The farmers need the integration of using improved resistance varieties, agrochemicals, and water supply for irrigation to tackle the red spider mite problem. Therefore, effective management should focus on understanding the right stages of the plant and the pest as well as prevailing climatic factors to prevent an outbreak. Furthermore, implementing low-cost agronomic practices and other effective technological interventions are suggested for sustainable management of the red spider mite

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Weighting of water resources vulnerability factors in the context of global environmental change in the Sebou basin: case of Fez, Ifrane and Meknes perimeters (Morocco)

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***Abstract*— *Water resources are at the center of all developments, whether it is a society or a living organism. It is rightly noted that the state of water resources around the world is no longer, what it once was because of the strong pressures resulting from global environmental change and human activities. Undertaking vulnerability assessments of water resources is a necessary and rapid need to spatially delineate the areas likely to be impacted. The main purpose of this study is to identify with water resource management experts a set of factors considered relevant, and to know the relative contribution of each factor in terms of vulnerability. To this end, the opinions of 32 water resource management experts were collected in order to define and weigh the factors of water resource vulnerability in the Sebou basin. The result of this survey shows that 25 factors divided into 5 components (hydrology, pollution, socioeconomics, environment, and eco-environment) of vulnerabilities are likely to affect the state of water resources in the area. The application of multiple factor analysis under R software for data processing to reduce dimensions has shown that 15 out of the 25 factors are the most important in terms of water resource vulnerabilities according to experts' opinion. The weights of these 15 factors and the 5 components of water resources vulnerability are different from each other, which highlights the relative nature of water resources vulnerability. This can help water managers to be more effective and relevant in water resource vulnerability analysis tools.*

***Keywords*— water resource vulnerability, Vulnerabilities, vulnerability factors, water resources, factor weighting, water resource management.**

I. INTRODUCTION

Water resources are at the center of all developments, whether it is a society or a living organism. It is accurately noted that the state of water resources around the world is no longer, what it was once. Rapid population growth, significant industrialization of societies, and global climate change are potential causes of water resource vulnerability [1](Pandey et al., 2010). Indeed, as the population and economy increase, as human-induced climate change accelerates, so does the pressure on regional water resources [2]. In a natural way, the distribution of water resources throughout the world is unevenly distributed [3], due to the physical and environmental characteristics of each locality, however the human dimension such as urbanization, and poor water management arevery important in the scarcity and vulnerability of water resources[1]. The notion of vulnerability in water resource management has a long history[1, 4]. However, Gleick [2]argues that there are many challenges to any effort to develop a water resources vulnerability assessment tool: the definition of vulnerability, the vulnerability indicators section, data availability and quality. Studies conducted around the world and in recent decades on water resource vulnerability assessments have presented several definitions of the term "vulnerability" and the definitions are still based on the environmental problems encountered, the objective of the study and the availability of data [4]. Several methods for assessing pressure on water resources

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have been developed to estimate this pressure quantitatively in the form of indices [2]. Padowski et al[3]estimate that many indices and indicators have been developed to improve understanding of water scarcity and identify areas at risk of vulnerability. Kanga et al[4]report that the methodological approaches to water resources vulnerability assessment developed in recent decades aim at a holistic conceptualization of the water resources system taking into account all components of vulnerability: these are the physical, environmental, socio-economic, institutional and governmental dimensions. It is imperative, nowadays, to recognize the links between socio-economic, cultural and institutional factors in a water resource system [5]. The assessment of water resource vulnerability depends on the context and specificities to each case study [5]. As a result, the factors used in the vulnerability assessment framework are so many and relative [4]. Winograd et al[6]argue that there is no single set of factors that is applicable in all contexts and that the selected vulnerability factors should be closely linked to the context of the study. Sonhs et al[7]have inventoried, through a qualitative study of the existing literature, the factors contributing to the vulnerability of household water in the Arctic. Kanga et al[4]also inventoried, with a systematic review of the international scientific literature, the tools (including factors) for the vulnerability of water resources at the local level. Several researchers [8, 9]have previously expressed the need to provide indicators for water resource vulnerability assessments. In Morocco, the problem of water stress is increasingly felt. Studies on the vulnerability of water resources have already been carried out [10, 11, 12, 13], but these are partial studies that focus only on the vulnerability of groundwater to pollution. The identification of water resources vulnerability factors in a participatory manner with water sector stakeholders can be a major asset in assessing the impact of different pressures on the entire water system in the area. To our current knowledge, there are no studies to assess the vulnerability of water resources in a multidimensional way of the water system in Morocco. The main purpose of this study is to identify with water resource management experts a set of factors considered relevant, and to know the relative contribution of each factor in terms of vulnerability.

II. MATERIALS AND METHODS

1. The study area

The study area is located in the large Sebou catchment area and extends over two aquifers: the Fès-Meknès aquifer and the aquifer of the Barren limestone plateau. It covers 7 provinces, including 64 municipalities, and covers an area of 5,849 Km^2. The economy is mainly based on agriculture and industry. Water resources are used for crop irrigation but also for drinking water supply to nearby cities. The use of agroinputs was already very high in the study area in 1996 and averaged 66.5% of farms. 51 potential sources of pollution are identified in the study area. Much of the study area has clayey textured soil, especially in the northwest, north and northeast. The eastern and central parts are mainly made up of sandy-clay textures. The western part of the study area consists of sandy-clay textured soils.The western part of the study area consists of sandy-silty textured soils and raw minerals. The deep aquifer of Fés-Meknès includes the highly fractureddolomitic limestone formations of the Lias. The thickness of this aquifer varies from a few metersatthe center to 760 m north of the study area. However, the water level is on average 50 m deep in the captive part of the water table and 250 m deep in the non-captive part. The nappe the Barren limestone plateau is juxtaposed with the Fès-Meknès water table and the basaltic aquifer of the Quaternary. The groundwater recharge is mainly provided by infiltration of rainfalls. Wells and boreholes are the means of exploiting groundwater in this area. Annual precipitation is highly variable. Average rainfall between 1988 and 2017 is 479 mm in the north and northeast and 800 mm in the south. The inventory of waterbodies in the study area shows some natural rivers and lakes: Fés river, Guigou river(flow rate: 0 to 54 m3/s), Boufekrane river, Tizguit river, Agay river, and Dayet (lake)Aoua. Figure 1 shows the location of the study area and its land use.

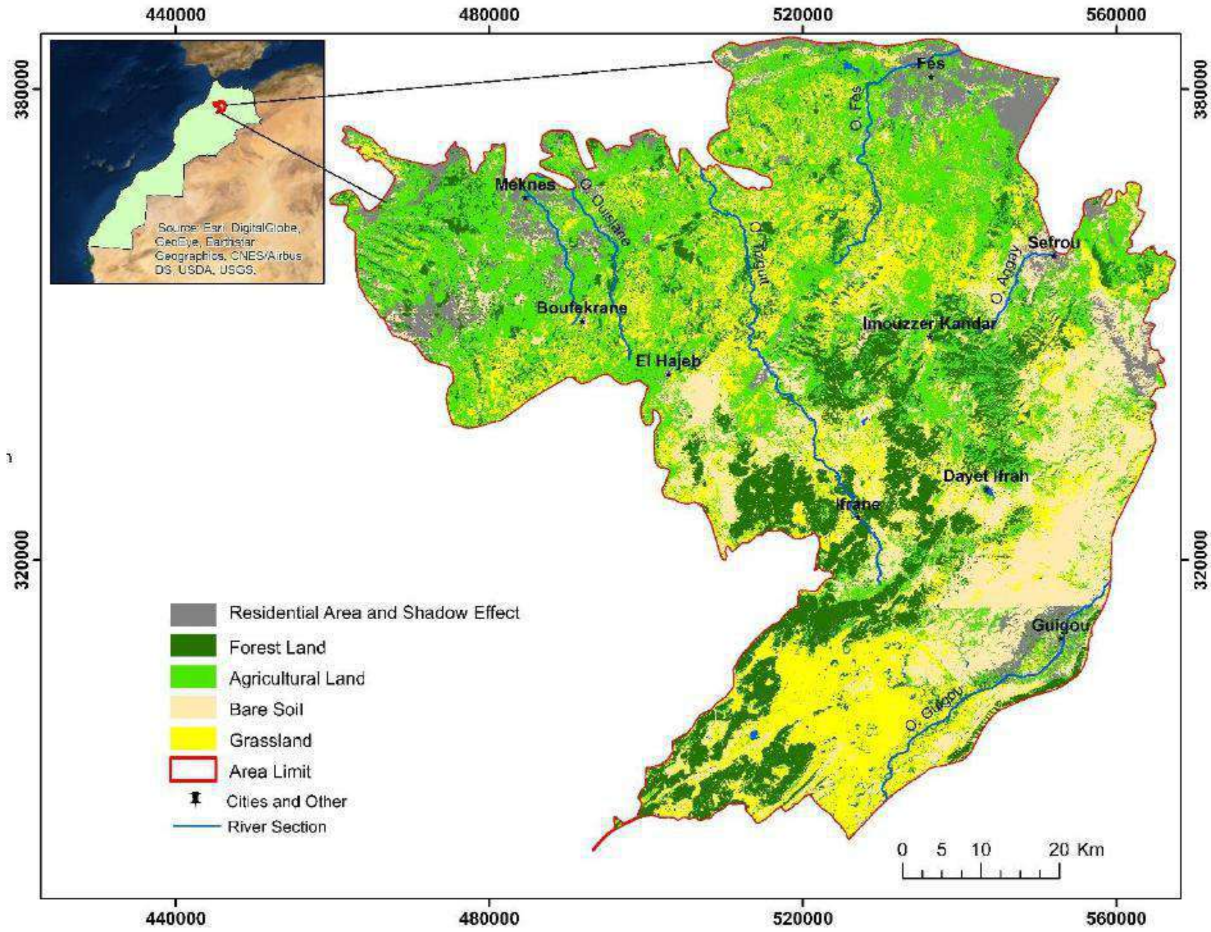

Fig.1:Study area and land use

III. METHODS

The vulnerability factors of water resources do not have the same impacts in terms of vulnerabilities. It is therefore necessary to prioritize them and assign them weights according to their relative contributions. A survey was conductedto identify vulnerability factors and to determine the relative importance of each factor. Several methods of investigating weight attributions through the survey exist in the scientific literature. Kanga et al [4] list several methods of assigning the following weights: the Delphi technique, participatory methods, the budget allocation process, public opinion, etc. The survey method used is very similar to the budget allocation process method reported by OECD[14]. It consists ofdefining the subject of the survey, then based on the definition, inventory a number of factors on a survey sheet, on which water management experts, are asked to assign weights to the various factors in terms of vulnerabilities based on the expert's personal experience. Experts are also asked to suggest other factors they consider important and to reallocate weights. A problem of the study area is summarized on the survey sheet in order to briefly present the study area for experts who are not familiar with the study area. To frame the study in relation to its objective, the notion of "water resources vulnerability" was defined.

1.1. The Survey Process

Four main steps were followed to carry out the survey: definition of the subject of the study, selection of experts, preliminary selection of factors, and assignment of weights by experts.

1.1.1. Define the subject of the study

The purpose of the survey is to assign weights to the importance of a factor in terms of the vulnerability of the water resources system. For the purpose of this survey, the vulnerability of water resources has been defined as follows: "the vulnerability of the water resources system, the degree of fragility or susceptibility with which human activities and natural factors affect water quality and quantity while taking into account society's ability to address these threats to the system in a sustainable manner".

1.1.2. Selection of experts

All water resources management institutions, universities and private firms working in the water sector were identified for the purpose of the survey. In each institution, the sheets are distributed while taking into account the expert's potential knowledge on the subject. The range of experts includes officials of the State Secretariat for Water, officials working in the private water sector, university professors, water engineers, and officials of the National Water and Electricity Office. A number of 58 experts were asked to express their opinions on the importance of vulnerability factors. Table 1 presents the category of experts who responded to the questionnaires and their numbers.

Table 1: Category of experts who participated in the survey

Categories of experts participating in the survey	Number
University Professors in the fields of water resources management	3
Engineers in water resource management working in private and publiccompanies	14
PhD students in hydrology, environmental and water management	13
Directors or heads of division of publicwater management agencies	2

1.1.3. Selection of factors

In the field of water resources vulnerability assessment, access to and availability of data is considered a challenge to which the actors involved in water management must respond. Gleick [2], Padowki et al [3] and Simha et al [5] reported that data availability is one of the major challenges in assessing water resource vulnerability. Therefore, the choice of factors was made according to whether the data of a factor can be collected or calculated over several years. Thus, 25 factors were defined in advance taking into account the objective of the study, the characteristics of the study area and how the notion of "water resource vulnerability" was defined. Appendix 1 presents the different factors, their descriptions and impacts on water. Five components of water resource vulnerability are defined in which several factors have been chosen for each component.

1.1.4. Assignment of weights by experts

To each expert addressed, it is to ask to distribute 200 points out of the 25 factors in an instinctive way and according to his personal experience in the management of water resources. Moreover, if there are other factors that the expert considers important but are not on the list, the expert is asked to incorporate them and reallocate the weights to the different factors of water resources vulnerability.

1.2. Determination of the average weights of the components of water resource vulnerability

Experts are not asked to directly weight the components of vulnerability. However, the factors were classified according to vulnerability components. To determine the weight assigned to a component, the weights of each factor constituting the vulnerability component are counted. Moreover, since the components do not have the same number of factors, the number of factors that makes up the vulnerability component divides the total weight for each component. Mathematically,

Let X_{ij} be the sum of the weights of the jth component of water resources vulnerabilities affected by the ith expert, and x_{ij} the weight of each factor j affected by the ith expert.

$$X_{ij} = \sum_{i=1,j=1}^{n} x_{ij} (1)$$

Let n_j be the number of factors containing the jth component of vulnerability,

$$\overline{X_{ij}} = \frac{\sum_{i=1,j=1}^{n} x_{ij}}{n_j} (2)$$

With $\overline{X_{ij}}$, the average weight of the jth component assigned by the ith expert.

Let Y_{ij} be the sum of the average weights of each water resource vulnerability component, then:

$$Y_{ij} = \sum_{i=1,j=1}^{n} \overline{X_{ij}} (3)$$

Let Z_{ij} be the normalized weight for each component of water resources vulnerability, with values between 0 and 1. The relative importance of each vulnerability component is calculated in percentage terms as follows:

$$Z_{ij} = \left(\frac{\overline{X_{ij}}}{Y_{ij}}\right), \text{ with } Z_{ij} \in [0, 1] \ (4)$$

1.3. Determination of the average weights of the factors of vulnerability of water resources

For each factor, the number of experts who participated in the experiment divided the sum of the weights. The values are then transformed between 0 and 1 by dividing the average for each factor by the total sum of the weights (200).

1.4. Multiple factor analysis and ascendant hierarchical clustering

The data collected were subjected to two types of multidimensional analyses. First, a multiple factor analysis (MFA) was performed, as the table of responses to the questionnaire is presented as Individuals X Groups of quantitative variables. The five groups of variables that have been constituted are as follows: Hydrology (6 variables), environmental physics (2 variables), socio-economics (10 variables), potential sources of pollution (4 variables) and eco-environment (3 variables). In addition, the variables have been reduced, in order to give them the same importance in the calculation of the MFAdimensions. At the end of the MFA, a hierarchical bottom-up classification of its results was carried out in order to see

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the profiles of the respondents. Finally, it should be noted that all these analyses were performed with the FactoMineR (version 1.34) and R (version 3.5.0) packages.

IV. RESULT AND DISCUSSION

Out of the 58 experts requested, 32 responded to the questionnaire, or 55%. This is because most experts find the survey difficult and time-consuming. The results of this study are presented in two parts: descriptive statistics of the data and the results of the multiple factor analysis.

1. Average weights of the components of water resource vulnerability

The average of each vulnerability component or factor group has been calculated to determine the relative importance of each group in terms of water resource vulnerability. Furthermore, they were transformed into values between (0 and 1). Table 2 presents the relative weight of each component of water resource vulnerability according to experts.

Table 2: Vulnerability components of water resources and their weights from the survey

Component	Hydrologic	Environment	Socioeconomic	Pollution	Eco environment
Mean Weight	8,76	7,25	7,07	8,79	8,98
Transformed Weight	0,21	0,18	0,17	0,22	0,22

The groups of factors "potential sources of pollution", the group "eco-environment" and hydrological factors have the highest values with 0.22;0.22; and 0.21 respectively. While the "physical factors of the environment" and the "socio-economic" factors presented the smallest values with 0.18 and 0.17 respectively. The values are transformed between 0 and 1 since several researchers who assessed the vulnerability of water resources in a multidimensional way transformed all the variables on this scale.In general, factor data are standardized between 0 and 1 also or between 0 and 100 by multiplying by 100. For example, Sullivan [15]ranked the final water resources vulnerability index between 0 and 100 after standardizing data on the same scale, with 0 means no vulnerability and 100 very high vulnerability, while for Plummer et al [16], the value 0 means high vulnerability and 1 no vulnerability. Pandey et al[1]and Alessa et al [17] ranked the final index of the outcome of the water resources vulnerability assessment on a scale of 0 and 1.Still in this same classification quantity for the final vulnerability of water resources, Wang and Li [18]classified the final index between 0 and 1.80 (extreme vulnerability).

2. Average weights of water resource vulnerability factors

Table 3 presents the mean, median, observed standard deviation, minimum and maximum of each variable from the survey. The largest averages are observed in the factors "Irrigated Areas (IA)", "Relative Annual Variability of Precipitation (RAVP)", "Number of Industries Discharging Wastewater (NIDW), "Population Density (PD)" and "Water Withdrawal for Industrial, Agricultural and Domestic Activities (WWA)". While the smallest averages are observed for the following factors : "Multidimensional Poverty Rate (MPR)", "Percentage of People Working in the Water Sector (PWWS)", "Illiteracy Rate (IR)", "Livestock Density (LD)", "Percentage of the Population with Access to the Toilet (PAT)" and "Net Activity Rate (NAR)". The means of the remaining variables are comparable and range from 7 to 9.

Table 3: Relative weights for each factor and its elementary statistics

Number	Factors	Mean	Median	Standard deviation	Minimum	Maximum
1	E	9,09	8,50	4,74	2,00	20,00
2	R	8,77	8,00	3,88	2,50	20,00
3	IC	8,44	8,00	4,54	3,00	20,00
4	GR	8,95	8,00	4,72	2,50	20,00
5	DC	8,59	8,00	4,61	2,00	20,00
6	RAVP	9,84	9,50	5,24	2,00	20,00
7	SWRC	7,09	7,50	2,79	1,00	15,00
8	FCR	7,38	7,50	2,70	1,00	15,00
9	PD	9,88	10,00	3,67	2,00	20,00

10	MPR	5,69	5,00	4,85	0,00	20,00
11	PWWS	4,16	5,00	2,14	0,00	10,00
12	WWA	11,88	10,00	5,01	5,00	25,00
13	LD	6,88	7,00	3,09	1,00	16,00
14	IR	5,94	5,00	4,30	0,00	20,00
15	ATW	7,31	8,00	3,87	1,00	20,00
16	AWS	7,22	8,00	3,61	1,00	17,00
17	UR	4,59	4,00	2,87	0,00	12,00
18	NAR	6,41	6,00	3,05	0,00	12,00
19	PAT	6,81	6,00	3,60	1,00	20,00
20	WEST	7,81	8,00	3,83	1,00	20,00
21	NIDW	10,38	10,00	2,98	5,00	16,00
22	NPS	8,97	10,00	2,82	3,00	15,00
23	AAL	9,53	10,00	4,28	1,00	20,00
24	IA	10,22	10,00	3,82	1,00	20,00
25	NTP	8,19	8,00	3,16	3,00	15,00

1. Evapotranspiration (E) 2. Runoff (R) 3.Infiltration Capacity (IC) 4. Groundwater Recharge (GR) 5. Dam Capacity (DC) 6. Relative Annual Variability of Precipitation (RAVP) 7. Soil Water Retention Capacity (SWRC) 8. Forest Coverage Rate (FCR) 9. Population Density (PD) 10. Multidimensional Poverty Rate (MPR) 11. Percentage of People Working in the Water Sector (PWWS) 12. Water Withdrawal for Industrial, Agricultural and Domestic Activities (WWA) 13. Livestock Density (LD) 14. Illiteracy Rate (IR) 15. Percentage of Access to Tap Water (ATW) 16. Access to Water and Sanitation (AWS) 17. Unemployment Rate (UR) 18. Net Activity Rate (NAR) 19. Percentage of the Population with Access to the Toilet (PAT) 20. Wastewater Evacuation by Septic Tank (WEST) 21. Number of Industries Discharging Wastewater (NIDW) 22. Number of Pollution Sources (NPS) 23. Areas of Agricultural Land (AAL) 24. Irrigated Areas (IA) 25. Number of Treatment Plants (NTP)

The medians are close to the averages for all factors. The standard deviations show a very large dispersion of dataforsome factors. The highest values of standard deviations are observed for"Relative Annual Variability of Precipitation (RAVP)", "Water Withdrawal for Industrial, Agricultural and Domestic Activities (WWA)", "Multidimensional Poverty Rate (MPR)", "Groundwater Recharge (GR)", "Evapotranspiration". Minimum and maximum values range from 0 to 25 for the entire dataset. Five factors have minimum values of 0 and 9 other factors have minimum values of 1; the factors having maximum values greater than or equal to 20 are 15 and are generally those with the highest standard deviation values.

3. Multiple Factor Analysis (MFA) and Cluster Analysis

3.1. Determination and analysis of retained components

The scree plot [19] was used in the investigation of the number of main components or dimensions to be used in interpreting the results of this survey. It is a graphical representation of the eigenvalues of the main components. When the curve undergoes a sudden variation at the level of a main component, and follows a continuity without significant variation, the test scree consists ofretaining the main components before the slope of the variation. Figure 2 shows the change in eigenvalues according to the main components. The red line in Figure 2 consists of applying the Kaiser criterion [20], which retains only Eigenvalues whose values are greater than 1, without losing any significant information on the source variables. The Kaiser criterion is also used to solve the problem of the number of main components to be retained [21]. The number of main components included in the multiple factor analysis is also a choice that depends on the number of variables in the survey. Bouroche and Saporta [22]assume that a first main axis that explains 45% of inertia with 11 variables is much more interesting than if the variables were 5; only the analysis of the significance of the main components and experience can define the number of main components retained. In Figure 2, the variation in the pace of the curve is observed on the fifth main component, and the application of the Kaiser criterion shows that the first 8 main components should be retained. However, the analysis of Table 4, which presents the correlations of variables with the main components, shows that only the first 4 main components show an interest for this study since beyond the fourth component, the variables no longer have a significant correlation. Therefore, only the first four main components are selected for this study and will be interpreted.

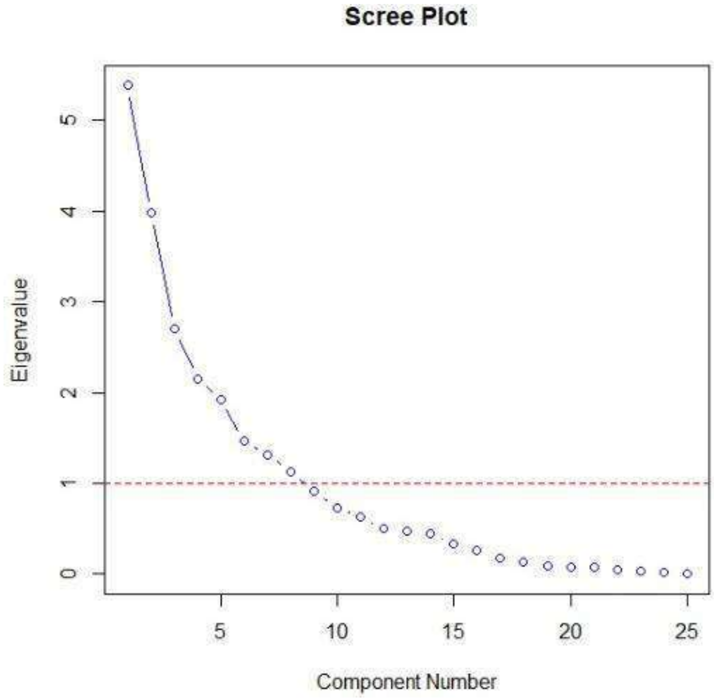

Fig.2: Scree plot for the 25 main components to be interpreted.

Table 4 shows the variation in inertia of the main components for the first 8 dimensions. The first 4 main components selected for this study explain 58.5% of the cumulative variability over the 25 main components. The other 21 main components explain only 41.85%, which means that the main components selected contain the essential information of the survey. The first 8 main components explain more than 81% of the information contained in the survey. The main plan (Dimensions 1 and 2) recovers one-third (36.5%) of the data set information.

Table 4:Eigen values of variables groups

	Dim.1	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**	**Dim.6**	**Dim.7**	**Dim.8**
Variance	2.4	1.9	1.567	1.124	0.874	0.758	0.592	0.549
% of var.	20.2	16.2	12.813	9.188	7.149	6.201	4.841	4.489
Cumulative % of var.	20.2	36.5	49.313	58.501	65.650	71.851	76.692	81.181

Table 5 presents the correlation coefficients with the main components. To facilitate the interpretation of correlations, Liu et al[23]applied the following classification: >0.75, 0.75-0.50 and 0.50-0.30 for “high”, “moderate”, “low” respectively. The factor groups of "potential sources of pollution" are strongly correlated to the first dimension, the factor groups "socio-economic" and "hydrological" are moderately correlated to the first main component. This first dimension can be interpreted as the axis of potential water pollution sources and socio-economic factors or the axis of anthropogenic vulnerability factors. On the second main component, the socio-economic factors group is highly correlated; the hydrological factors group is moderately correlated. This axis can be considered as the sensitivity factors of water resources. The group of hydrological factors is both moderately correlated with dimension 1 and 2, but does not contribute sufficiently to both axes compared to the group of factors "potential sources of pollution" and "socio-economic". The group of "environmental physics" and "eco-environment" factors are moderately correlated with the fourth main component, which can be considered as the axis of water resource vulnerability related to the physical component of the environment. The pollution factor is the one that contributes most to the formation of the first axis of the MFA, while dimensions 2 and 3 depicta high contribution from socio-economic and environmental factors.

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Table 5: Dimensions used to interpret the results.

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8
Hydro	**0.560**	**0.544**	0.127	0.053	0.303	0.063	0.030	0.147
Environment	0.414	0.386	**0.640**	0.159	0.028	0.162	0.005	0.067
Socioeconomic	**0.612**	**0.759**	0.123	**0.522**	0.281	0.240	0.362	0.210
Pollution	**0.802**	0.060	0.055	0.311	0.110	0.043	0.154	0.066
Eco-environment	0.090	0.238	**0.622**	0.078	0.152	0.250	0.040	0.058

Out of the 25 factors of vulnerability of water resources, 14 are positively and negatively correlated in a moderate way to the first two dimensions. On dimension 1, the "dam cover (DC)", "Relative Annual Variability of Precipitation (RAVP)", "Forest Coverage Rate (FCR)" factors are moderately and negatively correlated on the main level. In contrast to factors such as "Percentage of Access to Tap Water (ATW)" which are strongly and positively correlated to dimension 1, and factors such as "Percentage of the Population with Access to the Toilet (PAT)", "Wastewater Evacuation by Septic Tank (WEST)", Number of Industries Discharging Wastewater (NIDW), Number of Pollution Sources (NPS) which are moderately correlated to dimension 1. Figures 2, 3 and 4 present the graphical representations of dimensions 2, 3 and 4 with the main dimension providing the most information.

Table.6: Loadings of variables on the first eight components of data set

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8
E	-0.449	-0.110	0.322	-0.291	-0.399	-0.246	0.112	-0.191
R	-0.349	**-0.693**	-0.143	0.168	-0.125	-0.069	-0.179	-0.135
IC	-0.490	**-0.581**	0.093	-0.086	-0.415	-0.011	-0.160	0.105
GR	-0.335	**-0.731**	0.078	-0.050	-0.211	-0.012	-0.007	0.478
DC	**-0.606**	0.086	0.339	-0.045	0.411	0.111	-0.058	-0.279
RAVP	**-0.614**	-0.087	0.259	-0.098	0.457	-0.287	-0.035	0.045
SWRC	0.168	-0.296	**0.762**	0.269	0.080	0.406	0.022	-0.132
FCR	**-0.625**	**0.551**	0.262	0.297	0.147	0.003	0.071	0.226
PD	-0.135	0.323	0.031	-0.330	0.026	0.003	0.071	0.226
MPR	0.011	**0.746**	-0.105	0.301	-0.259	0.339	-0.246	0.003
PWWS	0.408	0.280	-0.239	0.225	0.355	-0.067	-0.366	0.299
WWA	0.324	0.211	-0.037	**-0.671**	0.010	0.409	0.139	0.126
LD	0.377	0.218	0.225	0.146	0.463	0.030	0.218	0.463
IR	-0.225	**0.796**	0.084	-0.232	-0.234	0.012	-0.115	-0.106
ATW	**0.800**	-0.064	0.045	0.116	-0.253	-0.132	-0.166	-0.012
AWS	0.463	0.166	0.057	**0.565**	-0.174	-0.023	0.233	-0.337
UR	-0.017	**0.575**	-0.338	0.407	-0.345	-0.050	-0.238	0.102
NAR	0.396	0.146	-0.251	0.031	0.095	-0.280	-0.363	-0.486
PAT	**0.704**	-0.223	0.293	-0.323	0.007	-0.280	-0.363	-0.486
WEST	**0.647**	0.173	0.149	-0.500	0.321	-0.170	-0.049	0.107
NIDW	**0.564**	0.564	-0.030	0.383	-0.304	-0.223	0.359	0.043
NPS	**0.609**	-0.105	0.046	0.348	0.157	-0.054	0.330	0.287
AAL	-0.037	-0.244	**-0.643**	-0.012	0.344	0.381	0.131	-0.281
IA	-0.292	-0.149	**-0.737**	-0.046	-0.145	0.412	0.177	0.132
NTP	-0.252	**-0.562**	-0.290	0.358	0.340	-0.321	-0.138	0.015

On dimension 2, "Runoff (R)", Infiltration Capacity (IC), Groundwater Recharge (GR), Number of Treatment Plants (NTP) factors are moderately and negatively correlated while the "Illiteracy Rate (IR)" factor is strongly correlated with this dimension. In contrast, factors such as "Multidimensional Poverty Rate (MPR)", "Unemployment Rate (UR)" which are positively and moderately correlated on dimension 2 (Table 6 and Figure 2). Dimension 3 is mainly explained by the Soil Water Retention Capacity (SWRC) factor with a strong and positive correlation coefficient on the one hand, and the Areas of Agricultural Land (AAL) and Irrigated Areas (IA) factors with moderate correlations on the other hand. Dimension 4 is mainly explained by the factor "Water Withdrawal for Industrial, Agricultural and Domestic Activities (WWA)" which has a negative and moderate correlation coefficient, and the factor "Access to Water and Sanitation (AWS)" which is moderately and positively correlated to this main component.

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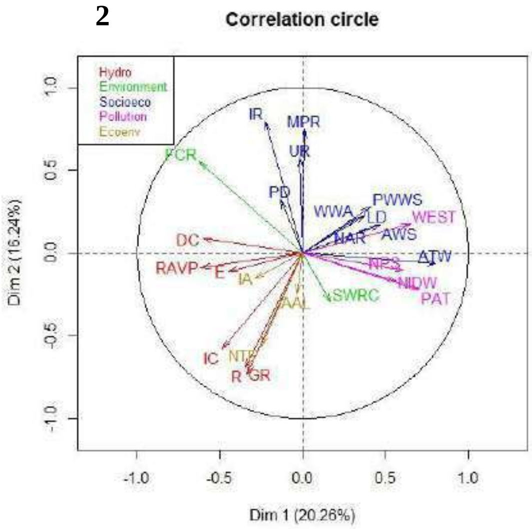

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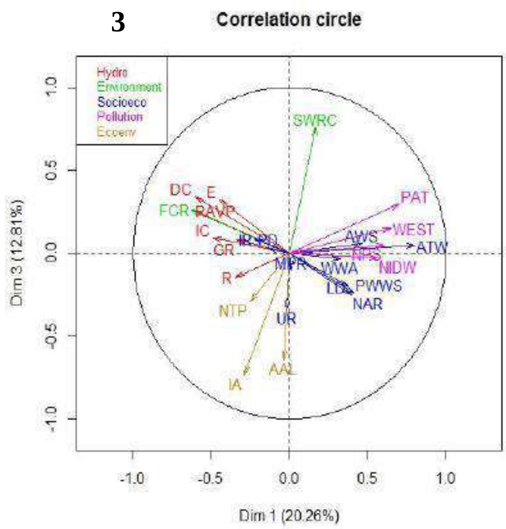

Fig.2 and 3: Representation of variables on dimensions 1, 2 and 3

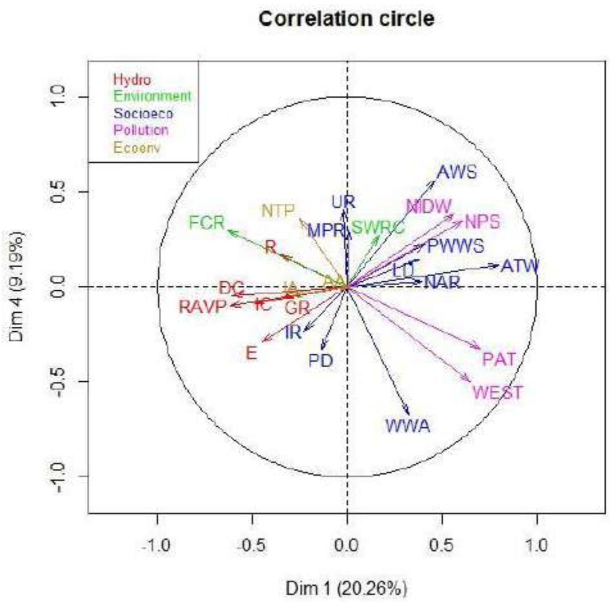

Fig.4: Representation of variables on dimension 1 and 4.

The projection of data in Table 6 on correlation circles (Figures 2, 3 and 4) shows opposite directions and senses for some factors along the dimensions. This opposition can be explained by the allocation of weights by experts. Indeed, multiple factor analysis makes it possible to establish linear relationships between variables. When an expert considers a factor very important, it would result in a depreciation of the weight of one or more factors. In any case, to assess the quality of representation on the correlation circle, the absolute value of its correlation coefficient is considered. Thus, out of the 25 vulnerability factors of water resources analyzed, 20 participate strongly to moderately in the formation of the different dimensions of the multiple factor analysis of this study (Table 6).

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Based on the classification criteria of Liu et al[23], it can be seen that there has been no effective reduction in factors. To reduce factors whose contributions are not as important, only factors with a correlation coefficient greater than or equal to 0.60 are considered. Table 7 presents the different groups of factors and the factors considered important with their transformed weights. Only 15 of the 25 source factors show some relative importance for experts in a holistic assessment of water resource vulnerability in this study. Kanga et al [4] found that on average 16 factors are used in integrated water resource assessments.

Table 7: Selected factors with their transformed weights after the application of MFA

Factor	Group	Correlation Coefficient	Factor weight	Group weight
DC	Hydrologic	-0.606	0,043	0,21
RAVP	Hydrologic	-0.614	0,049	0,21
FCR	Environment	-0.625	0,037	0,18
ATW	Socioeconomic	0.800	0,037	0,17
PAT	Pollution	0.704	0,034	0,22
WEST	Pollution	0.647	0,039	0,22
NPS	Pollution	0.609	0,045	0,22
IR	Socioeconomic	0.796	0,030	0,17
MPR	Socioeconomic	0.746	0,028	0,17
GR	Hydrologic	-0.731	0,059	0,21
R	Hydrologic	-0.693	0,044	0,21
SWRC	Environment	0.762	0,035	0,18
WWA	Socioeconomic	-0.671	0,059	0,17
AAL	Eco environment	-0.643	0,048	0,22
IA	Eco environment	-0.737	0,051	0,22

Each of the 5 factor groups presented at least one factor at the end of the correlation analysis. For the "hydrologic" group, these are Dam Capacity (DC), Relative Annual Variability of Precipitation (RAVP), Runoff (R), and Groundwater Recharge (GR). For the environmental physics group, the following factors are considered: Forest Coverage Rate (FCR), Soil Water Retention Capacity (SWRC). For the group of potential pollution sources, 3 factors are retained: Percentage of the Population with Access to the Toilet (PAT), Wastewater Evacuation by Septic Tank (WEST), Number of Pollution Sources (NPS). The socio-economic factor group is represented by Percentage of Access to Tap Water (ATW), Multidimensional Poverty Rate (MPR), Water Withdrawal for Industrial, Agricultural and Domestic Activities (WWA), and Illiteracy Rate (IR). Finally, the group of eco-environmental factors is presented by the following factors: Areas of Agricultural Land (AAL), Irrigated Areas (IA). The assessment of water resource vulnerability using several groups of factors is increasingly being used around the world. The former concept of water resource vulnerability assessment was based on the vulnerability of groundwater to pollution [24]. It should be noted that since the 2000s, the conceptualization of water resource vulnerability has taken on a new connotation by focusing on all natural and anthropogenic factors that are likely to directly or indirectly induce negative impacts on water. Kanga et al[4]reviewed the scientific literature on locally used water resource vulnerability tools, and found that 62.7% of the tools assess vulnerability in an integrated manner using physical factors, socio-economic factors, environmental and eco-environmental factors, institutional and government factors. Plummer et al [16] used 5dimensions of vulnerability for the integrated assessment of water resources vulnerability at the community level: the "water resources" (with all hydrological and qualitative factors), the "other physical environmental" (climate change, pressures on the environment, etc.), the "economic" dimension (factors related to the economy), the "institutions" (governance, conflicts, politics), and the "social" (culture, knowledge, etc.). The classification of factors into one or another vulnerability component (with the exception of the most obvious factors) is also relative and related to the researcher's understanding of the factors. Several researchers [3, 7, 8, 15, 16, 17, 25, 26, 27] have conceptualized water resource vulnerability with physical components (hydrology, hydrogeology, etc.).), socio-economic, environmental or eco-environmental, infrastructure, institutional and governance, etc., but the factors that describe these components are not necessarily the same. Water resource vulnerability assessment is seen by several researchers [15, 16,17,18,24, 28]as a relative

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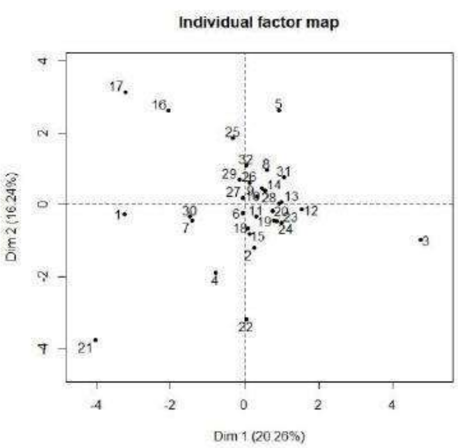
and often subjective notion in which each study seeks to deal with the environmental problem in its own study area. The number of factors to be taken into account is very often limited due to the availability and access of data. Many researchers [2, 3, 29]have previously pointed to data availability and quality as one of the challenges in assessing water resource vulnerability.

3.2. Studies of experts' opinions on factors and factor groups

To analyze the experts' opinions, the study of links and similarities or proximities between the experts was carried out. In order to better define the inter-expert opinion typology, an ascendant hierarchical clustering on the results of the MFAwas produced as well as its graphical representation in 3-dimensions. Figures 5, 6 and 7 show the dendrogram, the map of representation of individuals (2-dimensions), and the 3-dimensional distribution of groups of individuals on the main plane, according to their opinion classes. The analysis in Figure 5 shows that the majority of individuals are condensed towards the origin of the main plan. This means that the opinions of the majority of experts are close. However, some individuals are dispersed in the plan, and show that there is a heterogeneity in the opinions of experts. Figure 6 shows the dendrogram of the relationship between expert opinions. Five classes are distinguished and can be interpreted as 5 groups of opinions within experts on the factors of vulnerability of water resources. The 3-dimensional representation shows that classes 3 and 4 are more or less the closest groups of individuals among the 5 classes, and are the groups that contain most individuals. The diversity of expert opinions is not surprising. Indeed, each of the experts gives his point of view ona given factor according to his personal experience and in an instinctive way. In addition, in conceptualizing the vulnerability of water resources, the factors for assessing this vulnerability cannot be considered with the same importance. The choice of factors and their relative importance is always subjective even if they are related to the objective of the study, since from a perspective outside the study area; the environmental problem encountered may not be the same. In any case, there is no universal set of indicators that is suitable for all contexts; thus, the selection and weighting of indicators must be closely linked to the objectives [6]. IPCC[30]reports that many specialists in several fields have developed conceptual frameworks for vulnerability according to their areas of intervention based on the objectives to be achieved.

5



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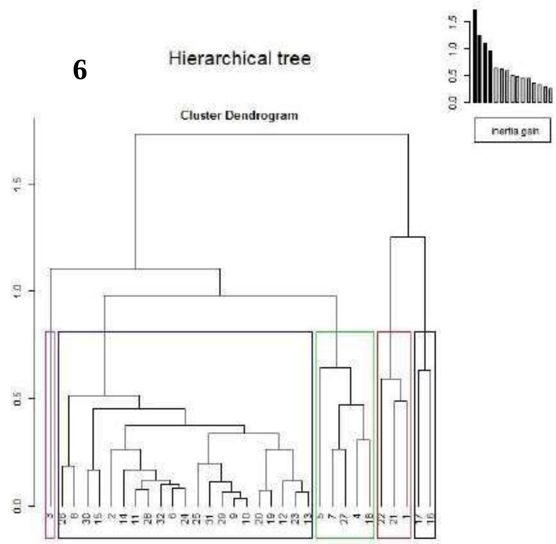

Fig.5 and 6: Representations of individuals on the main plane and dendrogram of the 5 classes of individuals.

Figure 7 shows the distribution of individuals in a 3-dimensional space in order to better visualize the proximities between individuals and between different classes. Class 3 and 4 are the closest in terms of opinions and contain more than 81% of individuals. Class 5 is the one whose opinion does not correspond to any of the classes and contains only one individual.

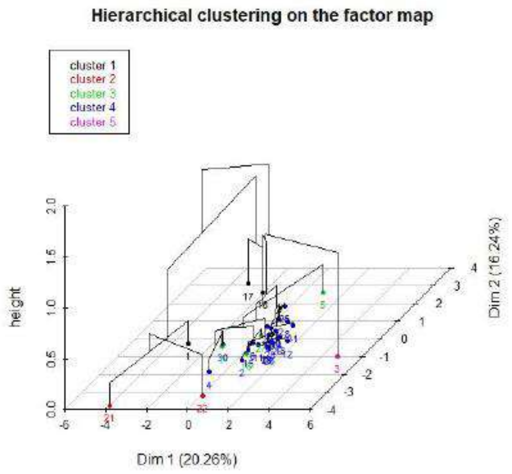

Fig.7: Representation of clusters in 3-D on the main plane

Table 8 presents the link between the 5 classes and the vulnerability factors, and examines the different opinions on the factors and their influences in terms of water resource vulnerabilities according to experts. With reference to the dendrogram (Hierarchical clustering), five classes of expert opinions are observed. Table 8 summarizes the characteristics of the classes of individuals with respect to factors and factor groups. Thus, the classes are summarized as follows:

Class 1: represents 9% of experts. It is the class whose individuals have the most diversified opinion in the sense that all five groups of factors studied are present, and is the class with the most factors. These experts strongly believe that environmental and hydrological factors affect vulnerability where eco-environmental and pollution factors play only a minor role. However, these same individuals have a mixed view of socio-economic factors. Thus, there are socio-economic variables NAR and ATW that do not affect vulnerability in the opinion of these expert groups, while the PD factor significantly affects vulnerability.

Class 2: represents 6% of expert individuals. They believe that hydrological and environmental factors lead to a high vulnerability of water resources, while socio-economic factors play a minimal role.

Class 3: represents 13% of expert individuals. For the later, only three factors significantly contribute to the vulnerability of water resources. These are the variables IC (Eco-environment), WWA (Socioeconomic) and SWRC (Environment). Thus, for them, the variables IC and WWA significantly affect the vulnerability of water resources while SWRC plays a minimal role.

Class 4: represents 69% of individuals. They believe that socio-economic and polluting factors induce a high vulnerability of water resources while hydrological factors play a minimal role, and these two groups of factors have already explained the majority of the contribution of the first two dimensions.

Class 5: 3% of the interviewees, or one individual. The later has the most diversified opinion (i.e., the type of factors) after those of class 1. Thus, for this expert, socio-economic and pollution factors significantly affect the vulnerability of water resources, while environmental and eco-environmental factors hardly affect it.

Table 8: Link between the cluster variable and the quantitative variables

Class	Variables	Groups	Influence	Class	Variables	Groups	Influence
1	FCR	Environment	+++	3	IA	Eco-environment	+++
	DC	Hydrology	+++		WWA	Eco-environment	+++
	IR	Hydrology	+++		SWRC	Environment	---
	RAVP	Hydrology	+++	4	NPS	Pollution	+++
	PD	Socioeconomic	+++		NAR	Socioeconomic	+++
	E	Hydrology	+++		LD	Socioeconomic	+++
	NAR	Socioeconomic	---		AWS	Socioeconomic	+++

	NIDW	Pollution	---		NIDW	Pollution	+++
	AAL	Eco-environment	---		WWA	Socioeconomic	---
	NPS	Pollution	---		GR	Hydrology	---
	ATW	Socioeconomic	---		IC	Hydrology	---
2	IC	Hydrology	+++	5	E	Hydrology	---
	GR	Hydrology	+++		PAT	Pollution	+++
	RAVP	Hydrology	+++		ATW	Socioeconomic	+++
	SWRC	Environment	+++		WEST	Pollution	+++
	LD	Socioeconomic	---		FCR	Environment	---
	NAR	Socioeconomic	---		IA	Eco-environment	---
	PD	Socioeconomic	---				
	UR	Socioeconomic	---				
	PWWS	Socioeconomic	---				

+++: influence; ---: non influence

V. CONCLUSION

The assessment of the vulnerability of water resources in a multidimensional way is increasingly being used around the world. The investigation to define the different components with their factors or indicators of this vulnerability, and their hierarchies is important for water resource modelers or managers. This research on the choice of factors and especially their weightings has shown that the notion of vulnerability of water resources to a relative connotation because experts do not consider the factors with the same importance in a given area. It is always very difficult when conceptualizing the vulnerability of water resources to define the factors at stake because there are several factors that can influence this vulnerability in one way or another. Again, even if these factors are known, it is conceivable that they do not have the same importance in the impact they can have on water resources. Therefore, survey methods to collect opinions on differences and their weights can be a major asset for water sector stakeholders. It is clear that the information from opinion surveys is subjective, but is very necessary because even other methods such as statistical methods to weight factors can also be subjective because the weights from these operations are linked to sourcedata that have a well-defined time scale. These vulnerabilities, defined and weighted in this way, are of great value in this context of global change in the study area, and perhaps elsewhere. It should be noted that the availability of data on these factors might limit the assessment of water resource vulnerability. Therefore, awareness raising and the creation of accessible databases on water resources in Morocco can help water managers be more effective and relevant in water resources vulnerability analysis tools.

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The Effect of Neem Leaves Powder (*Azadiractha Indica A. Juss)* and Storage Time to Rejected Corn Quality

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***Abstract*— *This research has aimed to determine the level of neem leaves powder (Azadirachta indica A. Juss) and the optimal storage time of rejected corn to decrease aflatoxin content, percentage of water content, and percentage of moldy seeds. This research uses a completely randomized experimental design (CRD) 6 x 4 factorial with three replications. Where factor A is the level of neem leaves powder (0, 0.5%, 1%, 1.5%, 2%, 2.5%) and factor B is the storage time (2, 4, 6 and 8 weeks). The analysis showed that there was no interaction (P> 0.05) between factor A and factor B to the content of aflatoxin, percentage of water content and percentage of moldy seeds, but respectively for factor A and factor B had a very significant effect (P <0.01) against aflatoxin content and percentage of water content. For a percentage of moldy seed, only factor B had a very significant effect (P <0.01). The results of the study can be concluding that the selected treatment found on neem leaves powder level of 1% with four weeks of storage time (A3B2). In this condition, a decrease in the aflatoxin content of rejected corn from 170 ppb to 53 ppb (a decrease of 68.43%) with a water content of 14.47% and 0% moldy seeds.*

***Keywords*— Aflatoxin, Neem leaves, Rejected corn, Storage time.**

I. INTRODUCTION

Corn is one of the most widely used agricultural commodities as raw material for animal feed, especially poultry. In 2018 Indonesia's corn production was estimated to reach 30,056 million tons of dry corn; this is higher than the production in 2017, only around 28.924 million tons of dry corn (ARAM I, BPS 2018). An increase in corn production from year to year.

Often the increase in corn production is not matched by proper post-harvest handling and the length of the distribution chain from farmers to collectors resulting in corn being easily damaged. Storage is one of the most critical post-harvest handling chains, with storage of corn availability being continuous. Storage must pay attention to factors such as corn water content, the relative humidity of the air, the temperature in the storage warehouse and the layout of the pile to avoid warehouse pests and fungus contamination

This contamination opportunity is quite significant because the tropical climate in Indonesia, which has high humidity and environmental temperature, is very supportive of the growth and development of mycotoxin-producing fungi (Rachmawati *et al*., 2004). Damage at this storage level will cause a decrease in the quality of corn, both qualitatively and quantitatively; this will affect the selling power of the corn.

Fungi that often contaminate corn during storage are fungi *Aspergillus sp.*, specifically *Aspergillus flavus* and *Aspergillus parasiticus*. Both these funagi produce mycotoxins in the form of aflatoxins. Aflatoxin is a secondary metabolite of *Aspergillus flavus* and *Aspergillus parasiticus.* A survey conducted by the UGM Faculty of Agricultural Technology in collaboration with the East Java Food Security Agency by taking 84 corn samples at the farmer level and 55 at the collector level found that 30% of corn at the farm level was contaminated by aflatoxin above two ppb and 10% was contamination with aflatoxin above 100 ppb with the highest value of 470 ppb. At the level of traders, 45% of aflatoxin polluted corn is above 20 ppb, while those above 100 ppb are 18% (Rahayu, 2010). Aflatoxin contamination in feed can reduce the bodyweight of broiler chickens (Al-Shawabkeh *et al*., 2009).

Aflatoxins consist of 4 types, namely aflatoxin B1, B2 G1, and G2. Research conducted by Bahri *et al*., (1994), on broiler feed, states that the most prevalent mycotoxin is aflatoxin, especially aflatoxin B1. Aflatoxin B1 is the most dangerous toxic compound and is categorization as an IA group carcinogenic compound. Chronic effects of AFB1 poisoning can cause a significant decrease in the body weight of broilers in feeding containing AFB1 200 ng / g for eight weeks. Supported by Muthiah *et al*., (1998) that AFB1 causes livestock health problems such as stunted growth and death so that livestock production decreases.

The results of mycotoxin experiment on local corn (Java, North Sumatra, Lampung, and South Sulawesi) and imports (USA and Argentina) from various feed mills in Indonesia that were tested by ELISA showed that AFB1 was detected in the concentration range of 19.1 - 87.4ng / g (Tangendjaja *et al*., 2008). Thus, the above conditions conflict with the SNI regarding the required aflatoxin content limit of 50 ng/g for feed (SNI, 2000). Aflatoxin will accumulate into the body tissues of livestock that consume, resulting in the presence of residues in livestock products that will endanger the health of consumers. Paulin *et al*. (2011) state that aflatoxin is also dangerous for the body because it can cause aflatoxin, which can be the forerunner to hepatitis B and liver cancer. Considering the effects that can be caused by contamination of food by aflatoxins are quite detrimental; thus, it is necessary to inhibit the biosynthesis of aflatoxin in corn in order to create food safety from animal origin.

One alternative in inhibiting aflatoxin biosynthesis is by giving neem leaves (*Azadirachta indica A. Juss*). Neem leaves (*Azadirachta indica A. Juss*) are known as natural anti-fungi and biopesticides. The use of natural biopesticides derived from plants is very safe to use because it can biodegrade, does not damage the environment with minimal side effects. Neem leaves extract reported to contain active ingredients azadirachtin, solanine, melantriol, and nimbin, which function as pesticides (Tjahjani and Rahayu, 2003). Compounds contained in neem leaves such as azadirachtin, solanine, nimbin, and nimbidin, where these compounds function as cell growth intruders that can result in fungal cell death (Syamsudin, 2007).

Bhatnagar and McCormick, (1988) reported that a mixture of neem leaf extract with a buffer of 0.01 potassium phosphate had shown to inhibit biosynthesis and aflatoxin production by *Aspergillus flavus* in vitro without affecting fungal growth. Research conducted by Bhuiyan *et al*., (2013) reported that the addition of neem leaf powder (*Azadirachta indica A. Juss*) by 20 g in 1 kg each of rice, corn, and wheat which were then stored for six months did not show aflatoxin contamination except in maize. However, the level of contamination in corn is also deficient at 0.8-3.2 ug/ kg. Extracts obtained from neem leaf and seed extracts can inhibit aflatoxin biosynthesis and inhibit fungal growth (Abyaneh *et al*., 2005; Allameh *et al*., 2002). Apart from being an antifungal and natural biopesticide, the addition of neem leaf powder (*Azadirachta indica A. Juss*) to broiler feed by 1, 2, 3 g/kg can increase the average body weight (Wankar *et al*., 2009).

With this problem, many corn farmers have sorted/rejected by feed mills. This resulted in maize not being utilized, and farmers suffered losses. Based on the above problems, it is necessary to do a research to find out the effectiveness of giving neem leaves to the rejected corn/mill sort in reducing the level of aflatoxin contamination so that the rejected corn can be reuse.

II. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were rejected/sorted corn from PT. Japfa Comfeed Indonesia Padang with 15% water content and 170 ppb aflatoxin content of 57.600 g and neem leaves powder as much as 720 g. The equipment used is an oven, a sifter, a blender, an analytical balance, a plastic bag, and a woven bag.

2.2. Method

The design used is a Completely Randomized Design (CRD) Factorial 6x4 with three replications. Factor A is the level of neem leaves powder consisting of 6 (six) levels, namely:

A1 : control (without neem leaves powder)
A2 : neem leaves powder 0,5 %
A3 : neem leaves powder 1 %
A4 : neem leaves powder 1,5%
A5 : neem leaves powder 2%
A6 : neem leaves powder 2,5%

Factor B is the storage time of corn consists of 4 (four) levels, namely:

B1 : 2 weeks
B2 : 4 weeks
B3 : 6 weeks
B4 : 8 weeks

Data analysis uses statistical analysis with an analysis of variance by the Completely Randomized Design (CRD) 6x4 factorial with three replications. If there are treatment differences, then the differences between treatments are tested with Duncan's Multiple Range Test / DMRT (Steel and Torrie, 1995).

2.3. Research Implementation

The neem leaves are dried in the sun or oven at a temperature of 600 C so that the water content is reduced, and then the neem leaves are grinding with a blender. Then filtering is done using a sieve.

Each treatment consisted of 800 grams of corn added with various levels of administration of neem leaf powder (0.5%, 1%, 1.5% 2%, 2.5%). The rejected corn is put into a container and then sprinkled with neem leaves powder according to the treatment until both are homogeneous. The rejected corn is put into a woven bag measuring 30 x 40 cm — storage at room temperature for 2, 4, 6, and 8 weeks.

2.4. Observed variables

1. Qualitative Calculation of Corn Contaminated Aflatoxin (UV light).

Observation of corn is done visually using a device equipped with UV light with a wavelength of 360 nanometers. As much as 800 grams of corn mashed with a grinding machine and placed in the tray then leveled. For the calculation of aflatoxin content, corn is brought to the darkroom then the tool is turned on. The tray containing refined corn is place under UV light, and the aflatoxin content is calculated.

2. Percentage of water content

To calculate the water content in corn can use the Mosture Tester Kett PM410 tool. Prepare a sample of corn each treatment as much as 100 g/replicate, then put in the tool evenly. The tool will work automatically, and on the monitor screen, the results show the moisture content in corn, and three repetitions are done.

3. Percentage of mouldy seeds

Moldy seed observations made visually with the sense of sight. The formula can calculate the percentage of moldy seeds::

$$\frac{\text{Weight of corn (week)} - \text{weight of whole seeds}}{\text{weight of corn during storage (week)}} \times 100\%$$

III. RESULT AND DISCUSSION

3.1. The effect neem leaves powder (*Azadirachta indica* A. Juss) and storage time to the aflatoxin content (ppb) of rejected corn.

The average aflatoxin (ppb) content of the rejected corn added by neem leaves powder and different storage times can be seen in Table 1.

Statistical analysis showed that there was no interaction between factor A (level of neem leaves powder) and factor B (storage time) to the content of aflatoxin (ppb) on rejected corn, but respectively for factor A and factor B had a very significant effect (P <0.01) of the aflatoxin content of rejected corn where the initial content of aflatoxin before the addition of neem leaves powder is 170 ppb.

Table 1. The average of aflatoxin content (ppb).

Level (%)	Storage time (week)				Average
	B1 (2)	B2 (4)	B3 (6)	B4 (8)	
A1 (0)	172,00	188,00	191,00	225,67	194,17[a]
A2 (0,5)	150,00	137,00	156,67	170,00	153,42[b]
A3 (1)	138,67	53,67	133,33	136,67	115,58[c]
A4 (1,5)	148,00	105,67	129,67	143,67	131,75[c]
A5 (2)	137,33	89,33	146,00	145,00	129,42[c]
A6 (2,5)	159,33	109,00	129,33	132,67	132,58[c]
Average	150,89[a]	113,78[b]	147,67[a]	158,94[a]	

Based on the results of the DMRT test (Duncan Multiple Range Test) on factor A showed that corn without neem leaves powder (control) the aflatoxin content was significantly (P <0.05) higher than all treatments, while the addition of neem leaves powder level is 0.5% (A2) the aflatoxin content was significantly (P<0.05) lower than adding 0% (control) but significantly (P <0.05) higher than adding 1-2.5% level of neem leaves powder (A3, A4, A5 A6). The addition of level 1- 2.5% (A3, A4, A5 A6) had a significantly different effect (P> 0.05) on the aflatoxin content. In factor B, the storage duration of 2, 6, and 8 weeks (B1, B3, and B4) were significantly (P <0.05) higher than the aflatoxin content of 4 weeks (B2).

In factor A, the highest aflatoxin content is found in corn without neem leaves powder (control / A1), which is then followed by a level of 0.5% (A2) neem leaves powder. Where there is no inhibition in the growth of *Aspergillus flavus* in producing aflatoxin in corn without neem leaves powder (control). Another case with the addition of 0.5% neem leaves powder (A2), in this treatment the level of neem leaves powder is relatively smaller among other levels, this causes no maximum inhibition of aflatoxin production.

At the level of neem leaves powder of 1-2.5% (A3, A4, A5, A6) low aflatoxin content can be caused by the ability of neem leaves to inhibit aflatoxin biosynthesis where the more doses are given, the higher the percentage of inhibitory power to aflatoxin production which was marked by a decrease in aflatoxin levels during the study. This is caused by the presence of active compounds contained in neem leaves. The active compounds in neem leaves extract do not inhibit the ability of fungal growth but interfere with the production of aflatoxin, wherein inhibition of aflatoxin production is associated with morphological changes in fungi (Abyaneh et al., 2005).

The addition of neem leaves powder in this study is believed to reduce the aflatoxin content in corn. This is supported by previous studies, among others conducted by Abyaneh *et al*., (2005) showing that the addition of neem leaves extract solution above 10% can effectively inhibit the production of aflatoxins from *Aspergillus flavus*. Furthermore, Ariwardhani's research (2008) showed that the influence of neem leaves extract was able to inhibit the production of aflatoxin as evidenced by the decrease in the content of aflatoxin on GAN (Glucose Ammonium Nitrate) media that had been inoculated with *Aspergillus flavus*. The effect of neem leaves extract on aflatoxin biosynthesis is related to morphological changes in the mycelium of the fungus *Aspergillus flavus*. Furthermore, the results of research by Zeringue Jr. and Bhatnagar (1994) showed that after incubation for three days, the production of aflatoxin decreased by 90% and the fungus biomass of *Aspergillus parasiticus* decreased by 51% compared with cultures without volatile compounds of neem leaves. In other words, by inhibiting fungal growth by neem leaves activity, aflatoxin production is also inhibited because aflatoxin is a secondary metabolite compound produced by the fungus *Aspergillus flavus*.

Neem leaves produce terpenoid compounds and phenolic compounds which function as antifungal. The terpenoid compounds in neem leaves such as azadirachtin, nimbidin, nimbin, nimbolide, and nimbidic acid are known to suppress the growth of pathogenic fungi by disrupting cell walls or inhibiting cell wall permeability so that essential components needed by pathogenic fungi such as proteins emerge from cell walls, cells then slowly die (Biswas, 2002., Koul *et al*., 2008 in Sekarsari *et al*., 2013). Terpenoid compounds can bind to protein and lipid molecules so that they can affect the physiological function of cell membrane proteins and protein enzymes (Utami, 2005). This is supported by Agustin *et al*., (2016) where nimbin and nimbidin compounds as antifungal substances in neem leaves extract can inhibit the growth of mycelium fungus A. porri.

Phenolic compounds, which are included in phenolic compounds are flavonoids and tannins. Phenolic compounds can inhibit aflatoxin production, this was stated by Kim et al. (2006) in which mitochondria play a role in the supply of acetyl-CoA which is a major precursor in aflatoxin biosynthesis. Damage to the mitochondrial respiration chain caused by the phenolic component is part of the inhibition of aflatoxin production. Phenolic compounds interact with cell membrane proteins that cause precipitation and denaturation of cell membrane proteins (Manitto, 1992). Damage to the cell membrane causes changes in the permeability of the membrane, resulting in fungal cell membrane lysis (Parwata and Dewi, 2008).

The mechanism of action of flavonoids in inhibiting fungal growth is by disrupting fungal cell membrane permeability. Hydroxyl groups found in flavonoid compounds cause changes in organic components and nutrient transport which will eventually lead to toxic effects on fungi (Jupriadi, 2011 in Nuryani 2016). Furthermore, Harboune (1987) states that flavonoid compounds enter fungal cells through holes in the cell membrane formed because phenol compounds have denatured cell membrane lipids. These protein compounds will be denatured by flavonoids through their hydrogen bonds. The ability of flavonoids to bind to proteins causes inhibition of cell wall formation so that hyphal growth is also inhibited because the cell wall composition needed is not met.

It can be concluded that the influence of neem leaves can cause damage to the fungal cell wall or lysis where this effect resembles some other antifungal compounds. Fungal cell walls are essential to protect cells from osmotic pressure changes, where if there is interference with the osmotic pressure it can increase cell vacuolation or even cause death in organisms due to osmotic shock. Chitin and glucan contained in the cell walls of *Aspergillus* species are molecules that are sensitive to antifungal compounds (Joklik, 1980 in Abyaneh, 2005). Lysis of *Aspergillus flavus* mycelium will cause deactivation or inhibition of aflatoxin production. Pitt (1993) states that the inhibition of aflatoxin production may be caused by enzymes released during lysis of the fungus mycelium. The same thing was said by Namazi *et al*. (2002) where damage to mycelium and conidium molds is one of the characteristics of the aflatoxin deactivation process.

In factor B (storage time), the aflatoxin content in corn with a storage period of 4 weeks (B2) is lower than other treatments (B1, B3, and B4), this is likely during four weeks storage is the best time of effectiveness of neem leaves in inhibiting the production of aflatoxin. Although it is not known exactly how many effective time intervals required by neem leaves to inhibit aflatoxin production due to the lack of research on the effect of neem leaves and storage time on the aflatoxin content of corn. According to Jay (1996) that the ability of mold to accumulate and aflatoxin depends on several factors, namely the genetic potential of the mold, environmental requirements (substrate, humidity, temperature, pH), and the length of contact between the mold and the substrate. This is what causes the longer storage time the higher the aflatoxin content, as seen in corn with eight weeks storage (B4). According to Susanto (2008), long-time storage will increase the content of aflatoxin, because with increasing time will provide opportunities for the fungus producing aflatoxin to produce secondary metabolites of aflatoxin.

Although the neem leaves are known for its anti-fungi properties which can inhibit the growth of fungus but the nature of the neem leaves in inhibiting the growth of this fungus by suppressing growth is not by stopping or killing the fungus. It can be interpreted that with long storage time, fungus spores can continue to grow and produce aflatoxin.

Furthermore, other factors that can affect the increase in aflatoxin content in corn during storage are temperature, humidity, and substrate. During the research, corn storage temperature ranged from 26-350C with humidity of 70-80%. This is supported by the opinion of Syarief *et al*, (2003) that the optimum temperature for producing aflatoxin is 25-350C and production yield varies based on the composition of the substrate. Below 12°C no metabolism produces aflatoxin. Even if there are tiny numbers, especially at a temperature of 15°C.

The results of Maryam's research (2006) that storage temperatures between 25-320C cause the growth of aflatoxin-producing fungi will increase by tens to thousands of ppb after being stored 28 days supported if storage techniques are not heeded. Aflatoxin-producing fungi can grow on a substrate that has fat content. According to Pater and Bullerman (1988), in general, the content of fat, protein, trace elements, amino acids and fatty acids in a material can encourage the production of aflatoxins by *Aspergillus flavus*.

The selected treatment based on the lowest aflatoxin content was in the A3B2 treatment (1% neem leaves powder level with 4 weeks storage time) which was 53.67 ppb (a decrease of 68.43% from the original aflatoxin content of 170 ppb fell to 53.67 ppb).

3.2. The effect neem leaves powder (*Azadirachta indica* A. Juss) and storage time to the percentage of water content (%) of rejected corn.

The average percentage of water content (%) of the rejected corn added by neem leaves powder and different storage times can be seen in Table 2.

Statistical analysis showed that there was no interaction between factor A (level of neem leaves powder) and factor B (storage time) to percentage of water content on rejected corn, but respectively for factor A and factor B had a very significant effect ($P < 0.01$) on the percentage of water content of rejected corn.

Table 2. The average percentage of water content (%)

Level (%)	Storage time (week)				Average
	B1	B2	B3	B4	
A1 (0)	14,70	14,50	14,37	14,43	14,50[a]
A2 (0,5)	14,57	14,40	14,13	14,17	14,32[b]
A3 (1)	14,70	14,47	14,27	14,01	14,36[b]
A4 (1,5)	14,50	14,43	14,10	14,04	14,27[b]
A5 (2)	14,50	14,40	14,17	14,07	14,28[b]
A6(2,5)	14,47	14,63	14,03	14,13	14,32[b]
Average	14,57[a]	14,47[b]	14,18[c]	14,14[c]	

Based on the results of the DMRT test (Duncan Multiple Range Test) on factor A showed that corn without neem leaves powder (control) the water content was significantly ($P < 0.05$) higher than all treatments, while the level of 0.5 - 2.5% (A2, A3, A4, A5 A6) neem leaves powder had no significant effect ($P > 0.05$) on water content during the study. For factor B, the water content at 2 weeks storage time was significantly different ($P < 0.01$) higher than 4, 6 and 8 weeks storage time and 4 weeks storage time was very significantly different ($P < 0.01$) higher than storage time of 6 and 8 weeks while storage time of 6 weeks was not significantly different ($P > 0.05$) with storage time of 8 weeks.

In factor A, the percentage of the water content of corn without neem leaves powder (control / A1) is higher than that of corn given with neem leaves powder with various levels. This can be caused by corn which is not given neem leaves, inhibition of the growth of the fungus Aspergillus flavus does not occur so that water levels increase because the fungus will undergo metabolic processes that will ultimately produce CO2, H2O, and energy. In contrast to controls, all treatments with the addition of neem leaves powder of various levels ie, from 0.5 - 2.5% (A2, A3, A4, A5, and A6) had the same effect when viewed from the results of the DMRT analysis. This can be caused by the neem leaves particles used in this study in the form of powder so that it can absorb water contained in corn.

The water content of corn rejected during storage in this study decreased from 2 weeks of storage (B2) to 8 weeks of storage (B4) as shown in Table 2. Where the initial storage water content ranges from 15%. Same to the results of research Widianingrum *et al*, (2010) in the first 4 weeks of corn storage the water content decreased from the water content at the beginning of storage. Decreased water content in corn during storage can be caused by the material still undergoing the process of respiration, transpiration, air concentration and the activity of microorganisms. The process of respiration is an overhaul of organic materials from food ingredients such as carbohydrates, proteins and fats to produce CO2 and H2O with a higher respiration rate and the longer the respiration rate increases, the process of reshuffle will increase which causes a decrease in water content (Ahmad, 2013).

The rate of respiration can be influenced by the availability of the substrate and the concentration of air (the availability of O2 and CO2). According to Setyadjit and Syaifullah (1994), high respiration can reduce the water content of food during storage. Higher concentrations of O2 in the storage space than CO2 concentrations cause the rate of respiration to be faster so that a decrease in the water content of the material, and vice versa if higher CO2 concentrations and O2 concentrations limit the rate of respiration of the material can be inhibited so that a decrease in water content can be prevented. It can be assumed that storage with average air concentrations causes the process of respiration to run generally at a rapid rate so that it will lose more water content.

The decrease in water content during storage in this study was also caused by transpiration. Transpiration is a process of losing water in the form of gas from living tissue. The transpiration occurs because of differences in temperature and relative humidity of corn stacks with the environment. From the temperature and Rh differences, the results of corn respiration will evaporate. Water in corn tends to move to areas where the humidity is smaller. Water that evaporates from corn is the result of respiration in which carbohydrates into simple sugars are then converted into water and carbon dioxide (Sutardi and Tranggono, 1990). The storage temperature in this study ranges from 26-350C. According to Suparjo (2010) at a high enough temperature which is around 25-270C, evaporation of the water content of the material will take place fairly quickly. This will encourage the material to release the free water it contains to retain moisture and prevent more significant water loss. The change in material water into the vapor phase is driven by an increase in temperature.

Another opinion, where the decrease in water content during storage can be caused by the growth and metabolic activity of microorganisms need water to transport nutrients or waste materials into and out of cells. All of these activities require liquid water. Water that undergoes crystallization and forms ice or chemically bound water in a solution of sugar or salt cannot be used microorganisms (Syarief *et al*., 2003). Woven bag has hygroscopic properties and can absorb water up to 34% (Hartanto, 2003).

3.3. The effect neem leaves powder (*Azadirachta indica* A. Juss) and storage time to the percentage of mouldy seeds (%) of rejected corn.

The average percentage of moldy seeds (%) of the rejected corn added by neem leaves powder and different storage times can be seen in Table 3.

Table 3. The average percentage of mouldy seeds (%)

Level (%)	Storage time (week)				Average
	B1	B2	B3	B4	
A1 (0%)	0,00	0,00	0,00	1,33	0,33
A2 (0,5%)	0,00	0,00	0,00	2,00	0,50
A3 (1%)	0,00	0,00	0,00	2,00	0,50
A4 (1,5%)	0,00	0,00	0,00	2,00	0,50
A5 (2%)	0,00	0,00	0,00	1,00	0,25
A6(2,5%)	0,00	0,00	0,00	2,00	0,50
Average	$0{,}00^b$	$0{,}00^b$	$0{,}00^b$	$1{,}72^a$	

Statistical analysis showed that there was no interaction between factor A (level of neem leaves powder) and factor B (storage time) to the percentage of moldy seeds on rejected corn. However, factor B had a very significant effect (P <0.01) on the reject moldy corn seeds.

Based on the results of the DMRT test (Duncan Multiple Range Test) on factor B, moldy seeds at 8 weeks storage time were significantly different (P <0.01) from 2, 4 and 6 weeks storage time. At the 8-week storage period (B4) the growth of the fungus looks very visually significant. There is a real correlation between storage time and fungus growth, so the longer the storage time the more mold spores are formed.

This is supported by opinion of Miller and Trenholm (1994) where the number of spores that infect and the storage time are one of the factors that influence the resistance of corn during storage. Although neem leaves contain active compounds known as antifungal with extended storage time, the effectiveness of neem leaves in inhibiting the growth of fungus spores decreases. Furthermore, the neem leaves are inhibiting by suppressing growth not by stopping or killing the fungus.

It can be interpreted that the fungus spores of Aspergillus flavus continue to grow with increasing storage time. Storage time that is too long will cause more significant damage due to the growth and proliferation of fungus contaminants. Research conducted by Nafiah

(2009) shows that the storage of corn for 6 weeks has a marked increase in the total changes in damaged corn kernels when compared to the previous week. This can also be one of the factors causing the growth of fungus/mold. Where the factors that influence the cracking of seeds are: changes in water content due to changes in weather, improper stripped and warehouse attacks. Damage to feed ingredients due to changes in water content is the most common case, making it easier for the growth of microorganisms, especially fungus. Microorganisms take and eat food substances from seeds or other raw materials that cause damage to the protective layer of material. In addition to causing physical damage due to its transient nature, microorganism paves the way for contaminating contaminants such as mycotoxin-producing molds which can increase damage to feed ingredients such as seed holes, crushed and broken.

The packaging method also affects the growth of the fungus during storage. The corn was packed in a woven bag measuring 40 x 25 cm during the study. Aprianie's research results (2009) show that the storage of material by packaging it in packaging material will be able to withstand the expenditure of heat energy produced by respiration from microbes from the packaging environment to the environment outside the packaging material. Respiratory energy that is retained by the packaging material will turn into heat and moisture that will accumulate during storage. After a few days, the condition inside the gunny sack is more humid than the outside air. Higher humidity than conditions outside the packaging material is also caused by a decrease in the water content of the material in the burlap sack so that Aspergillus flavus can grow and reproduce accurately.

IV. CONCLUSION

The use of neem leaves powder by 1% with 4 weeks storage time can reduce the aflatoxin content of rejected corn from 170 ppb to 53 ppb (a decrease of 68.43%) with a water content of 14.47% and moldy seeds 0%.

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Variation of carbon and major nutrients contents in two types of soil under stone bunds management in cotton-based cropping systems in the Sudanese zone of Burkina Faso

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***Abstract*— *In Burkina Faso, soil fertility decline is a major constraint in cotton-based farming systems. In this area, most of the soil fertility management are mainly focus on soil amendment with organic manure and the used of mineral fertilizers. In addition to these techniques, the present study on the use of stones bunds was conducted at Gombélédougou in order to limit erosion and improve availability of fertilizers provided to plants. Gombélédougou is in the district of Koumbia in Sudanese zone of Burkina Faso. About 605ha covering six (6) soil types were managed using stone bunds established along the contour lines. The spacing between the stone rows was 2.5 m. Concerning additional soil fertility management, it consisted in crops rotation (cotton//cereals); the application of mineral fertilizer at the dose of(110 ± 25 kg/ha for NPK (14-23-15) and 52.5 ± 15 kg / ha for urea (46%)) and organic manure (1787.50 ± 1390.96 kg / ha). The indicators for assessment of the effects of the stone bunds were evaluated using the variation of the carbon and of the major elements (nitrogen, phosphorus, potassium) contained in soil as well as the overall evolution of the fertility of these soils. The results showed that the stone bunds in combination with current fertilization techniques increased the soil carbon level by 0.04% and 0.15%, respectively in Lixisols (FLIP) and Cambisols(BEF) in one hand, and in the other hand, Nitrogen contents decreased from -0.01 to -0.02% and those in Phosphorus from -1.21 to -2.61 mg/kg in these soils. The stone bunds reduced significantly the transfer of sediments and nutrients from upper to the down slopes. As consequences soil fertility was improved in thelixisols located at the up slops at the detriment of Cambisolin the down slop. These results show that the stone bunds are more effective when producers combine an appropriate technique of organic (compost) and mineral fertilization.*

***Keywords*— cotton-based cropping systems, Gombélédougou-Burkina Faso, soil fertility, Stones bunds, Sudanian zone.**

I. INTRODUCTION

Soils degradation in cotton and cereals-based cropping systems area in Burkina Faso is a serious constraint and yield limiting factor. In these farming systems, cotton is a cash crop which is cultivated in rotation with steaple cereals (maize and sorghum) [1]. Therefore, this degradation treatment crops yields and consequently farmers´ incomes and food security. The cotton base-farming systems are located in the Sudanese zone with fairly good rainfall and less land pressure compared to the rest of the country. In addition, cotton producers have some facilities in getting mineral fertilizers. With regard of these advantages, soil fertility management have been focussed on mineral fertilization, crops rotation and fallow [2]. Currently, this area is experiencing internal population growth due to migration of populations coming from the other regions of the country for farmland. As consequences, the traditional soil fertility management systems are no longer able to address degradation issues. Alternative approaches in soil fertility management such

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as the development and the use of specifics fertilizers, conservation agriculture, crops residues recycling at the plot scale, have been developed to address the problem [3, 4, 5].

In the district of Koumbia as in most of the cotton production zones, Lixisols with sandy textural constitution and are the most dominant [6]. The topography is characterized by steep slopes (> 5%) with harsh and torrential rainfallexposing soil to heavy erosion risks. Despite of the situation depicted above, few actions such as establishing physical barriers on soil surface to mitigate runoff have been undertaken to address soil erosion as done in the Sahelian zones [7,8]. In Burkina Faso, soil erosion is a global constraint in agricultural lands and, in the Sahelian zones farmers have developed the settlement of stone bunds to address the issue since 1988. The use of stone bunds to control soil erosion and its adverse effects on soil nutrients (N, P and K) and carbon balance have been widely investigated [9, 10]. However, in the Sudanese zone (cotton production area), there are few integrations of stone bunds into soil fertility management packages despite the high risk of soil erosion. The few investigations focused on improving soil productivity using stone bunds were in forestry [11].

In the current context, with soil degradation and land pressure in the cotton production zones, it is consistent to move towards agricultural intensification while limiting the risk of erosion through the use of anti-erosion devices. Therefore, stone bunds appear as an alternative to experiment since this practice is unusual in the Sudanian zone as well as in the cotton-based cropping systems. The study reported here in was conducted at Gombélédougou in the district of Koumbia in Burkina Faso. It aimed to improve the organic and mineral status of soils by using stone bunds combined with organic and mineral fertilization. Carbon and nutrients (N, P, K) budget of different soil types were measured in order to assess the effectiveness of stone bunds on soil fertility management.

II. MATERIALS AND METHODS

2.1 Study site location

The study was conducted in the village of Gombélédougou, district of Koumbia(11°14’ 11’’ N Longitude, and 3°41’ 47’’ W Latitude) in Burkina Faso (Fig. 1) Gombélédougou has Sudanese climate with two distinct seasons. The rainy season lasts from juneto september and a dry season from october to may; In general,the rainfall distribution is irregular and the average rainfall ranged from 800 to 1100 mm.

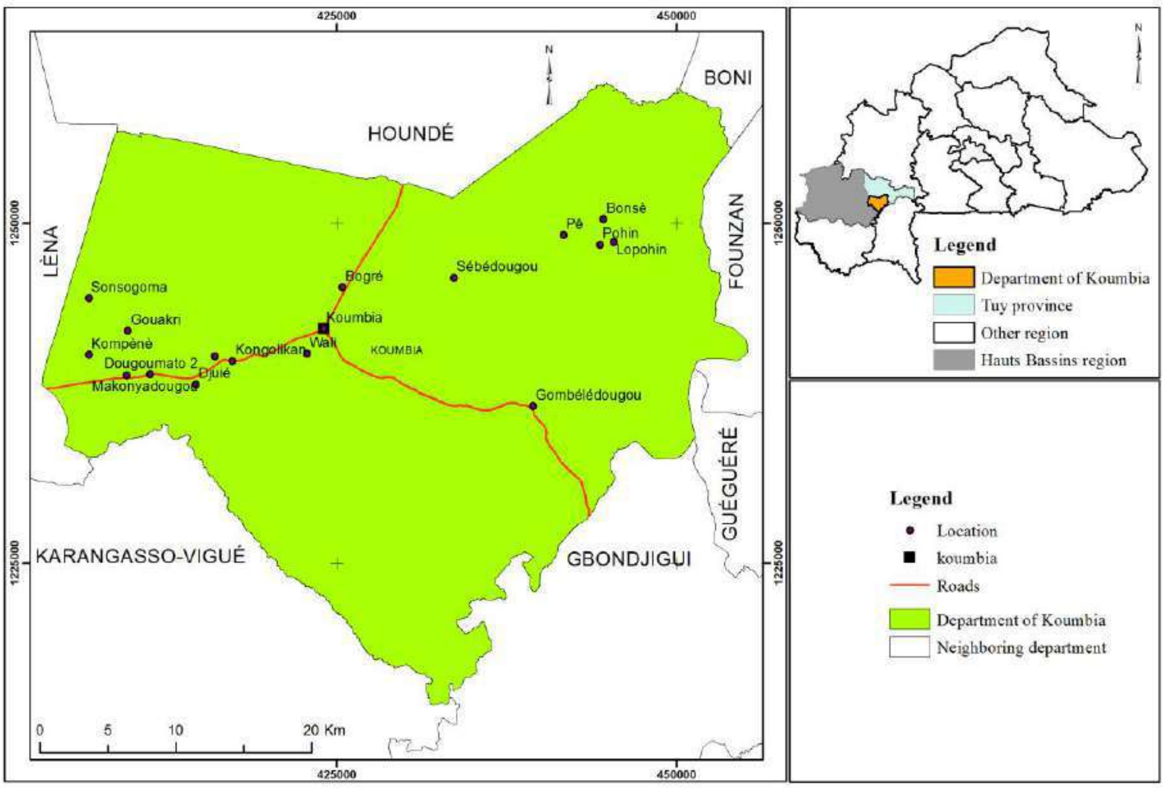

Fig.1: Land use map of the district of Koumbia

2.2 Treatments and background of the study site

The site presents a high level of soil degradation due to runoff and inconsistent soil fertility management practices. The stone bunds were hand constructed along the contour lines on 605.59 ha, using rock material from the surrounding mountains.The device includes main stones rows (40cm wide and 40 cm high) and stones minor rows (30 cm wide and 20 cm high) along the contour lines. Stone rows spacing were 5 meters between the consecutive main rows and 2.5 meters between the main row and the next minor row. The land use systems were mainly cotton based farming systems and fertility management techniques were mainly the application of organic manure in combination with NPK (14-23-15) and urea (46% N). The average application rates for these fertilizers were 1787.50 ± 1390.96 kg / ha for organic manure, 110 ± 25 kg / ha for NPK (14-23-15) and 52.5 ± 15 kg / ha for urea (46%).

2.3 Physical and chemical characteristics of the soil

At the baseline situation in 2016, physical and chemical characteristics of the soil were analysed after soil survey according to the standards [12, 13, 14]. In total, 40 soil samples (2 to 4 samples by soil type depending to the soil depths) were collected after the soil description in different soil types for the purpose of their characterization. Due to their unsuitability to farming activities, soil samples were not collected on the shallow soil (lithosolon rock or lateritic breastplate materials). In 2019, samples were collected at the same places as in 2016, using huger and at the soil 0-20 cm depth,for the purpose of the determination of soil fertility variation.

The soil parameters analysed were: total nitrogen (Nt), total phosphorus (Pt), assimilable phosphorus (Pass), total potassium (Kt) and available potassium (K dis), sulfur (S), and boron (B). The soil pH_{H2O}, organic carbon content (organic matter), the cation exchange capacity (CEC) and the sum of the exchangeable bases (S), These parameters were determined according BUNASOLS [15].

2.4 Determination of the average value of the chemical parameters and rating of the factors

For the determination of the average values of the chemical parameters and suitability classes of the different soil types, the following formula (I) and Table 1 were respectively used.

Average content of the parameter considered= $\frac{D1*C1+D2*C2+\cdots+Dn*Cn}{(D1+D2+\cdots Dn)}$ (I)

D (cm) = the thickness of the horizon

C = concentration of the chemical parameter considered

D1+D2+……. + Dn = depth the soil pit

Table1: Rating standards of parameters for the determation soil suitability

Parameters	Units	Very low/ unfavorable	Low	Medium	High	very high
Organicmatter (OM)	%	<0.5	0.5-1.0	1.0-2.0	2.0-3.0	> 3.0
Total Nitrogen (N)	%	<0.02	0.02-0.06	0.06-0.1	0.1-0.14	>0.14
Assimilable phosphorus (Pass)	mg/Kg	<5	5-10	10-20	20-30	>30
Availablepotassium (K)	mg/Kg	<25	25-50	50-100	100-200	>200

Source : BUNASOLS[13]

Table 2 gives the different soil fertility classes according to the standards of BUNSOLS [13]. Soil parameters considered for the rating were organic matter content, the sum of exchangeable bases and the pH_{water} [13].

Table 2 :Standard for evaluation of soil fertility

Classes	Very low	Low	Medium	High	Very high
Rating	< 4,4	4,5 – 7,5	7,6 – 10,5	10,6 – 13,5	> 13,6

III. RESULTS AND DISCUSSION

2.5 Soil resources of the study site

Details on soil types of the investigated site are summarized in Fig. 2. The most dominants soil in terms of proportion were the ferric epipetric plinthosols (53.17%) and endo/epipetric lixisols(30,83%).

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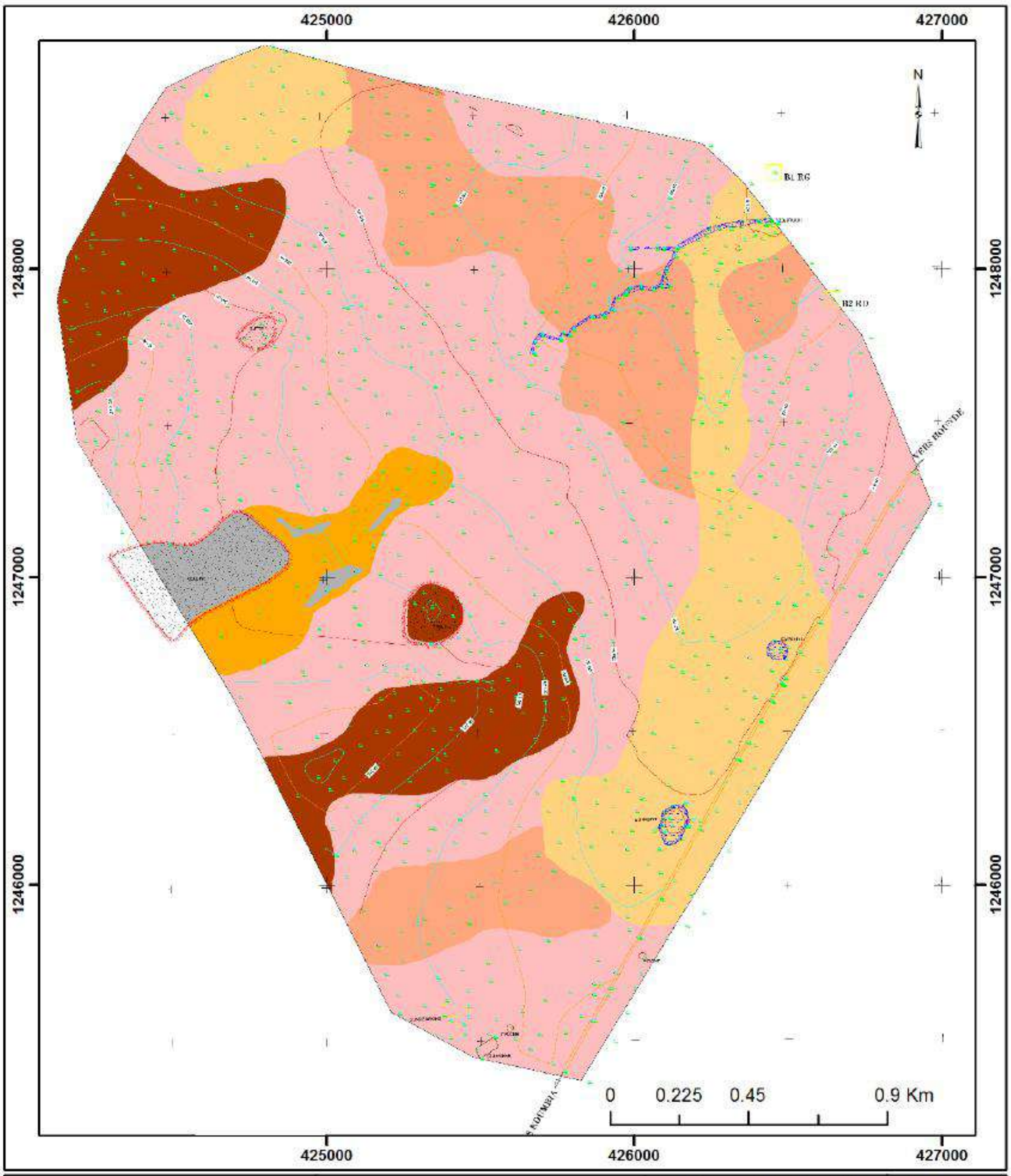

LEGEND

- Sampled profiles
- Topographic boundaries
- Water level contours of 2.5 m
- Water level contours of 5 m
- Main water level contours
- National roads
- water flow
- Hill
- stamped area

Soils Units	Soil types FAO/WRB, 2014 classification	Area ha	Area %
Lithosol sur roche	Epipetric Plinthosol	10.57	1.75
Lithosol sur cuirasse	Epipetric Plinthosol	66.05	10.91
So brun eutrophe tropical ferruginisé	Cambisol eutrique ferrique	20.26	3.35
Sol ferrugineux tropical lessivé induré profond	Lixisol endopétroplinthique -ferrique	86.5	14.28
Sol ferrugineux tropical lessivé induré peu profond	Plinthosol épipétrique, ferrique	321.97	53.17
Sol ferrugineux tropical lessivé à taches et concrétions	Lixisol gleyique, ferrique	100.22	16.55

Fig.2: Soil map of the site of Gombélédougou

Note: Epipetric Plinthosol = L/r; or L/c; Cambisoleutriqueferrique = BEF; Lixisolendoplinthique-ferrique= FLIP ; Plinthosol epipetrique-ferrique = FLIPP ; Lixisolgleyique, ferrique = FLTC

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2.6 Soil physical and chemical constitution

Soil physicochemical parameters were determined in 2016 as baseline situation. According to BUNASOLS [13], carbon, nitrogen and potassium in Eutric-ferric-cambisolwere "*medium*" and "*low*" in the other soil types (Table 3); indifferently to the soil type the available phosphorus was "*low*". Except the Eutric-ferric-cambisol with clay loamy textural constitution, the other soil had silty to clay-loam textural composition,

Table 3: Physicochemical characteristics of the different soil types in site of Gombélédougou at the baseline in 2016

		ferric epipetric plinthosols and endo/epipetric lixisols		
	BEF	**FLTC**	**FLIPP**	**FLIP**
Textural constitution	LA	LAF/LF	L	LA
Clay (%)	45.10	27.45 ± 11.09	25.49 ± 8.32	23.53 ± 2.77
Silt(%)	29.41	50.98 ± 8.32	39.22 ± 2.78	35.30 ± 2.78
sand(%)	25.49	21.57 ± 2.77	35.30 ± 11.09	41.18 ± 5.55
OM (%)	1.916	1.11 ± 0.30	1.21 ± 0.05	1.15 ± 0.11
C (%)	1.111	0.64 ± 0.17	0.70 ± 0.03	0.67 ± 0.07
N (%)	0.097	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01
C/N	11	11.00 ± 0.00	11.00 ± 1.41	11.50 ± 0.71
Pass(mg/kg)	4.24	4.27 ± 0.13	3.17 ± 1.01	3.16 ± 0.08
K_av(mg/kg)	51	41.50 ± 6.36	55.00 ± 43.84	47.50 ± 28.99
S total (S) %	0.4	0.68 ± 0.44	1.01 ± 0.18	0.72 ± 0.18
Total Br(B) %	0.04	0.05 ± 0.00	0.03 ± 0.01	0.04 ± 0.00
pH_{H2O}	5.7	5.28 ± 0.64	5.39 ± 0.18	4.93 ± 0.08

Note: BEF = Cambisoleutrique ferrique ; FLTC = Lixisolgleyique, ferrique ; FLIPP = Plinthosolepipetrique-ferrique ; FLIP= Lixisolendoplinthique-ferrique

2.7 Variation in soil organic carbon and major nutrients (N, P and K)content as affected by the stone bunds

2.7.1 Change in average organic carbon content

Regardless of the types of soil, stone bunds establishment and the application of organic manure contributed to accumulation of organic carbon soil (Fig. 3a). In fact, from 2016 to 2019, the increase in the soil carbon rate was ranged from 0.04% to 0.15%. Compared to CambisolEutric Ferric where the highest rate was recorded with 0.15% accumulation of organic carbon, this increase of soil carbon content in the ferric epipetric plinthosols and endo/epipetric lixisols was in the range of 0.04 and 0.08%. The accumulation of carbon recorded in the different soil types is linked to the mitigation of runoff by the stone bunds, and hence the lateral transfer of the sediments rich in organic matter from the upper to the down slops. These results are similar with those reported Zougmoré et *al*. [8] and Barry et *al*. [9] who found accumulation of soil organic carbon in soils under stone bunds management in the Sahelian zone of Burkina Faso.

Variation recorded in carbon accumulation between soil types could be explained by their textural constitution. Indeed, in addition to soil carbon loss through erosion, leaching is one of the main factors of soil carbon depletion in agriculturallands [16]. In the CambisolEutric Ferric leaching effects are lessened because of their high clay content 45.1%compared to the other soil types (Table 3).As consequence, carbon accumulation was found to be higher in the CambisolEutric Ferric compared to the other soil types.Also, by reducing runoff, the stone bunds promote water infiltration. In coarse textured soils (ferric epipetric plinthosols and endo/epipetric lixisols) this infiltration is favour the leaching of sediments from the upper soil layer to the down layers. In Cambisol Eutric Ferric, this process is reduced because the sediments are retained by the clay fraction at the soil upper layers.

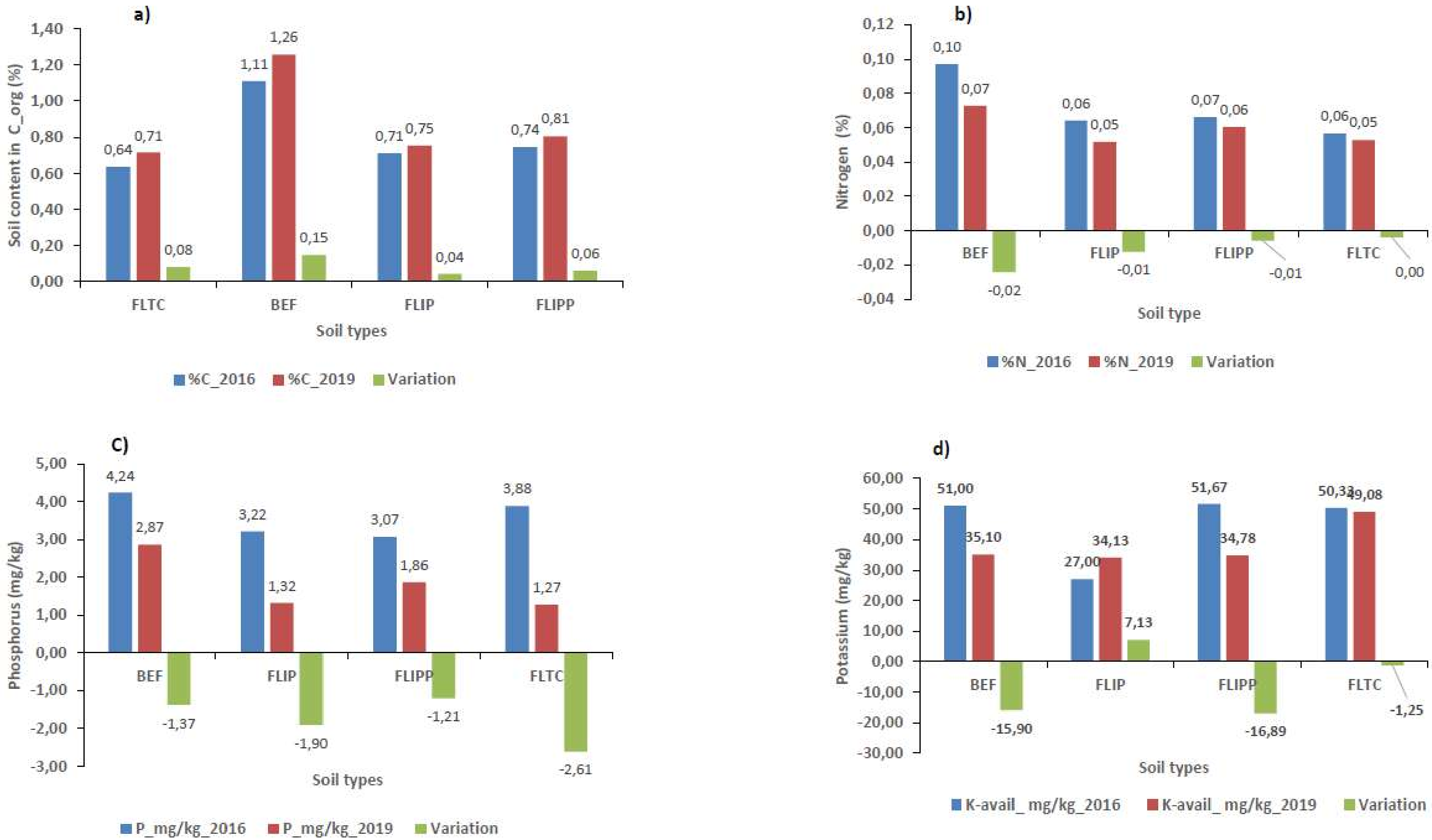

Fig.3: Variation in average carbon and major elements content (N, P and K) according to the soildifferent soil types from 2016 to 2019

Note: BEF = Cambisoleutrique ferrique ; FLTC = Lixisolgleyique, ferrique ; FLIPP = Plinthosolepipetrique-ferrique ; FLIP= Lixisolendoplinthique-ferrique

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2.7.2 Variation of soil major nutrients (N, P and K) contents

Figures. 3b and 3c indicated decreases of the global trends of nitrogen and phosphorus after three cropping campaigns despite the construction of stone rows and the implementation of soil fertility management practices. The decline of nitrogen was more severe in Eutric-ferric-cambisol (BEF) compared to the Endopetric/Epipetric lixisols (FLIP and FLIPP) and in the Ferric-gleyic-lixisol(FLTC) where the highest nitrogen loss was recorded(Fig. 3c). As for the phosphorus, the average decreases were rangedfrom-1.21 to -2.61 mg/kg. Regarding variation in average potassium content (Fig. 3d), only epipetric-lixisol (FLIP) recorded positive balance (+7.13 mg/kg) while other soil types experienced decreases ranging from -1.25 to -16.89 mg/kg. These declines of soil major nutrients content are due to nutrient outflow through crop yields and heightened by poor soil fertility management practices indeed, organic manure and mineral fertilizers which are the main sources of compensation of nutrients outflow from the farms lands were applied at the rate of 1787.50 ± 1390.96 kg/ha for organic manure and 110 ± 25 kg/ha for NPK (14-23-15) and 52.5 ± 15 kg / ha for urea (46%). Theses application rates fertilizers are below the recommendations: 2500 kg/ha for organic manure and 1500kg and 100 kg for NPK and urea respectively [17, 18]. In addition to low rate of application of organic manure, 30.5% of producers did not regularly apply organic manure to their plots for various constraints. These results confirm the inaccessibility of organic manure to producers in the majority of cotton base cropping systems reported by Dakuo et *al*. [19].

The increase in carbon content observed in the different types of soil despite the shortcomings noted in organo-mineral fertilization could be explained by the positive effects of the stone bunds on soil´s water content. Indeed, previous studies have shown a positive contribution of the stone bunds on soil water status [20, 21]. Water scarcity and its irregular distribution is a limiting factor in rain-fed agriculture; the improvement of soil water status by the stone bunds induces a good development of crops and consequently, higher demand of nutrients from soil including N, P and K.

2.8 Evolution of soil fertility from 2016 to 2019

A decline of fertility trend in Eutric-ferric-cambisol (BEF) was noticed after three cropping seasons; however, it did not led decrease of their fertility class (Fig. 5). This could be explained by the fact that the BEF have a higher intrinsic potential for crop growing and this potential was improved with soil water status improvement due to the establishment of the stone bunds as outlined above. In a context of poor compensation of the nutrients exported by crops, this results in a sharp drop in nutrients, which has repercussions on the rating of factors determining soil fertility. These results are consistent with those found by Koulibaly et *al*., [22] Coulibaly et *al*., [23], reporting soil fertility decline in cotton-based cropping systems with low levels of organic and mineral fertilizer.

In FLIP and FLTC, a significant increase of the fertility level was recorded, resulting in the change from the fertility class "Low" to "High". Concerning the FLIPP the soil fertility remained the same after the three production campaigns. In the context of investigation area where soil fertility decline in cultivated soils is a common feature, these records in the trends of soil fertility for the FLIP, FLIPP and FLTC can be considered as an improvement of soil quality. Thus, this can be explained in one hand by the fact that farmers are aware of the fragility of these and preferentially use organic fertilisers on these soil in detrimental of BEF considered as fertile soils. In the other and the establishment of stone bunds have led to the reduction of runoff and consequently the transfer of sediments rich in organic matter from the FLIP et les FLIPP, FLTC located in the up slops to the down slops where the BEF are found. Gebrernichael et *al*., [24] and Zougmoré et *al*., [25] also reported a reduction in sediment losses from 21% to 61% from up to the down slops after establishment of stone bunds in the Sahelian zone of Burkina Faso. Therefore, the trend in soil fertility observed in the different soil classes can be explained by the dynamics of the sediments.

IV. CONCLUSION

This study showed that the establishment of the stone bunds induced an increase of soil organic matter content with higher accumulation in the Cambisol eutrique ferrique (BEF). However, the general trend for major nutrients (N, P, K) decreased after three year of cultivation. With regard to the overall trends soil fertility, the mismanagement of soil fertility and the mitigation of sediment transfer through runoff from the up to the down slopes contributed to a decline of the level of fertility in Cambisoleutriqueferrique (BEF). Also, sediments retained by the stone bunds and the applications of organo-mineral fertilizers on the Lixisolgleyiqueferrique (FLTC); Plinthosol epipetrique-ferrique (FLIPP); Lixisolendoplinthique-ferrique (FLIP) improved the fertility status of these soils.

The establishment of stone bunds allow physical restoration of the soil without compensating soils´ nutrients exported through crops. However, the stone bunds represent an initiation of alternative to soil fertility

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management on the site of Gombélédougou. To be effective, additional actions such us efficient recycling of nutrients on the agricultural plots scale through the appropriated use of organic manure and mineral fertilizers are recommended.

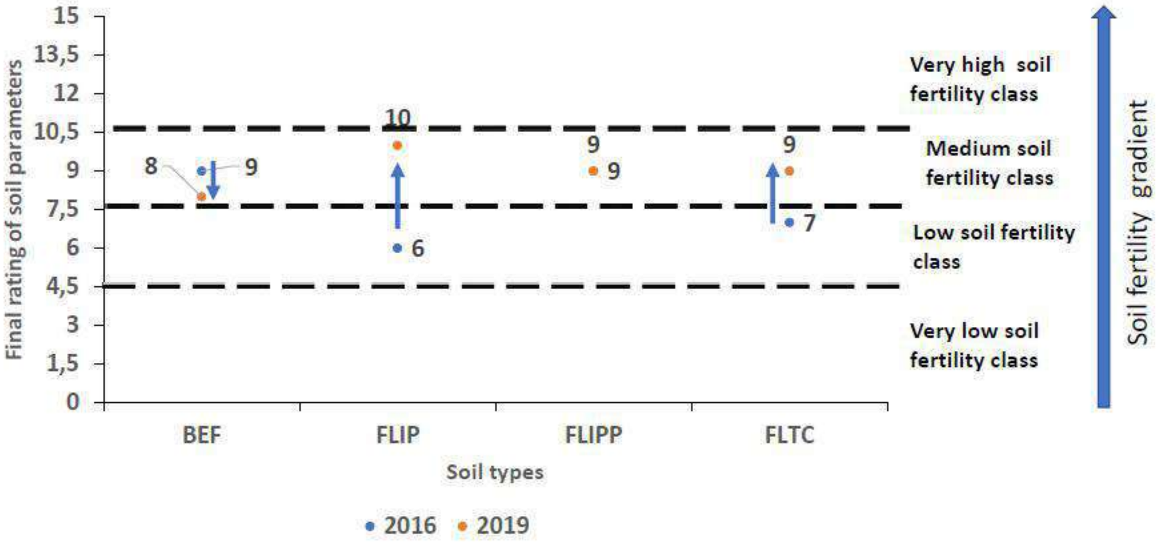

Fig.4: Evolution of fertility from 2016 to 2019 in the different types of soil

Note: BEF = Cambisoleutrique ferrique ; FLTC = Lixisolgleyique, ferrique ; FLIPP = Plinthosolepipetrique-ferrique ; FLIP= Lixisolendoplinthique-ferrique

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Study of Morphology and Physiology of Rice Seed IR Variety 42 (Oryza Sativa L) against aged moving with the SRI (The System of Rice Intensification) Method

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***Abstract*— *Germination is one of the main processes in plant development, where the age of seedling greatly influences the success of the process. The purpose of this study was to determine the morphological and physiological changes of rice seeds in various seedling transplants in the SRI method. The study began from September to October 2018 at the Andalas University Seed and Plant Physiology Laboratory and the Agricultural Product Technology Laboratory of the Andalas University. This study uses a Completely Randomized Design, where the treatments are 0 days of seedlings, 7 days of seedlings and 14 days of seedlings. The difference in radicle length and grain weight difference for each age moved by IR 42 seedlings SRI method.moving seeds can change the morphology of seeds from seed that looks dull marked by the presence of brown spots becoming fresh, shiny, but there are still a few brown spots. Changes in physiology of the age of IR 42 seedlings moving SRI method, the changes that occur is a decrease in the protein content of seedling age 0 days and 7 days after germination from 2.2181% to 0.8356% for four times the analysis process was carried out and the age of seedling 7 days after germination from 1.303% to 1.2588% for two times the analysis process was carried out, then there was a decrease in the content of starch analysis of seedling age 0 days and 7 days after germination from 15.4546% to 10.2812% for four times the analysis process carried out and the age of seedling transplants 7 days after germination from 11.9816% to 7.6806% for two times the analysis process was carried out, then there was a decrease in sugar content analysis of seedling age 0 days and 7 days after germination from 0.0771% to 0% for three times the analysis process was carried out and the age of seedling 7 days after germination was 0%.*
***Keyword*— endosperm, physiology, morphology, age of seedling, SRI method.**

I. INTRODUCTION

Rice (Oryza sativa L.) is an important food crop that has become a staple food for more than half of the world's population. In Indonesia, rice is the main commodity in supporting community food. Indonesia as a country with a large population faces challenges in meeting the food needs of the population. Therefore, food security policies are the main focus in agricultural development. Rice consumption in 2011 reached 139 kg capita-1 year-1 with a population of 237 million people, so that national rice consumption in 2011 reached 34 million tons. The need for rice continues to increase along with the rate of population growth which is faster than the growth of available food production, (BPS, 2011)

The obstacles and challenges faced in realizing national food security are competition in the use of land and water resources. Conversion of agricultural land for non-agricultural activities, especially in Java, causing agricultural production to decline. In this case, the agricultural sector faces challenges to improve efficiency and optimize the use of land resources. The increase can be done by increasing crop efficiency through the regulation of the planting system and streamlining the age of seedlings in nurseries. Setting the planting system and age of the right seeds, and the use of superior varieties of rice in addition to being effective in plant growth are also efficient in time and get optimal productivity.

To get the optimal level of production, seed is one of the most influential technological components. According to Kamil (1982), seedlings are young plants that are crucial for subsequent plant growth. One effort to achieve the above target is through an intensification program by applying appropriate production technologies and the use of efficient and profitable production facilities, including technology for the use of the number of seeds per family.

According to Djafar (2002), seedlings are one of the important factors in rice cultivation. Seedlings from superior varieties with good management from an early age will be able to face obstacles and competition in the field, so that they can produce high production. The quality of seeds planted is influenced by the age of seedlings planted before planting. The right age of seeds is one of the technologies that can determine rice production. According to De Datta (2000), transplanting seedlings at a younger age can reduce seedling damage, plants do not experience stagnation and plant growth is faster.

The use of rice seeds that are around 30 days old will give unfavorable results, because the seeds used are relatively old so they are slow to adapt to the environment, have non-uniform tillers, shallow roots and subsequently plant growth is imperfect (Abdullah et al., 2000) . While young seedlings adapt more quickly to the environment, forming deeper roots, so the plants are more resistant to lodging, drought tolerant, and able to utilize nutrients more effectively (Guswara and Kartaatmadja, 2001).

The age of transplanting seedlings must be appropriate to anticipate root development which generally stops at 42 days after seedling, while the number of productive tillers will reach a maximum at 49-50 days after seedling (Astri, 2007). Planting young seedlings has several advantages, among others, plants can grow better with a higher number of tillers and seedlings aged less than 15 days faster adapt and recover quickly from stress due to being moved from the nursery to the planted land (BPTP Jambi, 2009).

Kuswari and Alit (2003) The system of rice intensification (SRI) is a rice cultivation technique that is able to increase rice productivity by changing the management of plants, soil, water, and nutrients, proven to have succeeded in increasing rice productivity by 50% even in some places reach more than 100%. At this time no one has explained how the morphological and physiological changes in transferring rice seeds by SRI, in general, the planting system and age of seedlings in lowland rice plants are known to affect the growth and yield of lowland rice. Therefore this study was conducted by looking at the morphological and physiological changes of various ages moving the seeds of the SRI method.

II. RESEARCH METHODS

This research was conducted at the Seed Technology Science Laboratory, Faculty of Agriculture and the Agricultural Product Technology Laboratory of Andalas University. This research was conducted in September to October 2018.

The tools used in this study were, spectrophotometer, test tube stirrer, sparayer, stationery, paper labels. The materials to be used in this study are IR 42 rice seed varieties, paddy soil, plastic bottles, rubbing ash, aquades, soil, sand, Al (OH) 3, Aquadest, Na2CO3, K, boiling stones, luff-schoorl, KI 20 %, H2SO4 26.5%, Na-thiosulfate, H2SO4 (93-98% free N), Na2SO4-HgO (20: 1), NaOH-Na2S2O3, zinc, boric acid, methylene blue, HCl, erther, NaOH 45% , 70% alcohol, 96% and 100%, formalin, glacial acetic acid, xylol I, xylol II, xylol III, xylol IV, paraffin, safranin, and fastgreen.

This study uses descriptive methods and experimental methods. Descriptive method is used based on changes in seed anatomy. The experimental method is used based on observing physiological changes. This research was conducted in the form of a complete randomized design trial consisting of one factor with three replications. Factors in the experiment were the age of the seeds consisting of 3 treatments, namely:

A1 = 0 nursery days
A2 = 7 nursery days
A3 = 14 nursery days

In this study using 3 plastic cups (making it easier in the extraction process) each treatment so that there are 27 plastic cups for all experimental units. The data obtained were analyzed by analysis of variance. If the F calculated treatment is greater than the F table of 5%, it is followed by further Duncant Multiple Range Test (DNMRT) tests at the 5% significance level.

III. RESULTS AND DISCUSSION

Based on variance at the 5% level of seedling age treatment of IR 42 varieties the SRI method had a significantly different effect on the length of the radicles. These significant differences can be seen in Table 1 below.

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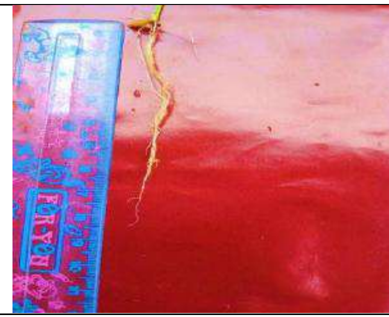
Table 1.Radicula lengths of some age-old transplanted S 42 IRS seedlings at 14 days after germination.

Seedling Age (Day)	Radicular Length (cm)
0	0 a
7	8,33 b
14	13,67 c
KK = 16,55%	

Based on the table above, it can be seen that the 14-day moving age shows radicle length (13.67 cm), significantly different from the 7-day treatment (8.33 cm), as well as the 0-day seed transfer age with an average (0 cm)). This is thought to be due to the availability of food substances in the endosperm that have been decomposed so that one of them occurs root elongation and leaf formation, This is in accordance with the opinion of Sutopo, (1993). That the stage of growth and development of sprouts is very dependent on the availability of food substances in endosperm. This is what causes the difference in root length in each seedling age treatment because of the longer days of seedling moving age, then the root length will increase as the availability of nutrients in the endosperm decreases.

Rice plants have the type of hypogeal germination in which the appearance of radicles is followed by elongation of the plumula, the hypocotyl does not extend above the soil surface while the cotyledons are in the seed coat below the soil surface. The cotyledons here, called scutellum, remain in the soil. According to Kuswanto, (1996).Scutellum functions as an organ that absorbs food from the endosperm and delivers it to the developing embryonic axis.

Table 2. Radicular length in various treatments

Age moved seedlings	Radicular Length (cm)	information
Day 0		The seeds have not yet released radicles
Day 7		The seeds have issued radicles with an average length of 8-10 cm.
Day 14		The seeds have secreted radicles with an average length of 10-15 cm.

During the germination process, the first thing to come out is the radicles. Furthermore, in these radicles outgoing lateral roots, together with the primary roots form the primary root system. This primary root system usually only functions for a while, and then dies. The function of the primary root system is then replaced by adventitious roots coming out of the first stem node and some of the overlying nodes. This adventitious root system (root fibers) is what guarantees subsequent plant life in terms of absorption of water and food from the soil and as a fixing device on the soil.

Germination will occur in the temperature range of 10 0C to 40 0C when the seed dormancy can be solved and the seeds absorb enough water (Yoshida, 1981). According to Salisbury (1985) embryonic axis growth occurs because of two events namely enlargement of existing cells and the formation of new cells at the point of growth of the radicles and plumules due to cell division. An increase in air temperature above the optimum limit will reduce root growth and nitrogen fixation which will cause low rice production (Prasad et al., 2000).

Protein Analysis

Based on the analysis of proteins that have been carried out at various ages moved seedlings IR 42 varieties in the SRI method can be seen in Figures 1 and 2 below.

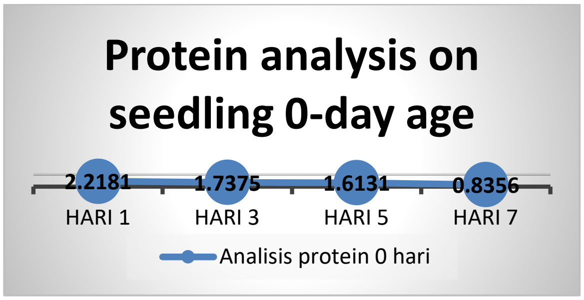


Fig.1.Analysis of age protein of 0 day seedling IR 42 variety in the SRI method.

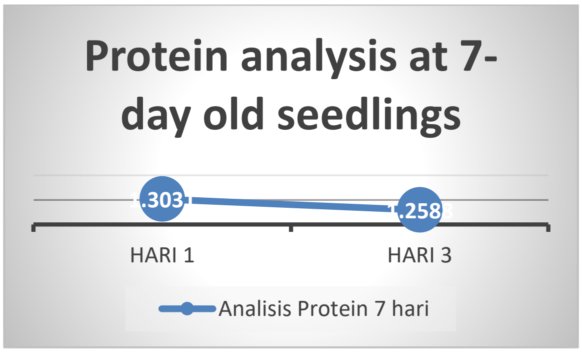


Fig.2. Protein analysis at 7 days old age of IR 42 seedling varieties using the SRI method

0 days age showed a protein content of 2.2181% for the first observation, then the observation was returned on the third day there was a decrease in protein content to 1.7375%, then on the 5th day observation the protein content decreased to 1.6131%, and the following day 7 protein content left only 0.8356%. Based on protein analysis that has been done on IR 42 rice varieties for 7-day nursery age, it was found that 1.3031% protein content, then again the protein content analysis on the third day decreased to 1.2588%. . Then for 14 days of IR 42 rice varieties were not carried out at all because there was no protein content remaining.

The amylase enzyme germinates to break down flour into maltose and maltose is hydrolyzed by maltase to glucose. Proteins are also broken down into amino acids. Then glucose compounds enter the metabolic process to produce energy or are converted into carbohydrate compounds that make up the structure of the body- Amino acids are assembled into proteins that function to structure cells and form new enzymes. Fatty acids are mainly used to make cell membranes. (Dwidjoseputro, 1983).

Protein is one of the main and important food reserves that accumulates in high amounts during the second stage of seed development, namely the mid-maturation stage, after the development of the zygote and before drying. Most of the protein is related to primary metabolism, which indicates a great need for this material for embryonic growth. Protein also plays an important role during seed development, is involved in the metabolism of sugars which provide a carbon source, and also in various biochemical activities of seeds (Li et al., 2012).

Transcription is the process of DNA replication to form RNA-d. Meanwhile, translation is the process of translating genetic information contained in RNA-d into a polypeptide amino acid sequence. In transcription, DNA is used as a model for protein synthesis.

Senthil and Gowri (2008) stated that rice seeds consist of endosperm and embryos, where embryos consist of plumules (leaf candidates) and radicles (primary root candidates). Rice seeds are orthodox seeds covered by palea and lemma (Manurung and Ismunadji, 1988). Protein is stored in the body of protein. The process of division and enlargement of cells depends on the formation of energy and growth component molecules originating from the food supply network.

Protein and fat molecules are important for protoplasmic growth, while complex molecules of polysaccharides and polyuronic acids for the formation of cell walls. Decomposition of the protein available in endosperm is what causes protein content to decrease. In general, there is a sudden increase in protein content that occurs within a few days during the period of cell expansion (Mandal and Mandal, 2000).

Protein is one of the main and important food reserves that accumulates in high amounts during the second stage of seed development, namely the mid-maturation stage, after the development of the zygote and before drying. Most of the protein is related to primary metabolism, which indicates a great need for this material for embryonic growth. Protein also plays an important role during seed development, is involved in the metabolism of sugars that provide carbon sources, and also in various biochemical activities of seeds (Li et al., 2012).

Most proteins are not active in seed metabolism and only function as food reserves to be used in embryo growth during germination. Only a small percentage of proteins are metabolically active, but they are very important for seed development and germination, which is to play a role in catalysis in all digestive metabolic processes, translocation, and utilization of food reserves, and all growth activities (Copelend, 1976).

Rice seed protein is a food reserve, so it will be mobilized during germination. Mobilization of protein in germinated seeds is associated with increased activity of protease enzymes that degrade proteins (Bewley and Black, 1983).. Protease enzymes are classified into endopeptidase and exopeptidase enzymes consisting of aminopeptidase and carboxypeptodase. Degradation of the protein catalyzed by the exopeptidase enzyme produces free amino acids, whereas endopeptidase produces a shorter peptide chain, then this peptide is further degraded by peptide hydrolase to free amino acids (Bewley and Black, 1983).

Albumin and globulin are the main constituents of seed protein reserves in dicotyledonous plants, whereas in monocotyledonous plants the main proteins are prolamins and glutelins (Mandal and Mandal, 2000). Proteins in seeds can be distinguished from other proteins, for example: (i) accumulating high amounts in seeds during the middle stages of ripening the seeds and used during germination; (ii) synthesized only in seeds (in cotyledons or endosperm) not in other tissues; (iii) has no other functional activity aside from being a food reserve; (iv) stored mainly in special storage organelles called protein bodies (Mandal and Mandal, 2000). The protein reserves in the seeds are synthesized mainly from photoasimylates in the form of sugars and from amino acids (Borek et al., 2009).

Starch Content Analysis

Based on the analysis of starch content that has been carried out at various ages moved seed IR 42 varieties in the SRI method can be seen in Figures 3 and 4 below.

Based on observations of the analysis of the content of starch content in IR 42 rice varieties for the age of 0 day seedlings were found 15.4546% for the first day analyzed, then the third day the analysis was conducted again found the results of a decrease in starch levels of 13.7199%, then the analysis was carried out again on the fifth day there was a decrease in starch content of 10.4704%, then on the seventh day a starch content analysis of IR 42 rice varieties was returned in 10.2812%.

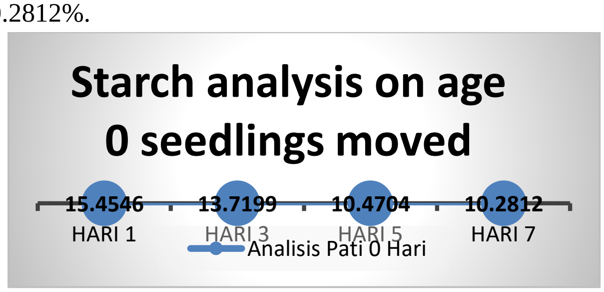


Fig.3.Analysis of starch content at 0 days old age of IR 42 seedling in the SRI method.

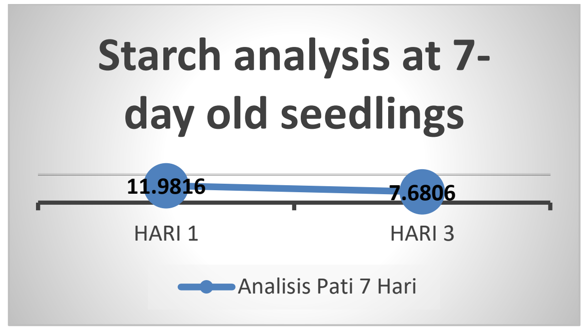


Fig.4.Analysis of protein in 7 days old age of IR 42 variety seedlings in the SRI method.

Based on observations of starch content analysis for 7 days nursery age found 11.9816% for the first day of analysis and re-analysis on the third day found the remaining starch content of 7, 6806%, the analysis process was stopped not carried out again, this is suspected not so much residual starch content is stored if it is associated with an analysis of protein content. This is in line with the process of analyzing starch content for the age of 14 days, the analysis process is not done at all because we also suspect the remaining content of starch can be seen at the age of the nursery 0 days and seven 7 days.

A decrease in food reserves can cause the substrate for respiration to decrease so that the energy obtained is not enough to carry out the physiological germination process. The ability of seeds in an effort to maintain food reserves (carbohydrates) causes the seeds can still store an energy supply that will be used by embryos to grow and develop.

In seeds, starch consists of two forms, namely amylopectin and amylose. The alpha amylase enzyme will break down amylopectin and amylose into dextrin, then the enzyme glucoamylase will convert dextrins into simple sugars such as maltose and glucose which will be used as fuel for respiration.

Decrease in seed starch content will be accompanied by an increase in grain weight. This can occur because a decrease in starch content is a series of catabolism associated with respiration in terms of providing energy and food reserves for seed growth. After the absorption of water by the seeds, growth enzymes become active.

According to Forgarty (1983) Carbohydrate overhaul carried out by the enzyme amylase from Aspergillus is also important for the growth of bacteria and yeast when soybeans undergo fermentation in salt solutions. A-amylase and glucoamylase are enzymes that have a role in the process of overhauling carbohydrates or starches. The α-amylase enzyme catalyzes the breakdown of the glycosidic α-1.4 bond from the starch molecule, while glucoamylase or amyloglucosidase hydrolyzes the α-1,4 and α-1,6 glycosidic bonds from the ends of the non-reducing sugar sequentially.

The alpha amylase and glucoamylase enzymes hydrolyze starch to glucose which has a simpler structure. These materials after overhauled then partly used directly as a constituent of growth in growth spots including growth at the root ends of the seeds. The main carbohydrates in rice are starch and only a small portion of starch, cellulose, hemicellulose, and sugar. Rice starch ranges from 85 - 90% of the dry weight of rice.

The quantity of starch decomposes in endosperm tissue by amylase is much greater than with phosphorylase, this shows the more important role of an-amylase in the breakdown of starch reserves in germination of rice seeds, it can be said that α-amylase is the main enzyme responsible for changes carbohydrate pattern in the seed after 4 days of germination.

Starch is the main source of carbohydrates in food in the form of polysaccharides stored in plant tissues, in the form of granules in leaf chloroplasts and in amyloplasts in seeds and tubers (Sajilata et al., 2006). Starch is a homopolymer composed of lots of glucose with glycosidic bonds. The glycosidic bond is a bond that joins two monosaccharides to form a disaccharide. Starch is composed of amylose which is a straight chain polymer and amylopectin which is a branched chain structure (BeMiller and Whistler, 2009). Amylase is a starch degrading enzyme that can be grouped into three enzyme groups, namely α-amylase, β-amylase, and glucoamylase. β-amylase (E.C 3.2.1.2) is an enzyme of the hydrolase group used in the process of saccharification of starch (a type of carbohydrate). Saccharification plays a large role in the breakdown of carbohydrate macromolecules.

The breakdown of carbohydrate macromolecules will produce short-chain (simple) carbohydrate molecules. The β-amylase enzyme is also called α-l, 4-glucan maltohydrolase E.C. 3.2.1.2. because it works on the α-1,4-glycosidic bond by inversing the configuration of the position of the number 1 C atom of the glucose molecule from α to β. This enzyme breaks the amylose and amylopectin bonds from outside the molecule and produces maltose from the non-reducing end of the polysaccharide chain. In the α-1,6glycosidic bond the activity of this enzyme will stop (Sadikin 2002). The main carbohydrates in rice are starch and only a small portion of starch, cellulose, hemicellulose, and sugar. Rice starch ranges from 85 - 90% of the dry weight of rice. The content of pentosan ranges from 2 - 2.5% and sugar 0.6 - 1.4% from broken skin rice (Winarno, 1997). Starch is composed of amylose which is a straight chain polymer and amylopectin which is a branched chain structure (BeMiller and Whistler, 2009).

Rice starch is composed of two carbohydrate polymers, namely amylose and amylopectin. Amylose is a starch with unbranched chemical structure and is a water-soluble fraction, while amylopectin is a starch with branched chemical structure, is insoluble in water, and tends to be sticky compared to the chemical properties of amylose (Haryadi, 2008).

Sugar Content Analysis

Based on the analysis of sugar content that has been carried out at various ages moved the seedlings IR 42 varieties in the SRI method can be seen in Figures 5 and 6 below.

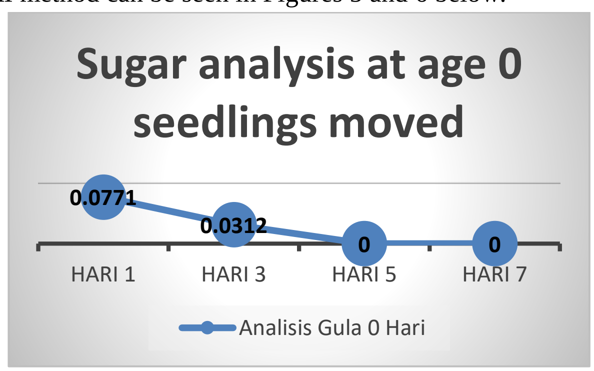


Fig.5.Analysis of sugar content at 0 day age seedlings IR 42 variety on the SRI method.

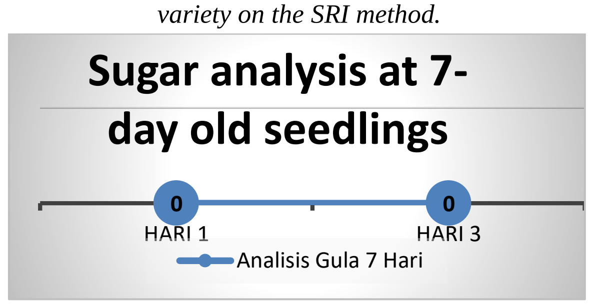


Fig.6.Analysis of sugar content at 7 days old seedling IR 42 variety on the SRI method.

Based on observations of the analysis of sugar content in IR 42 rice varieties for 0 day nursery age obtained 0.771% results for the first day of analysis, then on the third day there was a decrease in the remaining sugar content to 0.0312%, then on the fifth day the remaining sugar content remained only 0 %, so the analysis process is stopped because there are no more residual sugar content left.

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Based on the observation of the process of sugar content analysis in IR 42 rice varieties for 7 days nursery age, the residual sugar content was found to be only 0%, then the third day carried out the same process remaining 0% sugar content, so looking at the process of sugar content analysis not done anymore, along with this for the age of the 14th anniversary not done at all because not yet until the age of 14 days the remaining sugar content is no longer stored. In the process of digestion of seeds needed enzymes that function in turning starch into sugar (Kamil, 1979). During the age analysis process the rice seedlings moved along with reduced sugar content and reduced starch content in the seeds.

Sugar is a carbohydrate component found in seeds that has a defense against decreasing water content as well as protein. If there is a drying process due to water loss can be replaced by the presence of sugar contained in starch, because this can prevent membrane leakage by forming intracellular glass so that the solution becomes concentrated and the diffusion process can be blocked (Adimargono, 1997) The process of respiration as a catabolism process will break down food reserves in the seed that is converting glucose into energy needed by seeds to grow. After the seeds have absorbed water, the seed coat membrane will be permeable to allow oxygen absorption. Oxygen is used in the process of burning glucose.

The enzyme composition needed for the synthesis of sucrose from glucose, namely, hexokinase, phosphoglucoisomerase, phosphoglucomutase, UDP-glucose pyrophosphoryl-ase, sucrose synthetase and UDP-ATP-kinase. So that glucose from endosperm is synthesized into sucrose, by amylolytic activity and mobilized to the scutellum. Then, it is then transported to the embryonic axis, that is, the candidate bud and root candidate, for further metabolic purposes.

Hydrolysis is a decomposition reaction between a compound with water so that the compound breaks or breaks down (Kurniasih, et al., 2011). The more effective hydrolysis, the more glucose is produced (Arianie and Idiawati, 2010). Following this, the starch hydrolysis reaction forms glucose.

IV. CONCLUSIONS AND RECOMMENDATIONS

The results of research that has been carried out on the age of moving IR 42 seedlings using the SRI method are:

1. The difference in radicular length for each age of transfer of IR 42 seedlings in the SRI method.
2. There was a physiological change in the age of IR 42 seedlings moving SRI method, the change that occurred was a decrease in the protein content of seedling age 0 days and 7 days after germination from 2.2181% to 0.8356% for four times the analysis process was carried out and the age of transplanting seedlings 7 days after germination from 1.303% to 1.2588% for two times the analysis process was carried out, then there was a decrease in the content of starch analysis of seedling age 0 days and 7 days after germination from 15.4546% to 10.2812% for four times the analysis process was carried out and the age of seedling moved 7 days after germination from 11.9816% to 7.6806% for two times the analysis process was carried out, then there was a decrease in the content of sugar analysis of seedling age 0 days and 7 days after germination from 0.0771% to 0% for three times the analysis process was carried out and the age of seedling 7 days after germination was 0%.
3. Seedling age 14 analysis cannot be carried out because there is no longer stored protein content, starch content and sugar content to be able to be analyzed.

V. SUGGESTION

From the research that has been done it is advisable to use the age of moving seeds 7 days to 12 days, because the protein content, starch content, sugar content can still be stored in food reserves.

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Rice wheat cropping system in Nepal: Issues concerning sustainability

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Abstract— *Rice wheat cropping system has been widely adopted in Nepal since very long, but much less been studied about the sustainability aspects of this practice. South Asian countries-, as well as other rice-growing countries, have already experienced different ecological and environmental impacts of the long-term rice-wheat system. Also,climate change seems to inflict the rice-based farming system to a varied extent depending uponagroecological regions. In the meantime, the growth in yield of rice is plateauing to less than 1.5% although productivity is well below other South Asian countries. With the heightening consumption of agrochemicals and its subsequent impact on air, water and soil have to be taken into consideration. Rice-wheat system being heavy consumer of nutrient and waterraises question about the sustainable consumption of resources. The increasing dominance of hybrid varieties posses' threat of extinction of local landraces of rice. Apart from environmental and agronomic issues it also maybe affected by socio-economic issues of Nepalese farming families. Extensive studies are yet to be done, but practices like conservation agriculture and sustainable intensification practices are found to make system more resilient. The aim of this review is to discuss such issues associated with sustainability and propose some probable sustainability measures.*
Keywords— ***agrochemicals, climate change, sustainable, conservation agriculture.***

I. INTRODUCTION

The farming system in Nepal is rice-based, and rice-wheat cropping system (RWCS) is the most practised system in the country. RWCS covers about 0.5 million ha of land in the country and has share of 65.8% and 72.1% of total area and total production of cereal crops in the country (Khanal *et al.*, 2012). It covers about 37% of rice growing area and 85% of wheat growing area in the country (Tripathi *et al.*, 2010). Rice alone contributes to 20% of agricultural GDP of country and 53% of total cereal production in Nepal including one-third of total calorie intake of Nepalese population (Tripathi *et al.*, 2019). With the availability of hybrid and high yielding varieties all-round the country, this system is gaining more popularity.

Even though farmers prefer this cropping pattern across South Asia, these two crops have significant differences in field preparation techniques and water management system. Rice is normally grown in water stagnated field whereas following crop wheat requires well-drained soil condition. Under this system, Nepalese farmers cultivate paddy as a summer crop and planted during June/July whereas wheat is grown as a winter crop and planted in November following the harvest of paddy. However, there is wide variation in planting and harvesting time of both crops depending upon agroclimatic condition, irrigation facilities, cropping pattern and type of variety cultivated. Even thoughthe rice-wheat system is unavoidable cropping pattern for food security of Asia, there have been evidence of sustainability concerns associated with diverse expressions of the system. India and China have already experienced impingements of long-term intensive rice-wheat system on biotic as well as abiotic components of environment. Nepal too cannot be relieved from impediments of intensive RWCS regarding sustainability if we do not correct our steps. Though there exists variation in issues concerning sustainability of rice-wheat system across different rice-growing countries of Asia ultimately, they all are colligated with food security of whole world. There has not been significant research on sustainability aspects of RWCS in Nepal. This paper aims to identify the sustainability issues that are directly or indirectly associated with long-term intensive rice-wheat system and suggest some relevant amelioration practices for Nepal.

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II. SUSTAINABILITY CONCERNS

2.1 Impacts of Climate Change in Nepalese RWCS

Climate change is one of the most critical factors in the sustainability of the entire agriculture and farming system of today's world. Rice and wheat, both being highly critical with temperature and precipitation pattern, are affected to a significant degree by climate change. Nepal though it lies in tropical region, covers wide range of agroclimatic zones varying with altitudinal gradient. Nepalese agriculture is mostly rainfed, and big fraction of farmers practice subsistence farming. The irrigated portion of rice cultivable land in Nepal is only 56%, and rest is rainfed of which 39% is rainfed lowland and 5%is rainfed uplands (Tripathi *et al.*, 2019). The reliability of irrigation water is poor even for irrigated area.

It is projected that Nepal's mean annual temperature will increase by an average of 1.2 ℃ by 2030, 1.7℃ by 2050 and 3℃ by the end of this century (Adhikari *et al.*, 2017). The rise in temperature is closely associated with other issues like soil moisture deficit, the outbreak of diseases and insect-pest, incidence of new pests, diverse weed flora and virulence of pathogens (Bhatt *et al.*, 2015). The threshold daytime maximum temperature for rice is 29.9℃ in Terai. Since the maximum temperature in Terai is already high above the threshold, productivity is likely to decline (Adhikari *et al.*, 2017). It was found that for every 1℃ rises in average minimum temperature during September and January results decline in rice yield by 365 kg/ha and 38 kg/ha respectively (Pant, 2013). It was found that impact of the rise in minimum temperature is higher compared to rise in monthly maximum temperature. A study done by (Adhikari *et al*., 2017) showed that aberration in maximum temperature over the study period of 1990 to 2013 had negative consequences in rice yield in both hilly and Terai region of Nepal. Unprecedented rainfall during the harvesting period of both rice and wheat is another issue Nepalese farmer are facing in recent years. Drought is another impediment for agriculture in Nepal. 0.52 million ha of the area is affected by drought in Nepal (Tripathi *et al.*, 2019).

The spatial and temporal variation in precipitation pattern negatively impacts the rice-wheat cropping since 65% of cultivation is rainfed (Gahatraj *et al.*, 2018). The conventional method of rice cultivation requires about 1500 mm of water after transplanting to complete its life cycle before which seedling raising and puddling requires 50 mm of water (Karkaliya *et al.*, 2018). Studies have shown that average annual rainfall decreased at a rate of 10.21 mm/year and mean temperature increased at rate of 0.02℃/year between 1984 and 2014 in central part of southern Terai (Dhakal *et al.*, 2016). Terai region of Nepal has already experienced adverse impact of climate change on rice production. Temperature level has already crossed threshold level, and the growth rate of paddy production has been limited to 1.4% per year for past two decades (Adhikari *et al.*, 2017). Crop production nationwide was reduced by 12.5% in the year 2005 and 2006 due to early monsoon whereas winter drought of 2009 reduced wheat production by 14.5% (Dhakal *et al.*, 2016). The minimum optimum threshold temperature for wheat production in Nepal is 20℃, and annual increase of temperature in Nepal is 0.06℃ (Bhujel *et al.*, 2014). Studies done by (Parajuli and Devkota, 2016) at Bhairahawa showed that wheat production might increase with rising temperature to certain extent since annual average maximum temperature at area of wheat cultivation was 18.3℃. Extinction of indigenous varieties of rice such as aromatic rice varieties, stress-tolerant varieties etc. is new threat climate change possess in recent time.

2.2 Stagnation in Yield

The history of yield trend of rice and wheat doesn't seem to be so ameliorating in Nepal. Rice yield in Nepal was highest among south Asian countries in 1960s, but it is lowest in recent years (Tripathi *et al.*, 2019). The overall growth rate of rice yield was only 0.5% between 1958 and 1997 whereas wheat yield grew at a rate of 0.8% per annum between 1962 to 1998(Katkai *et al.*, 2001). Study done by Ladha *et al.*, (2001) for 20 years at Parwanipur, Nepal showed that even with full recommended dose of NPK and FYM, yield for rice and wheat were <3.15 tons/ha and <2.16 tons/ha respectively which were only 40-50% of potential attainable yields. Another long-term study was done by Regmi *et al.*, (2002) at Regional Agriculture Research Station, Bhairahawa, Nepal showed net loss of 62.3 and 15.2 kg/ha/year of potassium(K) in NPK and FYM treatments respectively at the end of 20 years. However, productivity of rice and wheat were increasing at rate of 1.65% and 2.52% between 1995 to 2014 (Gairhe *et al.*, 2018). But still productivity of rice and wheat were only 3.36 t/ha and 2.59 t/ha respectively in 2014 (Gairhe *et al.*, 2018) which is fairly below when compared with other South Asian countries.

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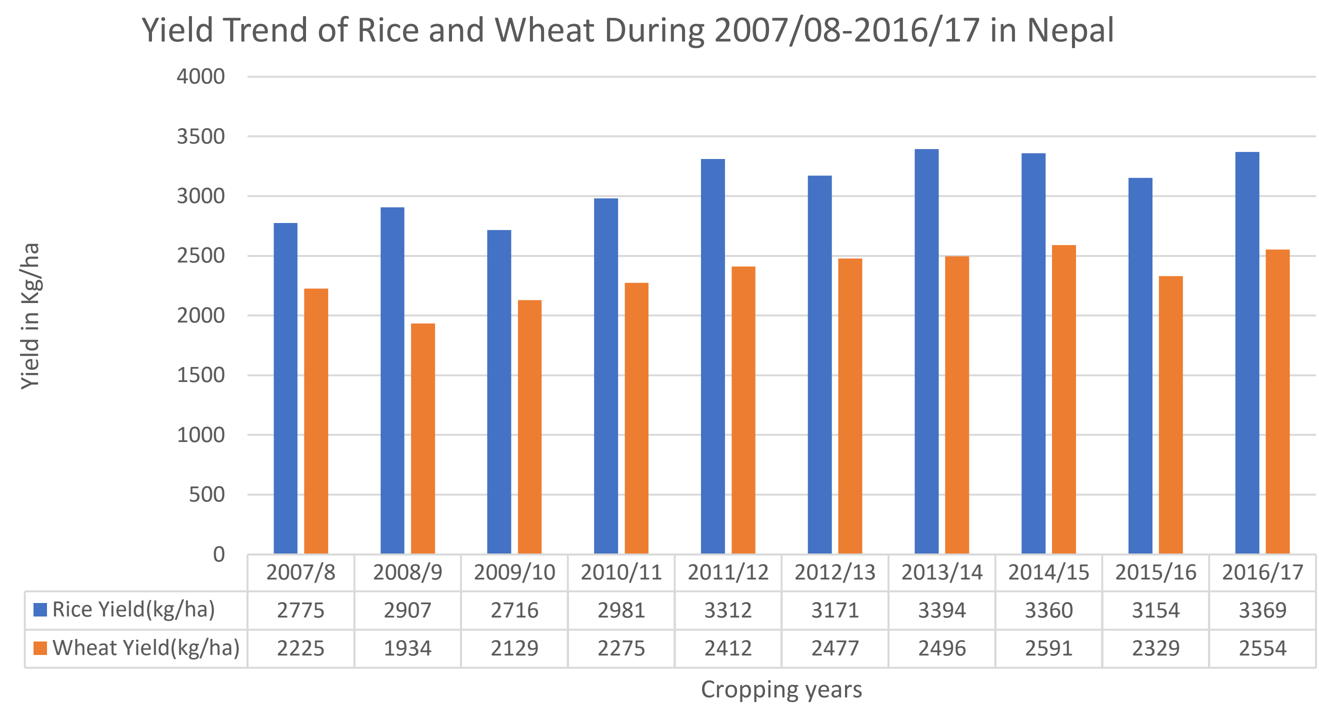

Source: MoALD 2018

Fig.1: Yield trend of rice and wheat in Nepal during 2007/8-2016/17

In recent years yield trend of rice and wheat seems to be plateauing although efforts are made to increase productivity to meet national production requirement. Incompatibility of rice and wheat varieties is also an important reason for sub-optimal production of wheat. Long duration varieties of rice mature lately and provide less turnaround time for seedbed preparation in wheat, forcing farmers for late sowing of wheat. Late planted wheat and long duration varieties of wheat mostly get hit by rainfall during harvesting time ultimately damaging the production. Naturally planting of wheat is delayed in an area where soil contains excessive moisture during rice harvesting period. Oritz-Monasterio *et al.*, (1994) observed 1% loss in yield potential for each day the planting of wheat was delayed beyond last week of November in IGP region. Also, Ishaq *et al.*, (2001) reported reduction of both water and nutrient use efficiencies of post ricewheat and sorghum by 38% and 22% respectively due to subsoil compaction in loamy soil. Stagnation in yield trend of major cereal crops raises question of food sufficiency in coming years for Nepal. It is necessary to enforce soil specific nutrient and water management practices to achieve stable growth rate in yield.

2.3 Impacts of Agrochemicals on Environment and Groundwater Pollution

Excessive application of synthetic fertilizers and other agrochemicals in rice-wheat system deteriorates the groundwater quality through runoff and leaching of chemical residues to groundwater (Karkaliya *et al.*, 2018). This leaching and runoff process make groundwater nitrate-rich. Extracting of such polluted groundwater for human consumption and agricultural application ultimately possess a threat to crop health as well as human and livestock (Bhatt *et al.*, 2016). Leaching of agrochemicals to groundwater aquifers can be serious in sandy soil. A study done by Pandey *et al.*, (2017) showed that consumption of chemical fertilizerincreased at the rate of 882.43 metric tons per year from 1991/91 to 2015/16 in Nepal and it was 42828 metric tons in 2016.

Rate of consumption of agrochemicals is significantly lower in Nepal compared to neighbouring countries. Application of synthetic fertilizersincreased at the rate of 0.7791 kg/ha per year from year 1994/95 to 2015/16 and average fertilizerconsumption in year 2015/16 was found to be 83.8 kg/ha across the country (Pandey *et al.*, 2017). Diwakar *et al.*, (2008) reported that pesticide consumption in Nepal is

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increasing by about 10-20% every year. Pesticide consumption in Nepal was only 142 gm/ha in 1995 which rapidly grew to 396 gm/ha in 2014 (Adhikari 2018). With these rates, threat to groundwater pollution in Nepal is lower compared to neighbouring countries like India and China where rates are significantly higher.

2.4 Yield Gap in Production

The yield of rice is lowest in Nepal compared to other South Asian countries. The differences in average potential yield and actual farm-level yield is high even for improved and hybrid varieties. The gap between potential yield and actual yield at farm level is 54% per unit of land for rice varieties (CDD 2015). This yield gap is associated with technological constraints, climate change, poor soil health, unscientific methodologies and resource unavailability. The technology involved in cultivation at farmer's field lags largely compared to technology at research stations. The package of practices involved at farmers field and research station have significant differences. This is more pronounced in hills and high hills where yield gap of rice varieties ranges to 107% and 158% respectively (CDD 2015).

2.5 Impacts of Longterm RWCS on Soil Health and Fertility

Soil fertility, soil organic matter content (OMC), its structure and its physicochemical properties are key players of sustainable RWCS. Soil undergoes major physical, chemical and biological changes throughout every cycle of rice-wheat cropping. The fertility status of Nepalese soil is declining due to poor OMC in more than 60% of the cultivated field and haphazard use of chemical fertilizers (Khanal *et al.,* 2012). Since the crop residues are used as fodder for cattle, residue recycling is poor in Nepal. This is one of major causes of declining soil OMC. Puddling of soil maybe crucial for water holding, weed growth suppression, reducing percolation losses and easy mechanical as well as manual transplantation of rice. But it is associated with many long-term changes in soil structure and physicochemical properties. Puddling done for reducing percolation loss also deteriorates soil physical properties by breaking down soil aggregates to form hardpans at shallow depth which leads to increased bulk density and decreases hydraulic conductivity in following wheat crop. Kukal and Aggarwal, (2003) reported that the puddling at normal depth of 10-12 cm develops sub-surface compaction layer at 14-20 cm which persists even during post-rice wheat seedbed preparation. Yield reduction of 8-9% was found in wheat following puddled rice when compared with wheat yield following non-puddled direct-seeded rice (DSR) (Zhou *et al.,* 2014). Waterlogging in such soil can result in oxygen stress, diminish redox potential and enhance accumulation of phytotoxins in the root zone, ultimately decreasing wheat yield (Ladha *et al.,* 2003). The subsurface pan creates moisture stress to lower root zone and even restricts root growth of wheat. Upon tillage after rice harvesting, medium to larger clods are formed with higher surface exposure permitting greater evapotranspiration from soil. Such tilth results in poor seed germination and seedling emergence of wheat (Chenkual and Acharya, 1990). Intensively tillage system that we currently practice is prone to loss of organic and inorganic soil nutrients through erosion and leaching. More extensive research is required to develop tillage system that would produce synergistic impacts on yield of both crop and aid in sustainable intensification of RWCS.

2.6 Greenhouse Gas Emission Status of Nepalese RWCS

Irrigated rice supports the accumulation of relatively higher amount of soil organic matter in rice-wheat system as compared to non-irrigated or upland condition. Water stagnated condition in rice prevents aerobic decomposition of soil organic matter (SOM). Therefore, CH_4 is produced through anaerobic decomposition of SOM from rice field. CH_4 emission in Indo-Gangetic plains normally didn't exceed 30 kg/ha CH_4 under mineral fertilization and 50 kg/ha CH_4 under organic, which is very low compared to Central China where CH_4 emission ranges from 200-900 kg/ha under mineral fertilization and up to 1100 kg/ha under organic system (Wassmann *et al.,* 2004). Average maximum CH_4 emission from rice field was found to be 28 kg/ha/season under rainfed condition from studies conducted at Nepal Agricultural Research Council (NARC), Khumaltar, Nepal (Malla, 2009). The same study showed CH_4 emission of 49.03 kg/ha/season from rice field supplied with 50% nitrogen + 15 cm stubble whereas minimum CH_4 emission in control plot was only 7.7 kg/ha. As for wheat crop it acts as sink for CH_4 and the major greenhouse gas is N_2O. during wheat crop season, biological nitrogen fixation is reduced and mineralization of soil organic matter is facilitated, this accelerates the loss of SOM. N_2O emission is high during dry soil state (wheat) than during water stagnated (rice) condition. This alteration of wetting and drying cycle in RWCS promotes N_2O emission.

According to World Resource Institute Climate Analysis Indicator Tool, emission from agriculture increased by 38% between 1990 to 2014, for which livestock sector was the largest contributor and rice cultivation was second one (USAID, 2014). The report also stated that agriculture sector was responsible for half (50.1%) of total emissions of Nepal

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in 2014. Synthetic fertilizers though represent small fraction (3%) of total emission from agriculture, but its share is increasing rapidly. As of 2014, Nepal's total CO_2 emission is 44.06 million metric tons which is0.09% of world total (USAID, 2014). There is no question that emission from Nepalese RWCS is not worth considering when compared to Indian and Chinese emission, but when it comes to potential impacts of global warming and climate change in agriculture, Nepal is on top of list.

2.7 Diverse Weed Flora

Excessive and uncontrolled weed pressure is an important factor for the yield gap in rice-wheat system. Higher weed density in rice-wheat system signifies intense competition for nutrient, light, water and even space for growth. Since both rice and wheat are cultivated intensively, overall decrease in productivity is significant. The dominance of weed is more experienced in dry seeded rice and zero tillage plots compared to puddled rice. Since the soil is supplied with enough water, micronutrients and fertilizers, it isn't easy to suppress the weeds. Manandhar *et al.,* (2007) reported reduction in straw yield by 13-38% and grain yield by 25-47% compared to weed-free control plots in experiment done at paddy field in Kirtipur, Kathmandu. Yield losses in wheat due to weed was estimated to be around 10-50% in various agroecological zones of Nepal (Ranjit *et al.,* 2009). There have been cases of persistence of *Phalaris minor* against isoproturon and 2,4-D. *Phalaris minor* is most hazardous weed for wheat in Nepal. In our current crop management system, weed seeds get mixed with harvested crop seed. So, there is long-term chance of existing hazardous weeds like *P. minor* unless we change our crop management practices.

2.8 A threatto Biodiversity Loss

Biodiversity loss is a critical environmental issue in most of the rice-growing countries in recent years. Since the practice of intensive industrial farming is increasing widely in many countries, the number of cultivated species is decreasing. Creating megafarms in vast area of land with purpose of cultivating only single or double crop can create serious biodiversity issues in local agroecosystem. The consequence of such farming system can range from imbalanced agroecosystem to species loss. The focus on major crops which have high market demand is reason behind decrease in number of cultivated species of crops. But this may also be closely associated with livelihood and socio-cultural aspect of farmers. In countries where farming is rice-based this issue is even more serious.

The productivity of cereal crops including (rice and wheat) is lowest in Nepal compared to other south Asian countries. To meet the nutrition requirement of ever-growing population it has become necessity to produce more from the limited cultivable lands. Accordingly, new high yielding, hybrid varieties were introduced in the country. Revolution of improved and hybrid varieties of rice and other major cereals pose threat to indigenous varieties. Before green revolution over 2500 landraces of rice were grown in Nepal but as of 2014 only 270 rice varieties are grown in different agro-ecological zones of which 157 are local, 59 are improved and 54 are hybrid varieties (CDD 2015). In terms of area local varieties cover only 10.2% of area whereas improved and hybrid varieties cover 82.4% and 7.4% respectively (CDD 2015). Though yield maybe high with new varieties, they are highly susceptible to diseases and pest outbreak. Also, indigenous varieties which were more tolerant to biotic and abiotic stress and belonged to local agroecosystem are now on the verge of extinction.

2.9 Depletion of Groundwater

Rice is a heavy consumer of water. Rice-wheat cropping system requires about 11,650 m^3/ha of water, out of which major portion 7650 m^3/ha goes for flooded rice (Bhatt *et.al.,* 2016). To produce 1kg of rice under irrigated condition, it requires around 5000 litres of water. Underground water has been a dependable source of irrigation all-round the year for intensive rice-wheat cultivation in South Asia. But the situation seems likely to be different under long-term scenario. Extraction of groundwater for agricultural as well as non-agricultural operations has increased sharply in Indo-Gangetic Plain (IGP). The per capita water availability of Nepal was 21,623 m^3 in 1950 which is projected to be 4,820 m^3 by 2020 (Bhatt *et al.,* 2016). According to Tiwari *et al.,* (2009) anthropogenic groundwater loss rate for the Ganga basin (India-Nepal) is highest (~22.56±3.8 km^3 per year) which is about 42% of total use in the region. Nepal's contribution to this huge depletion is obviously minor compared to India. There is no exact data for extraction of groundwater for agricultural and non-agricultural operations all round Nepal but the rate of extraction is increasing rapidly. This doesn't necessarily mean that reliability of underground water for irrigation in the near future should be doubted but it certainly means that the time has come for us to keep check and balance of ingoing and outgoing water from the ground. Consequences of groundwater depletion like drying of natural springs, watersheds and aquifers can be observed in Kathmandu valley as well as some districts of Terai region where groundwater has been extracted extensively. Environmental impacts will be harsh if future of intensively irrigated RWCS of South Asia relies largely upon

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underground water under current rate of groundwater recharge. Judicious water management is key to sustainability of rice-wheat system.

2.10 Outbreak of Pest and Diseases

The entire rice-wheat system is cultivated under high input environment. This system requires sufficient irrigations, agrochemicals (fertilizers and pesticides), micronutrient and organic inputs. This nutrient-rich environment acts as a catalyst for the growth of phytopathogens and insect-pest. The infestation of disease and insect pest adds unnecessary burden on cost of production, raising question about the sustainability of system. With excessive use of insecticides and other pesticides, insect-pest and disease are found to be resistant with current pesticides. New breeds of insect-pest are also found in many cases. This adds further challenge on sustainability of rice-wheat system. Higher dose of nitrogenous fertilizers aimed for increasing yield also promotes disease and insects in wheat.

2.11 Socioeconomic and Livelihood Factors

Nepalese farmers don't seem to be happy with the economic benefit of rice-wheat cultivation. The cost of production for per kg rice is increasing every year but farmers don't seem to be benefited from that. There is huge population of working age group who don't seem to be satisfied with agriculture profession and are shifting towards other occupations. In recent years there have been cases of large cultivable land being left uncultivated in many hilly villages due to lack of working population. The working-age group which used to provide labour force to agriculture are now leaving country for other jobs which could satisfy their economic needs. Also, farmers are shifting from cereal crops to vegetables, fruit and livestock where economic benefit is higher.

III. AMELIORATING RICE-WHEAT CROPPING IN NEPAL

3.1 Conservation Agriculture

According to Food and Agriculture Organization (FAO), CA is a farming system that promotes maintenance of permanent soil cover, minimum soil disturbances (zero tillage) and diversification of plant species. It is aimed at enhancing biodiversity and natural biological cycles in and out of the soil to improve water and nutrient use efficiency. The main motto of CA is sustainable food production. It relies on three main principles viz. minimum mechanical soil disturbances, permanent soil organic cover and species diversification. Intensive RWCS of Nepal which currently seems to be losing its sustainability can be brought on track by application of CA principles. Concept of CA differs widely from our current RWCS (especially puddled rice), but even partial application can bring positive changes. Although the initial yields might not compete with conventional method, the long-term benefits to soil health and entire agroecosystem are vital for our food security. CA is equally relevant in adapting Nepalese agriculture to climate change by establishing resiliency in the system. Intensification of rice-wheat system in sustainable manner is possible only if our agroecosystem remains functional and resilient. So, it is essential to put principles of CA into action for our long-term food security. The yield of rice maybe low under conservation agriculture but wheat yield is found to be significantly higher compared to conventional system. A four-year experiment done by Jat *et al.*, (2019) showed that under full CA-based basmati rice-wheat system, system productivity was higher by 36% and net returns by 43% compared to conventional.

3.1.1 Zero Tillage

Method of tillage is an important factor for determining oxygen fluxes in upper soil layer, which provides oxygen for aerobic decay of soil organic matter. In conventional system rice is transplanted in wet tillage (puddled) soil whereas following wheat crop is sown in dry tillage. Intensive tillage system in rice has been found to be detrimental to soil health and results in long term structural changes in soil. Sustainability of RWCS can't be ensured unless we modify our tillage system. Zero tillage supports minimum soil disturbances, prevents structural destruction and promotes water stable aggregates. In contrast to puddling it decreases bulk density and water penetration resistance (Zhou *et al.*, 2014).

Under zero tillage system seeds are directly placed to required depth and spacing using zero-till seed cum fertilizer drill or other heavy seeders depending upon presence or absence of crop residue in plots. Irrigation in zero tillage direct-seeded rice (ZTDSR) is based on soil matric potential, crop growth stage and soil properties. Jat *et al.*, (2019) reported 33% lower water use in fully CA applied plots of rice-wheat system compared to conventional. Flooding of rice as done in conventional system is avoided here. Moving from conventional to no-tillage system may bring positive changes in soil properties, micro fauna, microflora and weed flora ultimately affecting long term crop productivity and input use efficiency but further broad study is required for making the technology equally effective to rice also.

3.1.2 Species Diversity (Alternative Crop Rotation)

Zero tillage in RWCS promotes early planting of wheat, which creates opportunity to integrate legume crops or other green manure crop into the system, ultimately it maximizes

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system productivity and stabilizes soil health. Variation in cropping pattern in every cropping season changes the rhizosphere affected soil area by replenishing the depleted nutrients. Replacing rice with legume or other less hungry crop for a season can increase the sustainability of system either by fixing nutrients or by providing time for replenishment of depleted nutrients. Rice and wheat both crops are good hosts of nematodes whereas legume crops are resistant, therefore integrating legumes crops (cowpea, mungbean) during intervening period increases yield of both crops significantly compared to simultaneously rice-wheat. Furthermore *Bradyrhizobium sp*. and *Herbaspirillum sp*. are found to colonize the rice roots when grown in rotation with the legume, promoting rice growth and availability of plant nutrients especially nitrogen and phosphorous (Zhou *et al.*, 2014).

3.1.3 Soil Organic Cover and Residue Recycling

Organic soil cover is effective in regulating soil temperature, building healthy microbial ecology, reducing weed dominance and conserving moisture by reducing evapotranspiration. Under conventional agriculture entire crop residue is left in the field so that soil organic carbon and other nutrients can be recycled. Jat *et al.*, (2019) reported significant increase in soil organic content by 21% in zero tillage plots with residue recycling compared to conventional.

In rice-wheat system both crops heavily consume organic and inorganic nutrients of soil to produce biomass of crop with its residue. Practicing a cropping pattern with such heavy consumer crops for subsequent cropping season causes huge depletion in the organic and inorganic nutrient pool in soil. Unlike many Indian states where paddy straw is burned in field, Nepalese farmers neither burn paddy straw nor leave in field for decay. Instead it is used as fodder for cow and buffalo all over the country. Wheat straw is also used as fodder in many districts of Terai and hilly region although nutritional value of both is very low. So Nepalese rice-wheat system is in risk of depletion of organic and inorganic component of soil unless residue of corresponding biomass is replenished into soil. Applying of farmyard manure is effective way of replenishing the soil nutrient pool but at same time it is also responsible for increased CH_4 emission from rice-wheat system. So, it is vital to send crop residue back into the soil to replenish the depleted elements as well as to compensate the CH_4 emission from FYM through carbon sequestration. Green manure crop like *Sesbania sps.*, legume crops etc. are also effective. But the impact of applying crop residue is found to be higher compared to green manure crops. Incorporating green manure and crop residue in addition to synthetic fertilizers can have higher carbon sequestration ranging from 10-20% higher (Wassmann *et al.*, 2004).

3.2 Alternate Wetting and Drying (AWD)

AWD technique was developed by International rice research institute to cut off the water requirement for growing rice. In this system dislike conventional method of continuous flooding, cycle of draining and re-flooding is practiced depending upon crop stage and critical water requirement. Its main aim to maintain optimum water level at any stage of crop growth. Under the AWD technique, irrigation cycle begins after two weeks of transplantation. The rice field is left to dry to a point until there is optimum water for crop growth. The water content in soil is continuously monitored through level gauge (tensiometer). The field is re-flooded once the water level has gone below optimum level. AWD technique is practiced in all stages of crop growth except during flowering when field is kept flooding. A meta-analysis done by Carrijo *et al.*, (2017) showed that using mild AWD can reduce water consumption by 23% and it was found that soil properties can affect the AWD technique performance. The experiment done by Howell *et al.*, (2015) on dry season rice in central Terai of Nepal showed no significant difference in yield between treatment plots, although AWD plots received 57% less water requirement than conventional plots. In similar experiment Djaman *et al.*, (2018) observed 27.3% less water consumption with no yield loss compared to conventional. Apart from water use efficiency AWD system enhances tillering, root growth and helps cut off CH_4 emission from rice field by significant amount. Therefore, crop water use efficiency of RWCS can be improved by the application of AWD if required field infrastructures are developed. Farmers irrigating their field through pump water can have more benefit since they have more governance over irrigation water.

3.3 Climate-Smart Agriculture (CSA)

The main objective of CSA is to establish resilience and adaptive capacity in agro ecosystem as well as increase the productivity of system in sustainable manner. CSA techniques are oriented towards reducing greenhouse gas emission as possible. In a country like Nepal which has already experienced impacts of climate change in agriculture, it is essential to adapt its agriculture to changing climatic conditions to ensure food security for generations to come. In RWCS, CSA is primarily aimed at mitigating GHG's emission and adaptation of nutrient and water management practices with climatic variability. The technologies of CSA ranges from simple cultural management practices and adjustments to technical operations like weather forecast based irrigation, site-specific nutrient management (SSNM), adoption of System of Rice Intensification (SRI), need-based nutrient management and AWD practices.

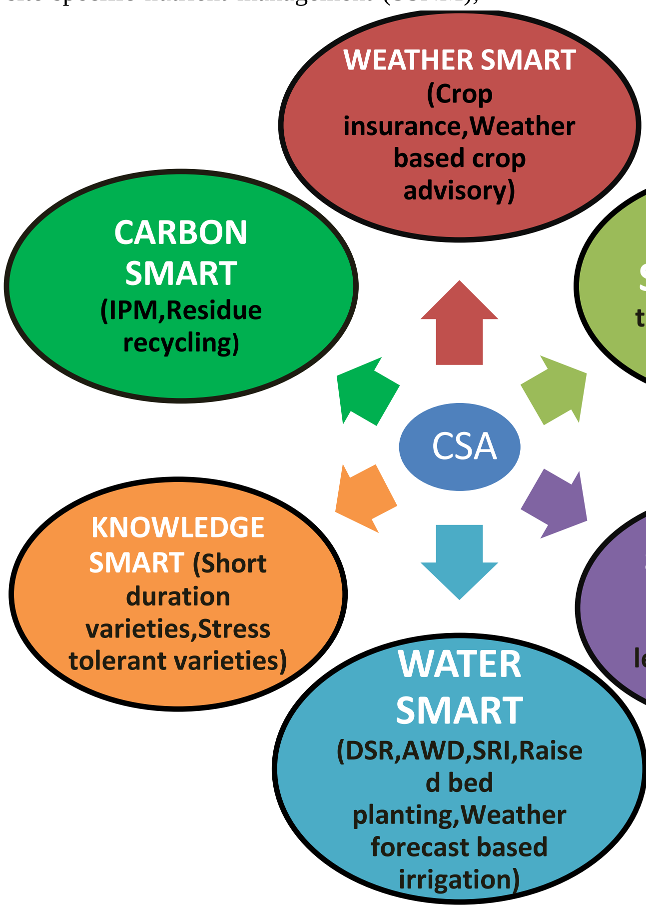

Fig.2: Climate Smart Agriculture for Sustainable Rice-Wheat System

IV. CONCLUSION

It's no doubt that RWCS is unavoidable cropping system for food security of Nepal but with growing concerns about sustainability all around the globe, Nepal needs to think critically about environmental impacts of system on long-term. The current trend of RWCS might lead to food insecurity in near future. It is essential to adopt crop diversification practices for sustainable farming system and building resilience in agriculture. Limited research has been done in Nepal about issues concerning sustainability of the rice-wheat system. Extensive studies have to be done to conclude about various aspects and changes have to made on policies as well as farm-level practices for sustainable food security.

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Effect of the corn-cowpea association on the organic carbon dynamics of the soils of two plots in real culture in northern Côte d'Ivoire

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***Abstract*— *Declining soil fertility is a major factor in low agricultural production and increases food insecurity in northern Côte d'Ivoire. The objective of this study is to understand the real influence of cowpea in the corn-cowpea association modalities on the evolution of organic carbon (C) and nitrogen (N) contents in the soil on the scale of plot compared to control soils without cowpea and fallow more than 30 years. In this perspective, two farmer field plots were conducted in a participatory manner with two groups of corn producers in the localities of Kolokaha and Sohouo (Department of Korhogo) in order to assess the effect of the semi density of cowpea (Vigna unguiculata L.) on the initial physical and chemical characteristics of the soil. In the open field, four (4) treatments corresponding to corn in pure culture (T0); corn + cowpea interposed online (T1-SNL); corn + cowpea in double intersected line (T2-SNDLI) and corn + cowpea staggered (T3-SNQ) were tested in three (3) completely randomized blocks with three repeats. Thirty-two (32) composite soil samples from natural fallows and T0, T1-SNL and T2-SNDLI treatments were taken and analyzed in the laboratory. The results show a clear improvement in the organic carbon and nitrogen levels, and above all an increase in the soil pH in the plots in associated crops. The combination of corn and cowpeas is an alternative to good soil fertility management and could be recommended in rural areas.*

***Keywords*— Soil fertility, association, semi density, yield, Côte d'Ivoire.**

I. INTRODUCTION

Depletion and degradation of the soil, as well as climatic variability, are responsible for the decline in agricultural yields and worsen the poverty of the populations in the north of Côte d'Ivoire. With the high land and population pressure observed in this area, farmers are forced to practice continuous cultivation and make the most of the available land (N'guessan et al., (2019a); N'goran et al., 2018), as is the case in the cotton zone of Burkina Faso reported by Coulibaly et al., (2012), Barro et al., (2016), Ouattara et al., (2016) and Coulibaly et al., (2017). Under these conditions, fertile land has become increasingly scarce in the cotton basin and farmers are unable to allow their soils to rest sufficiently. This has resulted in the depletion of agricultural soils in organic matter and their enrichment in sand (n'goran et al., 2018; n'guessan et al., 2019a) under the constraint of changes in use and agricultural intensification. However, soils, as a direct feeding mother of plants and indirect animals, constitute for farmers the major capital necessary to ensure the production necessary for their well-being and, for many, the main source of their diet. Today, farmers are worried about the depletion of their soils, say that the land no longer produces as before (n'guessan et al., 2019b). While it is recognized that soil organic matter (MOS) conditions many soil properties and its management is an essential component of the sustainability of agrosystems, it is thought that the introduction of a more productive and sustainable farming system in the peasant environment, based on maintaining ecosystem functionality, appears to be a solution to help restore soil fertility and productivity in northern Côte d'Ivoire. Because in the traditional cotton and maize-based farming systems that predominate in the dense area of Korhogo department, the supply of mineral fertilizers alone does not maintain soil fertility. In this area, legumes occupy only a marginal part of the cultivation systems, although they can play a very important role, whether they are grown in rotation or in combination. Several studies on legumes indicate that they can improve soil fertility by symbiotic nitrogen fixation in the air (Gbakatchetche et al., 2010, Barro et al., 2016; Ouattara et al., 2016; Coulibaly et al., 2017 and Kouassi et al., 2017), to produce quality fodder for animals (Zoundi et al., 2006; Bambara et al., 2008 and Ouattara et al., 2016) and provide income for farms. However, with the high land and demographic pressure observed in recent years in the northern part of Côte d'Ivoire, the use of soil restoration technique with tree legumes becomes problematic. The use of herbaceous legumes thus becomes an alternative to improve soil fertility. Among these, cowpea appears to be the best choice because it is the main source of protein and food for rural and urban populations in the area (N'guessan et al, 2019a) as well as for livestock. To do this, the research question is the maize-legume combination method that can reduce competition between crops and promote improved yields. This study, funded by KOREA-Africa Food - Agriculture Cooperation Initiative (KAFACI) as part of the KAFACI-Young Scientist Pilot Research Project (period 2017-2019) aims to develop with producers, organically efficient and technically acceptable associated maize/cowpea crop systems by conducting experiments in real-world crop conditions. This study was undertaken to assess at the plot level the impact of the corn-cowpea association on the organic carbon dynamics of agrosystem soils.

II. MATERIALS AND METHODS

2.1. Study site

The study was conducted in the villages of Kolokaha and Sohouo respectively located in the Sub-Prefectures of Sinématiali and Sohouo in Korhogo Department (Figure 1). These two villages are mainly populated by the Senoofo a natives whose main activity is agriculture and livestock. The general model of the region is a tabular set of iron breance with gentle fractures caused by garlands of hills and mounds with rounded reliefs placed on medium-height plateaus (Avenard et al., 1971). According to Beaudou and Sayol (1980), the geological substratum is made up of Calco-alkaline granites from the Precambrian. The soil cover of this region is characterized by the very large predominance of ferrallitic soils (Avenard et al., 1971). In terms of climate, the savannah district is bathed in a tropical Sudanese climate with two seasons, a dry season from November to April and a rainy season from May to October.

2.2. Sampling methodology

In each of the communities, the work was carried out after the maize harvest in December 2018, six (06) months after semi. Initially, each of the two demonstration plots had been gridded into 3 blocks and each block consisted of 3 sub-blocks subdivided into four 30 m^2 elementary plots measuring 6 m long and 5 m wide. On each elementary plot, there were 13 lines and on each line, 15 poquets. Each 30 m^2 elemental plot consisted of 195 corn plants per unit area on all three blocks. In the association system, maize and cowpea were placed in the elementary plots of each block and in blocks according to the schematic combination mode in Figure 2:

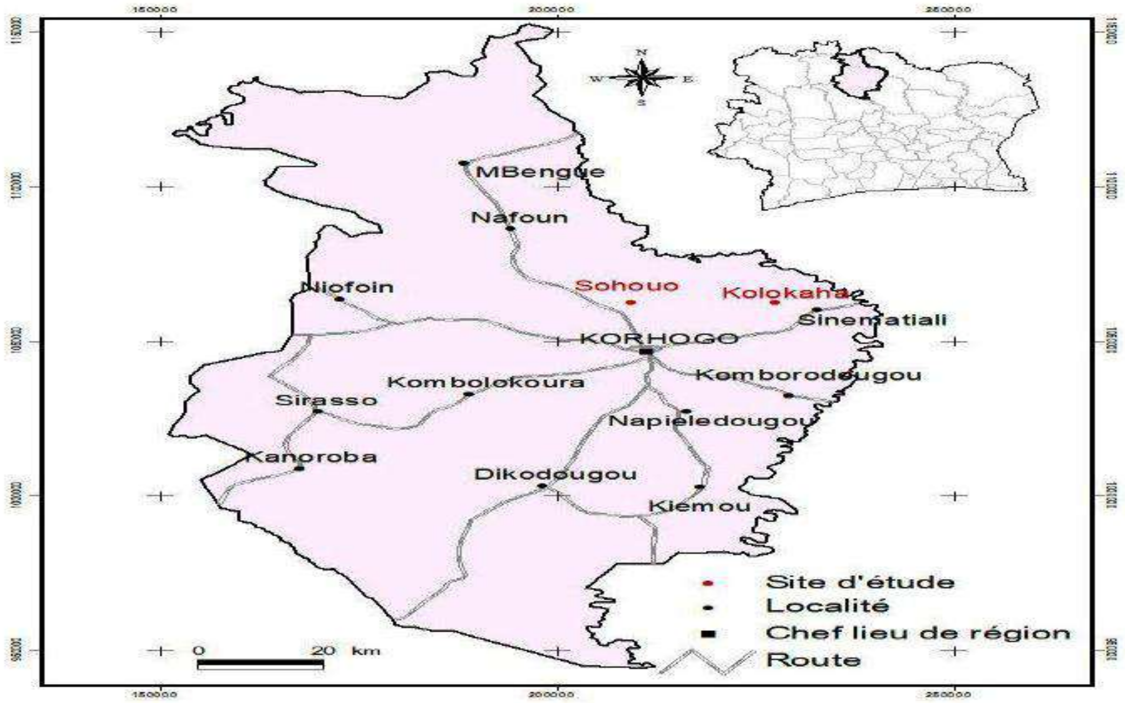

Fig.1: Map showing the location of the study areas.

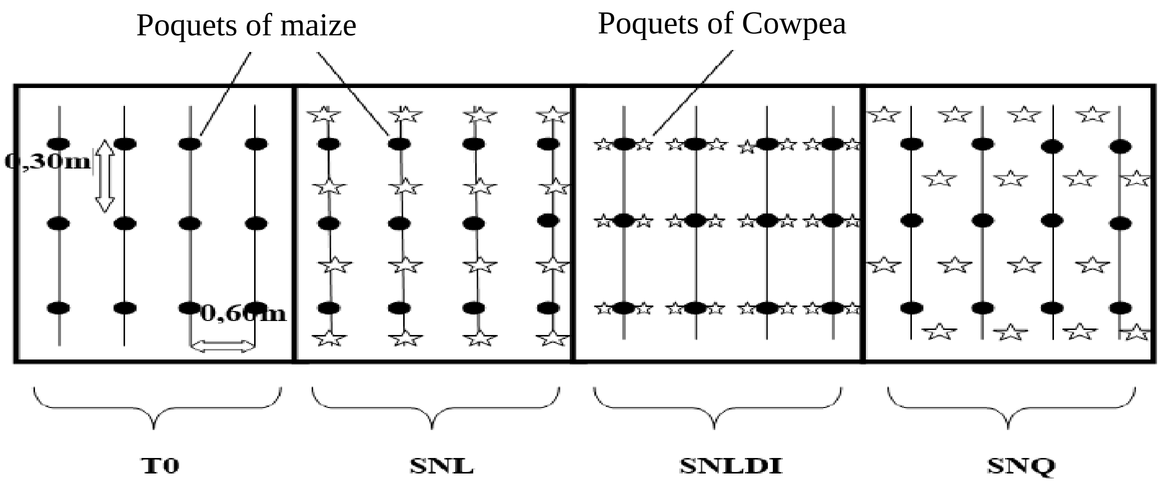

Fig.2: Maize and cowpea semi points in the association system

- **T0**: *maize seed in pure culture*
- **T1-SNL**: *corn-semi-cowpea seed interposed*
- **T2-SNLDI**: *semi-cowpea corn seed in double interbedded line*
- **T3-SNQ**: *semi-stale corn seed staggered*

There were 16 semi points when cowpea is sown in intercalated line (SNL) and quinconce (SNQ), or 16 cowpea plants per line for a density of 208 plants per elementary plot. In addition, when cowpea is sown in a double interspersed line (SNLDI), there were 32 plants on each line equivalent to a density of 416 plants/30m^2. All of these plots benefited from fertilizer inputs and insecticide treatments. For analysis purposes, soil sampling was conducted using the method described by Ouattara et al. (2011). It was carried out at the scale of the plot, considered a space of homogeneous physiognomy and cultural history in order to assess the impact of corn-cowpea associations on the initial characteristics of the soil. Also, given that changes under crop systems are taking place more rapidly in the surface layer (Azontonde et al., 1998; Koutika et al., 2001; N'guessan et al., 2015), in this study, we considered the layers (0 - 20 cm) as levels of soil sample collection for laboratory chemical analyses. With this in mind, four treatments were considered in this study at each of the two study sites. These are pure-grown corn treatments (T0); corn - cash in intercalated line (SNL); double-line cowpea corn (SNLDI) and a natural fallow more than 30 years old. On each of these elementary plots, 04 composite soil samples were compiled by processing and demonstration plot and on the natural fallow of 16 samples per locality for a total of 32 samples at all two sites. The samples were taken using an auger at the end of the growing season in December 2018.

2.3. Laboratory analysis

They were carried out in the soil and plant laboratory of the Higher School of Agronomy (E.S.A), of the National Polytechnic Institute Félix Houphouët-Boigny (INP-HB) of Yamoussoukro. The soil samples were first dried on newspaper at room temperature in laboratory B of Peleforo Gon Coulibaly University in Korhogo and then sieved with a 2 mm square mesh sieve. On these fine earth samples, the particle size analysis was carried out by the densimetric method using the Robinson pipette (Gee and Bauder, 1986). Five particle size classes have been separated: clays (0-2 Nm); fine stringers (2-20 Nm); coarse silt (20-50 Nm); fine sands (50-200 Nm); coarse sands (200-2000 Nm). The pH of the water was measured by electrometry, in a suspension of soil in water in a ratio of 1 / 2.5. The organic carbon (C) was measured according to the method of Walkley and Black (1934). As for total nitrogen, it was determined by the Kjeldahl method (Bremner, 1996). The exchangeable bases and the cation exchange capacity were measured in an ammonium acetate (CH3COOH 1N) extraction solution buffered to pH 7 (Thomas, 1982). The available phosphorus was determined by the modified Olsen Dabin method. As for total phosphorus, it was determined by colorimetry, after extraction with perchloric acid (Olsen and Sommers, 1982). With the analysis results, the structural stability index (St) and the beat index (IB) were calculated according to the formulas below. Pieri (1989) proposed the St index to characterize the structure of a soil. Indeed, the structure would be degraded for St <5 and good for St> 9. It is determined according to the following formula :

$$St = (MO \ \% \, x \, 100) \, / \, (A + L) \ \%$$

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For the beat, the index was calculated according to the formula :

$$IB = \frac{(1{,}5 \text{ x } \% \text{ fine stringers}) + (0{,}75 \text{ x coarse stringers})}{(\% \text{ clay} + 10 \text{ x } \% \text{ organic matter}} - C$$

- ✓ si pH < 7, C= 0 ;
- ✓ si pH > 7, C= 0,2 (pH – 7)

2.4 Statistical analysis

Comparison of soil character averages of different treatments began with variance analysis (ANOVA) at the 5% probability threshold. When a significant effect is noted between the different factors for a given parameter, the test of the smallest significant difference (ppds) was performed by the TUKEY method. All of these statistical tests were conducted using STATISTICA 7.1 software.

To better appreciate the evolution of soil parameters over time, the rate of evolution was calculated for each of them according to the following formula.

$$\boldsymbol{Evolution\ rate}\ (\%) = \frac{\boldsymbol{final\ value - initial\ value}}{\boldsymbol{initial\ value}} * \mathbf{100}$$

$\boldsymbol{final\ value}$: content of the element in the soil of any treatment

$\boldsymbol{initial\ value}$: element content in the reference soil

III. RESULTS

3.1 Granulometric characters of the soils studied

Table I shows the average proportion of the granulometric fraction (clay, fine silt, coarse silt, fine sand and coarse sand) of the soils of each study site based on treatments and natural fallow. On all two study sites, the results reveal that natural fallow soils are significantly richer in clay (A-28.33% in Kolokaha; A - 47% in Sohouo) compared to the soils of the two demonstration plots (A-19.83% in Kolokaha; A - 10.16% in Sohouo) having undergone the practice of continuous cultivation for several years. This represents an initial loss of clay of 30% and 78.38% respectively of the soil of the Kolokaha and Sohouo demonstration plots. The introduction of the associated maize-cowpea crop system on plots also resulted in a 34.44 per cent and 63.04 per cent reduction in the clay content in the soils of the T1-SNL and T2-SNLDI treatments in the Kolokaha plot. On the other hand, there was a slight trend in Sohouo to increase the proportion of clay in the soil of the SNLDI treatment by 34.64 per cent compared to a 14.76 per cent decrease in the soil of the SN treatment. For fine and coarse silt, there is no significant difference in Kolokaha between natural fallow soils and those from demonstration plots. Nevertheless, there was a slight enrichment of the soil of the demonstration plot of 20.75 per cent fine silt and 29.56 per cent coarse silt. In Sohouo, there is a marked difference in the proportion of fine and coarse silt from the natural fallow soil compared to the soil of the demonstration plot. Natural fallow soil is significantly richer in fine and coarse silt compared to the soil of the demonstration plot. The difference is 45.31% and 41.58%, respectively, of fine and coarse silt. In Kolokaha, the implementation of the maize-cowpea cultivation system had no effect, after 05 months of cultivation, on the proportion of fine and coarse silt of the soil of the elementary treatment plots (NLS). However, there is a downward trend of 9.63 per cent fine silt versus 5.63 per cent enrichment of coarse silt from the soil of these corresponding plots. In the elementary plots of SNLDI treatments, the proportion of fine and coarse silt is significantly lower than the corresponding values obtained at the Level of SNL treatments and the control plot without cowpeas. Thus, at the Kolokaha site, the decline in these elements was done with the semi density of the cowpea.In Sohouo, the basic plots SNL and SNLDI have a significantly higher proportion of fine silt than the cowpea-free control plot. With regard to fine sand and at the Kolokaha site, table I data show that prior to the installation of the crop, there is a similarity in its content in the soils of the demonstration plots (26.78 per cent). and under natural fallow (29.68%). The same is true for coarse sand content, although there is a 28.46 per cent enrichment trend in this element in the soil surface layer of the demonstration plot compared to natural fallow soil.

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Table I : Granulometric characteristics of soils of plots grown on baseline soil

		Clay (%)		Fine silt (%)		Coarse silt (%)		Fine sand (%)		Coarse sand (%)	
Traitements		**T**	**TE (%)**	**T**	**TE (%)**	**T**	**TE (%)**	**T**	**TE (%)**	**T**	**TE (%)**
Fallow		$28,33\pm0,85^{b}$	-	$8,00\pm1,47^{bc}$	-	$5,48\pm0,67^{bcd}$	-	$29,68\pm0,33^{ab}$	-	$28,5\pm0,84^{ef}$	-
Soil_T0	**KOL**	$19,83\pm0,62^{c}$	30,00	$9,66\pm0,94^{ab}$	20,75	$7,10\pm0,37^{b}$	29,56	$26,78\pm1,21^{b}$	9,77	$36,61\pm0,44^{e}$	28,46
SNL		$13,00\pm0,41^{d}$	34,44	$8,73\pm0,52^{ab}$	9,63	$7,50\pm0,99^{b}$	5,63	$30,4\pm1,24^{a}$	13,52	$40,36\pm1,89^{d}$	10,24
SNDI		$7,33\pm0,62^{g}$	63,04	$4,84\pm0,24^{d}$	44,56	$3,96\pm0,12^{cd}$	47,2	$19,76\pm1,11^{c}$	26,21	$64,10\pm1,02^{a}$	58,82
Fallow		$47,00\pm2,55^{a}$		$10,66\pm2,39^{a}$		$9,98\pm0,58^{a}$	-	$17,31\pm1,31^{de}$	-	$15,03\pm0,60^{g}$	-
Soil_T0	**SOH**	$10,16\pm0,85^{ef}$	78,38	$5,83\pm0,85^{cd}$	45,31	$5,83\pm0,63^{bc}$	41,58	$16,95\pm0,37^{e}$	2,08	$61,21\pm0,51^{ab}$	307,25
SNL		$8,66\pm0,62^{fg}$	14,76	$8,83\pm0,47^{ab}$	51,46	$3,28\pm2,45^{d}$	43,74	$19,55\pm0,72^{cd}$	15,34	$59,66\pm2,78^{b}$	2,53
SNDI		$11,66\pm0,47^{de}$	34,64	$8,16\pm0,24^{abc}$	8,27	$6,00\pm0,32^{bc}$	82,93	$29,43\pm1,40^{a}$	50,54	$44,73\pm1,37^{c}$	25,03
	ANOVA	-	-	-	-	-	-	-	-	-	-
	F	614,97	-	11,66	-	16,82	-	127,79	-	488,12	-
	P	< 0,001	-	< 0,001	-	< 0,001	-	< 0,001	-	< 0,001	-

In the same column, the means followed by the same letter are not significantly different at P <0.01, Fallow: Sol_T0: pure culture without cowpeas; SNL: SNL: treatment of interspersed online cowpea; SNLDI: treatment _semi cowpea in double intercal line, Kol: Kolokaha; SOH: Sohouo, T: element content or value; TE: Evolution rate.

In Sohouo, there is no significant difference between the fine sand proportion of the natural fallow soil and that of the demonstration plot before the cultivation was installed. On the other hand, there is initially a significant difference between the coarse sand content of the natural fallow soil (15.03%) demonstration plot (61.21%). This represents a coarse sand enrichment of 307.25% of the soil of the demonstration plot compared to the soil under natural vegetation. Five (05) months after the experiments were set up and at the Kolokaha site, the fine sand proportion of SNL treatments appeared significantly higher than the cowpea-free control soil with an enrichment rate of 13.52%. On the other hand, there was a 26.21% decrease in the proportion of fine sand (19.76 per cent) snite sNLDI plots with high cowpea semi density compared to the cowpea-free control soil. For coarse sand, its proportion increased with the presence and density of cowpea semi. Also, the values obtained at the level of SNL and SNLDI treatments are significantly higher than those obtained on the soil of control plots without cowpeas. Enrichment was 10.24% and 75.09%, respectively, in the soils of the SNL and SNLDI elementary plots compared to the soil of the cowpea-free control plot.

In Sohouo, the proportion of fine sand is significantly higher in the soils of the SNL and SNLDI elementary plots compared to the control soil without cowpeas. As a result, there was an enrichment of 15.34% and 73.63% of fine sand respectively in the soils of the SNL and SNLDI elementary plots. In contrast, the proportion of coarse sand decreased with the semi density of cowpea. The decrease was 2.53% and 26.92%, respectively, in the SNL and SNLDI elementary plots compared to the cowpea-free control plots.

Under current conditions of use, Table II data indicate that the soils of all treatments in each of the two demonstration plots, including those of natural fallows, are non-beating (IB -1.4). In terms of structural stability, the results also reveal that there is a significant difference between treatment soils and natural fallow. But the soils are very stable (Is 1) in the treatments of each of the two demonstration plots including those of natural fallows.

3.2. Physical-chemical characteristics of the soils studied

3.2.1. Organic carbon and soil acidity

Table III shows the pH, and total organic carbon content, nitrogen and soil phosphorus of the two demonstration sites, initially under continuous cultivation for several years compared to the natural fallow soil. Prior to the establishment of crops at all two study sites, the results show that the total carbon content is significantly higher (p -0.001) under natural fallow (T - 2.40% in Kolokaha; T - 2.55% in Sohouo) compared to the soils of the

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demonstration plots (T - 1.28% in Kolokaha; T - 0.93% in Sohouo). There was a 46.67 per cent and 62.37 per cent drop in total carbon soils in kolokaha and Sohouo cowpea-free demonstration plots compared to natural fallow soils. The same is true for nitrogen and phosphorus, which are higher in naturalfyding soils compared to soil levels in demonstration plots (Table III). At the end of the 2018 growing season, the combination of cowpea and maize in elementary plots had a significant effect on the carbon, nitrogen and soil acidity of the basic plots in pure cowpea cultivation. . In Sohouo, the carbon content increased in the surface layers of the soils of the elementary plots with the semi density of the cowpea. Indeed, carbon content (1.51%) nitrogen (0.09%) are significantly higher in elementary plots with high cowpea semi density (SNLDI) with maize compared to soil-level plots from pure cowpea-free elementary plots (C - 0.93%; N - 0.05 per cent.

Table I: Changes in the Structural Instability Index and the Weight Index

Traitments		**IB**	**TE (%)**	**ST**	**TE (%)**
Fallow	**KOL**	$0,24\pm0,05^{c}$	-	$0,5\pm2,47^{cd}$	-
SoIl_T0		$0,47\pm0,01^{\mathbf{b}}$	95,83	$0,9\pm0,48^{a}$	80,0
SNL		$0,51\pm0,04^{ab}$	8,51	$0,7\pm0,40^{bc}$	22,2
SNDI		$0,44\pm0,03^{\mathbf{b}}$	6,38	$0,6\pm0,10^{cd}$	33,3
Fallow	**SOH**	$0,26\pm0,05^{c}$	-	$0,8\pm0,24^{ab}$	-
SoIl_T0		$0,50\pm0,06^{\mathbf{bc}}$	92,3	$0,8\pm0,36^{ab}$	0,0
SNL		$0,6\pm0,09^{\mathbf{a}}$	20	$0,7\pm1,32^{bc}$	12,5
SNDI		$0,45\pm0,02^{\mathbf{b}}$	10	$0,4\pm0,34^{d}$	42,9
	ANOVA	-	-	-	-
	F	25,25	-	9,54	-
	P	< 0,001	-	< 0,001	-

In the same column, the means followed by the same letter are not significantly different at P <0.01, Fallow: Sol_T0: pure culture without cowpeas; SNL: SNL: treatment of interspersed online cowpea; SNLDI: treatment _semi cowpea in double intercal line, Kol: Kolokaha; SOH: Sohouo, T: element content or value; TE: Evolution rate.

This resulted in an increase of 63.53% and 68.75% respectively in the carbon and total nitrogen content in the soil layer of the SNLDI elemental plots. There are no significant differences in these parameters between soil-level values of low-density, semi-dented snare sunntos and pure-grown, cowpea-free plots. Nevertheless, there has been a 10.75% increase in total carbon in the soil layers of the sNL elementary plots compared to pure cowpea-free plots. In Kolokaha, there is no significant difference between the total carbon content of the soil layers of the elemental plots in associated cultivation compared to the soil values of pure cowpea-grown plots. However, there is a 7.81% increase in organic carbon in the soil layer of the low-density semi-SNL elemental plot. On the other hand, organic carbon falls by 27.34 per cent to the semi-density elementary plot (SNLDI) compared to the basic plot in pure cowpea-free cultivation. This trend of evolution of organic carbon content is identical to that of nitrogen. With regard to soil acidity and at each of the two study sites, there was no marked difference for this parameter between the soil layers of the elementary plots in associated cultivation of those of pure-grown, cowpea-free soils. In Sohouo, the results indicate that the pH is relatively higher in the low elementary plots (pH - 6.0) and high density (pH - 6.1) of semi cowpea compared to the soil of elementary plots in pure cowpea culture (pH - 5.7). Thus, there has been a tendency to raise the pH of the soil with the semi density of the cowpea. The improvement in soil acidity was 4.51% and 6.93% respectively in the surface layers of the soils of the low-fat (SNL) and high-density (SNLDI) soil plots of cowpea-free crops. At the Kolokaha site, soil acidity improved by 4.18% and 3.64%, respectively, in the SNL and SNLDI elementary plots compared to basic plots in pure cowpea-free cultivation. At the C/N ratio, the values are less than 15 in the soil layers of all the plots studied at the Kolokaha site. The lowest value (C/N - 9.63) is recorded in the soil surface layer of the SNL elementary plot.

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Table III: Evolution of organic carbon, nitrogen, phosphorus and soil pH content of different agrosystems.

Traitments	Sites	Organic carbon		Nitrogen		C/N ratio		pHeau		Phosphorus	
		C	TE (%)	N	TE (%)	C/N	TE (%)	pH	TE (%)	Pass	TE (%)
Fallow	KOL	2,40±0,53[a]	-	0,18±0,04[a]	-	13,42±1,32[cd]	-	5,90±0,65[ab]	-	63,66±10,87[a]	-
Soil_T0		1,28±0,14[ab]	46,67	0,10±0,01[b]	44,44	13,3±1,03[cd]	0,89	5,50±0,00[bc]	6,78	41,33±0,47[bc]	35,08
SNL		1,38±0,02[ab]	7,81	0,14±0,00[a]	40	9,63±0,22[e]	27,59	5,73±0,05[abc]	4,18	41,66±0,47[bc]	0,8
SNDI		0,93±0,02[c]	27,34	0,08±0,00[bc]	20	12,13±0,93[d]	8,8	5,70±0,00[abc]	3,64	45,66±3,09[bc]	10,48
Fallow	SOH	2,55±0,10[a]	-	0,16±0,00[a]	-	15,65±0,91[bc]	-	5,20±0,08[c]	-	49,33±0,47[b]	-
Soil_T0		0,93±0,07[c]	63,53	0,05±0,01[c]	68,75	18±2,32[a]	15,02	5,77±0,05[ab]	10,96	43,00±1,41[bc]	12,83
SNL		1,03±0,02[bc]	10,75	0,05±0,00[c]	-	19,51±2,05[a]	8,39	6,03±0,05[ab]	4,51	45,33±3,40[bc]	5,42
SNDI		1,51±0,03[b]	62,37	0,09±0,00[bc]	80	17,41±0,60[ab]	3,28	6,17±0,05[a]	20,59	37,33±4,50[c]	13,19
	ANOVA	-	-	-	-	-	-		-		-
	F	40,83	-	36,13	-	26,08	-	6,76	-	12,63	-
	P	< 0,001	-	< 0,001	-	< 0,001	-	< 0,001	-	< 0,001	-

In the same column, the averages followed by the same letter are not significantly different at P-0.01, Fallow: Sol_T0: pure cowpea-free cultivation; SNL: SNL: online _semi cowpea treatment; SNLDI: treatment _semi double intercale cowpea, Kol: Kolokaha; SOH: Sohouo, T: content or value in elements; TE: Rate of evolution.

On the other hand, on the Sohouo site, the C/N ratio is greater than 15 and there is no significant difference in this parameter between the values obtained in the elementary plots in associated culture (C/N of SNL - 19.51; SNLDI C/N - 17.41) compared to pure-grown plots (C/N - 18). As for phosphorus, its content remained almost invariable regardless of the study site and treatment. Nevertheless, there is a slight tendency in Kolokaha to enrich phosphorus with the semi density of cowpea. This slight increase in phosphorus was 0.8% and 10.48%, respectively, in the SNL and SNLDI elementary plots compared to pure cowpea-free plots. In Sohouo, the trend towards phosphorus enrichment was 5.42% in the soil of the basic SNL plots. Table V data indicate a 13.19% downward trend in phosphorus content in the soil layers of elementary semi-density plots of cowpea semi (SNLDI) compared to the soil layers of pure-grown elementary plots.

IV. DISCUSSION

The results of the study revealed a significant drop in the clay content and a decrease in the organic carbon stock in the soils of the demonstration plots before cultivation compared to soils under natural vegetation whatever the study site. These results are attributable to the practice of continuous cultivation of the land and the heavy use of pesticides and chemical fertilizers by farmers and confirm the work of N'guessan et al., (2019a and 2019b). They also corroborate the conclusions of N'guessan et al., (2015a), de Coulibaly et al., (2012) and Koulibaly et al., (2016) who made similar observations respectively in the north of Côte d'Ivoire. and in the cotton zone of Burkina Faso. In fact, in the agricultural landscape of the savannah district of the Ivory Coast, the mechanization of cotton and cereal farms intervenes in a privileged and systematic way for soil preparation. Because plowing is a particularly painful task to be carried out manually by producers. This is in line with the work of N'guessan et al., (2015a) who observed a compaction of cultivated soils in the departments of Ferkessédougou and Boundiali in the north of Côte d'Ivoire. According to the authors, the origin of the compaction of these soils is linked to their weak humiferous impregnation, to the increase in the apparent density with poor permeability and to the decrease in the hydraulic conductivity. Faced with this situation, the use of mechanization by harnessed or motorized cultivation has proven to be very effective in rural areas. In doing so, the clay content and the organic reserves of cultivated soils undergo numerous transformations over time under the constraint of changes in land use, the intensification of the use of resources and the vertical migrations of these elements. especially clay under the effect of infiltration water and their accumulation in depth. This result is in agreement with the work carried out in Tunisia by Annabi et al., (2009) which showed that the contents of total organic carbon (TOC) and total nitrogen (Ntotal) are closely related to the type of occupation of the ground. They observed that the forest soils of northern Tunisia contain 2.4% of TOC and 0.21% of Ntotal against 1.4 and 0.14% of TOC and Ntotal respectively for cultivated soils. This result also joins those of the works of Koulibaly et al., (2010) which obtained after 25 years of cultivation of the soil 44% reductions in carbon contents. Our results also agree with those of Tchienkoua (1999) who, in Cameroon, observed losses of 51% of total carbon, 43% of total nitrogen, 20% of phosphorus and 30% of fine sands after 3 years of setting in food crop cultivation. In Côte d'Ivoire, Ballo (2009) recorded, in the 0 - 20 cm layer, after twenty years of oil palm cultivation, losses of 70% of total carbon compared to the control soil under natural vegetation. Thus, the low richness of clay and organic elements in the soils of the demonstration plots reported by our results would thus be linked to their accelerated destruction during the execution of the plowing operations. It thus affects soil organisms directly by injuring, killing or exposing them to the risk of predation. This analysis agrees with those of annabi et al., (2009) who confirm that soil micro-organisms are the main driving force for destocking soil OM, since in the presence of favorable conditions (temperature, humidity, etc.), OM are the source of carbon and energy for these heterotrophic organisms. The tillage thus affects the biotic and abiotic factors of the soil, either directly by modifying the structural properties of the soil such as the arrangement of voids, aggregates, the connectivity of the pores, or indirectly by changing the aeration conditions, temperature and penetration of the soil by the roots. Our results revealed that the loss of clay and total carbon resulted in significant coarse sand enrichment of cultivated soils compared to reference soils. This has induced a particular structure to all cultivated soils in which, the solid constituents are piled up without any bond, for lack of colloids. Because of their size and the large spaces that separate them, these coarse sands make the soil filtering and light. According to Soltner (1992), reported by N'guessan et al., (2015b), when coarse sands dominate in a soil, they promote the penetration of water and air, retain little water and facilitate temperature exchanges. The soil heats up quickly in the dry season, and its constituents cannot clump together. This result corroborates the conclusions of Koné et al., (2008) and N’guessan et al., (2015b) who obtained similar results on soil quality in the humid savannah area of Côte d'Ivoire.

In the methods for combining cowpea corn, the results revealed a significant effect of cowpea on the organic carbon, nitrogen and soil acidity compared to the reference soil. In fact, in the associated crop plots, there was an increase in the organic carbon and total nitrogen content in the treatment of high semi-density of cowpeas compared to the values obtained at the level of the control plot without cowpea. The presence of cowpea also induced a significant increase in soil acidity in the treatments with low and high density of semi compared to the reference soil. It is likely that these qualitative changes in soil in the associated crop stimulate biological activity and modify a number of physical properties of the soil. These results are similar to those of Triomphe (1996), in North Honduras, who observed, in the long term, for a culture of corn associated with mucuna every year, an increase in the contents of C and N and exchangeable bases and absence of acidification. According to Latati et al., (2014) legumes can reduce nitrogen deficiency in the soil (N) by the symbiotic fixation of nitrogen in the air and change the soil pH.

V. CONCLUSION

At the end of this work, we can remember that the appropriate management of cultivated soils can allow carbon storage, in addition to the additional advantage of maintaining the resulting level of soil fertility. In Sohouo, the carbon (1.51%) and nitrogen (0.087%) contents increased by 62.36% and 74% respectively in the high density semi-cowpea treatments with corn compared to those obtained in reference ground level (C: 0.93%; N: 0.05%). Despite the increasing trend, no significant difference was noted for these parameters between the low-density semi-cowpea treatments associated with corn and the reference soil. As in Sohouo, there was an upward trend of 7.81% of organic carbon in the soil layer of the low density semi-SNL elementary plot. The presence of cowpea with maize raised the soil pH of the Kolokaha site by 4.18% and 3.64% respectively in the low and high density semi-cowpea treatments compared to the soil of elementary plots. in pure culture without cowpeas. The corn-cowpea association modalities thus constitute an alternative to the sustainable management of soil fertility in the north of Côte d'Ivoire.

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Pig Droppings: A Potential Biostimulatory Candidate for Bioremediation of Diesel Oil-Polluted Soil

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Abstract— *The effectiveness of pig droppings (PD) in enhancing bioremediation of diesel oil-polluted soil was investigated gravimetrically and spectrophotometricallyfor a period of 42 days. Polluted soil was amended with 5%, 10% and 15% (w/w) of PD. Loss of total petroleum hydrocarbon (TPH), microbial growth and germination indices were all monitored throughout the study period. At the end of 42 days, there was significant oil loss of 48.54% in the amended soil. Hydrocarbon-utilising bacterial (HUB) counts were higher in the amended option ranging from 4.2 x 10^6 ±0.69 to 10.9 x 10^6 ± 0.41 CFU/g. The HUB isolated from the oil-contaminated soil were identified tentatively as Bacillus cereus, Pseudomonas putida, Micrococcus variant, and Corynebacteriumsp and Staphylococcus sp. Similarly, fungal counts ranged from 3.0 x 10^5 ±0.21 to 10.9 x 10^5 ±0.33 CFU/g. Aerobic fungi isolated wereAspergillusniger, Aspergillusflavus, Fusariumsp, Cladosporiumsp and Penicilliumsp. Germination index of 53.4% was recorded in the amended option. Oil loss and microbial growth were significantly higher ((P≤ 0.05) in the amended option than the control option. Poultry droppings, therefore can offer a good alternative in bioremediation of diesel oil-polluted soil.*

***Keywords*— Bacteria, fungi, pig droppings, bioremediation, diesel oil, pollution.**

I. INTRODUCTION

The global reliance on petroleum products for source of energy has markedly engendered pollution of the aquatic and terrestrial systems [1]. Environmental pollution due to petroleum products always occurs due to accidental spills or anthropogenic mishap. The impact of hydrocarbon pollution can have serious and far-reaching effects on all life forms. Therefore, several strategies had been developed over the years to combat the menace of incessant oil spills.The scale of hazards imposed on the natural environment depends on the surface of the area contaminated by the petroleum products, their chemical composition, and the depth at which pollutants occur [2]. For this reason an increasing attention has been directed towards the research fornew strategies and environment-friendly technologies to be applied in the remediation of soil contaminated by petroleum hydrocarbons. Physical and chemical approaches to hydrocarbon remediation are expensive and ecotoxic [3]. Bioremediation technology which involves the use of life forms to detoxify or remove pollutants through the mechanisms of biodegradation has been found to be ecofriendly, noninvasive and relatively cheaper [4]. Microbial remediation which involves the use of microorganisms to clean up contaminated soils has gained wide acceptance. However, while organisms utilize hydrocarbons as carbon source, they need other nutrients such as nitrogen and phosphorus for their growth and activities. It stands to reason, therefore, that insufficiency of nutrients in the right proportions is the bane of microbe-based bioremediation strategies. Knowledge of the aforementioned fact induced the scientific community to look out for materials that can enhance the growth and activities of hydrocarbon-utilising microbes. Many agricultural wastes have been used to stimulate the activities of different hydrocarbon-utilising microbes [5, 6, 7]. Specifically, few works have been done on using pig droppings to stimulate the bioremediation of diesel oil-polluted soil. This work was

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therefore designed to assess the biostimulatory potential of PD in enhancing bioremediation of diesel-oil polluted soil.

II. MATERIALS AND METHODS

Collection and Processing of Samples

Soil sample used in this was collected from an agricultural land from different sites in Obukpa, Nsukka, South east, Nigeria at a depth of 0-30 cm. The soil sample was air dried and sieved through a 2 mm mesh. Diesel oil was bought from TOTAL filling station in Nsukka metropolis while the pig droppings (PD) was collected from a household farmer in Nsukka, Southeast, Nigeria.

Physicochemical Analyses Soil and Pig Droppings

Physicochemical properties of soil and pig droppings were determined using standard methods [8]: Particle size distribution of the soil, total nitrogen, available phosphorus of the soil sample and total phosphorus of the pig droppings, pH, moisture and total organic carbon(TOC) were all determined. Triplicate determinations were made for each assay.

Determination of the Extraction Efficiency of Different Solvents for Diesel Oil

Three different organic solvents namely dichloromethane, n-hexane and diethylether were used to extract diesel oil and their extraction rates were determined. The best solvent in terms of extraction efficiency for diesel oil was later used for the bioremediation assay. The extraction efficiency was determined gravimetrically. Briefly, forty grammes of the soil sample was transferred into a 250 mL flask and polluted with 4 mL of diesel oil. A 4 mL quantity of diesel oil was used so as to simulate a 10% pollution condition that would be studied in the present work. A 100 mL quantity of the three organic solvents was added separately to each polluted soil sample set-up and the set-ups shaken for six hours at 180 rpm. The solution was then filtered using a Whatman No 4 filter paper and the weight of the extracted oil recorded. The extraction efficiency of the organic solvents for diesel was then determined by weight difference following the formula [9]. The experiment was carried out in triplicates.

$$\text{Extraction efficiency} = \frac{\text{Weight of 4 mL diesel oil} - \text{Weight of oil extracted from soil}}{\text{Weight of 4 mL diesel oil}} \times 100$$

Soil Preparation for Bioremediation Study

A 1 kg quantity of soil was air –dried for two days and sieved with 2 mm mesh size. Soil sample was then placed in sterile polythene bags and 10 % (v/w) of diesel oil was added separately, thoroughly mixed, and left undisturbed for 48 hours. After two days, 5%, 10% and 15% of pulverized PD were respectively introduced separately to diesel oil-polluted soils and thoroughly mixed. Soil contaminated with 10% v/w diesel oil without PD amendment served as control. The moisture content of the soil was adjusted to 60% water holding capacity by the addition 50 mL of sterile distilled water (three times weekly) and the set-up kept at room temperature (28±2ºC). The experiment was set up in triplicates.

Determination of the level of diesel oil loss from polluted soil

Periodic sampling from each polythene bag was carried out every seven days in order to determine the residual diesel oil. A slight modification of the gravimetric and spectrophotometric methods [6] was employed in the determination of residual diesel oil present in both the control soil and amended options: Composite polluted soil samples weighing ten grammes were put in a 100 mL flask and 50 mL of n-hexane was added. N-hexane was used owing to its highest extraction efficiency for diesel oil among other solvents(see result section). The set-ups were shaken with a rotary shaker at 180 rpm for 10 hours to allow for an efficient and complete oil extraction with n-hexane. The mixture was then filtered with a whatman No 4 filter paper. A two-step filtration was done to ensure complete extraction of the liquid phase. The filtrate was diluted by adding 50 mL of n-hexane to 1 mL of the extracted diesel oil and the absorbance of the solution measured at 460 nm (Shimadzu UV 1800) using n-hexane as blank. The total petroleum hydrocarbon (TPH) was estimated by extrapolating from a standard curve derived from different concentrations of fresh diesel oil diluted with n-hexane. Percent remediation (R) was calculated using the following formula:

$$\mathrm{R} = \frac{TPHi - TPHr}{TPH} \times 100$$

Where TPHr and TPHi are residual and initial TPH concentrations

Enumeration and Identification of Heterotrophic Microbes

Ten grammes of soil sample from the amended options and the control option was introduced into 90 mL of distilled water and shaken vigorously for proper mixing of the sample. A 0.1 mL aliquot of the appropriate dilution of the suspension was inoculated on sterile nutrient agar plates by the spread plate method for aerobic heterotrophic bacteria[10]. The nutrient agar medium was supplemented with 50 μg/mLnystatin to suppress the growth of fungi. The agar plates were incubated at 35°C for 24 h after which colony forming units (CFU) per gram of soil samples were calculated. Three replicate samples from each oil-polluted soil were withdrawn every 7 days for the enumeration of total aerobic heterotrophic bacteria (AHB). Hydrocarbon utilizing bacteria (HUB) in the soil samples were enumerated by plating on Bushnell Hasmedium, pH 7.4, using the vapour phase transfer method as described11]: A filter paper saturated with sterile diesel oil was aseptically placed on the inside of the cover of inverted inoculated petri dishes and incubated at 28°C for 7 days. Distinct colonies of hydrocarbon-utilizing bacteria were picked and pure isolates obtained by repeated sub-culturing on nutrient agar. The bacterial isolates were characterized using microscopic techniques and biochemical tests such as catalase, urease, oxidase, starch hydrolysis, spore forming, H_2S production, motility, citrate utilization and methyl-red tests.

For the isolation and enumeration of fungi, 0.1ml of the appropriate dilution of each of the set-ups was inoculated into Sabouraud Dextrose Agar (SDA) plates and incubated at 28±2ºC for 4 days. Colony counts were taken and pure isolates obtained by repeated sub-culturing on SDA plates. The fungal isolates were characterized by slide culture and microscopic techniques and identified by the schemes [12].

Seed Germination Toxicity Test

The polluted soil amended with PD and unamended control soil were subjected to seed germination test, post remediation period following the method [13]. Seeds of *Phaseolus vulgaris* (common bean) were used. Briefly, 40 g of thoroughly-mixed remediated soil samples from both the control soils and the amended soil was placed in 100×15 mm petri-dish. Six viable seeds were placed evenly throughout each Petri dish and covered with 10 g of dry sand. The moisture content of the set-ups were maintained at 60% water holding capacity. Triplicate determinations was made for each assay. At the end of 10 days, the number of seeds that germinated from the surface of the soil was counted and root length measured to the nearest centimeter using a metre rule. The results were evaluated using the formula [14] with slight modification. Soil neither polluted nor amended served as the positive control soil while polluted soil without amendment served as negative control.

- Germination index (%) =(SG×LR)/100
- SG=(ET/CG) × 100
- LR=(LRT/LRC) ×100

  Where SG= number of seed germination, LR=root length (elongation), ET=number of seeds that germinated on treated soil, CG=number of seeds that germinated on positive control soil, LRT= root length on treated soil, LRC=root length on positive control soil.

Statistical Analysis of Data

The data obtained in the present study were subjected to one-way analysis of variance (ANOVA). Relationship between variables and comparison of means of the different treatments were tested for level of significances at $P \leq 0.05$ using least square difference and post-hoc multiple comparison tests. The data analysis was performed using SPSS.

III. RESULTS

Physicochemical Properties of Soil and Pig Droppings

The physicochemical properties of the soil and pig droppings used in this study are presented in Table 1. The percentage organic nitrogen content of the soil was 0.042 and available phosphorus content of 10.64 %. Other parameters of the soil in percentage are: organic carbon 2.49, moisture 10.38 and the pH is 4.9. The pig droppings has a pH 10.0 and nitrogen content of 0.238

Table 1: Physicochemical properties of soil and pig droppings

Parameter	Non-polluted Soil	Pig droppings
pH	4.90±0.37	10.0±0.03
Nitrogen	0.042±0.02	0.238±0.04
Organic carbon	2.49±0.45	22.94±0.09
Phosphorus (PPM)	10.64±0.5	22.40±0.07
Moisture (%)	10.38±0.3	8.69±0.229
Clay (%)	71.00 ± 0.41	-
Silt (%)	19.50± 0.06	-
Sand (%)	9.50± 0.08	-
Texture	Clayey Loam	

Extraction Efficiency of Solvents for Diesel Oil

The amount of diesel oil (in percentage) that was extracted by three different solvents namely: n-hexane, dichloromethane and diethylether six hours after polluting soil with 10% (v/w) diesel oil were 80.72%, 80.68%, 80.56%, respectively.

Bioremediation of Diesel Oil:

The level of bioremediation in unamended control soil and soil amended with 5 %, 10% and 15% (w/w) PD over a 42-day period are presented in Figure 1. Percentage oil (TPH) loss in theamended option ranged between 25.83± 2.07 and 46.67±1.32 across all amendment levels. Oil loss ranging from 19.93% ±0.96 to 32.22%± 0.6 was recorded in the unamended control soil Percentage oil (TPH) loss in the PD-amendment option ranged between 25.83± 2.07 .

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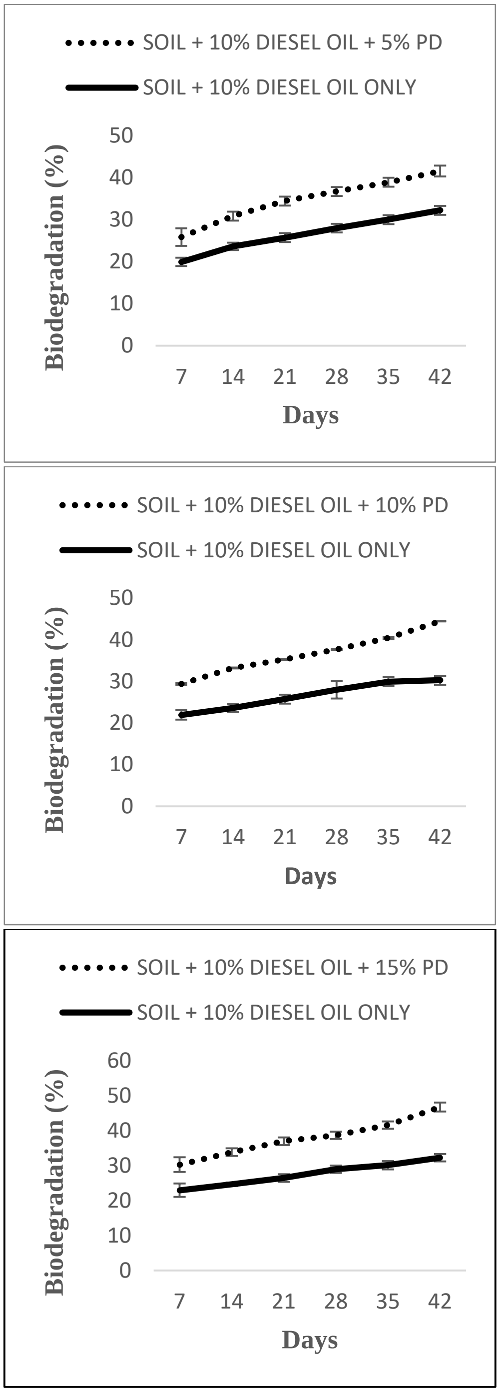

Fig.1: Bioremediation of Diesel Oil in Oil polluted soil amended with 5%, 10% and 15% (v/w)

Enumeration and Identification of Microorganisms

Active aerobic heterotrophic bacteria count:

Active aerobic heterotrophic bacterial (AHB) counts in both the control soil and polluted soil amended with 5%, 10% and 15% (w/w) PD 15%presented in Figure 2. AHB counts ranged between 6.8±0.2 and 23.8 ± 0.14 CFU/g across all amendment levels. AHB counts recorded in the unamended control soil ranged between 1.0 ± 0.06x 10^7 and 20 ± 0.9 x 10^7 CFU/g.

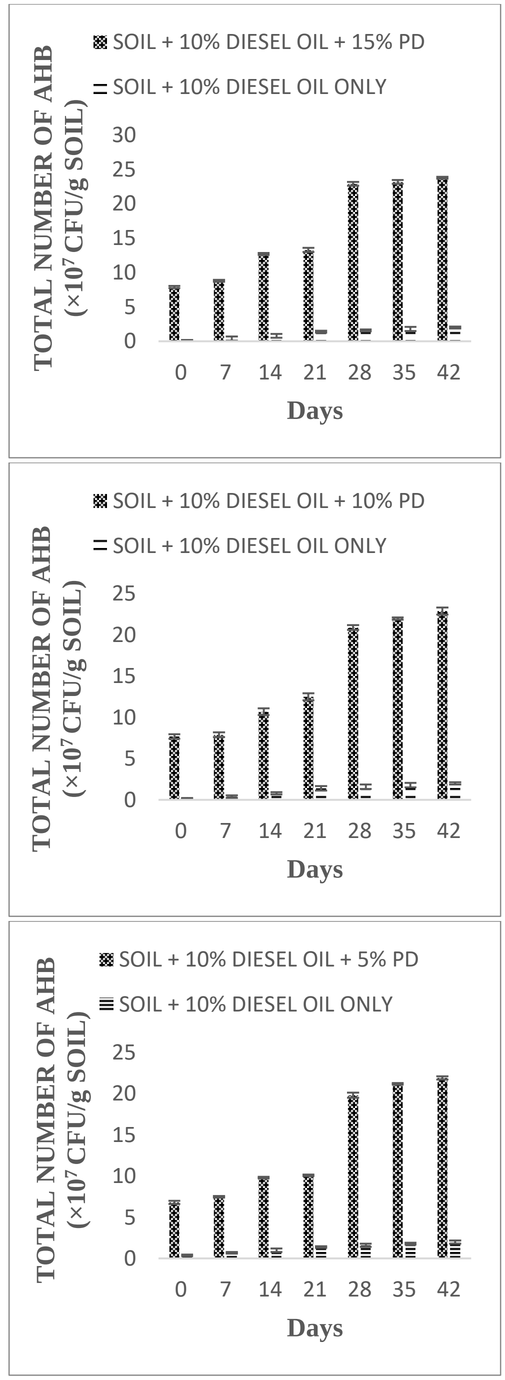

Fig.2: Active Aerobic Heterotrophic Bacteria (AHB) population in oil polluted soil amended with 5%, 10% and 15% (w/w)

Hydrocarbon-utilising bacterial count:

The total number of hydrocarbon-utilising bacteria in unamended control soil and polluted soil amended with 5, 10% and 15% (w/w) PD is recorded in Figure 3 ranging from 4.8 ±0.2 x 10^6 to 12.2 ± 0.23 x10^6 CFU/g while unamended control HUB population ranged from 0.5 ±0.08 to1.5 ± 0.03 CFU/g.

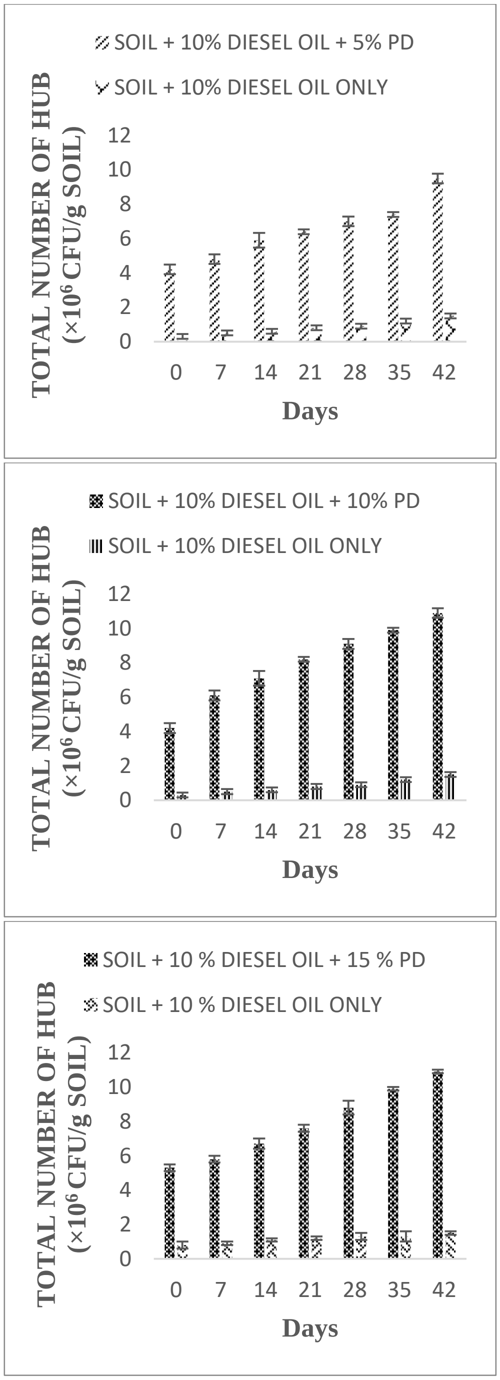

Fig.3: HUB population in oil polluted soil amended with 5%, 10% and 15% (w/w) PD

Identities of Bacterial isolates

The microscopic and biochemical characteristics of the isolated hydrocarbon-utilising bacteria are presented in Table 2. The HUBs are identified tentatively as *Bacillus licheniformis*, *Pseudomonas putida*,*Corynebacterium*sp., *Micrococcus varians*, *Staphylococcus aureus and Bacillus cereus*

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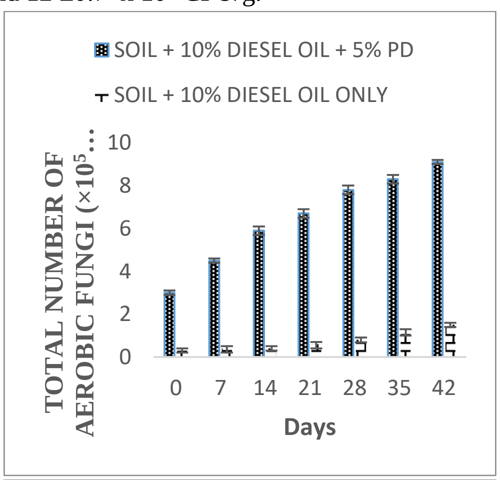
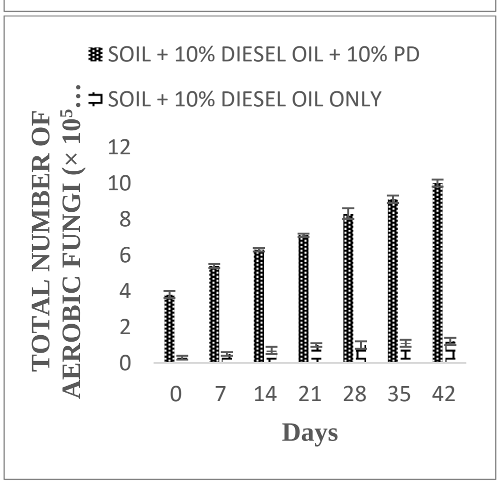
Table 2: Microscopic and Biochemical Characteristics of Bacterial Isolates

Gram reaction	Catalase test	Oxidase test	H_2S	Starch hydrolysis	Methyl red	Urease test	Citrate utilisation	motility	Spore-formation	Probable identity
+	+	-	-	+	+	-	-	+	+	*Bacillus*sp
+	+	+	+	-	+	+	+	-	-	*Micrococcus*sp
+	+	-	-	-	-	-	+	-	-	*Staphylococcus*sp
-	+	+	-	+	-	+	+	+	-	*Pseudomonas*sp
+	+	+	-	-	-	-	-	-	-	*Corynebacterium*sp
+	-	-	-	+	-	-	-	+	+	*Bacillus*sp

Aerobic Fungal Count

The total number of aerobic heterotrophic fungi in the control option and the PD-amended options is presented in Figure 4. Aerobic fungal counts in the PD-amended option ranged from 3 ±0.1 to 10.4 ±0.2. Aerobic fungal counts recorded in the unamended control ranged from 0.3 x 10^5 ± 0.62 and 12 ±0.7 x 10^4 CFU/g.





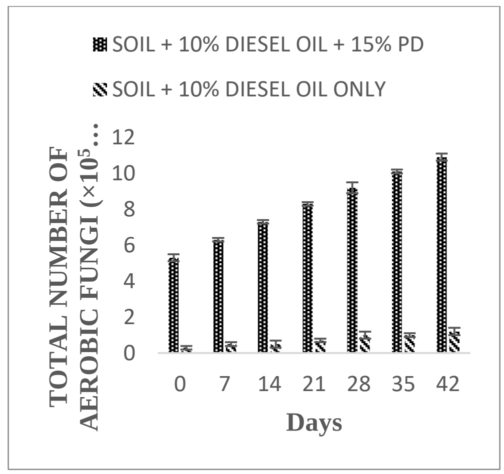


Fig.4: Active Aerobic fungal population in oil polluted soil amended with 5%, 10% and 15% (w/w) PD

Identities of Fungal isolates

The cultural and microscopic characteristics of the isolated hydrocarbon-utilising fungi are presented in Table 3. Fungi isolated predominantly were identified tentatively as*Aspergillusniger*, *Aspergillusflavus*, *Fusarium*sp., *Cladosporium*sp. *and Penicillium*sp

Table 3: Cultural and Microscopic Characteristics of Fungal Isolates

Cultural characteristics	Microscopic Characteristics	Probable Identity
Dark brown, powdery, flat spread on the surface of the solid medium with reverse	septate and branched hyphae with conidia in chains	*Aspergillus*spp
Yellow, powdery, flat spread on the surface of the solid medium with colourless reverse	Septate and branched hyphae with conidia in chains	*Aspergillus*spp
Grey colonies that were large with white border. Colourless or white reverse	Long conidiophores consisting of broom-like conidia in chains	*Penicillium*spp
Whitish and cottony mycelium with pinkish pigments at the centre. Brown reverse side	segmented canoe-shaped spores and branched conidiophores	*Fusarium*spp
Powdery, slow-growing, blackish-brown colonies, olivaceous-black reverse. Conidiophores and conidia equally pigmented	Branched and shield-shaped conidia in chains	*Cladosporium*spp

Seed Germination Profile of Remediated Soil

The seed germination parameters of soil after a 42-day bioremediation period is presented in Table 4. GI ranged from 15.8 to 53.4 across all amendment levels. Negative and positive control had percentage GI of 2.8 and 100, respectively. LR (%) ranged from 47.7 to 80.0 across all amendment levels. Positive and negative control had percentage LR of 100 and 16.9, respectively. Positive and negative controls had % SG of 100 and 16.7 respectively while % SG ranged from 33.3 to 66.1 across all amendment levels.

Table 4: Seed Germination Parametres

Soil preparations	SG	LR (cm)	SG(%)	LR(%)	GI (%)
5% PD	2.0	3.1	33.3	47.7	15.8
10% PD	3.0	4.0	50.0	61.5	30.7
15% PD	4.0	5.2	66.1	80.0	53.4
Negative control	1	1.1	16.7	16.9	2.8
Positive control	6	6.5	100	100	100

Key: number of seeds that germinated, LR= root length, GI= germination index

IV. DISCUSSION

This work was carried out against the backdrop of the insufficiency of the use of microbes alone in the bioremediation of diesel oil-polluted soil. It was rightly reported [15] that despite the presence of microbes in hydrocarbon-contaminated soils, their levels might not measure up to that needed for bioremediation of the site, hence the need to stimulate their growth and activities. Lack of nutrients in the right proportions have been identified as the bane of several biostimulation-based bioremediation [16]. Addition of organic wastes to improve the activities of hydrocarbon-degrading microbes has therefore been widely demonstrated [16, 3, 6].

It has been a common practice by the industrial and household populations to dispose of organic wastes indiscriminately. As can be noted from the present study, PD has potential for stimulating the activities of hydrocarbon-utilising microbes in bioremediation processes. In the present study, the percentage organic nitrogen content of the soil is relatively low (**Table 1**). Organic nitrogen was identified as a panacea to realizing an efficient biostimulation strategy [5]. Also, positive nitrogen amendment in polluted soils has been recorded [6]. This is due to the fact that while microbes utilize hydrocarbons in spills as carbon source, they need adequate concentrations of nutrients such as nitrogen and phosphorus to synthesize important macromolecules such as proteins and nucleic acids. Hence, addition of PD with a higher nitrogen (**Table 1**) and phosphorus content enhanced the activities of the diesel oil-utilising microbes. Furthermore, the pH of the experimental soil was low (fairly acidic) (**Table 1**) and therefore low for an effective biodegradation. Low pH was reported to affect biodegradation of pollutants [17]. In this study, however, the fairly acidic experimental soil was neutralized by the alkaline

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organic waste, **PD** with a pH of 8.7 since bacterial remediation rates tend to be fastest at neutral pH[3].

The result of the extraction efficiency experiment clearly indicated that n-hexane was the best choice in extracting diesel oil under the experimental conditions employed in the current study. This is due to the fact that the highest amount of diesel oil was extracted with n-hexane among other solvents such as dichloromethane and diethylether used in this study.

Percentage oil loss (bioremediation) increased tremendously from the first week to the sixth week in all the amended options and the control option. However, highest oil loss was observed at 15% amendment level (**Figure 1**). This obviously could be due to an enhanced nutrient level present at the highest amendment level. There was still a notable oil loss in the unamended control soil. Comparative studies on different hydrocarbon pollutants and different amendments obviously recorded different levels of oil loss in both the amended and unamended options [17, 6, 3, 11]. Natural bioattenuation by the indigenous hydrocarbon degraders, photovolatilization and sorption might have been the contributory factors in the oil loss observed in the control option. Similar trend has been documented [18].

Despite the fact that biostimulation-based bioremediation has been widely published as potent in enhancing bioremediation experiments [18, 10, 11], the converse has been proven that natural attenuation was more potent than biostimulation in a similar study in Hong Kong [19]. It was also reported that nutrient addition did not significantly enhance bioremediation of polluted soil [20]. However, it was asserted that different soils have different inherent microbial potential to degrade hydrocarbons [21] and the outcome of bioremediation of polluted soil depends on the type of oil and extent of pollution, properties of oil as modified overtime by physical and chemical processes, the organisms and habitats exposed and the nature of the exposure [22].

Heterotrophic levels and activities of indigenous flora is a bioindicator of the impact of nutrients embedded in organic wastes.In this study, active aerobic bacterial (AHB) counts increased notably throughout the 42-day study period (**Figure 2**) in both the control and amended options. However, AHB counts were significantly higher ($P \leq 0.05$) in the amended options. A similar study on diesel degradation using cowpea chaff [23] recorded a lower AHB counts of 8.0 $x10^6$ – 30.0x 10^6 CFU/g. similarly, HUB counts were significantly higher ($P \leq 0.05$) in all the amended options when compared with the control option (**Figure 3**). It was observed, however that AHB counts was greater than HUB counts. Similar trend has been documented [24]. Hydrocarbon-utilising bacteria are therefore a group of AHBs that evolved possibly as a result of frequent hydrocarbon spills. The HUB isolated in this study were identified tentatively as *Corynebacterium*sp., *Bacillus licheniformis*, *Micrococcus varians*, *Pseudomonas putida*, *Staphylococcus aureus and Bacillus cereus* (**Table 2)**. These bacteria have been widely reported [7, 24, 25] as having hydrocarbon-utilisation attributes.Fungal counts increased also within the study period but the counts were not significantly higher ($P \leq 0.05$) (**Figure 4**) in the amended option than the control option as was noted in their bacterial counterparts. Also, fungi isolated in the present study were identified tentatively as*Fusarium*sp., *Aspergillusniger*, *Aspergillusflavus, ,Cladosporium*sp. *and Penicillium*sp. (**Table 3)** These have also been reported by several researchers [25, 26] as being implicated in hydrocarbon degradation

The observation of higher fungal and bacterial counts in the amended options was possibly due to amendment with PD. Organic wastes such as PD are a reservoir of different hydrocarbon-degrading bacteria and fungi with inherent hydrocarbon-degrading attributes. It stands to reason therefore, that organic amendment is 'uncontrolled bioaugmentation'.The bacterial actors were higher in counts than fungi. It was argued in a similar study [27] that despite the fact that fungi and bacteria are the major actors in hydrocarbon remediation, bacteria are more versatile and hence may play more significant role in bioremediation of oil-polluted soil.

Generally, in similar literatures, reports on bioremediation results and microbial counts have been widely divergent. Several factors such as nature and type of organic wastes, soil structure, length of bioremediation period and constituents of the hydrocarbon pollutant may be responsible for the consistently observed variations.

Seed germination studies have been proposed as a criteria for the assessment of the efficiency of a bioremediation process [13]. In the present study, the highest germination index was noted in the amended option and at the highest amendment level (**Table 4**). The germination index recorded in the present study followed the same pattern of result as seen in microbial counts and bioremediation levels (**Table 4**). There was 100% germination in the positive control soil while 1% germination was recorded in the negative control soil (**Table 4**). This was probably due to absence of oil pollution in the positive control soil and oil pollution in the negative control soil. Soil pollution with hydrocarbons was reported by different researchers [28, 29] as having adverse effects on plant development parametres. Growth of all seeds of *Moringaolifera* was recorded in the positive control option [30]. A 99.6% germination was also noted in the positive control option [29]. *Phaseolus vulgaris* normally germinates within 8-10 days but the germination was delayed to 19-21 days owing to slightly heavy pollution simulated in this study (10%) which had not been fully remediated (48.54%) as at the end of the 42-day study period.

There was a significant difference in bioremediation level and microbial counts (P≤0.05) between PD-amended soil and control even at 10% and 15% level but not at 5% amendment level.

V. CONCLUSION

From the foregoing, it was found that polluted soil amended with pig droppings obviously enhanced oil loss from polluted soils with concomitant increase in microbial counts. Pig droppings, therefore can be considered a good alternative biostimulatory candidate for bioremediation of polluted soil.

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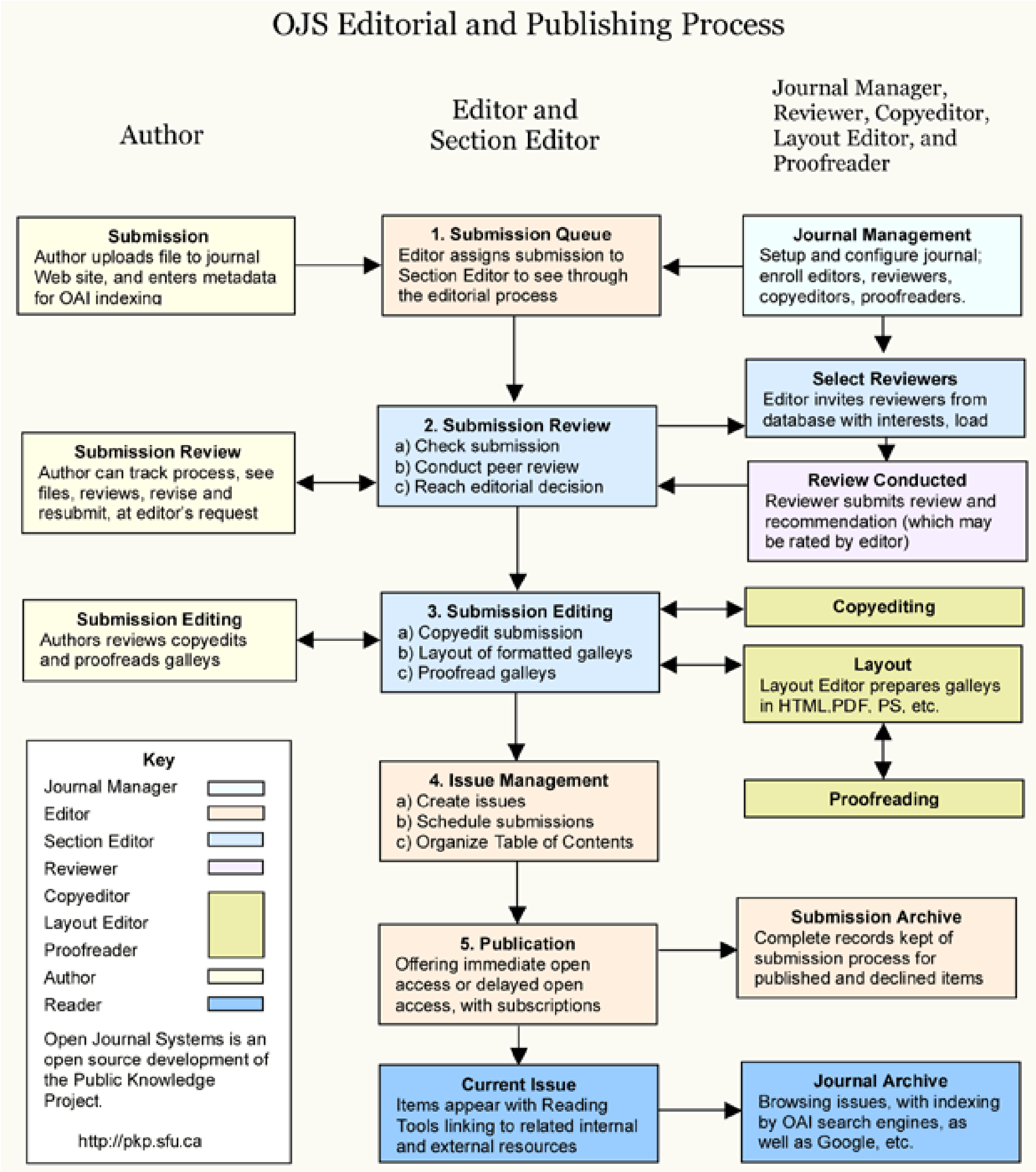

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