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Evaluation of the shelf-life of biocontrol agents enhanced with additives for use in tomato cultivation

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Abstract— *This study investigated the shelf life of biocontrol agents amended with additives and their efficacy were evaluated on growth parameters and wilt incidence in tomato. Under in vitro conditions 13 biocontrol agents were evaluated for their efficacy against Ralstonia solanacearum using the agar well diffusion technique and it was observed that Pseudomonas fluorescens (Pf 3) and T. koningii (DMA -8) were most effective in inhibiting the growth of R. solanacearum. Talc based formulations were prepared for biocontrol agents and it was observed that treatments with 2 per cent tryptone and 2 per cent peptone supplemented with 2 per cent glycerol enhanced the shelf life of Pf 3 and treatments with 2 per cent yeast extract and tryptone supplemented with 2 per cent glycerol increased the shelf life of Bacillus spp. (Bc) in the formulation. Biocontrol formulations were also evaluated in the greenhouse and their effect was studied on the wilt incidence and growth parameters. It was revealed that the combined application of Bc1+Pf 3 enriched with 2 kg FYM significantly reduced the wilt incidence and R. solanacearum population density and also increased the shoot length, number of branches/plants, number of fruits/plant and yield of tomato.*

Keywords— ***Biocontrol agents, shelf life, Tomato and talc based formulations***

HIGHLIGHTS

Nutrient-amended talc formulations extend the shelf-life and efficacy of biocontrol agents, making biological disease management practical for field use.

Integrating bioagent-enriched FYM in tomato cultivation represents a sustainable alternative to chemical bactericides, reducing chemical input, environmental residue, and enhancing crop health.

I. INTRODUCTION

Bacterial wilt disease caused by *Ralstonia solanacearum* have been reported to affect more than 200 plant species of more than 53 botanical families (Denny 2006). Tomato a very important vegetable crop grown in the hill state of Himachal Pradesh in 13,185 ha area with production of 5,39,540 tonnes (Anonymous 2021). Tomato grown in the sub-tropical and sub temperate hills of Solan, Sirmaur, Palampur and Mandi Districts of Himachal Pradesh is very susceptible to the bacterial wilt caused by *R. solanacearum*. In the absence of host plants, *R. solanacearum* can survive in soil or water for a long time. Continuous cultivation of tomato on the same piece of land over the years has also aggravated the disease associated with the crop especially soil borne diseases like Bacterial wilt. Farmers are advised to practice field sanitation, crop rotation, and the use of bactericides but these methods have only given a moderate level of success in managing the disease and the use of chemicals also pose significant issues, such as environmental pollution and the generation of pesticide residues. These concerns have raised doubts about the safety of agricultural products. Biological control using bacterial antagonists has emerged as an essential method for plant disease management (Kumar 2017). This makes the management of diseases less dependent on the use of hazardous chemicals and has a strong effect on the management of soil-borne phytopathogens. Therefore, biological control methods that use antagonistic fungal and

bacterial agents is an attractive approach (Mandal et al. 2017).

There are some inconsistencies associated with biocontrol agents like they exhibit variability and limited effectiveness, primarily because their lifespan is shorter than that of chemical pesticides. The effectiveness of biological control largely relies on the method employed to deliver biocontrol agents to the plants. A formulated product, therefore, should be easy to prepare and stable during transportation and storage. In addition, it should have abundant viable propagules with good shelf life. Talc is used as a raw material in the soapstone industry, is inert in nature and is readily available at low cost. Therefore, it is used as a carrier for formulation development. The present study investigated the effect of using talc-based formulation on the shelf life of two bioagents (*Bacillus subtilis* and *Pseudomonas fluorescens)* up to 150 days of storage. These bioagents formulations were then evaluated to check their efficacy on wilt incidence.

II. MATERIALS AND METHODS

1. Isolation, identification, purification and maintenance of *R. solanacearum*

The plants showing typical symptoms of vascular discoloration caused by *R. solanacearum* were collected and brought to bacteriological research laboratory. A confirmatory test was conducted by aseptically cutting the wilted plants showing discolored vascular tissues into small pieces of 3-4 mm size using a sterilized scalpel blade and suspending them in a test tube containing clean water. After 5-10 minutes it was observed that clean water turns turbid due to oozing of the bacterial cells and hence, confirmed the presence of the bacteria. After preliminary diagnosis again 3-4 mm small bits were cut and surface sterilized in 1.0 per cent sodium hypochlorite for 30 seconds followed by three subsequent washings of sterile water to remove traces of sodium hypochlorite. The infected bits were then suspended in a test tube containing sterilized distilled water for 10 minutes. The oozing of the bacterial cells from the tissue took place, turning the water in the test tube milky. Then a loopful of bacterial cell suspension was streaked onto three petri plates containing Kelman's 2, 3, 5 triphenyl tetrazolium chloride agar (TZC) medium under aseptic conditions. The inoculated plates were incubated at 28 ± 1^0C and after 24-36 hrs. the plates were examined for the development of well separated irregularly shaped, fluidal, dull white colonies with slight red or pink center. It was purified by picking the highly virulent colonies producing extracellular polysaccharide (EPS) and having a pink center and streaked on the surface of TZC medium contained in Petri plates.

2. Preparation of Talc based formulation

i) Culturing of bacteria

Mass multiplication of *P. fluorescens* was done by inoculating King's B broth with 0.1 per cent of bacterial culture (from late log phase) in 250 ml medium in 500 ml flasks and incubated for 48 hrs. on a rotary shaker at 200 rpm (28ºC). *B. subtilis* was grown in nutrient broth under similar condition for mass multiplication.

ii) Formulation of treatments

The formulation was initially prepared as described by Vidhyasekaran *et al*. (1997) using a mixture of 10 g of carboxymethylcellulose (CMC) in 1 kg of talc powder. The pH was adjusted to 7.0 by adding calcium carbonate and modified with nutrients. Nutrients includes 1 or 2 per cent bacteriological peptone or yeast extract or tryptone. Nutrients fortified with 1 or 2 per cent glycerol were also evaluated. The talc powder ($3MgO\ 4SiO_2.\ H_2O$) was incurred from Loba Chemie Pvt. Ltd. Mumbai and then autoclaved at 121^0C for 30 min on two successive days and was mixed with 0.2 per cent CMC prior to soil application. The formulations were prepared by mixing 200 ml culture broth of different isolates of *P. fluorescens*, containing a minimum population of 9 x 10^8cfu/ml with 500g of sterile talc powder (pre-mixed with 15 g calcium carbonate + 10 g of carboxymethylcellulose (CMC) + nutrient). Calcium carbonate was used to adjust the pH to 7.0. The control treatments were talc + calcium carbonate + CMC + culture broth and talc + culture broth. The resulting mixture was thinly spread over sterilized aluminium trays and dried overnight under sterile conditions. The mixture was packed in polypropylene bags, sealed and stored at room temperature (25 ± 2°C).

iii.) Preparing talc-based formulation of *Trichoderma* spp -

Mass multiplication of *Trichoderma* spp was done by inoculating PDA broth with bits from fully grown culture *Trichoderma* spp. and incubated at 25 ± 2°C for 7 days. of in 250 ml medium in 500 ml flasks and incubated for 48 hrs. on a rotary shaker at 200 rpm (28ºC). After 7 days of incubation 400 ml of broth was mixed with 1 kg of earlier sterilized talc powder. The soil was mixed with the *Trichoderma* spp. formulation at 5, 10, 15 g/kg of soil.

iv.) Population count

To estimate the viable population in the talc-based formulations, 1.0 g of the talc mixture was ground in a mortar and pestle. The resulting solution was mixed with 10 ml of sterile water and stirred for 20 minutes. Serial dilutions up to 10^8 cfu were prepared. From each dilution, 0.1 ml aliquots were spread on King's B agar (for *P. fluorescens*) and nutrient agar (for *Bacillus* spp.). Plates

were incubated for 24 hours, and observations on survival were recorded. Subsequent observations were made every 30 days, up to 150 days.

v.) Survival of *P. fluorescens* and *Bacillus* spp. in talc based powder formulation amended with additives

The talc-based formulation of *P. fluorescens* and *Bacillus* spp. were taken and amended with following ingredients

T_1	Talc + 1% peptone + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_2	Talc + 2% peptone + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_3	Talc + 1% peptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_4	Talc + 2% peptone + 1% $CaCO_3$ + 0.1% carboxy methylcellulose
T_5	Talc + 1% tryptone + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_6	Talc + 2% tryptone + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_7	Talc + 1% tryptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_8	Talc + 2% tryptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_9	Talc + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_{10}	Talc + 1% yeast extract + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_{11}	Talc + 2% yeast extract + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_{12}	Talc + 1% yeast extract + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_{13}	Talc + 2% yeast extract + 1% $CaCO_3$ + 0.1% carboxymethylcellulose
T_{14}	Talc + calcium carbonate + CMC + culture broth
T_{15}	Talc + culture broth
T_{16}	Talc alone

vi.) Enrichment of organic manure

Two gram of talc formulation of bioagents *viz. Bacillus* spp.and *P. fluorescens* were mixed with 2 kg of farm yard manure (FYM) and kept for 15 days maintained optimum moisture conditions and once in every two days the manure was thoroughly mixed. The pot experiment was conducted in Department of Plant Pathology, CSKHPKV, Palampur to evaluate the bio-efficacy of various talc based formulations against *R. solanacearum* by adopting a complete randomized design which comprised of twelve treatments including an untreated control which was replicated thrice.

vii.) Development of bioformulations and their mode of application

The sick soil was taken from the farm of Department of Plant Pathology; subsequently the bioformulations were developed and then applied in the manner as specified below.

T_1	Sick soil
T_2	T_1+ application of 1kg enriched farm yard manure with *Bacillus* spp.
T_3	T_1+ application of 1kg enriched farm yard manure with *Pseudomonas fluorescens*
T_4	T_1+ application of 1kg enriched farm yard manure with *Bacillus* spp. + *Pseudomonas fluorescens*
T_5	T_1+ application of 2kg enriched farm yard manure with *Bacillus* spp.
T_6	T_1+ application of 2kg enriched farm yard manure with *Pseudomonas fluorescens*
T_7	T_1+ application of 2kg enriched farm yard manure with *Bacillus* spp. + *Pseudomonas fluorescens*
T_8	T_1+ application of 1kg farm yard manure
T_9	T_1+ application of 2kg farm yard manure
T_{10}	T_1+ Streptocyclin @ 0.1g in 1 litre of water and used for drenching
T_{11}	T_1+ application of 1kg enriched farm yard manure with *Trichoderma* spp.

The inoculation was done as per rapid method of screening given by Kishun and Chand (1988) in which the seedlings were pricked using a sterilized needle and a drop of bacterial inoculum (10^8cfu/ml) was kept at the leaf axil of third or fourth expanded leaf from the top of seedling and pricked with the help of a sharp sterilized dissecting needle. The seedlings inoculated with sterilized distilled water in a similar manner was served as control.

viii.) Treatment of tomato seedlings

Five gram of talc formulation of bioagents *viz*., *Bacillus* spp*., P. fluorescens*, was added to 1 liter of water. The seedlings were treated by dipping in these suspensions for 30 minutes before transplanting.

ix.) Estimation of *R. solanacearum* population density

The rhizosphere soil after 45 days of termination of the experiment was enumerated by serial dilution technique and

spread plate method on triphenyl tetrazolium chloride (TTC) agar. Three replicates were maintained for each dilution. The cfu from each plate were counted and population density of *R. solanacearum* per g of soil was calculated as follows:

$$No.\,of Colonies/g\ soil = \frac{\text{Average No. of colonies in a dilution}}{\text{Dry weight of soil (g)}} X\ Dilution\ factor$$

Wilt incidence

After termination of the experiment, the wilt incidence was recorded using the following formula:

$$\text{Wilt incidence (\%)} = \frac{\text{Number of wilted plants}}{Total\ number\ of\ plants\ observed} X\ 100$$

x.) Analysis of yield attributes

Data on average fruit weight (g)/plant, No. of branches/plant at 50 per cent flowering, shoot length (cm)/plant at 50 per cent flowering, No. of fruits/plant, yield (kg)/plant and dry plant weight (g)/plant of the treated tomato plants were recorded to evaluate the efficacy of bioagents.

III. RESULTS AND DISCUSSION

Survival of potential *P. fluorescens* (Pf 3) isolated from talc-based powder formulations amended with additives

The data pertaining to viable populations of *P. fluorescens* in talc-based powder formulations amended with additives are presented in Table 2. The changes in population were monitored over a 150-day period. In almost all the treatments in which the plants were amended with nutrients, there was a gradual increase in the *P. fluorescens* (Pf 3) population for up to 90 days, which ranged from 0.00 to 10.94 × 10^6 cfu/g, 0.00 to 11.22 × 10^6 cfu/g, 0.00 to 11.74 × 10^6 cfu/g, and 0.00 to 11.80 × 10^6 cfu/g on the 1st, 30th, 60th and 90th days, respectively. At 90 days, the highest population of 11.80 x 10^6 cfu/g was observed in talc amended with 1% tryptone and glycerol. A decrease in the population was observed from 90 days onward in all treatments, ranging between 0.00 and 11.32 x 10^6 cfu/g and between 0.00 and 10.97 x 10^6 cfu/g on the 120th and 150th days, respectively. With respect to the nonamended talc formulations, a decrease in the population was observed after 30 days, and a population of 0.00 cfu/g was observed on the 30th day in the talc alone group, followed by an increase of 8.54 x 10^6 cfu/g in the talc + broth group. Similarly, a decrease in the population density of 0.00 to 9.16 x 10^6 cfu/g, 0.00 to 8.74 x 10^6 cfu/g, 0.00 to 8.12 x 10^6 cfu/g, and 0.00 to 7.44 x 10^6 cfu/g were detected on the 60th, 90th, 120th and 150th days, respectively.

The results of the present study revealed that treatment with 1 per cent tryptone, 2 per cent yeast extract and 2 per cent peptone supplemented with 2 per cent glycerol improved the survival of Pf 3. These results are in agreement with the findings of Kumaresan *et al*. (2005), who also developed talc-based formulations of several isolates of PGPR belonging to the species *Bacillus subtilis* and *Pseudomonas* spp. and reported that these formulations had a shelf life of more than 4 months.

Survival of potential *Bacillus* spp. (Bc 1) isolated from talc-based powder formulations amended with additives

The data with respect to viable populations of *Bacillus* spp. in talc-based powder formulations amended with additives are presented in Table 3. The changes in population were monitored over a 150-day period. In almost all the treatments in which the plants were amended with nutrients, *Bacillus* spp. (Bc 1) increased for up to 90 days, ranging between 0.00 and 9.54 × 10^6 cfu/g, 0.00 and 9.90 × 10^6 cfu/g, 0.00 and 9.92 × 10^6 cfu/g, and 0.00 and 9.90 × 10^6 cfu/g on the 1st, 30th, 60th and 90th days, respectively. On the 90th day, the highest population of 9.90 x 10^6 cfu/g was observed in talc amended with 2% yeast extract and glycerol. A decrease in the population was observed from 90 days onward in all treatments, ranging between 0.00 and 9.86 x 10^6 cfu/g and between 0.00 and 9.82 x 10^6 cfu/g on the 120th and 150th days, respectively. For the nonamended talc formulations, a decrease in the population was observed after 30 days, and a population of 0.00 cfu/g was observed on the 30th day for talc alone, followed by a decrease of 7.56 x 10^6 cfu/g in talc + broth. Similarly, a decrease in the population was observed, ranging between 0.00 and 7.82 x 10^6 cfu/g, 0.00 to 7.80 x 10^6 cfu/g, 0.00 to 7.55 x 10^6 cfu/g, and 0.00 to 7.44 x 10^6 cfu/g on the 60th, 90th, 120th and 150th days, respectively.

The perusal study (Table 3) revealed that all the talc formulations amended with nutrient sources for 150 days had a population of 8.80 x 10^6 cfu/g or more. The population declined to below 7.50 x 10^6 cfu/g only in the nonamended treatments. Treatment with 2 per cent yeast extract or tryptone supplemented with glycerol increased the shelf life of the Bc 1 isolate. Schmidt *et al*. (2001) reported that the biological control activity of *B. subtilis* increased by adding peptone (0.25%) to the medium and that of *Erwinia herbicola* increased by adding glucose (0.5%) or high concentrations of peptone (1-2%).

Table 2: Survival of potential Pseudomonas sp. (Pf 3) in talc-based formulations amended with additives

Treatment	**Days (cfu × 10^6 per g of talc)**					
	1*	**30***	**60***	**90***	**120***	**150***
T_1 - Talc + 1% peptone + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.60 (3.25)	9.84 (3.29)	9.93 (3.30)	9.98 (3.31)	9.34 (3.21)	8.93 (3.15)
T_2 - Talc + 2% peptone + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	10.83 (3.43)	10.86 (3.44)	10.90 (3.45)	10.94 (3.45)	10.69 (3.42)	9.87 (3.29)
T_3 - Talc + 1% peptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.40 (3.22)	9.44 (3.23)	9.47 (3.23)	9.48 (3.23)	8.97 (3.15)	8.03 (3.00)
T_4 - Talc + 2% peptone + 1% $CaCO_3$ + 0.1% carboxy methylcellulose	8.76 (3.12)	8.92 (3.15)	9.49 (3.23)	9.53 (3.24)	8.77 (3.12)	8.35 (3.05)
T_5 - Talc + 1% yeast extract + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.93 (3.30)	10.24 (3.35)	10.36 (3.37)	10.38 (3.37)	9.62 (3.25)	9.40 (3.22)
T_6 - Talc + 2% yeast extract + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	10.27 (3.35)	11.04 (3.47)	11.65 (3.55)	11.66 (3.55)	11.30 (3.50)	10.89 (3.44)
T_7 -Talc + 1% tryptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.02 (3.16)	9.39 (3.22)	9.68 (3.26)	9.80 (3.28)	9.24 (3.20)	8.22 (3.03)
T_8 - Talc + 2% tryptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.33 (3.05)	8.46 (3.07)	8.67 (3.11)	8.82 (3.13)	8.25 (3.04)	8.03 (3.00)
T_9 - Talc + 1% tryptone + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	10.42 (3.37)	10.51 (3.39)	10.76 (3.42)	10.80 (3.43)	10.09 (3.33)	9.33 (3.21)
T_{10} - Talc + 2% tryptone + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	10.94 (3.45)	11.22 (3.49)	11.74 (3.56)	11.80 (3.57)	11.32 (3.51)	10.97 (3.46)
T_{11} - Talc + 1% yeast extract + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.20 (3.19)	9.69 (3.27)	10.26 (3.35)	10.19 (3.34)	9.45 (3.23)	8.56 (3.09)
T_{12} - Talc + 2% yeast extract + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.67 (3.26)	9.82 (3.28)	9.87 (3.29)	9.80 (3.28)	9.44 (3.23)	8.24 (3.04)
T_{13}- Talc + 2% $CaCO_3$ + 0.1% carboxymethylcellulose	8.77 (3.12)	8.80 (3.13)	9.16 (3.18)	8.74 (3.12)	8.12 (3.02)	7.44 (2.90)
T_{14} -Talc + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.75 (3.12)	8.91 (3.14)	8.20 (3.03)	7.85 (2.97)	7.60 (2.93)	7.22 (2.86)
T_{15} -Talc + culture broth	8.69 (3.11)	8.54 (3.09)	7.86 (2.97)	7.44 (2.90)	6.75 (2.78)	3.76 (2.18)
T_{16} -Talc alone	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.	0.06	0.06	0.05	0.06	0.05	0.05
SE(m)	0.02	0.02	0.01	0.02	0.02	0.02

* = mean of three replications

cfu – Colony forming unit

The values in parentheses are square root-transformed value

Combined effect of different talc-based formulations and seedling treatments on yield

It is evident from the table 4 that all the treatments showed significantly better results than the control. All the antagonistic treatments, consisting of either single or combined application of *Bacillus* spp. and *Pseudomonas fluorescens* to seedling roots and soil, resulted in significantly greater tomato fruit yields than the

uninoculated control. Among all the treatments, the highest average number of fruits per plant was recorded in the T_7 treatment, which was enriched with Bc 1+Pf 3 isolates along with 2 kg FYM with 8.7 fruits per plant, followed by treatment T_7, which was enriched with Bc 1+Pf 2 along with 2 kg FYM with 7.7 fruits per plant, and the minimum average number of fruits per plant was recorded in the T_1-Sick soil, i.e., 1.3. The maximum average yield was also recorded in the T_7 treatment, which was enriched with Bc 1+Pf 3 along with 2 kg FYM with a yield of 296 g per plant, followed by treatment T_7, which was enriched with Bc 1+Pf 2 along with 2 kg FYM with a yield of 238 g per plant. Thus, these two treatments were found to be significantly superior to the other treatments in terms of the yield of tomato fruits. The lowest average yield (32.30) was obtained in the T_1-Sick soil. It is apparent from the above results that the combined application of *Bacillus* spp. and *Pseudomonas fluorescens* with 2 kg of FYM explicitly substantiated the yield-promoting effects. The results of the present investigation are also in agreement with those of Wydra and Semrau (2005).

Table 3: Survival of potential Bacillus sp. (Bc 1) in talc-based formulations amended with additives

Treatment	Days (cfu × 10^6 per g of talc)					
	1*	30*	60*	90*	120*	150*
T_1 - Talc + 1% peptone + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.40 (3.06)	8.50 (3.08)	9.02 (3.16)	9.10 (3.17)	8.99 (3.16)	8.80 (3.13)
T_2 - Talc + 2% peptone + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.99 (3.16)	9.48 (3.23)	9.65 (3.26)	9.66 (3.26)	9.60 (3.25)	9.20 (3.19)
T_3 - Talc + 1% peptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.48 (3.07)	8.68 (3.11)	9.14 (3.18)	9.16 (3.18)	9.12 (3.18)	9.00 (3.16)
T_4 - Talc + 2% peptone + 1% $CaCO_3$ + 0.1% carboxy methylcellulose	9.29 (3.20)	9.62 (3.25)	9.82 (3.28)	9.84 (3.29)	9.80 (3.28)	9.65 (3.26)
T_5 - Talc + 1% tryptone + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.58 (3.09)	8.80 (3.13)	9.78 (3.28)	9.79 (3.28)	9.78 (3.28)	9.76 (3.28)
T_6 -Talc + 2% tryptone + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.54 (3.08)	8.61 (3.10)	9.79 (3.28)	9.78 (3.28)	9.72 (3.27)	9.70 (3.27)
T_7 -Talc + 1% tryptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.51 (3.08)	8.65 (3.10)	9.20 (3.19)	9.18 (3.19)	9.15 (3.18)	9.10 (3.17)
T_8 - Talc + 2% tryptone + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.50 (3.08)	8.72 (3.11)	9.20 (3.19)	9.14 (3.18)	9.11 (3.18)	9.00 (3.16)
T_9 - Talc + 1% yeast extract + 1% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.52 (3.24)	9.79 (3.28)	9.90 (3.30)	9.88 (3.29)	9.69 (3.26)	9.46 (3.23)
T_{10} - Talc + 2% yeast extract + 2% glycerol + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.54 (3.24)	9.90 (3.30)	9.92 (3.30)	9.90 (3.30)	9.86 (3.29)	9.82 (3.29)
T_{11} - Talc + 1% yeast extract + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.48 (3.23)	9.82 (3.28)	9.84 (3.29)	9.80 (3.28)	9.75 (3.27)	9.50 (3.24)
T_{12} - Talc + 2% yeast extract + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	9.49 (3.24)	9.84 (3.29)	9.85 (3.29)	9.84 (3.29)	9.70 (3.27)	9.58 (3.25)
T_{13}- Talc + 2% $CaCO_3$ + 0.1% carboxymethylcellulose	8.40 (3.06)	7.84 (2.97)	7.82 (2.97)	7.80 (2.96)	7.55 (2.92)	7.44 (2.90)

T_{14} -Talc + 1% $CaCO_3$ + 0.1% carboxymethylcellulose	8.40 (3.06)	7.73 (2.95)	7.66 (2.94)	7.58 (2.92)	7.29 (2.88)	7.22 (2.86)
T_{15} -Talc + culture broth	8.38 (3.06)	7.56 (2.92)	7.52 (2.91)	7.46 (2.90)	7.25 (2.87)	7.00 (2.82)
T_{16} -Talc alone	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.	0.05	0.07	0.06	0.05	0.05	0.06
SE(m)	0.01	0.02	0.01	0.01	0.01	0.02

* = mean of three replications. Values in parentheses are square root-transformed values.

cfu – Colony forming unit

Table 4 Effect of potential microbe-based formulations on yield

Treatment	**Avg. no. of fruits/plant***	**Yield(g)/plant***
T_1 - Sick soil	1.3	32.3
T_2 - T_1+ 1 kg FYM with Bc 1	5.7	140.0
T_2 - T_1+ 1 kg FYM with Bc 2	5.3	140.4
T_2 - T_1+ 1 kg FYM with Bc 3	5.0	138.6
T_3 - T_1+1 kg FYM with Pf 1	5.3	140.0
T_3 - T_1+ 1 kg FYM with Pf 2	5.7	140.7
T_3 - T_1+ 1 kg FYM with Pf 3	6.0	140.7
T_4 - T_1+ 1 kg FYM with Bc 1+Pf 1	6.7	159.6
T_4 - T_1+ 1 kg FYM with Bc 1+Pf 2	6.7	159.6
T_4 - T_1+ 1 kg FYM with Bc 1+Pf 3	7.0	224.8
T_4 - T_1+ 1 kg FYM with Bc 2+Pf 1	6.3	140.0
T_4 - T_1+ 1 kg FYM with Bc 2+Pf 2	6.3	140.4
T_4 - T_1+ 1 kg FYM with Bc 2+Pf 3	6.7	159.6
T_4 - T_1+ 1 kg FYM with Bc 3+Pf 1	7.0	224.8
T_4 - T_1+ 1 kg FYM with Bc 3+Pf 2	7.0	224.8
T_4 - T_1+ 1 kg FYM with Bc 3+Pf 3	7.7	234.4
T_5 - T_1+ 2 kg FYM with Bc 1	6.0	140.0
T_5 - T_1+ 2 kg FYM with Bc 2	6.3	140.4
T_5 - T_1+ 2 kg FYM with Bc 3	6.3	140.0
T_6 - T_1+ 2 kg FYM with Pf 1	6.3	140.7
T_6 - T_1+ 2 kg FYM with Pf 2	6.7	159.6
T_6 - T_1+ 2 kg FYM with Pf 3	6.7	159.6
T_7 - T_1+ 2 kg FYM with Bc 1+Pf 1	7.0	224.8
T_7 - T_1+ 2 kg FYM with Bc 1+Pf 2	7.7	238.0
T_7 - T_1+ 2 kg FYM with Bc 1+Pf 3	8.7	296.0
T_7 - T_1+ 2 kg FYM with Bc 2+Pf 1	5.3	140.4
T_7 - T_1+ 2 kg FYM with Bc 2+Pf 2	5.7	140.7
T_7 - T_1+ 2 kg FYM with Bc 2+Pf 3	6.7	159.6
T_7 - T_1+ 2 kg FYM with Bc 3+Pf 1	5.7	140.7
T_7 - T_1+ 2 kg FYM with Bc 3+Pf 2	5.3	140.0
T_7 - T_1+ 2 kg FYM with Bc 3+Pf 3	5.3	140.0

T_8 - T_1+ 1 kg FYM	5.0	138.6
T_9 - T_1+ 2 kg FYM	5.3	140.0
T_{10} - T_1+ Streptocyclin @ 0.1 g/liter of water	7.0	224.8
CD (P=0.05)	0.33	2.60
SE(m)	0.63	0.02

* = mean of three replications, Pf - *Pseudomonas fluorescence*, Bc – *Bacillus* spp.

Combined effect of different talc-based formulations and seedling treatments on wilt incidence

The data depicting the combined effect of different talc-based formulations and seedling treatments on wilt incidence are presented in Table 5. All the treatments of bioagents consisting of either single or integrated application of *Bacillus* spp. and *P. fluorescens* exhibited a significantly high degree of reduction in the incidence of bacterial wilt, which ranged from 31.56 to 65.78% over that of the uninoculated control. The greatest reduction in wilt incidence was observed in treatment T_7, which was enriched with Bc1+ Pf 3 along with 2 kg FYM, which was statistically at par with treatment T_7, which was enriched with Bc1+ Pf 2 isolates along with 2 kg FYM, and the disease control was up to 65.78%. The minimum reduction in wilt incidence over the control was observed in treatment T_8, which was enriched with 1 kg FYM in comparison to the uninoculated control and the disease (20.14%). The results explicitly suggest a greater level of protection from bacterial wilt; thus, the most efficient isolates could be used for biological control. *P. fluorescens* is an effective control measure for managing bacterial diseases, and its application reduced the incidence of bacterial wilt from 85% in the untreated control group to 30% after treatment with *P. fluorescens* (Vanitha *et al*. 2009).

Table 5 Effect of different talc-based formulations and seedling treatments on wilt incidence

Treatment	**Wilt Incidence (%)**	**Disease control (%)**
T_1 - Sick soil	97.40 (81.06)	-
T_2 - T_1+ 1kg FYM with Bc 1	66.66 (54.71)	33.34 (35.25)
T_2 - T_1+ 1kg FYM with Bc 2	64.43 (53.37)	35.56 (36.59)
T_2 - T_1+ 1kg FYM with Bc 3	64.44 (53.37)	35.56 (36.59)
T_3 - T_1+1kg FYM with Pf 1	48.88 (44.34)	51.12 (45.62)
T_3 - T_1+ 1kg FYM with Pf 2	48.88 (44.34)	51.12 (45.62)
T_3 - T_1+ 1kg FYM with Pf 3	44.43 (41.78)	55.56 (48.17)
T_4 - T_1+ 1kg FYM with Bc 1+Pf 1	35.55 (36.58)	64.45 (53.38)
T_4 - T_1+ 1kg FYM with Bc 1+Pf 2	35.55 (36.58)	64.45 (53.38)
T_4 - T_1+ 1kg FYM with Bc 1+Pf 3	35.55 (36.58)	64.45 (53.38)
T_4 - T_1+ 1kg FYM with Bc 2+Pf 1	66.66 (54.71)	33.34 (35.25)
T_4 - T_1+ 1kg FYM with Bc 2+Pf 2	66.66 (54.72)	33.34 (35.25)
T_4 - T_1+ 1kg FYM with Bc 2+Pf 3	50.00 (44.98)	50.00 (44.98)
T_4 - T_1+ 1kg FYM with Bc 3+Pf 1	48.88 (44.34)	51.12 (45.62)
T_4 - T_1+ 1kg FYM with Bc 3+Pf 2	48.88 (44.34)	51.12 (45.62)
T_4 - T_1+ 1kg FYM with Bc 3+Pf 3	50.00 (44.98)	50.00 (44.98)
T_5 - T_1+ 2kg FYM with Bc 1	64.44 (53.37)	35.56 (36.59)
T_5 - T_1+ 2kg FYM with Bc 2	64.44 (53.37)	35.56 (36.59)
T_5 - T_1+ 2kg FYM with Bc 3	66.66 (54.72)	33.34 (35.25)
T_6 - T_1+ 2kg FYM with Pf 1	50.00 (44.98)	50.00 (44.98)
T_6 - T_1+ 2kg FYM with Pf 2	44.43 (41.78)	55.56 (48.17)
T_6 - T_1+ 2kg FYM with Pf 3	44.44 (41.79)	55.56 (48.17)
T_7 - T_1+ 2 kg FYM with Bc 1+Pf 1	35.55 (36.58)	64.45 (53.38)
T_7 - T_1+ 2kg FYM with Bc 1+Pf 2	33.33 (35.24)	66.70 (54.73)

T_7 - T_1+ 2kg FYM with Bc 1+Pf 3	33.33 (35.24)	66.70 (54.73)
T_7 - T_1+ 2kg FYM with Bc 2+Pf 1	73.33 (58.89)	26.67 (31.07)
T_7 - T_1+ 2kg FYM with Bc 2+Pf 2	73.33 (58.88)	26.67 (31.08)
T_7 - T_1+ 2kg FYM with Bc 2+Pf 3	73.33 (58.88)	26.67 (31.07)
T_7 - T_1+ 2kg FYM with Bc 3+Pf 1	50.00 (44.98)	50.00 (44.98)
T_7 - T_1+ 2kg FYM with Bc 3+Pf 2	44.44 (41.78)	55.56 (48.17)
T_7 - T_1+ 2kg FYM with Bc 3+Pf 3	44.44 (41.79)	55.56 (48.17)
T_8 - T_1+ 1kg FYM	77.77 (61.85)	22.23 (28.11)
T_9 - T_1+ 2kg FYM	73.33 (58.89)	26.67 (31.08)
T_{10} - T_1+ Streptocyclin @ 0.1g/ litre of water	50.00 (44.98)	50.00 (44.98)
T_{11} - T_1+ 1kg FYM with *T. koningii* (DMA -8)	64.43 (53.37)	35.56 (36.59)
T_{11} - T_1+ 1kg FYM with *T. harzianum* (TH-11)	64.44 (53.37)	35.55 (36.59)
T_{11} - T_1+ application of 1 kg FYM with *T. harzianum* (TH-5)	66.66 (54.71)	33.34 (35.25)
CD (P=0.05)	2.22 (1.57)	2.08 (1.23)
SE(m)	0.78 (0.55)	0.73 (0.43)

* = mean of three replications; Pf - *Pseudomonas fluorescence*, Bc – *Bacillus* spp.; The values in parentheses are angularly transformed values.

IV. CONCLUSION

The study concludes that nutrient-amended talc-based formulations of Bacillus subtilis and Pseudomonas fluorescens significantly enhance their shelf life and viability for up to 150 days. Application of these optimized biocontrol agents, particularly in combination with farmyard manure (FYM), resulted in a marked reduction in bacterial wilt incidence and improved tomato yield. Integrating these biocontrol agents with organic amendments offers a sustainable and effective strategy for managing soil-borne diseases in tomato, minimizing chemical input and promoting better crop health. The approach facilitates practical field use of biological products, supporting safer, residue-free agriculture and improved farmer outcomes.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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