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Research Article

Biocontrol of *Rhizoctonia solani* root rot of chilli by *Bacillus subtilis* formulations under pot conditions

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ABSTRACT: The chilli crop suffers massive yield loss due to root rot caused by *Rhizoctonia solani*. An increase of 10×10^5 root colonizing units/cm was obtained as a result of *Bacillus subtilis* in vitro root colonisation assay post germination. Shelf life studies of the formulations revealed stable population level of the biocontrol agent upto 180th day (30°C – 1.6×10^8; 4°C – 1.9×10^8) in talc and upto 150th day in lignite (30°C – 1.5×10^8; 4°C – 1.3×10^8). Soil, seed, foliar spray and dip treatment methods of *B. subtilis* and chlorothalonil brought about a considerable enhancement of all biometric parameters and reduced disease incidence compared to the untreated control. In comparison to the untreated control (50 g and 21 g fresh and dry weight, respectively), highest plant fresh weight (76.84 g) and dry weight (34.17 g) was achieved by the Seed application method. Comparison of plant height revealed maximum values 70 cm (soil application) and 77 cm (dip treatment) with *B. subtilis* application which was analogous to chlorothalonil treatment (56.5 cm with soil application and 70.33 cm with dip treatment) which was considerably superior to the untreated control (58.2 cm with dip treatment and 61 cm with soil application, respectively). Root dip treatment showed considerable increase in root length with *B. subtilis* (33 cm) and chlorothalonil (28.5 cm) when compared to untreated control (15 cm). Growth promotion was better with Root dip application while disease control was achieved better with seed application. A 66% and 84% reduction in incitation of disease was noticed with soil and seed application methods, respectively.

KEY WORDS: *Bacillus subtilis*, *Rhizoctonia solani*, bio formulation, shelf life, biocontrol, chilli, pot studies

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INTRODUCTION

Rhizoctonia solani (Julius Kuhn) causes damping-off disease of seedlings, root and stem rot in chilli (*Capsicum annuum* L.) leading to massive yield loss. It induces root rot in mature plants and leads to wilting and death of chilli plants. *R. solani* is a soil borne pathogen which persists in soils and organic debris. Fungal pathogen management relies on avoiding saturated soil conditions (Sherf and MacNab 1986) and usage of superior-quality seeds subjected to thiram or captan fungicide treatment. It can affect the chilli crop at multiple stages such as seed decay, pre- and post emergence damping-off, wirestem, root rot, and hypocotyl or tap root with necrotic spots on them (Sherf and MacNab 1986). Literature supports biological control as an effective and sustainable management strategy against *Rhizoctonia* diseases (Brewer and Larkin 2005). Research carried out by Mao *et al.* (1998), Ramamoorthy *et al.* (2002), Sid Ahamed *et al.* (2003) and Nakkeeran *et al.* (2006), are an evidence to numerous reports on the biological control of several chilli diseases on different cultivars however, the biocontrol of damping-off caused by *R. solani* on *S. melongena* and *Capsicum* sp. has not been reported (Abheysinghe, 2009).

Bacillus subtilis (Ehrenberg) is known to control varied plant pathogens both at pre-harvest and post harvest conditions in a safe and sociable manner (Collins *et al.,* 2003; Rodger, 1989; Sharga and Lyon, 1998). Diseases such as fruit rot and die back of chilli are controlled by *B. subtilis* (Jayalakshmi *et al.,* 1998).

Biological control of various crop diseases has been attempted using Bacillus-based formulations, however, reports of their application on chilli pathogens has been very limited. *B. subtilis* (JN032305) used in the present study, revealed a broad spectrum of mycolytic activity against nine potent pathogens of chilli through the production of lytic enzymes as recorded in the earlier reports (Ashwini, *et al.,* 2013) . Pot level studies regarding the biocontrol potential of *B. subtilis* against *R. solani* root rot of chilli was attempted in this study.

MATERIALS AND METHODS

The research work was conducted at Jain University, Bangalore in the year 2013.

Microorganisms

The biological control agent (*B. subtilis*) employed in the present study was formerly isolated from chilli rhizospheric soils of Indian Institute of Horticultural Research (IIHR). It exhibited a broad spectrum of lytic enzyme-based inhibitory activity against several potent chilli pathogens (Ashwini and Srividya, 2012; Sasirekha *et al.,* 2012).

In vitro root colonisation

The root colonization potential of the antagonist was tested by using a modified method as specified by the relevant literature (Montealegre *et al.,* 2003; Patten and Glick 2002). For each treatment, 15 chilli seeds were surface sterilised with ethyl alcohol (70%, v/v for 5 min) followed by sodium hypochlorite rinse (1%, v/v for 1 min) and sterile distilled water (2X) wash. The processed seeds were subjected to treatment with 1 mL aliquot of *B. subtilis* (24 h) nutrient broth culture which was diluted to get a viable count of 100 colony forming units (cfu)/ml. To enable seed coat binding, the treated seeds were placed in sterile petri dishes with 9 cm diameter containing a large moistened (with sterile distilled water) filter paper disc and maintained for 1 h at room temperature. To permit root development, the treated and the untreated control seeds were later incubated for 4–5 days at 30°C in an environment devoid of light. For assessing root colonization, 1 cm of the root from each set of treated seeds was aseptically excised, serially diluted in sterile water blank for enumeration by viable count and expressed as cfu/cm root.

Bioformulation of *Bacillus subtilis* and study of its shelf life

Bacillus subtilis was cultured in nutrient broth for 48 h, diluted to obtain a load of 9×10^8 cfu/mL and was used to prepare the formulation of talc/lignite-base (Vidhyasekaran and Muthamilan, 1999). For further investigations, talc formulation with a bacterial load of $2.5–3 \times 10^8$ cfu/g and moisture content of 10% was used.

For further analysis, the talc/lignite formulations were stored under the temperatures, 30°C and 4°C. To assess the population density of viable cells of the antagonist in the formulation, 1g samples were withdrawn from covers selected randomly, subjected to serial dilutions and plated on nutrient agar medium. Similarly, the shelf life of the formulation was monitored every month (Anitha and Rabeeth, 2010).

Compatibility study of *Bacillus subtilis* with fungicide

Bacillus subtilis was evaluated for its tolerance against chlorothalonil at field application levels of 2 g/l. The required concentration of the filter sterilized fungicide was solubilised in sterilized distilled water and mixed with nutrient agar just before pouring into the Petriplates. Upon solidification, the isolate was streaked onto the plates incubated at 28±2°C for 72 h. Response of the *B. subtilis* isolate to fungicide tolerance was recorded (Papavizas and Lewis, 1981) as follows:

- = no growth; + = growth.

Pathogen

Rhizoctonia solani was cultivated on potato dextrose agar (Difco) and maintained at 28°C for 10 days. The surface of the *R. solani* colony was scraped into 10 mL of sterile distilled water using a sterile scalpel to obtain conidial suspensions and the mycelial debris was removed by filtering through four layers of cheese cloth. The total spore count was set to 2.1×10^6 conidia/mL and enumerated using haemocytometry.

Pot studies

The seeds of chilli cultivar *Arka Shweta* (source Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru) were surface sterilized and sown in potting medium (red soil: sand: cow dung manure at 1:1:1 w/w/w) taken in a plastic tray (55 cm × 35 cm × 15 cm), filled into the pots (5 cm × 15 cm × 10 cm) and 2 seedlings per pot were transplanted at the two leaf stage. The evaluation of the rate of disease incidence and bio control was performed by using these cultivated plants (Lamsal *et al.,* 2012).

Treatments

The experimental set up comprised of seven treatments (seed and soil application): T1 = *R. solani*, T2 = chlorothalonil, T3 = *B. subtilis*, T4 = chlorothalonil + *R. solani*, T5 = *R. solani* + *B. subtilis* and T6 = chlorothalonil + *B. subtilis*. T7 = control (untreated). For foliar spray and root dip application method T1 = control (untreated), T2 = *B. subtilis*, T3 = chlorothalonil.

Chilli plants were subjected to four treatment methods such as, seed application (20 g/kg of seeds), root dip (20 g L^{-1} for 1 h), treatment of soil (10 g/pot) and spray on the foliage (20 g L^{-1}) with *B. subtilis* in talc formulation. During transplantation of the seedlings to the pots, they were subjected to soil treatment while the foliar spray treatment was given at the maximum stage of growth, prior to flowering

and at fruit maturation stages. These plants were subjected to seed application (2 g/kg of seeds), root dip (1 g L^{-1}), treatment of the soil (2 g/pot) and spray of the foliage (2 g L^{-1}) with chlorothalonil, the standard fungicide.

For seedling dip treatment, solution was prepared @ 1Kg biopesticide in 40 L of water, poured into a tray and roots of the seedlings were dipped for 30 minutes duration. Later seedlings were removed from the solution and used for transplanting into the pots. The plants were grown and disease incidence was recorded as per standard protocols.

Rhizoctonia solani spores were used to challenge inoculate the soil and seed treatments whereas root dip and foliage spray methods were exposed to normal environmental conditions of infection (Rini and Sulochana, 2006). For the sake of comparison, controls inoculated with the pathogen and normal controls were maintained. Regular water monitoring was done and the plants were evaluated after 90–120 days of sowing for the analysis of disease incitation.

For the disease incitation, *R. solani* spores (10^6 conidia/ml) were inoculated into 25-day-old seedlings after 5 days of transplanting and the spore suspension was also sprayed over the canopy (2 ml/plant).

For the evaluation of disease incidence, the plants were observed after 100 days of growth. The infection percentage was obtained by with the help of the following formula (Sultana *et al.,* 2006).

$$\% I = \frac{\text{No: of plants infected}}{\text{No: of plants infected in control}} \times 100$$

All the treatments were performed in triplicates in factorial completely randomized design.

Observations

The height of the Plant, fresh and dry weight, root and shoot length and fruit yield were the plant growth parameters evaluated to study the positive influence on chilli plant growth. For the purpose of disease control study, number of disease inflicted plants and the quality of the root and fruit was assessed. The plants were dried at 60°C until the weight stabilized and then the dry weight was recorded. The root length, length of the shoot and weight of five fresh seedlings were collected and the average was calculated. After recording the fresh weight of five seedlings, the seedlings were dried until the weight stabilized and the dry weight was expressed in grams.

Experimental design and statistical analysis

A randomized block pattern in triplicates was utilized in the experiment. The observed data were analyzed statistically at 5% (p = 0.05) significance level and the Duncan Multiple Range Test (DMRT) was used to compare the mean values by using SPSS (Statistical Package for the Social Sciences, later modified to Statistical Product and Service Solutions) software (version 10). The least value in a column is depicted by superscript alphabet 'a' and ensuing readings in ascending order are denoted in the alphabetical list in increasing order. The same superscript letter(s) in a column showed that the readings were not different considerably (Sutanu *et al*., 2014).

RESULTS AND DISCUSSION

Root colonisation assay (*in vitro*)

After 15 days of treatment, the root colonization competency of *B. subtilis* was analyzed. 2×10^5 cfu/cm of the root was the heightened counts obtained with *B. subtilis* treatment when compared to the un-inoculated control. Augmentation in viable counts from 10^2 to 4.5×10^6 cfu/cm root was recorded with *B. subtilis* treatment however, the control revealed 10^4 cfu/cm root length. Favorable microbes and their products are delivered through root colonization. Jedabi and Awatif (2009) have reported similar elevation in root colonization assay for different species of Bacillus after four days of germination, when compared to the control with 100 cfu/cm root length count. Several other research groups have reported similar results on lowered disease incitation and enhanced growth through heightened root colonization (Bais *et al.,* 2004; Basha and Ulaganathan 2002; Marten *et al.,* 2000). Thus, the study identifies the elevated root colonisation ability of *B. subtilis* better than the untreated control.

Shelf life study of *Bacillus subtilis* in talc/lignite-based formulation

Viability retention for a considerable period is the basic requirement for any bioinoculant. This criteria was fulfilled by both the formulations as indicated by the population statistics (cfu/g) (Fig. 1). Though a gradual decline in the counts was observed in the lignite formulation, the reduction was significant only after four months of storage where the cfu reduced severely but at the end of two and three months storage, the fall in cfu level was not considerable. The talc formulation exhibited an insignificant gradual decline in shelf life. Population statistics study revealed appreciable viability retention for 180 days under both storage temperatures. Stable antagonist population was retained in

talc. Talc formulation exhibited a stable population level till the end of six months with 1.6×10^8 and 1.9×10^8 at 30°C and 4°C, respectively, however, in lignite the stability was retained till with 1.5×10^8 and 1.3×10^8, respectively. *Bacillus sp*. consortia in Talc reported considerable control in rice sheath blight and enhancement in plant yield when compared to single strains under field conditions (Nandakumar *et al.,* 2001). Talc and peat based formulations of *P. chlororaphis* and *B. subtilis* have exhibited similar results in the control of turmeric rhizome rot (Mathiyazhagan *et al.,* 2004). The work of Salaheddin *et al.,* (2010) revealed a reduction in population level in talc formulation of *B. subtilis* after 60 days of storage. Further investigations were carried out with talc-based formulations owing to their more effective shelf life and ease of usage. As reported by prior studies *B. subtilis* sustained well in talc formulation, however, this strain demonstrated enhanced population stablility than earlier reports.

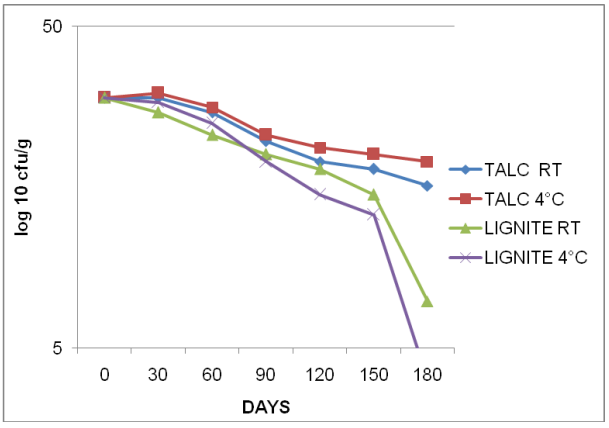

Fig. 1. Viability studies of *Bacillus subtilis* formulation.

Compatibility study of *Bacillus subtilis* with the fungicide

Bacillus subtilis was compatible with chorothalonil (Kavach), used to control root rot in solanaceous crops (Fig. 2). This compatibility affords an opportunity for the integration of chemical with biological agents. Fungicides may influence the pathogen as well as the antagonist negatively, hence, such a study, would provide valuable data on the selection of fungicides and fungicide resistant antagonists. Beneficial rhizospheric microflora can be established by combined usage of the fungicides and biocontrol agents (Papavizas and Lewis, 1981) moreover, the inhibitory effect of antagonist can be elevated by fungicide application (Kay and Stewart, 1994; Naar and Kecskes, 1998). The results are in concordance with many authors who reported the compatibility of fungicides with biocontrol agents in various crops (Utkhede *et al*., 2002; Sendilvel *et al.,* 2004; Anand *et al.,* 2007).

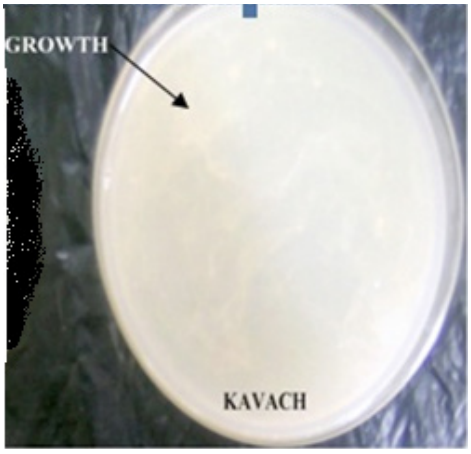

Fig. 2. Compatibility of the fungicide chlorothalonil (Kavach) with *Bacillus subtilis.*

Green house studies

Soil analysis

Routine soil analysis is done to establish the nutritional status of soil and fertilizers. Evaluation of all elements necessary for plant growth can be performed through standardized protocols. The analysis of the soil (Table 1) as a part of the present study indicated the suitability of the soil for the cultivation of chilli without supplementation with any micro nutrients. Earlier soil analysis reports are in conformity with the present results (Nirmal *et al.,* 2003). However, addition of Nitrogen, Phosphorus and Potassium is necessary for good chilli plant growth and yield.

Table 1. Physico chemical nature of the experimental soil

Soil classification	Typic Haplustert
Soil Texture	Loamy sand
Bulk density	1.41 g cm^3
pH	5.8
E.C	0.27 dS m^{-1}
Org. C	5.24 g kg^{-1}
Mineralizable N	242 kg ha^{-1}
Bray's P	27.3 kg ha^{-1}
Exchangeable K	149 kg ha^{-1}
Exchangeable Ca	1240 kg ha^{-1}
Exchangeable Mg	278 kg ha^{-1}
Extractable S	11.36 ppm
DTPA Zn	0.620 ppm
DTPA Cu	0.371 ppm
DTPA Mn	4.32 ppm
DTPA Fe	56.4 ppm

Disease control (%)

Biological control of plant disease causing agents can

be more successful if antagonists with varied mechanism of action can be employed. The at hand pot culture investigations were carried out with talc formulations of the antagonist alone and in conjunction, against chilli root rot disease (Fig. 3–7). Relative to the control and fungicide treatment, *B. subtilis* treatment significantly enhanced plant rigor and yield of chilli plants in all the four applications as revealed by the post-harvest biometric observations (Table 2–5).

The influence of treatment of soil with *B. subtilis* on plant growth parameters such as growth and yield enhancement and inhibition of *R. solani* is recorded in Table 2, Fig. 3.

Assessment of plant growth parameters such as height of the plant, root and shoot length, fresh and dry weight and yield (per plant) revealed considerably enhanced values in *B. subtilis* application (T3) relative to the control (T7). In comparison to all other treatments, *B. subtilis* treatment (T3) exhibited significant enhancement in plant growth parameters namely height of the plant (70.33 cm), root length (26.0 cm), shoot length (44.33 cm), fresh weight (59.67 g), dry weight (30.5 g) and yield (51.39 g/plant). Significant decline in all growth parameters was observed with the chilli plants treated with *R.solani* (T1). A considerable enhancement in plant growth parameters was obtained when the chilli plants were treated with both the pathogen and *B. subtilis* (T5) when compared with only the pathogen treatment (T1). Likewise, chlorothalonil (Kavach) treatment (T2) and the untreated control (T7) exhibited comparable plant growth parameters. Compatibility of the antagonist with the fungicide (T6) was supported by the considerable augmentation recorded with all biometric parameters of chilli plants.

Table 2. Efficacy of *Bacillus subtilis* treatment of soil in reduction of chilli root rot disease in pot trials

Treatments	Plant Height (cm)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Yield (g)
R.solani (T1)	38.33^{a}	13.17^{a}	25.17^{a}	29.00^{a}	11.00^{a}	25.71^{a}
Chlorothalonil (T2)	56.50^{b}	21.50^{c}	35.00^{bc}	52.33^{b}	20.33^{b}	39.13^{bc}
B.subtilis (T3)	70.33^{d}	26.00^{d}	44.33^{e}	59.67^{c}	30.50^{c}	51.39^{d}
R.solani + Chlorothalonil (T4)	55.75^{b}	22.58^{c}	33.17^{b}	47.00^{b}	22.00^{b}	39.23^{bc}
B.subtilis + *R. solani* (T5)	61.33^{c}	20.67^{c}	39.00^{cd}	51.83^{b}	21.33^{b}	42.11^{cd}
Chlorothalonil + *B.subtilis* (T6)	64.50^{c}	22.00^{c}	42.83^{de}	53.83^{bc}	20.00^{b}	45.09^{d}
Control (untreated)(T7)	61.00^{b}	16.67^{b}	44.33^{e}	49.00^{b}	31.00^{c}	35.40^{b}
SEM±	00.09	00.08	00.11	00.15	00.18	00.15
F	**	**	**	**	**	**
CD $P = 0.01$	00.35	00.32	00.40	00.55	00.52	00.56

T7 shows *R.solani* un-inoculated healthy plants. Two plants per pot represented each replicate. Means with the same alphabet superscript in each column are considerably undifferentiated by DMRT ($P < 0.05$); data were $\sqrt{x} + 0.5$ transformed. * Significant. The least observation in a column is represented by superscript alphabet 'a' and succeeding observations in increasing series are represented by ascending order of alphabets.

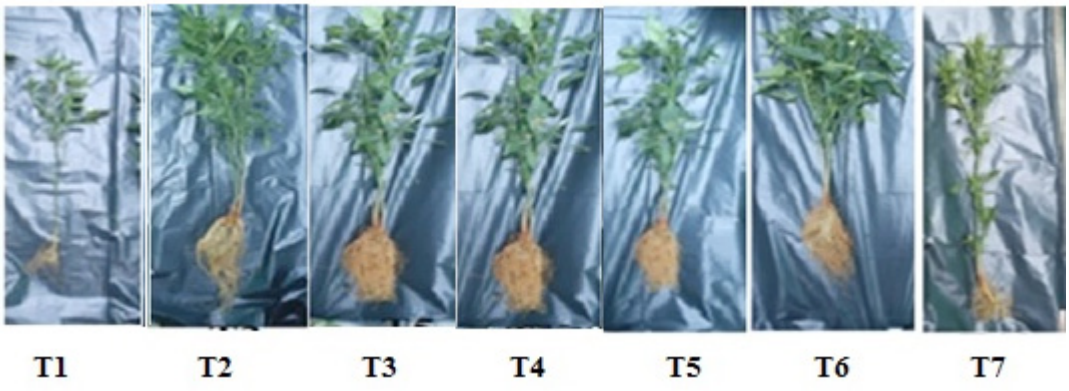

Fig. 3. Evaluation of chilli plant growth parameters of soil treatment.

Table 3 reveals the results of the influence of *B. subtilis* treatment of chilli seeds on plant growth parameters, yield enhancement and reduction of *R. solani* disease incitation. Similar to soil treatment results, there was an enhancement of all the growth parameters and yield in *B. subtilis* applications (T3) in comparison to the control (T7) (Fig. 4). *B. subtilis* treatment (T3) alone was markedly higher over all other treatments as revealed by, plant height (75.50 cm), root length (26.17 cm), shoot length (49.33 cm) fresh weight (76.83 g), dry weight (34.17 g) and yield (52.41 g/ plant). Though the biometric parameters were negatively influenced by (T1) pathogen treatment alone, simultaneous inoculation of the plant with the pathogen and antagonist (T5) brought about an overall enhancement in all the values. Effect of Chlorothalonil treatment (T2) on growth parameters was similar to results of the untreated control (T7). Compatibility of chlorothalonil and *B. subtilis* was revealed by the results of simultaneous application of both to the plants.

Table 4, Fig. 5 sums up the results obtained with the spray application of *B. subtilis* formulation on the chilli foliage and its influence on biometric parameters and yield. *B. subtilis* (T2) inoculated treatments exhibited superior influence on plant growth parameters in comparison to chlorothalonil treatment (T3) and untreated control (T1), though there was no significant influence on shoot length between the treatments. Assessment of the results obtained revealed superiority of *B. subtilis* treatment (T2) in terms of the height of the plant (70.12 cm), root length (25.5 cm), fresh weight (44.34g), dry weight (20.167 g) and yield (47.39 g/ plant) as compared to the other two treatments (T1 & T3).

Table 3. Efficiency of *Bacillus subtilis* seed treatment in the lowering of chilli root rot disease in pot trials

Treatments	Plant Height (cm)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Yield (g)
R.solani (T1)	37.33^{a}	13.33^{a}	24.00^{a}	31.83^{a}	10.67^{a}	27.67^{a}
Chlorothalonil (T2)	53.50^{b}	20.83^{cd}	32.67^{b}	57.50^{c}	16.67^{b}	33.79^{b}
B.subtilis (T3)	75.50^{e}	26.17^{e}	49.33^{e}	76.83^{d}	34.17^{c}	52.41^{e}
R.solani + Chlorothalonil (T4)	49.83^{b}	18.50^{bc}	31.33^{b}	49.67^{b}	18.17^{b}	41.65^{c}
B.subtilis + *R. solani* (T5)	58.83^{c}	19.83^{cd}	39.00^{c}	52.33^{bc}	18.67^{b}	43.52^{cd}
Chlorothalonil + *B.subtilis*(T6)	66.50^{d}	22.17^{d}	44.33^{d}	53.67^{bc}	16.50^{b}	47.78^{de}
Control (untreated) (T7)	61.00^{c}	16.67^{b}	44.33^{d}	49.00^{b}	31.00^{c}	35.40^{b}
SEM±	00.08	00.09	00.07	00.13	00.15	00.15
F	**	**	**	**	**	**
CD $P = 0.01$	00.29	00.34	00.27	00.48	00.58	00.57

T7 represents *R.solani* uninoculated healthy plants. Two plants per pot represented a single treatment. Means with the same alphabet superscript in each column are considerably undifferentiated by DMRT ($P < 0.05$); data were $\sqrt{x} + 0.5$ transformed. * Significant. The least observation in a column is represented by superscript alphabet 'a' and succeeding observations in increasing series are represented by ascending order of alphabets.

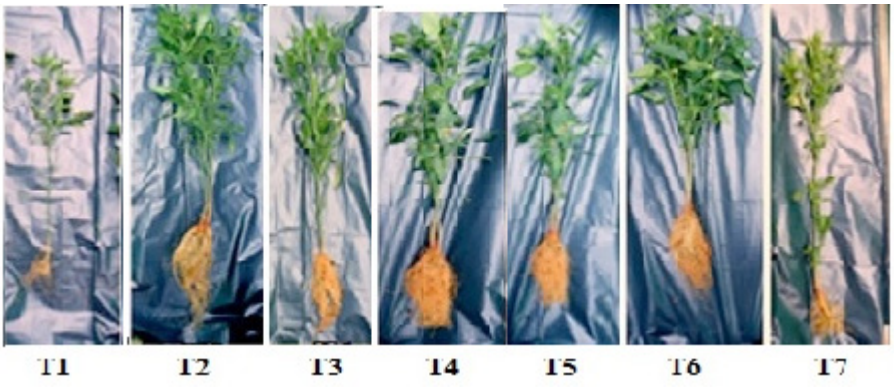

Fig. 4. Effect of seed treatment on chilli plant growth parameters.

Table 4. Efficiency of foliar spray *Bacillus subtilis* treatment in the reduction of natural root rot disease incitation in chilli

Treatments	Plant height (cm)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Yield (g)
Control (T1)	61[a]	16.67[a]	44.33[a]	41.33[a]	19.37[a,b]	36.85[a]
B.subtilis (T2)	70.12[b]	25.5[c]	44.34[a]	48.67[b]	20.167[a,b]	47.39[b]
Chlorothalonil (T3)	60.33[b]	18.00[c]	42.33	37.78[b]	15.67[b]	37.89[b]
SEM±	0.148	0.132	-	-	-	0.307
CD *P* = 0.01	0.329	0.295	-	-	-	0.684
Significance	*	*	NS	NS	NS	*

Control plant (T1) indicates uninoculated healthy plants. Two plants per pot represented each replicate. Means with the same alphabet superscript in each column are considerably undifferentiated by DMRT ($p < 0.05$); data were $\sqrt{x} + 0.5$ transformed. * Significant, NS-Non significance.

Table 5. Efficiency of *Bacillus subtilis* in the lowering of natural chilli root rot disease in pot trials using root dip treatment

Treatments	Plant height (cm)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Yield (g)
Control (T1)	58.2[a]	15.83[a]	42.6[a]	48.87[a]	14.4[a]	36.91[a]
B.subtilis (T2)	77.66[c]	33.33[c]	44.30[b]	62.8[c]	33.11[c]	72.02[c]
Chlorothalonil(T3)	70.33[b]	28.50[b]	41.83[a]	56.44[b]	22.74[b]	63.86[b]
SEM±	0.036	0.084	0.050	0.187	0.304	0.761
CD *P* = 0.01%	0.0824	0.188	0.112	0.416	0.677	1.69
Significance	*	*	*	*	*	*

T1 represents uninoculated healthy plants. Two plants per pot represented each replicate. Means with the same alphabet superscript in each column are considerably undifferentiated by DMRT ($p < 0.05$); data were $\sqrt{x} + 0.5$ transformed. * Significant.

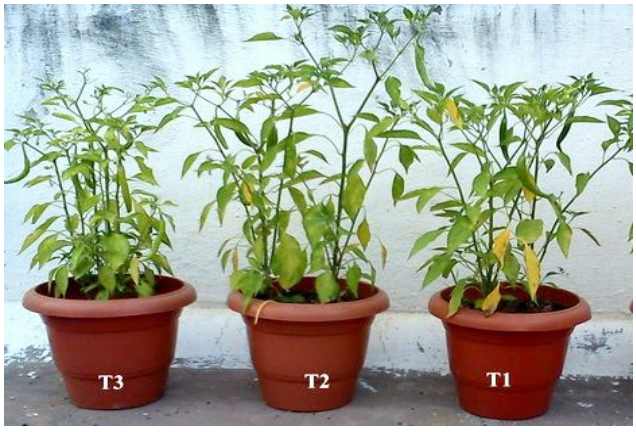

Fig. 5. Evaluation of plant growth parameters of foliar spray treatment.

Fig. 6. Evaluation of plant growth parameters after dip treatment.

Table 5, Fig. 6 summarizes the results obtained for the root dip treatment of *B. subtilis* formulation on biometric parameters. *B.subtilis* (T2) treatment exhibited superior values in terms of increase in plant height (77.66 cm), root length (33.33 cm), shoot length (44.30 cm) fresh weight (62.81 g), dry weight (33.11 g) and yield (72.02 g/plant) in comparison to the untreated control (T1) and fungicide treatment (T3).

Study of natural infection incitation is also equally important, hence the spray on the foliage and root dip treatments were not challenge inoculated, instead natural infection with *Rhizoctonia solani* was assessed. Though the plants did not exhibit any root rot like symptoms, the control exhibited leaf spots development. The benefit of treating the chilli plants with *B. subtilis* was noticed with regard to plant rigor improvisation and stability.

The evaluation of the four application methods, revealed the superiority of *B. subtilis* dip treatment on length of the plant (77 cm, 1.26 fold increase), root length (33 cm, 1.86 fold), dry weight (33 g, 1.57 fold) and fruit yield (72 g, 2 fold) in comparison to the untreated control (Fig. 6).

Fig. 7. Comparison of plant vigour of antagonist treated chilli plant with untreated control.

Treatment of the chilli plants with *R. solani* alone (T1) drastically reduced the plant biometric parameters, however co-inoculation with *B. subtilis* and the pathogen *R.solani* enhanced the growth in terms of better plant length (61.33 cm, 1.6 fold; 58.33cm, 1.56 fold), root length (20.67 cm, 1.56 fold; 19.83 cm, 1.48 fold) (Fig. 8), shoot length (39cm, 1.55 fold ; 39cm, 1.62 fold), dry weight (21.33g, 1.94 fold; 18.67g, 1.75 fold) and chilli fruit yield (42.11 , 1.64 fold; 41.65g, 1.5 fold). Soil and seed treatments recorded comparable results (Table 2 & 3) and further indicated the superiority of *B. subtilis* treatment to chlorothalonil treatment also. The Statistical evaluation ($p \leq 0.05$) of the results by analysis of variance (one-way) exhibited that *B. subtilis* application on chilli plants either alone or upon co-inoculation with the pathogen exhibited an increase in biometric parameters when compared to the untreated control. Treatment with *B. subtilis* recorded statistically significant enhancement than chlorothanlonil in soil, seed and spray on the foliage methods and analogous results with the root dip treatment method.

Dual benefit of effective retention of plant vigour and reduction of disease incitation was obtained by all the four application methods. Pot studies data analysis (Table 2 & 3) revealed the efficiency of *B. subtilis* talc formulation in reduction of disease incitation as compared to the pathogen inoculated control in both seed and soil treatment methods. A 67% reduction in disease incidence was observed by soil treatment method whereas 83 % reduction was obtained with seed treatment. The evaluation of the results indicated statistically considerable effect of *B. subtilis* treatment in comparison to chlorothalonil treatment.

Fig. 8. Effect of treatments on root length of chilli plant after harvest.

B. subtilis and *Pseudomonas fluorescens* consortia foliar spray, brought about a comparable reduction in bacterial blight disease of cotton as reported by Salaheddin *et al.* (2010). Soil application of different species of *Bacillus* was attempted by Lamsal *et al.* (2012) and a 40% reduction in anthracnose incidence was achieved which was lower by 10% according to the values obtained in the present study. Efficient reduction in the stem blight of *Phyllanthus amarus* was achieved by root dip treatment of seedlings with talc-based formulation of *B. subtilis* (BSCBE4) or *Pseudomonas chlororaphis* (PA23) (Mathiyazhagan *et al.,* 2004). Combined seed application of *B. subtilis* CA32 and *Trichoderma harzianum* RU01A brought about a 50% reduction in Fusarium wilt disease incidence as reported by Abeysinghe (2007). However, in comparison to the above reports, seed treatment of the present study has been able to demonstrate a significantly higher 83 % reduction in disease incidence which clearly supports the enhanced ability of seed and soil application methods of *B. subtilis* in comparison to the others quoted in the literature.

Additionally, the present study recorded the enhancement of plant growth parameters such as, shoot and root length, fresh biomass and total dry matter in chilli plants treated with *B. subtilis*. The results of the present research are in accordance with prior reports on the usage of rhizospheric Bacillus species in effective soil borne disease control (Williams and Asher 1996). The study by Choudhary and Johri (2009) has recorded the results of the evaluation

and elucidation of the characteristics of induced systemic resistance by employing Bacillus species. The results of diverse green house studies and field trials on a varied series of host plants have supported the efficacy of induced systemic resistance by specific strains of *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* (Kloepper *et al.,* 2004). Ryu *et al.,* (2003) have showed the the activity of unstable compounds 2, 3-butanediol and acetoin synthesized by *B. subtilis* strain GB03 and *B. amyloliquefaciens* strain IN937a in growth promotion of *Arabidopsis thaliana.* A significant increase in aerial plant parts of tomato and chilli plants as a result of combined application of *Pseudomonas* sp. and *B. subtilis* have been reported by Sundaramoorthy and Balabaskar (2012). The present research work summarizes that seed application was best with regard to lowering of disease incidence whereas dip treatment was best in terms of increase in most of the plant growth parameters.

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