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Biocontrol efficacy of *Trichoderma* isolates against tomato damping off caused by *Pythium* spp. and *Rhizoctonia solani* (Kuhn.)

Markidahun Biam and Dipali Majumder

Abstract
Damping-off of tomato is one of the worst diseases of tomato nurseries in Meghalaya. Pathogens can kill both germinating seeds and young seedlings. Management of damping off by fungicides is not the most desirable mean to manage the disease. Integration of biocontrol agents, specially the *Trichoderma* spp., which can reduce doses of fungicides is considered as better management strategy against the damping off of pathogens. Considering the fact, present investigation was formulated and aimed on to evaluate bio-efficacy of *Trichoderma* formulation against *Pythium* spp. and *Rhizoctonia solani* Kuhn. Cause damping off in tomato. The bio priming of tomato seeds with 4 *Trichoderma* isolates (TR 55, TR 66, TR 122 and TR 136) showed considerable increase in germination percentage and vigour index over control, with highest germination percentage and vigour index recorded in TR 55 (75.13% and 47.99% respectively). Among the treatments (seed, soil and seed plus soil) highest reduction in incidences of pre-emergence and post emergence damping-off was obtained with the isolate, TR 55 (seed plus soil treatment) in both the pathogens *i.e.*, *Pythium* spp. and *R. solani* Kuhn. The rhizosphere colonization of *Trichoderma* spp. showed that the rate of colonization increases up to 45 days after sowing and reduced at 60 days after sowing in all the treatments. Colonization was highest in TR 55 (3.73×10^6) followed by TR 122 (3.63×10^6), TR 66 (3.50×10^6) and TR 136 (3.37×10^6). Plant height, number of leaves and flowers per plant, dry and fresh weight, root length and yield of tomato was significantly superior in *Trichoderma* treated plants and low in control. Viability of *Trichoderma* in talcum powder showed that microbial count was highest initially but a gradual decline was recorded with the increase in the storage time.

Keywords: bio-efficacy, damping off, *Pythium* spp, *Rhizoctonia solani*, *Trichoderma*

1. Introduction
Damping off in tomato is one of the worst diseases caused by a variety of fungi, including the fungal-like organism leads to severe damage to crop yield worldwide, by destroying tomato germinating seeds and young seedlings in the nursery (Agrios, 2005) [2]. Infact, damping off is a seedling disease common to most of the vegetables like brinjal, chilli, cabbage, etc grown from transplanting or even when directly planted. In the field, garden, or planter box, tomato seedlings often fail to come up, or die soon after they have emerged from the soil. Seeds may rot before they germinate, shoots may be decayed before they emerge, or stems of seedlings may be attacked near the soil line, causing young plants to collapse. These diseases often are collectively referred to as "damping-off" (Thakur and Tripathi, 2015) [30]. Damping off disease is mainly caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn, which are responsible for seed decay as well as pre- and post-emergence damping-off of tomato seedlings. Pre-emergence damping-off is a term used to describe the rot of seeds, or the death of the seedlings, before the hypocotyls has been broken and the seed coat emerge from the soil. Post-emergence damping-off affects seedlings that have already emerged from the soil. These seedlings may develop a dark stem rot near the soil surface which will cause them to fall over and die as the rotted area shrivels (Rehman *et al*., 2012) [27]. Most of these fungi can also cause cuttings to rot (Abd-El-Khair *et al*., 2010; Kamala and Indira, 2011) [1, 15]. These fungi are found in practically all soils and pose a large threat to plant propagation. Usage of chemical fungicides is the one strategy proposed nowadays in the field. However, this strategy was not proved to be successful as it can be harmful to the microflora present in the soil as well as other organisms, environmental pollution and fungicidal resistance among pathogen (Alwathnani and Perveen 2012) [3]. To combat this problem, alternative strategy is the application of biocontrol agents such as

rhizobacteria and fungi to manage the soil borne diseases. One such biocontrol agents used is *Trichoderma* spp. includes *T. hamatum*, *T, viride* and *T. harzianum*. Damping-off of tomato caused by *Pythium* spp. and *R. solani* was reduced by the application of talc based formulation of *Trichoderma* spp. and *Pseudomonas fluorescence* in nursery beds before sowing. Besides reducing the pre - and post-emergence damping off these antagonists' increased the root length, shoot length and biomass production of tomato seedlings (Rajendraprasad *et al.*, 2017) [25, 31]. These antagonists significantly reduced the population of *Pythium* spp. and *R. solani* in soil. Damping-off is an important disease of tomato, causing significant losses in nurseries where young susceptible transplants are produced. In this present investigation a pot culture experiment was conducted in the polyhouse to study the efficacy of *Trichoderma* isolates against *Pythium* spp. and *R. solani*.

2. Material and Methods

2.1 Seed germination test

The potential antagonistic and plant growth-promoting activity of the four native *Trichoderma* isolates previously identified (table 1) *viz*., *T. hamatum* strain CEN693 (TR 55), *T. hamatum* strain US10g (TR 66), *T. hamatum* strain DIS 326F (TR 122) and *T. harzianum* (TR 136) were confirmed initially by seed germination test and tomato seedling vigor index before evaluated under pot experiment.

The bio priming of seeds of tomato was done with these four potent isolates of *Trichoderma*. The mycelia inoculum were prepared by taking mycelia disc of 5 mm diameter from young growing region of 4 days old culture of test isolates of *Trichoderma* and inoculated into Erlenmeyer flasks (100 ml) containing 50 ml PDB. Inoculated flasks were incubated at 28±1 °C for 3-4 days inside BOD incubator. Mycelial mat were harvested by passing through Whatman No. 42 filter paper and homogenized with a stirrer. The required concentration of mycelial inoculum were prepared by adding sterilized distilled water and used for seed germination test. Seeds of tomato were washed thoroughly with distilled water, air dried and finally dipped into 10^{-1} suspension of bioagents for 1 min. Stirring was done to ensure uniform coverage of seeds with the bioagents. The treated seeds were then spreaded on a cleaned blotter paper and allowed to air dry. The treated seeds were seeded into petriplates lined with double layered moist blotter paper and covered with upper lid of petriplate lined with moist blotter paper and incubated for one week at 28±1 °C. Germination rate was calculated by using the formula below (Farooq *et al*., 2005) [10]:

$$\text{Seed germination rate} = \frac{\text{No. of germinated seed} \times 100\%}{\text{Total No. of seeds}}$$

The tomato seedlings were raised from the seeds for which germination was tested and further assessed at the 5th day after incubation for root and shoot lengths. The germination rate, root and shoot lengths were used to calculate for vigor index using the following formula (Farooq *et al*., 2005) [10]:

Vigour index of seedlings = [Root length (cm) + shoot length (cm)] x germination (%)

2.2. Mass production of *Pythium* spp. and *R. solani*

The pathogens *Pythium* spp. was cultured as described by Jayaraj *et al*. (2006) [14] on broken maize-sand medium (broken maize 75.0 g; sand 7100 g; tap water 720.0 ml) and *R. solani* Kuhn on rice bran – sand mixture (Ngullie and Daiho, 2013) [20] filled in polypropylene bags (600 g) then sterilized at 1.4 kg cm^2 for 45 min and inoculated with mycelial discs (5 mm diameter) taken from one-week-old PDA culture of the fungus pathogens. The mouth of polypropylene bags were sealed with the help of cotton plug duly tied with fine but stiff thread and incubated at 28±1°C for 15 days inside BOD incubator with periodical mixing to avoid formation of clump. When the medium were fully covered with test fungus, it was immediately inoculated into the pot containing sterilized soil in the net house.

2.3. Mass production of *Trichoderma* isolates

The selected potent isolates of *Trichoderma* were mass multiplied in talc powder substrate following the methods of Pan and Bhagat, 2007 [22]. The talc powder were mixed thoroughly and filled into the double layered polypropylene bags, plugged with cotton plug and tied with fine thread and finally sterilized at 121 °C for 15-20 min for 2 consecutive days. The mycelial suspension (1 x 10^5/ml conidia) were inoculated into polypropylene bags containing talc powder and incubated at 28 ± 1° C for 21-25 days with periodical mixing to avoid formation of clump and to get uniform growth and sporulation of *Trichoderma* in the said medium. The appearance of green colouration throughout polypropylene bag is the indication of mature culture of *Trichoderma* and was used immediately in pot culture test.

2.4. Pot culture experiment

In vivo efficacy of potent isolates of *Trichoderma* were evaluated against *Pythium* spp.*and R. solani* Kuhn. under net house condition with three different treatments *viz*., seed treatment, soil treatment and combination of the seed and soil treatment at CPGS, CAU (Imphal), Umiam, Meghalaya. Each of the treatment were conducted by planting 10 tomato seeds per pot and replicated 3 times. Two days before sowing, potting soils were inoculated with *Pythium* spp. and *Rhizoctonia solani* separately at the rate of 5 g/kg soil. Simultaneously after 2 days of pathogen inoculation, soils were inoculated with potent *Trichoderma* isolates at 5 g/kg soil, and then pots were watered for 7 days before sowing. The details of treatments in the pot culture experiment are as follows:

T_1-	Seed treatment with *Trichoderma* @ 5 g (talc powder) (1 x 10^8cfu/g)/kg seed
T_2-	Soil treatment with *Trichoderma* @ 25g (talc powder) (1 x 10^8cfu/g) / pot
T_3-	$T_1 + T_2$
Control-	Without *Trichoderma* isolates (non – treated control)

Disease incidence of pre-and post-emergence and survival (%) of tomato plants were recorded after 15, 30 and 45 days, respectively using the standard procedure (Omokhua, 2011), as follows:

$$\text{Percentage of disease incidence (pre-emergence)} = \frac{\text{Number of seeds not germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Percentage of disease incidence (post-emergence)} = \frac{\text{Number of seedling infected}}{\text{Total number of seeds germinated}} \times 100$$

Biological control efficacy was calculated by using the following formula (Zhang *et al*., 2012):

$$\text{Biological control efficacy} \frac{\text{Disease incidence of control} - \text{disease incidence of treatment group}}{\text{Disease incidence of control}} = \text{x } 100$$

Plant growth promotion traits like plant height, root length, fresh and dry weight, number of leaves and flowers were recorded at the end of the experiment.

2.5 Rhizosphere colonization

Multiplication of *Trichoderma* in potting soil was recorded in terms of colony forming unit (CFU). The rhizosphere soil was collected by gently uprooting the test crops and brushing the soil adhered to roots after 15 days. The observation was recorded at 15 days interval upto 60 days. The rhizosphere soil from three plants were collected from each pot and was mixed thoroughly in each case and the rhizosphere population of *Trichoderma* spp. was estimated by soil dilution plate technique (Dhingra and Sinclair, 1995) [8].

2.6 Efficacy of talc-based *Trichoderma* formulation under *in vitro* condition

Proliferation of potential *Trichoderma* isolates was studied by taking talc as a carrier. Preparation of the talc based formulation was done by using the protocol given by Vidhyasekaran and Muthamilan (1995). To study the colonization of the antagonist, 1 gm of the formulation was suspended in 100 ml sterile distilled water in 250 ml flasks by thorough shaking and was serially diluted and 0.1 ml from 10^4dilution was spread on PDA plates with a sterilized glass spreader. Five replications were maintained. The colonies of the antagonists were counted with the help of a colony counter after 24 hr of incubation. Viability of antagonist for duration of three months was also observed.

3. Results and Discussion

3.1 Seed germination test

The bio priming of seeds of tomato (variety Hybrid-017) was done for potent isolates of *Trichoderma* spp. *viz*., *T. hamatum* strain CEN693 (TR 55), *T. hamatum* strain US10g (TR 66), *T. hamatum* strain DIS 326F (TR 122) and *T. harzianum* (TR 136) and results are presented in table 2. Table 2 indicated that TR 55 recorded the highest root (14.15±0.29 cm), shoot length (10.9±0.56 cm) and germination percentage 92% followed by TR 122, TR 66 and TR 136. The least was observed in control (with 6.85±0.35cm root length, 4.85±0.24cm shoot length and 62% germination percentage). The vigour index was highest in TR 55 (2306), followed by TR 122 (1990.5), TR 66 (1768.1), TR 136 (1576.5) and least was observed in control (724).

Pot culture experiment

Bio-control of damping off disease on tomato variety Hybrid-017 by seed treatment with *Trichoderma* @ 5 g (talc powder) (1 x 10^8cfu/g)/kg seed (T_1), soil treatment with *Trichoderma* @ 25g (talc powder) (1 x 10^8cfu/g) / pot (T_2) and combination of both (T_3) were studied. The effect of different treatments on pre and post emergence damping off and their ability to reduce the damping off incidence caused by *Pythium* spp. and *R. solani* under artificially inoculated conditions in net house was studied and the results are present here under.

Pre emergence damping off incidence and biological control efficacy of *Trichoderma* isolates against *Pythium* spp. and *R. solani*

From Table 3, it is observed that all the treatments significantly reduced the disease incidence over control. The pre-emergence damping off incidence caused by *Pythium* spp. ranged between 20 to 50 percent in various treatments. The minimum pre emergence damping off incidence was recorded in the seed + soil treatment with *T. hamatum* strain CEN693 (TR 55) with 20% disease incidence (DI) when compared to control (62%). Among the treatments, the lowest damping off incidence was recorded in T_3 (seed + soil treatment). Among the isolates, the lowest damping off incidence was observed in TR 55 with DI 30.67 percent, followed by TR 122 TR 66 and TR 136 with DI of 33.33, 38.67 and 43.33 percent, respectively. The highest pre-emergence damping off incidence was recorded in control (untreated pot) with 62% DI. Again it was observed that the biological control efficacy (BCE) was significantly difference among the four isolates. The highest BCE was recorded in TR 55 with BCE of 65.28%, followed by TR 122, TR 66 and TR 136 with BCE of 56.33%, 50.31% and 35.15 %, respectively.

Likewise pre-emergence damping off incidence caused by *R. solani* ranged between 28 to 48 percent in various treatments with minimum pre emergence damping off incidence recorded in the treatment T_1 (seed treatment with *T. hamatum* strain CEN693) and T_3 [seed + soil treatment with *T. hamatum* strain CEN693 (TR 55)] with incidence of 28 percent when compared to control (64 percent). Among the isolates, the lowest damping off incidence was observed in TR 55 with DI 39.60 percent, followed by TR 122, TR 66 and TR 136 with DI of 34.00, 37.33 and 44.00 percent, respectively. The highest pre-emergence damping off incidence was recorded in control (untreated pot) with 64% DI. Among the treatments, the lowest damping off incidence was recorded in T_3 with DI of 39.60 percent, followed by T_1 and T_2 with 40.80% and 45.60 % DI, respectively. The highest biological control efficacy (BCE) was recorded in TR 55 with BCE of 57.14%, followed by TR 122, TR 66 and TR 136 with BCE of 45.53%, 35.41% and 28.55 %, respectively. Among the treatments, the highest BCE was recorded in T_3 with BCE 44.12%, followed by T_2 (33.29%) and T_1 (22.56%).

Post emergence damping off incidence caused by *Pythium* spp. and biological control efficacy of *Trichoderma* isolates against *Pythium* spp. and *R. solani*

Post emergence damping off disease incidence (DI) caused by

Pythium spp. on tomato plants treated with TR 55, TR 122, TR 66 and TR 136 was recorded at 15, 30 and 45 days after sowing (DAS). From table 4, it was observed that at 15, 30 and 45 DAS, all 4 *Trichoderma* isolates could inhibit the pathogen *Pythium* spp. upto 67.40 % (seed + soil treatment with TR 55). The lowest post-emergence damping off incidence at 15, 30 and 45 DAS was observed in TR 55 (with 12.11%, 26.26% and 25.35% DI, respectively) and the highest disease incidence was observed in control (with 25.32%, 42.83% and 49.60% DI, respectively). Again at 15, 30 and 45 DAS, among the treatments, the lowest post-emergence damping off incidence was recorded in T_3 (16.24%, 26.62% and 32.38 % DI, respectively). Highest biological control efficacy (BCE) was recorded in TR 55 (65.28%), followed by TR 122 (56.33%), TR 66 (50.31%) and TR 136 (33.15%). Among the treatments, the highest BCE was recorded in T_3 with BCE 43.92 %, followed by T_1 (40.53%) and T_2 (38.59%).

In the case of post emergence damping off disease incidence (DI) caused by *R. solani* on tomato plants, all 4 *Trichoderma* isolates could inhibit the pathogen *R. solani* upto 68.27 % (seed + soil treatment with TR 55) (Table 5). The lowest post-emergence damping off incidence at 15, 30 and 45 DAS was observed in TR 55 (with 14.58%, 14.29% and 15.10% DI, respectively) and the highest disease incidence was observed in untreated pot (with 39.25%, 34.35% and 47.10% DI, respectively). Among the treatments at 15, 30 and 45 DAS, the lowest post-emergence damping off incidence was recorded in T_3 (22.95%, 21.04% and 24.78% DI, respectively), followed by T_2 (21.38%, 22.75% and 26.90% DI, respectively) and T_1 (23.37%, 23.21% and 28.82% DI, respectively). The highest biological control efficacy (BCE) was recorded in TR 55 (65.61%), followed by TR 122 (53.28%), TR 66 (46.21%) and TR 136 (26.45%). Among the treatments, the highest BCE was recorded in T_3 with BCE 41.56%, followed by T_2 (38.58%) and T_1 (34.80%).

3.2 Effect of biocontrol agents on growth parameters of tomato variety hybrid-017

The influence of *Trichoderma* isolates on the growth parameters such as root length, plant height, numbers of leaves, number of flowers, fresh and dry weight and yields were studied in pots under greenhouse conditions and the results are presented here under Table 6 and 7.

It was observed that the plant height recorded after 45 DAS ranged between 92.20 cm to 127.20 cm when compared to control (90 cm). The highest plant height was observed in TR 55 (107 cm), followed by TR 122 (103.06 cm), TR 66 (98.33 cm) and TR 136 (96.60 cm). Among the treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment), maximum plant height was recorded in T_3 (109 cm), followed by T_2 (94.72 cm) and T_1 (93.28 cm).

The number of leaves recorded after 45 DAS ranged from 32 to 57.20 numbers, when compared to control with 30 numbers (Table 4). Maximum number of leaves/plant was recorded in TR 55 (45), followed by TR 122 (43.20), TR 66 (36) and TR 136 (34.46). Among the treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment), the highest number of leaves was recorded in T_3 (43.64), followed by T_2 (36.20) and T_1 (33.36).

The number of flower recorded after 45 DAS ranged from 18 to 34.40 when compared to control (17). Maximum numbers was observed in TR 55 (25.33), followed by TR 122 (21.73), TR 66 (20.53) and TR 136 (20.06). Among the treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment), highest flower numbers was recorded in T_3 (25.08), followed by T_2 (19.20) and T_1 (18.52).

Root length of tomato plant was recorded after 45 DAS ranged between 15.04 cm to 22.80 cm when compared to untreated pots (11 cm). Maximum root lenght was recorded in TR 55 (18.35 cm), followed by TR 122 (16.67 cm), TR 66 (16 cm) and TR 136 (15.81 cm). Among the treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment), maximum root length was recorded in T_3 (17.42 cm), followed by T_2 (14.82 cm) and T_1 (14.46 cm).

The fresh weight of tomato plants increased in all the treatments compared to control (300 g). The highest fresh weight was recorded in TR 55 (450.67 g), followed by TR 122 (432 g), TR 66 (366.67 g) and TR 136 (347.33 g). Among the treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment), maximum fresh weight was recorded in T_3 (438 g), followed by T_2 (364 g) and T_1 (336 g).

The dry weight of tomato plants increased in all the treatments compared to control (53.32 g). The highest dry weight was recorded in TR 55 (68.86 g), followed by TR 122 (67.17 g), TR 66 (56.29 g) and TR 136 (55.34 g). Among the treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment), maximum dry weight was recorded in T_3 (67.94 g), followed by T_2 (58.04 g) and T_1 (54.60 g).

The variation in the yield of tomato plants due to different *Trichoderma* isolates and different treatments (T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment) was found to be statistically significant. The yield increased in all the treatments compared to control (1.4 kg). The highest yield was recorded in TR 55 (2.25 kg), followed by TR 122 (1.76 kg), TR 136 (1.74 kg) and TR 66 (1.69 kg). Among the treatments, maximum yield was recorded in T_3 (2.07 kg), followed by T_2 (1.65 kg) and T_1 (1.58 kg).

3.3 Rhizosphere colonization

Multiplication of *Trichoderma* in potting soil was recorded in term of colony forming unit (CFU) and observation recorded at 15 days interval upto 60 days after sowing and results are presented in table 8. It is evident from table 8 that the mean population of *Trichoderma* isolates was found to be increased steadily up to 45 days and reduced at 60 DAS. At 15, 30, 45 and 60 days after sowing (DAS), maximum population was recorded in TR 55 with peak population (3.73×10^6cfu/g at 45 DOI) followed by TR 122, TR 66 and TR 138 (Table 8).

3.4 Efficacy of talc-based *Trichoderma* formulation under *in vitro* condition

Proliferation of four potential *Trichoderma* isolates *viz*., TR55, TR66, TR122 and TR136 was studied by taking talc as a carrier. The colonies of the antagonists were counted with the help of a colony counter after 24hr of incubation for duration of three months at 15 days interval and observed that population was decreasing with increasing storage time (Table 9). Maximum population was recorded in TR 55 (13.1×10^9cfu/g at 45 days of incubation) and least in TR 138 (3.34×10^9cfu/g at 90 days of incubation) (Table 9).

4. Discussion

In the present study, the bio priming of seeds of tomato (variety Hybrid-017) done for potent isolates of *Trichoderma* spp. *viz*., *T. hamatum* strain CEN693 (TR 55), *T. hamatum* strain US10g (TR 66), *T. hamatum* strain DIS 326F (TR 122) and *T. harzianum* (TR 136) and results showed that they have significantly increased shoot and root length, germination percentage and vigor index compared to untreated control.

Several researchers have reported the biological seed treatments for protection of seed and control of pathogens causing seedling diseases (Mastouri, 2010; Islam *et al.* 2011; Doni *et al*. 2014; Pandey, 2017) [17, 13, 19, 9]. Reddy (2012) bio-primed sweet corn seeds with *Trichoderma harzianum* and reported that seed priming alone or in combination with low dosage of biocontrol agents improved the rate and uniformity emergence of seed and also reduced damping-off disease. Balakrishnan *et al*. (2017) reported that the paste formulaton of *T. harzianum* has significantly increased seed germination, shoot length, root length, seedling mean, dry matter production, vigour index in blackgram, chilli, cotton, sunflower, and tomato.

All three treatment *i.e*., T_1 (Seed treatment), T_2 (soil treatment) and T_3 (seed + soil treatment) were significantly superior over untreated control for both pre and post emergence damping off (caused by *Pythium* spp. and *R. solani*). However T_3 showed the best mode of application of *Trichoderma* for optimum management of pre and post-emergence damping off. TR 55 (*T. hamatum* strain CEN693) showed the least pre and post emergence damping off disease incidence and highest biological control efficacy followed by TR 122, TR 66, TR 136 and least in untreated control. A number of previous studies made by other researchers also support and reflect the same findings (Gravel *et al.*, 2005; Shabir and Rubina, 2010; M-Uddin *et al*., 2011). Thakur and Tripathi (2015) [30] effectively controlled the pre- and post-emergence damping-off of Tomato [cv. Solan lalima] by seed and soil treatment with *T. harzianum* as compared to untreated control. Also, Pandey (2017) [24] reported pre and post emergence damping off disease reduction in neem seedlings with application of *Trichoderma koningii* and *T. harzianum* either singly or with arbuscular mycorrhizal fungi. Uma Devi *et al*. (2017) [31] treated tomato seeds with *Trichoderma harzianum* and results indicated that the application of *Trichoderma* isolates in the pot experiment tended to reduce the incidence of pre- and post-emergence of damping-off disease of tomato compared to control, which support the present findings.

The overall growth of tomato was observed to be significantly increased in all four *Trichoderma* isolates compared to untreated control. Plant height ranged between 92.20 cm to 127.20 cm, number of leaves per plant ranged from 32 to 57.20, number of flowers ranged from 18 to 34.40 and root length of tomato plant ranged between 15.04 cm to 22.80 cm. The fresh and dry weight of tomato plants was recorded highest in TR 55. The yield increased in all the treatments compared to control (1.4 kg) with maximum yield in TR 55 (2.25 kg). The potential of *Trichoderma* spp. to enhance plant height, root length, fresh and dry weight and yield of crop plants was reported by several workers (Rehman *et al.*, 2012) [27]. Rajendraprasad *et al*. (2017) [25, 31] reported that the combination of *Trichoderma* isolates and potential bacterial treatment proved effective in increasing the shoot and root weight, fresh and dry weight and yield of tomato plants.

The rhizosphere competence of the potent isolates of *Trichoderma* spp. showed variation in the levels of colonization. It is evident from the result that the mean population of *Trichoderma* isolates was found to be increased steadily up to 45 days and there after declined. Increase in the rate of colonization upto certain period and reduction later may be result of the decrease in the amount of nutrients available to the antagonist. Rhizosphere competence of antagonistic fungi in root zone of many crops is a vital for successful management of plant diseases by biocontrol agents as they are expected to come in contact and establish within the rhizosphere zone of plant earlier than any other microorganisms, thus they will provide a protective cover for root tips and hairs which otherwise vulnerable to attack by several plant pathogenic fungi. McLean *et al.,* (2005) [18] reported that *Trichoderma* populated more near the stem base or collar region and root tip or root hairs than middle sections of root systems, which are the most vulnerable plant parts to be attacked by pathogens. The effectiveness of *Trichoderma* as seed treatment is probably determined not only by their biocontrol qualities but also by their abilities to multiply in the rhizosphere when applied to soil. In the present findings there were increased in the rhizosphere population of *Trichoderma* spp. with the advancement of crop age upto 45 DAS and thereafter the population declined marginally which was also reported by other workers like Aziz *et al*., (1997) [4] and Yang *et al*., (2011) [32]. Benitez *et al.* (2004) [6] reported that during active growth phase, the different root exudates serve as source of nutrients to introduced antagonist and also found that *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, than many other soil microbes. Sathiyaseelan *et al*., (2009) [28] reported that *Trichoderma viride* is highly rhizosphere competent and able to colonize and grow on roots as they develop. Prabha *et al*., (2015) [23] studied the competence of *Trichoderma viride* as biocontrol agents against soil borne disease on onion and reported that the combination of *Trichoderma viride* and *Fusarium oxysporum* showed increased levels in all observed characteristics of crop and rhizosphere soil than individual one.

Viability test of talc based *Trichoderma* formulation for three months at different days of incubation showed variation in the mean population of all four *Trichoderma* isolates. The mean population was found to be increased steadily upto 45 days and thereafter declined. These findings are in consistent with the findings of Das *et al*. (2006) [7] who reported that talc based formulation exhibited gradual declining trend in multiplication and sporulation of *T. harzianum* after 30 days onwards. Jayaraj *et al*. (2006) [14] studied seven different formulations (talc, lignite, lignite + fly ash-based powder formulation, wettable powder, bentonite paste, polyethylene glycol-paste and gelatin-glycerin-gel) and observed that the population of *Trichoderma harzianum* strain M1 propagules was optimum in all the formulations up to three months of storage. Gupta and Dohroo (2014) [12] reported that microbial count of *T. harzianum* and *Bacillus subtilis* was highest initially but a gradual decline was recorded with the increase in the storage time.

5. Conclusion

Biopriming of tomato seeds with TR 55, TR 66, TR 122 and TR 136 showed higher seed germination and vigor index compared to the control (springled with water only). Application of *Trichoderma* isolates through seed (T_1), soil (T_2) and soil plus seed (T_3) results in lower pre and post emergence damping off (caused by *Pythium* spp and *R. solani*) disease incidence and showed higher biological control efficacy than the untreated control. However, *Trichoderma* isolates applied through both seed and soil (T_3) were found significantly superior in disease biological control efficacy and also in enhancing overall growth and yield of tomato plants. Also, results indicate that among the isolates, TR 55 (*T. hamatum* strain CEN693) showed superior in disease biological control efficacy as well as in increasing growth parameters and yield of tomato plants. Results showed

that all four isolates *i.e.*, TR 55 (*T. hamatum* strain CEN693), TR 122 (*T. hamatum* strain DIS 326F), TR 66 (*T. hamatum* strain US10g) and TR 136 (*T. harzianum*). TR 55 (*T. hamatum* strain CEN693) are highly rhizosphere competent with TR 55 showing the highest mean population of 3.73×10^6cfu g-1at 45 DAS. Talc based *Trichoderma* formulation microbial count was highest initially but a gradual decline was recorded with the increase in the storage time. So, from the present findings recommendation could be made for further evaluation of the native *Trichoderma* isolates TR 55, TR 66 and TR 122 under different climatic condition of the state for development of effective *Trichoderma* formulation as a component of integrated disease management practice to manage damping off of tomato in the nursery beds in Meghalaya.

Table 1: List of *Trichoderma* species used in the present study

Sl. No.	Code Name Given	*Trichoderma* spp
1.	TR 55	*T. hamatum* strain CEN693
2.	TR 66	*T. hamatum* strain US10g
3.	TR 122	*T. hamatum* strain DIS 326F
4.	TR 136	*T. harzianum*

Table 2: Bio priming of tomato seed with *Trichoderma* isolates

Sl. No.	*Trichoderma* isolates	Root Length(cm) (R)	Shoot Length(cm) (S)	Germination Percentage (G)	Vigour index(V)* V= (R+S) X G
1.	TR 55	14.15±0.29^{a} (3.76)	10.9±0.56^{a} (3.29)	92±2.00^{a} (75.13)	2306±78.86^{a} (47.99)
2.	TR 66	11.85±0.38^{c} (3.44)	8.74±0.25^{bc} (2.95)	86±2.45^{ab} (68.31)	1768.1±32.40^{c} (42.04)
3.	TR 122	13.15±0.29^{b} (3.62)	9.5±0.32^{b} (3.08)	88±2.00^{ab} (69.93)	1990.5±21.94^{b} (44.61)
4.	TR 136	10.85±0.29^{d} (3.29)	7.9±0.13^{c} (2.81)	84±2.45^{b} (66.68)	1576.5±66.08^{d} (39.67)
5.	Control	6.85±0.35^{e} (2.61)	4.85±0.24^{d} (2.19)	62±2.00^{c} (51.97)	724±15.28^{e} (26.92)
SE(m)		0.007	0.015	2.68	1.58
CD (p=0.05)		0.112	0.164	6.94	1.68

*Means of three replications

Values in parentheses are $\sqrt{x+0.5}$ transforsmed value

Table 3: Effect of *Trichoderma* isolates on Percentage of Incidence and Biological control efficacy of Pre-emergence damping off disease of tomato under net house condition

Trichoderma isolates	Percentage of pre-emergence disease incidence (DI)								Biological control efficacy (BCE) over control							
	Pythium spp				*R. solani*				*Pythium* spp				*R. solani*			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
TR 55	34.00	38.00	20.00	30.67	28	36	28	30.67	64.46	63.99	67.40	65.28	39.23	62.85	69.33	57.14
TR 66	46.00	44.00	26.00	38.67	36	42	34	37.33	45.71	55.25	49.97	50.31	23.42	31.42	51.50	35.41
TR 122	38.00	40.00	22.00	33.33	34	38	30	34.00	50.40	57.28	61.33	56.33	29.51	52.85	54.22	45.53
TR 136	50.00	50.00	30.00	43.33	42	48	42	44.00	32.39	35.63	37.43	35.15	20.66	19.33	45.66	28.55
Control	62.00	62.00	62.00	62.00	64	64	64	64.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Mean	46.00	46.00	32.00	41.00	40.80	45.6	39.60	42.22	38.59	42.43	43.22	41.41	22.56	33.29	44.12	33.32
	Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)	
SE(m)	1.93	1.49	3.34		2.21	1.71	3.803		0.76	0.59	1.32		0.90	0.70	1.57	
CD(p=0.05)	5.46	4.23	9.46		6.25	6.84	10.83		2.16	1.67	3.74		2.56	1.98	4.44	

*Means of five replications;

Where- $\mathbf{T_1}$ = Seed treatment, $\mathbf{T_2}$ = Soil treatment and $\mathbf{T_3}$ = Seed + Soil treatment

Analyzed by two-way analysis of variance (2 way factor ANOVA).

Table 4: Effect of *Trichoderma* isolates on Disease Incidence and their Biological control efficacy against Post-emergence damping off of tomato caused by *Pythium* spp. under net house condition

Trichoderma isolates	Percentage (%) of disease incidence (post-emergence) *Pythium* spp												Biological control efficacy (post-emergence) over control (%)			
	15 DAS				30 DAS				45 DAS							
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
TR 55	12.95	11.85	11.54	12.11	24.60	28.50	25.62	26.26	25.00	26.11	24.95	25.35	63.99	64.46	67.40	65.28
TR 66	17.04	17.06	14.25	16.12	28.35	32.51	23.53	28.15	32.60	36.00	28.82	32.47	49.97	45.71	55.25	50.31
TR 122	14.65	16.26	12.48	14.46	27.41	30.10	26.96	28.12	26.80	29.31	26.51	27.54	57.28	50.40	61.33	56.33
TR 136	19.60	20.70	17.85	19.38	33.16	33.78	19.51	28.81	36.20	38.28	32.83	35.77	31.42	32.39	35.63	33.15
Control	24.47	26.40	25.10	25.32	42.40	48.60	37.51	42.83	48.40	51.60	48.80	49.60	00.00	00.00	00.00	00.00
Mean	17.74	18.45	16.24	17.48	31.18	34.69	26.62	30.83	33.80	36.26	32.38	34.14	40.53	38.59	43.92	41.01
	Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)	
SE(m)	0.23	0.18	0.40		0.25	0.19	0.45		0.46	0.35	0.79		0.74	0.57	1.28	
CD(p=0.05)	0.65	0.50	1.14		0.72	0.56	1.25		1.30	1.00	2.25		2.90	1.62	3.63	

*****Means of five replications;

Where, $\mathbf{T_1}$ = Seed treatment, $\mathbf{T_2}$ = Soil treatment and $\mathbf{T_3}$ = Seed + Soil treatment

Analyzed by two-way analysis of variance (2 way factor ANOVA).

Table 5: Effect of *Trichoderma* isolates on Disease Incidence and their Biological control efficacy against Post-emergence damping off of tomato caused by *R. solani* Kuhn. under net house condition

Trichoderma isolates	Percentage of disease incidence (post-emergence) *R. solani* Kuhn.												Biological control efficacy (post-emergence) over control			
	15 DAS				30 DAS				45 DAS							
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
TR 55	16.06	14.40	13.28	14.58	16.00	13.99	12.88	14.29	20.00	18.80	15.10	17.96	64.00	64.58	68.27	65.61
TR 66	23.40	20.10	17.53	20.34	18.18	20.51	20.79	19.82	25.66	24.40	19.60	23.22	41.71	45.71	51.20	46.21
TR 122	19.70	16.28	15.56	17.18	17.66	21.71	16.70	18.69	22.20	21.45	16.73	20.12	48.97	54.22	56.66	53.28
TR 136	21.30	24.00	19.20	21.50	26.80	24.60	22.15	24.51	26.55	28.26	22.48	25.76	19.31	28.38	31.66	26.45
Control	36.42	32.13	49.20	39.25	37.40	32.96	32.70	34.35	49.71	41.60	50.00	47.10	00.00	00.00	00.00	00.00
Mean	23.37	21.38	22.95	22.57	23.21	22.75	21.04	22.33	28.82	26.90	24.78	26.83	34.80	38.58	41.56	38.31
	Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)	
SE(m)	0.34	0.26	0.59		0.34	0.26	0.60		0.34	0.26	0.59		0.08	0.62	1.38	
CD(p=0.05)	0.93	0.74	1.66		0.98	0.76	1.70		0.97	0.75	1.69		2.26	1.75	3.93	

*****Means of five replications;

Where- **T_1** = Seed treatment, **T_2** = Soil treatment and **T_3** = Seed + Soil treatment

Analyzed by two-way analysis of variance (2 way factor ANOVA).

Table 6: Plant growth promotion of tomato by *Trichoderma* isolates under net house condition

Trichoderma Isolates	Plant height (cm)				Number of leaves/plant				Number of flowers/plant				Root length (cm)			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
TR 55	96.40	97.40	127.20	107.00	36.80	41.00	57.20	45.00	20.00	21.60	34.40	25.33	15.68	16.56	22.80	18.35
TR 66	92.20	93.60	109.20	98.33	32.00	33.20	42.80	36.00	18.00	19.00	24.60	20.53	15.06	15.12	17.82	16.00
TR 122	95.60	99.00	114.60	103.06	35.40	42.80	51.40	43.20	19.00	19.00	27.20	21.73	15.50	15.60	18.90	16.67
TR 136	92.20	93.60	104.00	96.60	32.60	34.00	36.80	34.46	18.60	19.40	22.20	20.06	15.04	15.80	16.60	15.81
Control	90.00	90.00	90.00	90.00	30.00	30.00	30.00	30.00	17.00	17.00	17.00	17.00	11.00	11.00	11.00	11.00
Mean	93.28	94.72	109.00	99.00	33.36	36.20	43.64	37.73	18.52	19.20	25.08	20.93	14.46	14.82	17.42	15.56
	Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)	
SE(m)	1.09	0.84	1.89		0.69	0.54	1.20		0.57	0.44	0.99		0.31	0.24	0.53	
CD(p=0.05)	3.09	2.39	5.35		1.97	1.52	3.41		1.62	1.25	2.80		0.87	0.67	1.52	

*****Means of five replications;

Where- **T_1** = Seed treatment, **T_2** = Soil treatment and **T_3** = Seed + Soil treatment

Analyzed by two-way analysis of variance (2 way factor ANOVA).

Table 7: Yield, dry and fresh weight of tomato under net house condition

Trichoderma Isolates	Fresh weight/plant (g)				Dry weight/plant (g)				Yield/plant (kg)			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
TR 55	370	410	572	450.67	61	64.16	81.42	68.86	1.92	1.83	3.00	2.25
TR 66	328	340	432	366.67	51.86	48.22	68.80	56.29	1.52	1.65	1.91	1.69
TR 122	354	428	514	432	54.30	68.70	78.52	67.17	1.53	1.62	2.14	1.76
TR 136	328	342	372	347.33	52.58	55.86	57.58	55.34	1.55	1.75	1.92	1.74
Control	300	300	300	300	53.30	53.30	53.38	53.32	1.41	1.40	1.40	1.40
Mean	336	364	438	379.33	54.60	58.04	67.94	60.19	1.58	1.65	2.07	1.77
	Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)		Bioagents (A)	Treatments (B)	Interaction (AxB)	
SE(m)	5.92	4.58	10.36		0.89	0.69	1.55		0.47	0.36	0.82	
CD (at 5%)	16.76	12.98	29.03		2.54	1.96	4.40		0.13	0.10	0.23	

Means of five replications;

Where- **T_1** = Seed treatment, **T_2** = Soil treatment and **T_3** = Seed + Soil treatment

Analyzed by two-way analysis of variance (2 way factor ANOVA).

Table 8: Rhizosphere colonization of *Trichoderma* isolates at different days after sowing (DAS)

Sl. No.	*Trichoderma* isolates	Mean population (× 10^6cfu g-1) at different days after sowing (DAS)			
		15	30	45	60
1.	TR 55	0.78^{a} (0.88)	2.07^{a} (1.44)	3.73^{a} (1.93)	2.89^{a} (1.70)
2.	TR 66	0.69^{b} (0.83)	1.87^{b} (1.36)	3.50^{c} (1.87)	2.56^{c} (1.60)
3.	TR 122	0.71^{ab} (0.84)	1.92^{b} (1.38)	3.63^{b} (1.90)	2.72^{b} (1.65)
4.	TR 136	0.64^{b} (0.80)	1.70^{c} (1.30)	3.37^{d} (1.83)	2.46^{d} (1.56)
SE(m)		0.001	0.00	0.00	0.00
CD (p=0.05)		0.046	0.027	0.027	0.021

Means of three replications

Values in parentheses are transformed values for mean population at different days after sowing (DAS)

Table 9: Viability test of Talc based *Trichoderma* formulation at different days of incubation by serial dilution plate technique

Sl. No.	*Trichoderma* isolates	Mean population (× 10^9cfu g-1) at different days of incubation (DOI)					
		15	30	45	60	75	90
1.	TR 55	5.41[a] (2.32)	8.68[a] (2.94)	13.1[a] (3.61)	9.29[a] (3.04)	6.52[a] (2.55)	4.48[a] (2.11)
2.	TR 66	4.71[b] (2.17)	7.07[c] (2.65)	10.85[c] (3.29)	7.32[c] (2.70)	5.76[c] (2.40)	3.73[b] (1.93)
3.	TR 122	5.28[a] (2.29)	7.67[b] (2.76)	11.44[b] (3.38)	8.46[b] (2.90)	6.13[b] (2.47)	4.23[a] (2.05)
4.	TR 136	4.39[c] (2.09)	6.3[d] (2.51)	9.34[d] (3.05)	6.97[d] (2.64)	5.46[d] (2.33)	3.34[c] (1.82)
SE(m)		0.001	0.001	0.004	0.002	0.002	0.004
CD (p=0.05)		0.04	0.04	0.079	0.056	0.053	0.081

Means of three replications

Values in parentheses are transformed values for mean population at different days of incubation (DOI)

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