Int.J.Curr.Microbiol.App.Sci (2019) *8*(2): 566-579

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 02 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.802.064

In vitro Efficacy of Native *Trichoderma* Isolates against *Pythium* spp. and *Rhizoctonia solani* (Kuhn.) causing Damping-off Disease in Tomato (*Solanum lycopersicum* Miller)

Markidahun Biam*, Dipali Majumder and Heipormi Papang

Department of Plant Pathology, College of Post Graduate Studies, Central Agricultural University, Umiam-793103, Meghalaya, India

**Corresponding author*

Keywords

Meghalaya, Damping off, *Trichoderma* spp., *Pythium* spp., *Rhizoctonia solani*, Tomato, per cent inhibition, PGPR and biocontrol potential

Article Info

Accepted:
04 January 2019
Available Online:
10 February 2019

A B S T R A C T

The present study was conducted to evaluate the *in vitro* efficacy of native *Trichoderma* spp. against *Pythium* spp. and *Rhizoctonia solani* (Kuhn.) causing damping-off disease in Tomato. One hundred eighty soil samples were collected from 11 districts of Meghalaya and ninety seven *Trichoderma* isolates were obtained. Rapid screening against damping off pathogens (*Pythium* spp. and *R. solani*) of tomato revealed that 20 isolates showed maximum antagonism and subsequently 20 best isolates were selected for further analysis. Isolate TR 55 isolated from tomato rhizosphere was found to be the most effective isolate against both *Pythium* spp. and *R. solani* Kuhn, showing an inhibition percentage of 89.26% and 87.41% respectively, followed by other isolates like TR 66, TR 122 and TR 136. Screening for the PGPR and biocontrol potential of the twenty potential isolates revealed that all isolates were found positive for IAA production, ACC deaminase production and phosphorous solubilization, whereas 17 positive for chitinase production, 16 isolates were found positive for siderophore and ammonia production and 13 isolates were positive for HCN production.

Introduction

Tomato (*Solanum lycopersicum* Miller) is one of the most important temperate vegetable crops throughout the world and is also widely cultivated in Meghalaya, India. Tomato production has a major role in global horticulture, ranking second in importance next to potato in many countries (Sharma *et al.*, 2014). The main advantage of tomato farming is that it can be grown either in the field or under greenhouse conditions. Tomatoes are a good source of potassium, vitamin C, vitamin A and excellent antioxidant lycopene. They help in fighting cancer, reducing heart disease and are also good for eye health and digestion (Nahar and Ullah, 2012).

China is leading producer among Asian countries, followed by India. In India, tomato is cultivated as one of the leading vegetable

crops, covering an area of 8.65 lakh hectares with a total production of 168.26 lakh tonnes having productivity of 19.60 tonnes ha-1 (Anon, 2014). Bihar, Karnataka, Odisha, Maharashtra, Himachal Pradesh, West Bengal, Tamil Nadu, Uttar Pradesh and Gujarat are major tomato growing states (Ghinaiya and Pandya, 2017) and among them Karnataka is the largest producer state in India. These States account for 91% of the total production of the country. Meghalaya accounts for 36.60 million tonnes of tomato production, which is 0.16 % of country's total tomato production (Anon, 2018).

Although tomato is commercially grown across the globe, there is no place where the plant is free from diseases. One of the major causes of seedling loss is damping-off, a disease that is mainly caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn, which are responsible for seed decay as well as pre-emergence and post-emergence damping-off of tomato seedlings. Most of these fungi can also cause cuttings to rot (Thakur and Tripathi, 2015).

Management of damping off by fungicides is not the most desirable means of disease management, for several important reasons. Fungicides are heavily regulated and additionally, they are expensive, cause environmental pollution, and may induce pathogen resistance (Lamichhane *et al*., 2017). Since cultural practices alone are not always sufficient to effectively manage the disease, alternative strategies are needed. Therefore, management of plant pathogens using microbial bio inoculants has been considered as a potential management strategy for integrated disease management. Many researchers have demonstrated the potential of *Trichoderma* spp. in management of damping-off diseases of crop plants caused by *Pythium* spp. (Lamichanne *et al.,* 2017; Majeed *et al.,* 2018; Al-shemamary *et al.,* 2018) and *Rhizoctonia solani* (Asad *et al.,* 2014; Abbas *et al.,* 2017; Mangariello *et al.,* 2018). *Trichoderma* spp. are widely distributed all over the world and found in all natural habitats especially in those containing forest humus layer (Wardle *et al*., 1993) as well as in agricultural orchard soils (Roiger *et al*., 1991) and soil consisting of organic matter (Papavizas, 1985). It is known to be one of the best candidates of BCA against wide range of plant pathogens.

Therefore, in the present investigation major emphasis was to study the bio-efficacy of native *Trichoderma* isolates against damping off caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn.

Materials and Methods

Isolation, identification and maintenance of pathogen

Pythium spp. and *R. solani* Kuhn. were isolated from naturally infected tomato, showing damping off symptoms (soft rot and wire stem symptom for *Pythium* spp. and *R. solani* respectively) and pathogenicity test was carried out in SCP, CPGS, CAU, Umiam, Meghalaya. Diseased samples collected from farmers' fields were brought to laboratory and isolations were done. With repeated isolations, *Pythium* spp. and *R. solani* Kuhn were consistently found with the infected seedlings of tomato. *Pythium* spp. cultures isolated from the infected tomato seedlings were identified based on the types of fungal mycelium and filamentous sporangia with terminal, smooth and spherical oospores as compared with the old cultures available in the Laboratory. Also *R. solani* Kuhn was identified based on the hyphae that tend to branch at right angles and a septum near each and a slight constriction at the branch are diagnostic. The fungus was purified by hyphal tip cut method. The purified culture was

maintained on PDA slants at 4˚C in refrigerator.

Collection and isolation of *Trichoderma* from different locations of Meghalaya

Soil samples from root rhizosphere, coal mines, jhum fallows, manure compost and submerged areas were collected from 11 districts of Meghalaya. Isolation of *Trichoderma* was done by dilution plate method using PDA (Dhingra and Sinclair, 1995). One gram of soil was suspended in 250 ml Erlenmeyer flasks with 100 ml sterilized distilled water. Samples were shaked for 20-30 minutes on a rotary shaker at 250 rpm and dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made for each soil samples. An aliquot of 0.1 ml of substrate suspension was dispensed on PDA. The Petriplates (90mm in dia) were incubated at 28 ± 1ºC for 24 hours. Morphologically distinct colony was isolated, purified and grown in pure culture on PDA. The obtained fungal isolates were grown on PDA slants and kept at 4°C until being used. Isolated *Trichoderma* was grown on Malt Extract Agar (MEA) medium and identified based on characters *viz.* conidiophores, phialides and conidia (Rifai, 1969; Bisset, 1992). Microscopic examination was carried out by mounting the culture in lactophenol cotton blue.

Rapid screening of *Trichoderma* isolates

Isolates tentatively identified as *Trichoderma* were exposed to rapid screening of *Trichoderma* isolates against *Pythium* spp. and *R. solani* Kuhn. by dual culture technique on PDA medium on the basis of their relative growth rate measured as a function of incubation period. Mycelial discs of 5mm diameter was picked up from the margin of young 3-4 days old culture of *Trichoderma* and the respective pathogens were inoculated at the peripheral region of the Petriplates (90mm in dia) at equal distance from the centre and incubated for 5-6 days at 28±1°C. The relative growth rate of test antagonist and the pathogen were observed and recorded. The most efficient *Trichoderma* isolate was sorted out as potent isolate against the respective pathogen. These isolates were multiplied and maintained as mentioned earlier for long term preservation and preserved at 4°C in PDA slants for subsequent use.

The *Trichoderma* isolates were rated on the basis of their ability to suppress the mycelia growth of pathogen following the methods of modified Bell's scale (Bell *et al.*, 1982).

S_1: The antagonist completely overgrew the pathogen (100% over growth)
S_2: The antagonist overgrew at least 2/3rd growth of the pathogen (75% overgrowth)
S_3: The antagonized colonized half of the growth of pathogen (50% overgrowth)
S_4: The pathogen and antagonist (locked at the point of contact)
S_5: The pathogen overgrew the antagonist
The experiment was conducted with three replicates per treatments.

Efficacy of *Trichoderma* isolates against *Pythium* spp. and *R. solani* Kuhn

The potential *Trichoderma* isolates were further evaluated for their antagonistic potential *in vitro* against *Pythium* spp. and *R. solani* Kuhn. through dual culture technique (Ramanathan *et al*., 2013).

Dual culture technique

For mycelial growth inhibition of test plant pathogens by the *Trichoderma* spp., both pathogens (*Pythium* spp, *R. solani* Kuhn.) and antagonists were inoculated at peripheral region opposite to each other in sterilized Petriplates (90 mm dia) containing 20 ml

sterilized PDA medium and incubated at 28±1°C. Plates inoculated with the pathogens only served as the control. Observation for the dual inoculation of the *Trichoderma* spp. and the pathogen was taken till the growth of the pathogen fully covered the plate. The experiment was replicated three times. The suppression effect of all *Trichoderma* spp. isolates were evaluated in terms of Percentage Inhibition in Radial Growth (PIRG) of *Pythium* spp. and *R. solani* based on the following formula (Gaigole *et al*., 2011).

$$PIRG = \frac{R1 - R2}{R1} \times 100\%$$

R1 = Radial growth of *Pythium* spp. and *R. solani* in the absence of the antagonist in the respective plate (control)

R2 = Radial growth of *Pythium* spp. and *R. solani* in the presence of the *Trichoderma* isolates (treatment)

Screening of isolated *Trichoderma* for *In vitro* plant growth promoting and biocontrol potential

Chitinolytic enzyme assay

The strains of *Trichoderma* isolates were determined for chitinolytic activity on chitin detection medium (Thakar and Saraf, 2015).

Preparation of colloidal chitin

5.0 g of chitin was added to 60 ml of conc. HCl (acid hydrolysis) by constant stirring using a magnetic stirrer at 4°C and kept in refrigerator overnight. The resulting slurry was then added to 200 ml of ice-cold 95% ethanol and kept at 26 °C overnight (ethanol neutralization). Then it was centrifuged at 3,000 rpm for 20 min at 4°C. The pellet was repeatedly washed with sterile distilled water by centrifugation at 3,000 rpm for 5 min at 4 °C until the smell of alcohol vanished. The final colloidal chitin was stored at 4 °C until further use.

Chitinase detection medium

The final chitinase detection medium per litre comprised of 4.5 g colloidal chitin, 0.3 g magnesium sulphate, 3.0 g ammonium sulphate, 2.0 g potassium dihydrogen phosphate, 1.0 g citric acid monohydrate, 15 g agar, 0.15 g bromocresol purple and 200ul of tween-80. The pH of the media was maintained at 4.7 and autoclaved at 121 °C for 15 min. The fresh culture plugs of *Trichoderma* isolates to be tested for chitinase activity were inoculated into the sterile plates containing chitinase detection medium and incubated at 28 ± 2 °C for 2–3 days and observed for the coloured zone formation. Formation of purple coloured zone was observed and recorded.

Siderophores production test

The ability of *Trichoderma* spp. to produce iron-binding compounds of siderophore-type was detected in solid medium by universal Chrome Azurol S (C.A.S) assay (Srivastava *et al*., 2013)

Preparation of the C.A.S. (Chrome Azurol S) Blue Agar

One litre of C.A.S blue agar was prepared using 60.5 mg C.A.S dissolved in 50 ml distilled deionized water and mixed with 10 ml iron (III) solution (1 mM $FeCl_3.6H_2O$, 10 mM HCI). Under stirring, this solution was slowly added to 72.9 mg Hexadecyl tri methyl-ammonium bromide (HDTMA) dissolved in 40 ml water. The resultant dark blue liquid was autoclaved for 20 min. Also autoclaved a mixture of 750 ml water, 15 g agar, 30.24 g Pipes and 12 g of a solution of 50% (w/w) NaOH to raise the pH to the pKa

of Pipes (6.8). The dye solution was finally poured along the glass wall and agitated with enough care to avoid foaming. Petri dishes (9cm in diameter) were prepared with 30 ml of PDA medium for culturing *Trichoderma* spp. After solidification, the medium was cut into halves, one of which was replaced by C.A.S. blue agar (15 ml). The halves containing culture medium were inoculated with 5 mm discs of seven days old culture of *Trichoderma* strains. The inoculum was placed as far as possible, from the borderline between the two media. The plates were incubated at 28 ± 2°C for 7 days in the dark. Colour-changed from blue to purple or dark purplish- red (magenta) in the C.A.S.-blue agar, starting from the borderline between the two media was considered positive for siderophore production. The experiment was carried out in triplicates. The control plates of C.A.S.-agar uninoculated were incubated under the same conditions as described above. The experiment was conducted with three replicates per treatments.

HCN production

Production of HCN was detected by inoculated different isolates of *Trichoderma* spp. separately onto the PDA medium amended with 4.4 g/ml glycine and lid of plate was covered with the soaked Whatsman no.1 filter paper in 0.5% picric acid and in 2% sodium carbonate, then incubated for 5-7 days at 28±1°C. Change in colour of filter paper from deep yellow to orange and finally to orange brown to dark brown indicated the positive reaction. The experiment was conducted with three replicates per treatments (Dixit *et al*., 2015)

Indole-3-acetic acid (IAA) estimation test

Quantitative estimation of IAA was done through addition of tryptophan in the potato dextrose broth (PDB) for *Trichoderma* spp. and incubated at 25±1°C for seven days and filtered with Whatman No. 2 filter paper, then 1 ml filtrate was mixed with 2 ml Salkowski reagent (2% 0.5M $FeCl_3$ in 35% perchloric acid) in a test tube (Gravel *et al*., 2007). The mixture was incubated at room temperature for 20 minutes. Pink colour producing samples was considered as positive reaction and absorbance was measured at 540 nm by spectrophotometer. A standard curve was prepared using IAA and the presence of IAA in the culture filtrate was quantified. The IAA produced was compared to the standard graph and expressed as µg/ml (Dixit *et al*., 2015).

Phosphate (P) solubilization

Solubilization of P was tested quantitatively using 20 ml Pikovskaya's broth medium (PKV) amended with 5 g/l tricalcium phosphate (17% P) then inoculated with a mycelial disc of seven days old culture of *Trichoderma* spp. and incubated at 28±1°C on a shaker for 3-4 days. Uninoculaed PKV broth served as control in each case. Each experiment was done in triplicate set. Mycelial growth was filtered through Whatman No. 42 filter paper and 50 µl of resultrant filtrate was added with 500 µl of ammonium molybdate solution and shaked well. An addition of 2ml distilled water, 13µl chlorostannous acid and 2.5 ml distilled water was made. Blue colour intensity was recorded by spectrophotometer at 600nm. The available phosphorus in the culture filtrate was calculated from a standard curve prepared using various concentration of standard K_2HPO_4 solution and the results were expressed in µg/ml (Rudresh *et al.*, 2005).

ACC deaminase production test

The ACC deaminase production of the *Trichoderma* isolates was screened using the methods described by Jasim *et al.,* (2014). For

this, the isolates were inoculated on to Difco (DF) salts minimal medium (potassium dihydrogen phosphate 4 g/L, disodium hydrogen phosphate 6 g/L, magnesium sulfate heptahydrate 0.2 g/L, ferrous sulfate heptahydrate 0.1 g/L, boric acid 10 µg/L, manganese(II) sulfate 10 µg/L, zinc sulphate 70 µg/L, copper (II) sulfate 50 µg/L, molybdenum (VI) oxide 10 µg/L, glucose 2 g/L, gluconic acid 2 g/L, citric acid 2 g/L, agar 12 g/L) amended with 0.2 % ammonium sulphate (w/v). The fungal growth in this media after 4-7 days of incubation was considered as positive result. The experiment was conducted with three replicates per treatments.

Ammonia production

Trichoderma isolates was tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water separately and incubated for 48-72 h at 36 ± 2°C. Nessler's reagent (0.5 ml) was added in each tube. Development of yellow to brown colour indicated for positive test (Thakkar and Saraf, 2015). The experiment was conducted with three replicates per treatments.

Results and Discussion

Soil samples collected from different locations of Meghalaya were tested. The isolates showing the lime green to greenish colour sporulation with highly fluffy growth and sparse to compact colony after 7-10 days of incubation were selected. The selected isolates were grown on Malt Extract Agar (MEA) to observe conidiophores, phialides and conidia. Based on taxonomic keys provided by Rifai (1969) Bisset (1992), it is evident that altogether ninety seven (97) *Trichoderma* isolates were isolated from 180 soil samples collected.

Rapid screening of *Trichoderma* isolates was done against *Pythium* spp. and *R. solani* Kuhn. by dual culture in potato dextrose agar and the results showed that 77 isolates attained S_4 stage (the pathogen and antagonist locked at the point of contact), 8 isolates showed S_3 stage (the antagonized colonized half of the growth of pathogen i.e. 50% overgrowth) and remaining 12 isolates showed S_2 stage (the antagonist overgrew at least $2/3^{rd}$ growth of the pathogen i.e. 75% growth) from 4-7 days after inoculation on the basis of modified Bell's scale (Plate 1).

The study of antagonistic potential of 20 screened isolates of *Trichoderma* spp. against damping-off pathogens which showed S_2 and S_3 stage on the basis of modified Bell's scale revealed that all 20 isolates showed an inhibition percentage of more than 65%. Among which 4 *Trichoderma* isolates *viz.* TR 55, TR 66, TR 122 and TR 136 were most effective in inhibiting *Pythium* spp. with percent inhibition of 89.26%, 88.15%, 88.89% and 87.78% respectively, whereas only 2 isolates *viz.* TR 55 and TR 122 were found effective against *R. solani* with percent inhibition of 87.41% and 86.48% respectively (Table 1).

The result (Table 2, Plate 2) showed that out of 20 screened isolates, only 16 isolates were positive for siderophore production as indicated by colour-changed from blue to purple or dark purplish- red (magenta) in the C.A.S.-blue agar. Also 16 isolates were positive for ammonia production indicated by development of yellow to brown colour in Nessler's reagent whereas 17 isolates were positive for chitinase and 13 isolates were positive for HCN production as evidenced by the change in the colour of filter paper. However all 20 screened isolates grew on Difco (DF) salts minimal medium showing their ability to produce ACC deaminase.

Table.1 *In vitro* efficacy of *Trichoderma* isolates against *Pythium* spp and *R. solani*

Sl. No.	*Trichoderma* isolates	Growth (cm)		Per cent inhibition over control	
		***Pythium* spp**	***R. solani***	***Pythium* spp**	***R. solani***
1.	TR 12	2.00±0.06^{d} (1.41)	1.90±0.06^{ij} (1.37)	77.78±0.64^{e} (61.88)	78.89±0.64^{de} (62.65)
2.	TR 24	2.43±0.07^{c} (1.56)	2.07±0.09^{gh} (1.43)	72.96±0.74^{i} (58.67)	77.04±0.98^{fg} (61.37)
3.	TR 36	2.80±0.06^{b} (1.67)	2.33±0.03^{cde} (1.52)	68.89±0.64^{j} (56.10)	74.07±0.37^{jk} (59.39)
4.	TR 40	2.23±0.07^{c} (1.49)	2.07±0.03^{gh} (1.43)	75.19±0.74^{f} (60.12)	77.04±0.37^{fg} (61.37)
5.	TR 55	0.97±0.03^{h} (0.98)	1.13±0.03^{o} (1.06)	89.26±0.37^{a} (70.87)	87.41±0.37^{a} (69.22)
6.	TR 64	1.97±0.07^{d} (1.40)	1.97±0.07^{hi} (1.40)	78.15±0.74^{e} (62.13)	78.15±0.74^{ef} (62.13)
7.	TR 66	1.07±0.03^{h} (1.03)	1.43±0.03^{l} (1.19)	88.15±0.37^{a} (69.86)	84.07±0.37^{b} (66.47)
8.	TR 74	2.00±0.00^{d} (1.41)	1.73±0.03^{k} (1.31)	77.78±0.00^{e} (61.87)	80.74±0.37^{c} (63.97)
9.	TR 78	1.83±0.09^{de} (1.35)	2.40±0.06^{c} (1.54)	79.63±0.98^{de} (63.18)	73.33±0.64^{l} (58.91)
10.	TR 82	1.93±0.09^{d} (1.39)	2.23±0.07^{def} (1.49)	78.52±0.98^{e} (62.40)	75.19±0.74^{hij} (60.12)
11.	TR 87	2.37±0.03^{c} (1.54)	2.18±0.04^{efg} (1.47)	73.70±0.37^{h} (59.14)	75.74±0.49^{ghi} (60.49)
12.	TR 88	1.57±0.03^{fg} (1.25)	1.53±0.03^{l} (1.23)	82.59±0.37^{bc} (65.34)	82.96±0.37^{b} (65.62)
13.	TR 106	1.70±0.12^{ef} (1.30)	2.30±0.00^{m} (1.51)	81.11±1.28^{cd} (64.26)	74.44±0.00^{ijk} (59.63)
14.	TR 109	2.33±0.03^{c} 1.53)	2.12±0.10^{fg} (1.45)	74.07±0.37^{fg} (59.39)	76.48±1.13^{gh} (61.00)
15.	TR 112	1.63±0.09^{f} (1.28)	2.63±0.03^{b} (1.62)	81.85±0.98^{bc} (64.79)	70.74±0.37m (57.25)
16.	TR 116	1.83±0.07^{de} (1.35)	2.35±0.05^{cd} (1.53)	79.63±0.74^{de} (63.17)	73.89±0.56^{jk} (59.27)
17.	TR 122	1.00±0.00^{h} (1.00)	1.22±0.02^{no} (1.10)	88.89±0.00^{a} (70.53)	86.48±0.19^{a} (68.43)
18.	TR 136	1.10±0.06^{h} (1.05)	1.82±0.06^{jk} (1.34)	87.78±0.64^{a} (69.55)	79.81±0.67^{cd} (63.30)
19.	TR 143	1.47±0.03^{g} (1.21)	2.37±0.03^{cd} (1.53)	83.70±0.37^{b} (66.19)	73.70±0.37^{jk} (59.14)
20.	TR 171	1.63±0.09^{f} (1.28)	2.70±0.06^{b} (1.64)	81.85±0.98^{bc} (64.79)	70.00±0.64^{mn} (56.79)
21.	Control	9.00±0.00^{a} (3.00)	9.00±0.00^{a} (3.00)	0.00±0.00^{k} (0.36)	0.00±0.00^{o} (0.36)
SE(m)		0.002	0.001	0.72	0.44
CD (p=0.05)		0.06	0.051	1.40	1.10

*Mean of three replications

Note: Figures in parentheses are square root transformed values for growth and arc sine transformed values for per cent inhibition over control.

Table.2 PGPR and biocontrol efficacy of *Trichoderma* spp.

Sl. No.	*Trichoderma* spp	Chitinase production	Siderophore Production	HCN Production	Ammonia production	ACC deaminase activity	IAA produce (ug/ml)	Phosphorous production (ug/ml)
1.	TR 12	+	++	-	-	+	1.47	0.68
2.	TR 24	+++	++	-	+	+	0.84	0.21
3.	TR 36	+	+	-	-	+	2.97	0.53
4.	TR 40	++	+++	-	++	+	3.22	0.22
5.	TR 55	++	+++	+	+++	+	4.96	0.72
6.	TR 64	+++	++	+	+++	+	1.81	0.41
7.	TR 66	++	+	++	++	+	2.14	0.75
8.	TR 74	+	-	+	+	+	2.45	0.09
9.	TR 78	+	++	-	-	+	2.51	0.23
10.	TR 82	-	++	-	+	+	1.53	0.03
11.	TR 87	++	++	+	+	+	1.44	0.32
12.	TR 88	+	+	++	+	+	4.70	0.58
13.	TR 106	+++	+	+	+	+	0.48	0.98
14.	TR 109	+	-	+	+	+	0.48	0.74
15.	TR 112	+	+++	++	+	+	1.94	0.39
16.	TR 116	-	-	-	++	+	0.33	0.32
17.	TR 122	+++	++	++	+++	+	0.72	0.25
18.	TR 136	+	++	++	+++	+	0.99	0.79
19.	TR 143	++	-	+	-	+	0.58	0.59
20.	TR 171	_	++	+	+	+	0.87	0.29

*Mean of three replications
(+) indicates light color, (++) indicates dark color and (+++) indicates very dark color
(-) indicates absence

Plate.1 Rapid Screening of *Trichoderma* spp. following modified Bell's scale

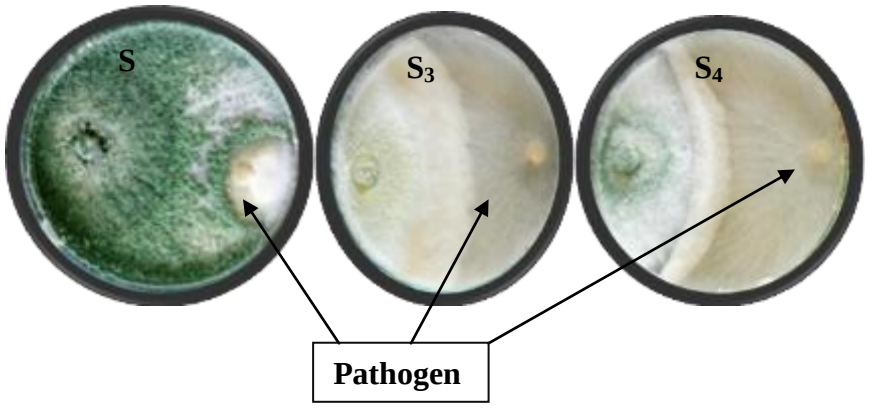
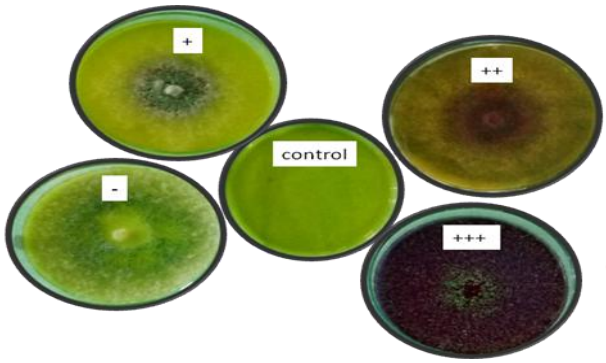
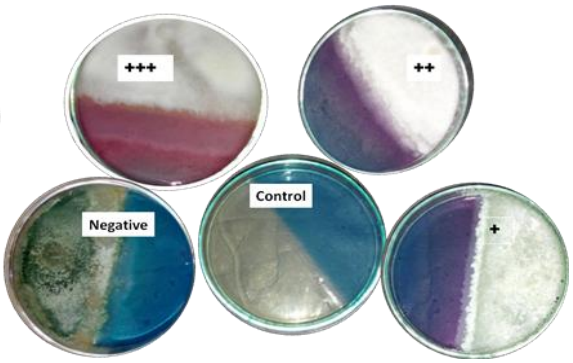


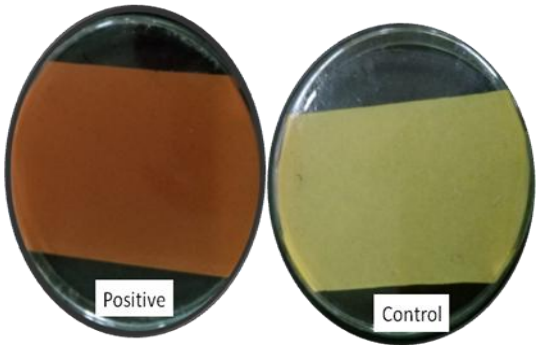
Plate.2 PGPR and biocontrol efficacy test of *Trichoderma* isolates



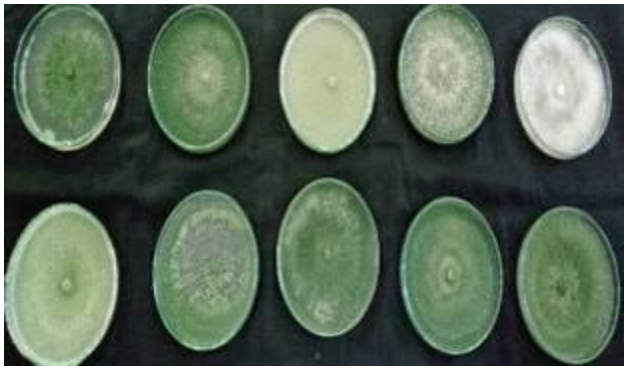
Chitinase production test



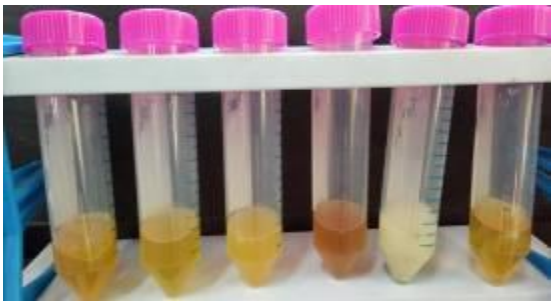
Siderophore production test



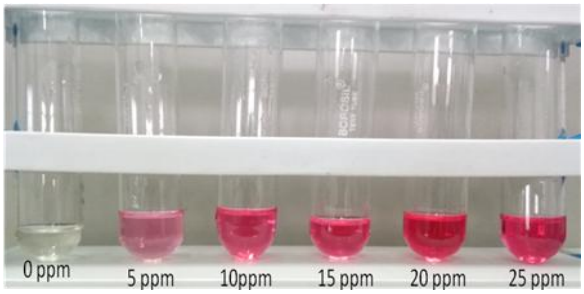
HCN production test



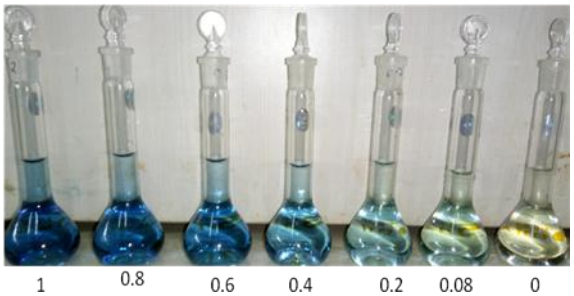
ACC deaminase production test



Ammonia production test



IAA production test



Phosphorous solubilization

Screening for plant growth promoting traits of 20 isolates revealed that all produced IAA with TR 55 showing highest production (4.96 ug/ml) followed by others like TR 88 (4.70 ug/ml), TR 40 (3.22 ug/ml) and least in TR 116 (0.33 ug/ml). Also all produced phosphorous with highest production in TR 106 (0.98 µg/ml) followed by TR 66 (0.75 ug/ml), TR 55 (0.72 ug/ml) and the least was observed in TR 82 (0.03 ug/ml).

From 180 soil samples collected from all 11 districts of Meghalaya, 97 *Trichoderma* isolates were obtained which showed that *Trichoderma* isolates are predominant in different habitat *i.e*., crop rhizosphere, compost manure, sacred forest, coal mine and lime stone. The predomination of *Trichoderma* in natural soils, decaying wood, plant materials, crop rhizosphere were reported by several workers (Kredics *et al*., 2012; Kumar *et al*., 2012; Rai *et al*., 2016; Jaisani and Pandey, 2017).

Out of 97 isolates screened by dual culture against *Pythium* spp. and *R. solani* Kuhn., only 20 isolates colonized more than half of the growth of pathogen i.e. 50% overgrowth. TR 55 isolated from tomato rhizosphere was found to be the most effective isolate against both *Pythium* spp. and *R. solani* Kuhn, showing an inhibition percentage of 89.26% and 87.41% respectively, followed by other isolates like TR 66, TR 122 and TR 136. The antagonism of *Trichoderma* spp. against *Pythium* spp. and *R. solani* were widely reported (Goud *et al*., 2015; Kotasthane 2015; Waghunde *et al.,* 2016; Kumari *et al.*, 2016; Naik *et al.*, 2017; Rajendraprasad *et al.*, 2017) which supported the antagonism of *Trichoderma* spp. against *Pythium* spp. and *R. solani* Kuhn. during the present investigation.

All 20 isolates screened for their PGPR and biocontrol potential such as HCN, ammonia, siderophore, IAA, chitinase, ACC deaminase production and phosphate solubilisation showed that all 20 screened isolates were found positive for ACC deaminase production and 17 positive for chitinase production. Out of 20 screened isolates tested for other functional attributes (determining antagonistic potentials), 16 isolates were found positive for siderophore and ammonia production, whereas 13 isolates were positive for HCN production. Screening for plant growth promoting traits of 20 isolates revealed that all produced IAA and Phosphorous with values ranging from 0.33 to 4.96 µg/ml and 0.03 to 0.98 µg/ml respectively. The production of lytic enzymes by *Trichoderma* species is known as one of the major mechanisms for biocontrol activity against phytopathogenic fungi, involvement of chitinase in control of phytopathogens was reported (Harman *et al*., 2004a; Harighi *et al*., 2007, Azad *et al*., 2015, Thakar and Saraf 2015). HCN, siderophores and ammonia are produced by many *Trichoderma* spp. and are believed to play a role in biological control of pathogens (Rawat and Tiwari, 2011; Qi and Zhao, 2013, Zhang *et al*., 2016). Phosphate solubilizing efficiency of different isolates of *Trichoderma* was observed by many workers like Tallapragada and Gudimi (2011); Sarawanakumar *et al*., (2013); Promwee *et al*., (2014): Borges Chagas *et al*., (2015), Franca *et al*., (2017). ACC deaminase production of *Trichoderma* spp was reported by several workers (Viterbo *et al*., 2010; Hermosa *et al*., 2012; Aban *et al*., 2017). Aban and his co-workers also reported IAA production and phosphate solubilisation by *Trichoderma yunnanense* and *Trichoderma simmonsil* which is similar to the present findings.

In conclusion, many *Trichoderma* isolates were obtained from the crop rhizospheric soils in the 11 districts of Meghalaya, India. Via screening test, 20 isolates showed the best antagonism against damping off pathogens

(*Pythium* spp. and *R. solani*) of tomato seedlings. Screening of 20 isolates for their PGPR and biocontrol potential found that almost all revealed their ability of HCN, ammonia, siderophore, IAA, chitinase, ACC deaminase production and phosphate solubilisation. However, out of the 20 isolates, 4 *Trichoderma* isolates *viz.* TR 55, TR 66, TR 122 and TR 136 were found to be the most effective, so these 4 potent isolates need further evaluation in field condition to develop effective bio-formulation against damping off of tomato.

References

Aban, J. L., Barcelo, R. C., Oda, E. E., Reyes, G. A., Balangcod, T. D., Gutierrez, R. M, and Hipol, R. M. 2017. Auxin Production, Phosphate Solubilisation and ACC Deaminase Activity of Root Symbiotic Fungi (RSF) from *Drynaria quercifolia* L. *Bull. Env. Pharmacol. Life Sci*. 6(5): 18-23.

Abbas, A., D. Jiang, and Fu, Y. 2017. *Trichoderma* spp. as Antagonist of *Rhizoctonia solani*. J. Plant Pathol. Microbiol. 8:402. doi:10.4172/2157-7471.1000402.

Anonymous, 2014. http:// www. indiastat. com/ agriculture/ 2/ vegetable/ 17427/ stat. Aspx.

Anonymous, 2018. Monthly Report Tomato. Horticulture Statistics Division Department of Agriculture, Cooperation and Farmers Welfare, Ministry of agriculture and Farmers Welfare, New Delhi.

Asad, S. A., A. Tabassum, A, Hameed, F. Hassan, A. Afzal, S.A. Khan, R. Ahmed and Shahzad, M. 2015. Determination of lytic enzyme activities of indigenous *Trichoderma* isolates from Pakistan. Brazilian Journal of Microbiology. 46(4): 1053-1064.

Asad, S.A., N. Ali, A. Hameed, S.A. Khan, R. Ahmad, M. Bilal, M. Shahzad, and Tabassum, A. 2014. Biocontrol efficacy of different isolates of *Trichoderma* against soil borne pathogen *Rhizoctonia solani*. Pol J Microbiol. 63(1): 95-103.

Bell, D.K., H.D. Wells, and Markham, C.R. 1982. In-vitro antagonism of *Trichoderma* spp. against six fungal plant pathogens. Phytopathol. 72:379-382.

Bissett, J. 1992. *Trichoderma atroviride.* Canadian J. Botany. 70 (3): 639-641, 10.1139/b92-082.

Borges, C. L. F., J. A. F. Chagas, C. M. Rodrigues, L. D. Miller, and Orozco, C. B. S. 2015. Evaluation of the phosphate solubilization potential of *Trichoderma* strains (Trichoplus JCO) and effects on rice biomass. Journal of Soil Science and Plant Nutrition. 15 (3): 794-804.

Dhingra, O.P., and Sinclair, J.B. (1995). Basic plant pathology methods, 2nd edn. CRC press, Bocca Raton, America.

Dixit, R., R. B. Singh, and Singh, H.B. 2015. Screening of antagonistic potential and plant growth promotion activities of *Trichoderma* spp. and fluorescent *Pseudomonas* spp. isolates against *Sclerotinia sclerotiorum* causing stem rot of French bean. Legume Research. 38 (3): 375-381.

Franca, V. C. D., C.K. Kupper, M. Rosa-Magri, T. Gomes, and Rossi, F. 2017. *Trichoderma* spp. Isolates with potential of phosphate solubilization and growth promotion in cherry tomato. Pesquisa Agropecuaria Tropical. 47: 360-368. 10.1590/1983-40632017v4746447.

Gaigole, A.H., 2011. *Trichoderma viride* are having ability to inhibit soil borne pathogen of different crops like *Rhizoctonia solani* and most

commonly used fungal bio control agent and have long been known as effective antagonist against plant pathogen. Asiatic J. Biotechnol. Resources. 2: 461-465.

Ghinaiya, H.K., and Pandya, J.R. 2017. Association and occurrence of seed-borne fungal pathogens (SFP) in tomato (*Solanum lycopersicum* L.) Asian J. of Appl. Sci and Technol. 5(1): 06-07.

Goud, T. S., R. A. Raju, S. Karri, and Kumar, Y.S. 2015. Production and antagonistic effect of Trichoderma spp. on pathogenic microorganisms (*Botrytis cinerea*, *Fusarium oxysporium*, *Macrophomina phasealina* and *Rhizoctonia solani*). African J. Biotechnol. 14: 668-675. 10.5897/AJB2014.13904.

Gravel, V., H. Antoun, and Tweddell, R.J. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil. Biol. and Biochem. 39: 1968-1977.

Harighi M.J., M.R. Zamani., Motallebi, M. 2007. Evaluation of antifungal activity of purified chitinase 42 from *Trichoderma atroviride* PTCC5220. Biotechnol 6:28-33.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004a. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Rev. Microbiol., 2: 43-56.

Hermosa, R., A. Viterbo, I. Chet, and Monte, E. 2012. Plant-beneficial effects of Trichoderma and of its genes. *Microbiol*. 158:17–25. DOI 10.1099/mic.0.052274-0

Jaisani, P., and Pandey, R.N. 2017. Morphological and molecular characterization for identification of isolates of *Trichoderma* spp. from rhizospheric soils of crops in middle Gujarat. Indian Phytopath. 70(2): 238-245. doi:10.24838/ip.2017.v70.i2.71652

Jasim, B., A.A. Joseph, C.J. John, J. Mathew, and Radhakrishnan, E.K. 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of *Zingiber officinale*. 3 Biotech. 4(2): 197–204. doi: 10.1007/s13205-013-0143-3.

Kotasthane, A., T. Agrawal, R. Kushwah, and Rahatkar, V. 2015. *In-vitro* antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. European J. of Plant Pathol. 141(3): 523-543.

Kredics, L., M. Laday, P. Kormoczi1, L. Manczinger, G. Rakhely, C. Vagvolgyi, and Szekeres, A. 2012. Genetic and biochemical diversity among *Trichoderma* isolates in soil samples from winter wheat fields of the Great Hungarian Plain. Acta Biologica Szegediensis. 56(2):141-149.

Kumar, K., N. Amaresan, S. Bhagat, K. Madhuri, and Srivastava, R.C. 2012. Isolation and Characterization of *Trichoderma* spp. for Antagonistic Activity Against Root Rot and Foliar Pathogens. Indian J Microbiol. 52(2): 137–144. doi: 10.1007/s12088-011-0205-3.

Kumari, A., R. Kumar, S. Maurya, and Pandey, P.K. 2016. Antagonistic Potential of Different Isolates of *Trichoderma* against *Rhizoctonia solani*.

Lamichhane, J. R., C. Durr, A.A. Schwanck, M. H. Robin, J. P. Sarthou, V. Cellier, A. Messean, and Aubertot, J.N. 2017. Integrated management of damping-

off diseases. A review. Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, pp. 37 (2): 25.

Majeed, M., M.G. Hassan, M. Hassan, F.A. Mohuiddin, S. Paswal, and Farooq, S. 2017. Damping Off in Chilli and Its Biological Management-A Review. J. Curr. Microbiol. App. Sci. 7(4): 2175-2185.

Manganiello, G., A. Sacco, M.R. Ercolano, F. Vinale, S. Lanzuise, A. Pascale, M. Napolitano, N. Lombardi, M. Lorito, and Woo, S.L. 2018. Modulation of Tomato Response to *Rhizoctonia solani* by *Trichoderma harzianum* and Its Secondary Metabolite Harzianic Acid. Front Microbiol. 9: 1966. doi: 10.3389/fmicb.2018.01966.

Nahar, K., and Ullah, S.M. 2012. Morphological and physiological characters of tomato (*Lycopersicon esculentum* Mill) cultivars under water stress. Bangladesh J. Agril. Res. 37(2): 355-360.

Naik, M.K., Y.S. Amaresh, Ravikiran, D.S. Ashwathanarayan, M.G. Patil, Mahadevaswami, D. Nair, and Shruthi, P. 2017. Morphological, Molecular Characterization of *Trichoderma* species Isolated from Different Rhizosphere Soils and Its Anti-Pathogenic Properties. Imperial J. of Interdisciplinary Research. Vol-3, Issue-4. http://www.onlinejournal.in

Papavizas, G.C., 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Rev. of Phytopathol. 23: 23-54.

Promwee, A., M. Issarakraisila, W. Intana, C. Chamswarng, and Yenjit, P. 2014. Phosphate Solubilization and Growth Promotion of Rubber Tree (*Hevea brasiliensis* Muell. Arg.) by *Trichoderma* Strains. J. Agricul. Science. 6. 10.5539/jas.v6n9p8.

Qi, W., and Zhao, L. 2013. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. J Basic Microbiol. 53(4): 355-64. doi: 10.1002/jobm.201200031.

Rai, S., P.L. Kashyap, S. Kumar, A.K. Srivastava, and Ramteke, P.W. 2016. Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *SpringerPlus*, *5*(1): 1939. doi:10.1186/s40064-016-3657-4.

Rajendraprasad, M., B. Vidyasagar, G.U. Devi, and Rao, S.R.K. 2017. Biological control of tomato damping off caused by *Pythium debaryanum*. International J. of Chemical Studies. 5(5): 447-452.

Ramanathan, G., M. Saran Sundar, and Vinodhkumar T. 2013. Evaluation of antifungal activity of metabolites from *Trichoderma* species against fungal phytopathogens. International J. of Science Innovations and Discoveries, 3(5): 528-538.

Rawat, R., and Tewari, L. 2011. Effect of abiotic stress on phosphate solubilization by biocontrol fungus Trichoderma sp. Current Microbiology, 62(5), 1521-1526. http://dx.doi.org/10.1007/s00284-011-9888-2

Rifai, M.A., 1969. A revision of the genus *Trichoderma*. Commonow. Mycol. Inst. Mycol. pp. 116-156.

Roiger, D.J., S.N. Jeffer, and Caldwell, R. W. 1991. Occurrence of *Trichoderma* species in apple orchard and woodland soil. Soil Biology and Biochemistry 23, 353-359.

Rudresh, D.L., M.K. Shivaprakash, and Prasad, R.D. 2005. Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. in relation to P uptake and growth and yield

parameters of chickpea (*Cicer arietinum* L.). Can. J. Microbiol. 51: 217–222.

Saravanakumar, K., A.V. Shanmuga, and Kandasamy, K. 2012. Effect of *Trichoderma* on soil phosphate solubilization and growth improvement of *Avicennia marina*. Aquatic Botany. 104. 10.1016/j.aquabot.2012.09.001.

Sharma, P., M. Sharma, M. Raja, and Shanmugam V. 2014. Status of *Trichoderma* research in India: A review. Indian Phytopath. 67 (1): 1-19.

Srivastava, M.P., R. Tiwari, and Sharma N. 2013. Effect of different cultural variables on siderophores produced by *Trichoderma* spp. International Journal of Advanced Research. 1(7): 1-6.

Tallapragada, P., and Gudimi, M. 2011. Phosphate solubility and biocontrol activity of *Trichoderma harzianum*. Turkish Journal of Biology. 35. 10.3906/biy-0911-4.

Thakkar, A., and Saraf, M. 2015. Role of Volatile Metabolites from *T. citrinoviride* in Biocontrol of Phytopathogens. Int. J. Res. Chem. Environ. 5(1): 86-95.

Thakur, N., and Tripathi, A. 2015. Biological Management of Damping-Off, Buckeye Rot and Fusarial Wilt of Tomato (cv. Solan Lalima) under Mid-Hill Conditions of Himachal Pradesh. Agricultural Sciences. 6: 535-544.

Viterbo, A., U. Landau, S. Kim, L. Chernin, and Chet, I. 2010. Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent Trichoderma asperellum T203. FEMS-*Microbiol*. *Lett*. 305: 42–48

Waghunde, Rajesh, Shelake, Rahul, N. Sabalpara, and Ambalal. 2016. Trichoderma: A significant fungus for agriculture and environment. African J. of Agricultural Research. 11: 192-196. 10.5897/AJAR2015.10584.

Wardle, D.A., D. Parkinson, and Waller, J.E. 1993. Interspecific competitive interaction between pairs of fungal species in natural substrates. Ecologia 94, 165-172.

Zhang, S., Y. Gan, and Xu, B. 2016. Application of Plant-Growth-Promoting Fungi *Trichoderma longibrachiatum* T6 Enhances Tolerance of Wheat to Salt Stress through Improvement of Antioxidative Defense System and Gene Expression. *Frontiers in plant science*, *7*, 1405. doi:10.3389/fpls.2016.01405.

How to cite this article:

Markidahun Biam, Dipali Majumder and Heipormi Papang. 2019. *In vitro* Efficacy of Native *Trichoderma* Isolates against *Pythium* spp. and *Rhizoctonia solani* (Kuhn.) causing Damping-off Disease in Tomato (*Solanum lycopersicum* Miller). *Int.J.Curr.Microbiol.App.Sci.* 8(02): 566-579. doi: https://doi.org/10.20546/ijcmas.2019.802.064