Bio-efficacy of *Trichoderma* Formulation Against Damping-off Caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn. on Tomato (*Solanum lycopersicum*)

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In

Plant Pathology

by

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November 2019

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This is to certify that the thesis entitled **Bio-efficacy of *Trichoderma* Formulation Against Damping-off Caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn. on Tomato (*Solanum lycopersicum*)"** submitted by **Mrs. Markidahun Biam [Regn. No. CAU/179-H/08(B)**] submitted to Central Agricultural University, Imphal-795 004 (Manipur) in partial fulfilment of the requirements for the award of the degree **Doctor of Philosophy (Agriculture)** in the subject of **Plant Pathology** has been approved by the Student's Advisory Committee after oral examination jointly with an External Examiner.

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I hereby declare that the thesis entitled **"BIO-EFFICACY OF *Trichoderma* FORMULATION AGAINST DAMPING-OFF CAUSED BY *Pythium* spp. AND *Rhizoctonia solani* KUHN. ON TOMATO (*Solanum lycopersicum*)"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any other degree, diploma, associateship, fellowship or any other similar title.

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Dated/__Umiam the…………2019 *(****Markidahun Biam****)*

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LIST OF ABBREVIATIONS AND SYMBOLS

Analysis of variance	-	ANOVA
At the rate of	-	@
Base Pair	-	bp
Beta	-	β
Biological Control Efficacy	-	BCE
Biological Oxygen Demand	-	BOD
Centimeter	-	cm
Colony forming unit	-	cfu
Critical difference	-	C.D.
Days After Sowing	-	DAS
Days of incubation	-	DOI
Degree Celcius	-	^{o}C
Deoxy Ribonucleic Acid	-	DNA
Diameter	-	dia.
Disease Incidence	-	DI
Edition	-	Ed.
et alibi	-	*et al*.
et cetera	-	etc.
Figure	-	Fig.
Gram	-	g/gm
Hectare	-	ha
Hour	-	hr
id est	-	*i.e*.
Kilogram	-	kg
kilo Pascal	-	kPa
Less than	-	<

Litre	-	L
Meter	-	m
Microgram	-	µg
Microliter	-	µl
Micrometer	-	µm
Micromolar	-	µM
Milligram	-	mg
Milliliter	-	ml
Millimeter	-	mm
Millimolar	-	mM
Minute	-	Min.
More than	-	>
Nanogram	-	ng
Nanometer	-	nm
Number	-	No.
Parts Per Million	-	ppm
Per	-	/
Per cent	-	%
Picomole	-	pmol
Rotation Per Minute	-	rpm
Serial	-	Sl.
Species	-	sp
Standard error of mean difference	-	S.E. (d)
Two-third	-	$2/3^{rd}$
Unit	-	U
Videlicet	-	*viz.*
Weight per volume	-	w/v

ABSTRACT

Damping-off is one of the most prevalent and damaging disease of tomato nurseries in Meghalaya, caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn. These pathogens can kill both germinating seeds and young seedlings. Management of damping off by integration of biocontrol agents, specially the *Trichoderma* spp., not only can reduce doses of fungicides but will be a 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 P*ythium* spp. and *Rhizoctonia solani* kuhn. cause damping off in tomato. Out of 180 soil samples collected from different habitats (crop rhizosphere, compost manure, forest, jhum areas, pig manure, coal mine and lime stone) of 11 districts of Meghalaya, 97 *Trichoderma* isolates were identified based on their morphology. Dual culture assays of the twenty screened isolates against damping-off pathogens revealed that the 4 *Trichoderma* isolates *viz.* TR55, TR66, TR122 and TR136 were found highly effective in inhibiting *Pythium* spp. with percent inhibition of 89.26, 88.15, 88.89 and 87.78 respectively, whereas only 2 isolates *viz.* TR55 and TR122 were effective against *R. solani* with percent inhibition of 87.41 and 86.48, respectively. Isolate TR55 recorded as the best antagonist against both pathogens causing damping off. All 20 screened isolates were positive for ACC deaminase production whereas 17 were positive for chitinase production. Isolate TR 106 and TR 136 were the best in chitinase production with purple colour zone of 9 cm in diameter. 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 promotion traits of 20 isolates revealed that all the isolates produced IAA and Phosphorous with values ranging from 0.33 to 4.96 µg/ml and 0.03 to 0.98 µg/ml, respectively. Presence of antibiotic biosynthetic genes in the 20 isolates by using gene specific primers detected that 12 isolates were positive for β-1,6-Glucanase (Tvbgn3), 10 isolates for Trichodiene synthase (tri5), 14 isolates for Serine protease (prb1) and 17 isolates for Endochitinase (ech42). Only 4 *Trichoderma* isolates showed presence of all the 4 antibiotic biosynthetic genes tested. Observations of the anamorphic characteristics of the *Trichoderma* isolates showed variation in anamorphic characters *viz.*, size of phialides, phialospore and conidiophores among the isolates. The sequence analysis of the internal transcribed spacer regions 1 and 2 (using universal primer ITS1 and 4) of the rDNA of the 10 potential isolates showed that they belonged to 2 *Trichoderma* species *viz.*, *T. hamatum* (TR 55, TR 66, TR 87 and TR 122) and *T. harzianum* (TR 64, TR 88, TR 106, TR 109 and TR 136) with amplicon at 600bp in all isolates. Dendogram was generated using alignments containing the ITS region revealed formation of two main clusters with closeness ranging from 13 to 96 per cent. The biopriming of tomato seeds with 4 *Trichoderma* isolates (TR55, TR66, TR122 and TR136) showed considerable increase in germination percentage and vigour index over control, with the highest germination percentage and vigour index recorded in TR55 (75.13 per cent and 47.99 per cent, 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, TR55 (seed plus soil treatment). It also exhibited increase in the growth parameters such as root length, plant height, number of leaves, number of flowers, fresh as well as dry fruit weight and yields of tomato; variety Hybri-017 in the treated crops as compared to the untreated control. The rhizosphere colonization of 4 potent isolates of *Trichoderma* spp. *viz.*, TR55, TR66, TR122 and TR136 showed that their 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). Native *Trichoderma* isolates TR55, TR66 and TR122 performed better in terms of functional attributes *viz.*, disease suppression, growth promotion, rhizosphere colonization, self-life in talc formulation, could be further evaluated under different climatic condition of the state for development of effective *Trichoderma* formulations found effective can be recommended as a component of integrated disease management practice to manage damping off of tomato in the nursery beds in Meghalaya.

Key words: Damping off of, *Trichoderma* spp., *Pythium* spp., *Rhizoctonia solani,* Phylogeny and Meghalaya

Chapter - 1

Introduction

Tomato (*Solanum lycopersicum* Miller) is an important temperate vegetable crop cultivated throughout the world, also widely cultivated in Meghalaya, India. In addition, there are certain indigenous vegetable crops that are grown in the State like tree tomato (*Cyphomandra betacea*). It is native to Peruvian, the region of Mexico. Tomato production has a major role in global horticulture, ranking second in importance next to potato in many countries (Sharma *et al.*, 2014). Tomato cultivation has become one of the special farming in the Ri-Bhoi belt of Meghalaya, where most of the farmers grow tomatoes during both of the season *i.e.*, Kharif and Rabi. Tomato farming has recently been introduced to the high altitude places of East Khasi Hills Distrct. Farming of tomato is spreading to the different areas of the district in the higher places, mainly due to the high rate of economic returns achieved from tomatoes farming which are harvested during off-season *i.e.*, from June to October. One of the main advantage of tomato cultivation is that it can be grown both in the field as well as under greenhouse conditions. Tomatoes are one of the good source of potassium, vitamin C, 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).

Among Asian countries China is no.1 producer of tomato, followed by India. In India, tomato is cultivated as a major vegetable crop, covering an area of 8.65 lakh hectares and produced a total production of 168.26 lakh tones with productivity of 19.60 tonnes/ha (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 these states Karnataka is the largest producer. These States account for 91 per cent of total production of our country. Meghalaya accounts for 36.60 million tonnes of tomato production, which is 0.16 per cent of country's total tomato production (Anon 2018).

Although tomatoes are commercially grown all over the globe, there's no place where the crops are free from diseases. One of the major loss of seedling is due to damping-off disease that is caused by different types of fungi, including the fungal-like organism (member of Straminopila). Damping-off is one of the worst diseases of tomato occurring in the nursery and can kill both germinating seeds and young seedlings (Agrios, 2005). Infact, damping off is a seedling disease common to

many vegetables like brinjal, chilli, cabbage, etc cultivated by 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 germinating, 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). Pre-emergence damping-off is a term used to describe the rot of seeds, or death of the seedlings may occur before the hypocotyls broke 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). They may also rot from the tips of the roots. This rot will progress up the seedling until the stem is rotted. The seedlings that can survive until they are a bit older before they are infected may develop "wire-stem", a condition in which the base of the stem is partially invaded by the fungus. There is often a discolored and slightly shriveled or constricted area at or just below the soil line (Broders *et al.*, 2009). Although the plant lives for a while, it is stunted, pale, often wilt and eventually die. Brown or white fungal growth may be seen on the surface of the potting mixture or on the seedlings themselves (Manoranjitham *et al.*, 2001).

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. Most of these fungi can also cause cuttings to rot (Abd-El-Khair *et al.*, 2010; Kamala and Indira, 2011). These fungi are found in all soils and pose a large threat to plant propagation. The *Pythium* species are fungal-like organisms, commonly referred to as water molds, (belong to kingdom Straminopila; phylum of Oomycota; class of Oomycetes; subclass of Peronosporomycetidae; order Pythiales and family of Pythiaceae). They are worldwide in distribution and found to be associated with a wide range of habitats ranged from terrestrial to aquatic environments, in the cultivated or in fallow soils, in the plants, in saline water or in fresh water. Genus *Pythium* falls under one of the biggest oomycete genus. It consists of not less than 130 recognized species of different kind which are obtained from different areas of the world (Paul *et al.*, 2006; Bala *et al.*, 2010; Robideau *et al.*, 2011). Important species of *Pythium* that caused damping off disease in India are *P. aphanidermatum*, *P. ultimum* and *P. debaryanum* (Parveen and Sharma, 2015). *Pythium* diseases of vegetables as well as the other crops that are grown in the field are considered as important limiting factor in their successful

cultivation throughout the world and responsible for losses of multibillion dollar worldwide (Van West *et al.*, 2003; Parveen and Sharma, 2015). The genus *Rhizoctonia solani* (perfect state *Thanatephorous cucumeris*) is a genus of anamorphic fungi that does not form vegetative spores and it can be present as mycelium, sclerotia or basidiospores and “Rhizoctonia” means “root killer”. It belongs to kingdom Fungi; division Basidiomycota; class Agaricomycetes; order Cantharellales and family Ceratobasidiaceae (Kendrick and Carmichael, 1973). It produces shade of brown, thread-like growth called hypha (Leach and Garher, 1970). It is characterized by the diameter of the vegetative hyphae (measures as 8-12 μm), there is constriction at the point of branching, and matured hyphae branch at right angle (Parmeter and Whitney, 1970). Sclerotia, the primary survival structure of the pathogen, formed in soil are irregular-shaped, brown to black in colour, survive for several years in soil (Sherwood, 1970; Guttierez *et al.*, 1997). Some other fungi also responsible for damping off are *Phytophthora, Glomeralla, Thielavia, Phoma, Fusarium, Sclerotinia, Sclerotium, Botrytis*, etc (Singh *et al.*, 1995).

Management of damping off involves cultural practices and use of chemical protectants. But the best method to manage this disease is to adopt preventive measures. One way to prevent this disease is to keep the fungi that cause damping off disease out of the flats of seeds. Management of damping off by fungicides is not the most desirable method of disease management, due to several important reasons. Fungicides are heavily regulated and additionally, they are expensive, cause environmental pollution, and may induce pathogen resistance (Larson, 1987; Lamichhane *et al.*, 2017). Consequently, management of damping off rely on cultural ways like thin planting, use of light soil in nursery beds and nursery soil solarization (Paulitz, 1997). Since cultural practices alone are not enough to effectively manage damping off disease, alternative management methods are needed. Therefore, management of pathogens causing plant diseases using microbial bio-inoculants have been considered as a potential management strategy for integrated disease management. Integrating of biological control agents with low doses of fungicides has the potential to manage plant pathogens with minimal impact on the environment (Chet and Inbar 1994). Therefore, exploitation of biological control agents (BCA’s) for management of plant diseases has been increasing. Many investigators have demonstrated the potential of *Trichoderma* spp. in management of damping-off diseases of plant which are caused by *Pythium* spp. (Lifshitz *et al.,* 1986; Le *et al.*, 2003; Abdelzaher, 2004; Alwathnani, 2012; Lamichanne *et al.,* 2017; Majeed *et al.,*

2017) and *Rhizoctonia solani* (Elad *et al.,* 1982; Askew and Laing, 1994; Lewis *et al.*, 1998; Asad *et al.,* 2014; Abbas *et al.,* 2017; Manganiello *et al.,* 2018).

Trichoderma species have been investigated as biological control agents (BCAs) for over 70 years (Hjeljord and Tronsmo, 1998). It was described first in 1794 by the mycologist C.H. Persoon (Persoon, 1794). It was classified as imperfect fungi and belongs to the kingdom Fungi; division Dueteromycota; sub-division Dueteromycotina; class Hyphomycetes; class Hyphomycetes; order Monilliales and family Moniliaceae (Gams and Bisset, 1998, Kulkarni and Sagar, 2007). *Trichoderma* spp. are found to be largely distributed all over the globe especially in the natural habitats 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). *Trichoderma* is found to be one of the best candidates of BCA against wide range of plant pathogens. *Trichoderma* spp. were found to have ability to reduce several plant diseases by inhibiting plant pathogens mainly found in the roots, through antagonistic and mycoparasitic potential (Viterbo *et al.*, 2010; Naher *et al.*, 2014; Mukherjee *et al.*, 2014). It reduces growth, survival or infections caused by pathogens by different mechanisms like competition for nutrients and space (Harman *et al.*, 2004), siderophores production (Lorito *et al.*, 1993; Wilhite *et al.*,1994), tolerance to stress through enhanced root and plant development (Inbar *et al.*, 1994), solubilization of potassium, phosphorus, zinc, nickel and cadmium (Altomare *et al.*, 1999; Nongmaithem *et al.*, 2016), sequestration of inorganic nutrients and induced resistance (De Meyer *et al.,* 1998), secretion of extracellular hydrolytic enzymes (Di Pietro *et al.*, 1993; Schirmbock *et al.*, 1994), production of the cell wall degrading enzymes (Chowdhury *et al.*, 2014; Fahmi and Ragaa, 2016), production of antibiotics (Dennis and Webster, 1971 a, b; Claydon *et al.*, 1987) and other secondary metabolites like pyrones, kaninginis, viridians, azaphilones, nitrogen heterocyclic compounds, butenolides, hydroxyl-lactones, isocyano metabolites and diketopiperazines peptibols that are toxic to the fungal pathogens (Vinale *et al.*, 2014; Mukherjee *et al.*, 2017).

However, there is still considerable interest in finding more efficient native fungal biocontrol agent especially within *Trichoderma* spp., which showed different biocontrol ability. It is critical to obtain *Trichoderma* isolates which showed higher antagonistic potential. These can be obtained by critical selection of isolates having higher ability to produce extra cellular lytic enzymes chitinase and β-1, 6-glucanase enzymes. The exact establishment of the identity and cultural characteristic of *Trichoderma* to the level of species is the first step in utilizing the full potential of

fungi in a specific application. The morphological characters of *Trichoderma* had been described by Rifai (1969) and Bissett (1991). They emphasized the difficulties inherent in defining morphological species of *Trichoderma*. It is extremely difficult to characterized *Trichoderma* spp. on morphological basis, hence molecular methods including DNA sequencing and genealogical concordance phylogenetic species recognition using several unliked genes are needed to give accurate identification of *Trichoderma* spp. (Druzhinina *et al*., 2006). *Trichoderma* is monophyletic (Kullnig-Gradinger *et al*., 2002), with teleomorphs in the genus Hypocrea. The introduction of molecular analysis of several strains showed that the characterization based on the morphological data had been to a great extent, erroneous resulting in reclassification of several isolates and species (Rehner and Samuels, 1995; Kuhls *et al*., 1996).

Molecular data derived from enzymes and DNA have been useful to characterize *Trichoderma* more efficiently than traditional methods (Lieckfeldt *et al*., 1998). More than 200 *Trichoderma* spp. are taxonomically well defined and more than 7 *Trichoderma* spp. have been sequenced (*T. ressei, T. asperellum, T. citrinoviride, T. longibrachiatrum, T. atroviride, T. virens*). The most important biocontrol species are *T. harzianum*, *T. virens* (syn. *Gliocladium virens*), and *T. viride* (Mukherjee *et al*., 2017). However, molecular data have shown very high level of heterogenesity in these isolates and other species within the genus, and the heterogeneity of *T. harzianum* has been documented (Hermosa *et al*., 2000; Samuels *et al*., 2002; Chaverri *et al*., 2003; Manter and Vivanco, 2007; Manganiello *et al*., 2018). Similar results have been attained with sequence analyses of ITS1 and ITS4 (Raj and Singh, 2014; Wu *et al*., 2017). Characterization of the potential biocontrol agents employed by molecular method using Internal Transcribe Spacer-Polymerase Chain Reaction (ITS-PCR) helps in determining the diversity and identification as ITS region evolves very fast and even within a genus it may vary among species. Therefore, the sequences of these regions can be used for identification of closely related species (White *et al*., 1990; Kannangara *et al*., 2017).

Another critical factor which determines the success of biological control agent is the shelf life and the ability of the bioagents to establish in the targeted niche (Abdel-Fattah *et al*., 2007). For this development of acceptable easily prepared and cost effective formulations for delivery is important. For mass multiplication of bioagent through solid state and liquid fermentation technology an enormous quantity of spore biomass is needed (Dominguesa *et al.,* 2000). Various substrates like talc based formulation, vermiculite-wheat bran based formulation, coffee huskbased formulation, press mud based formulation (Singh *et al*., 2007; Jegathambigai *et al*., 2007; Jin and

Custis, 2011; Sabalpara, 2014) and banana waste based formulations (Kumar *et al*., 2014) have been investigated by so many researchers and are found to be promising carriers.

Considering the importance of *Trichoderma* spp. as effective bio-control agent against damping off of tomato crop, the present research work is aimed to study the bio-efficacy of *Trichoderma* formulation against damping off caused by *Pythium* spp. and *Rhizoctonia solani* with the following objectives:

1. Screening of isolated *Trichoderma* for functional attributes under *in vitro*.
2. To study the plant growth promotion and biocontrol potential of *in vitro* screened *Trichoderma* isolates against *Pythium* spp. and *Rhizoctonia solani* Kuhn under *in vivo* conditions.
3. To study the efficacy of talc-based *Trichoderma* formulation under *in vitro*.

Chapter - 2

Review of Literature

Available literatures related to the present investigation are being reviewed under the following headings.

2.1. Tomato and its importance

The tomato is a major vegetable crop that has achieved tremendous popularity over the last century (Wener, 2017). It is grown in practically every country of the world - in outdoor fields, greenhouses and nethouses. Tomato comes from the kingdom *Plantae*, family *Solanacaea* and the genus, *Solanium*. Its botanical name is *Lycopersicon esculentum* (Sharma *et al.*, 2014). The tomato belongs to the nightshade family. It originated in America and was spread around the world following the Spanish colonization of the Americas. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes and sauces, and in drinks (Bhowmik *et al.*, 2012). While it is botanically a fruit, it is considered a vegetable for culinary purposes. The fruit is rich in lycopene which is a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of health complications and diseases. Also researchers reported that including tomato in every diet have many health benefits as it is a good source of potassium, vitamin C and A. They help in fighting cancer, reducing heart disease and are also good for eye health and digestion (Nahar and Ullah, 2012). The total global area under tomato is 46.16 lakh ha and the global production is to the tune of 1279.93 lakh tonnes. 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 (Anon, 2014). In Meghalaya, tomato is cultivated during both Kharif and Rabi seasons. The production of tomato from Meghalaya has been reported upto 36.60 million tonnes, which is 0.16 per cent of country's total tomato production (Anon, 2018). The highest production of tomato in the state is usually during June to October and derived high rate of economic returns due to its off-season cultivation and non-availability in the neighbouring states.

The yield per hectare in Meghalaya is low as compared to other parts of the country due to attack of several viral and soilborne diseases, that are responsible for damaging the quantity and quality of the crop every year. Among the soilborne

fungal diseases, damping-off of seedlings and wilt of adult plants all caused by several species of *Fusarium, Pythium, Rhizoctonia* and *Verticillium (*Lucas *et al*., 1997; Jiskani, 2007; Shinde *et al*., 2016), are widely prevalent throughout the world.

2.2. The Pathogens

Rhizoctonia was first described by De Candolle (1815) as a root destroying fungus. Then again it was observed and described by Julius Kuhn on diseased potato tubers and named it *Rhizoctonia solani*. *Rhizoctonia* is from Ancient Greek, *rhiza* which means "root" and *ktonos*, means “murder". *Solanum*, Latin for nightshade, is the genus of the potato (Gonzalez *et al*., 2011). *Rhizoctonia solani* was found to be a parasite of more than 165 species of plants, including the tomato and the pathogen prefers warmer, wet climates for infection and growth (Peltier, 1916). *R. solani* is soil borne pathogen and does not produce any asexual spores. It can develop in both cultured and non-cultured soils and survive in the soil for many years in the form of sclerotia (Mohamed *et al.,* 2015), hence is identified only from mycelial characteristics or DNA analysis (Moussa, 2002). Its hyphal cell are multinucleated and produced colourless mycelia initially when young but becomes brown to deep brown/yellow coloured as they grow and mature when grown on an artificial medium. The hyphae are 4–15 μm wide and tend to branch at right angles. A septum near each hyphal branch and a slight constriction at the branch are diagnostic (Gaigole *et al.* 2011). It causes numerous diseases in crop plants *viz*., damping-off, black spot and root rot diseases (Dev and Dawande, 2012)

Pythium was observed to be associated with the root rot and root injury of number of crops (Trow, 1901). *Pythium* is considered *as* saprophyte, as once established it can survive for many years in the soil, even in the absence of host plants, either as saprophytes or as living resting structures that are capable of enduring adverse conditions (Menzies 1963). *Pythium* produces filamentous sporangia with reniform biciliate zoospores. The oogonia are terminal, smooth, and spherical; oospores are smooth and spherical (Deadman, 2017). Antheridia are stalked or intercalary; usually one or, rarely, two antheridia are formed per oogonium. It requires higher temperatures (32–37°C) for pathogenicity (Zappia *et al*., 2014). *Pythium* is ubiquitous in soil and in water, distributed worldwide, and with very diverse host ranges. They include some of the most important and destructive plant pathogens, causing losses of seeds, pre-emergence and post-emergence damping-off, rots of seedlings, roots, basal stalks, decays of fruits and vegetables during cultivation, storage, transit or at the market, and cause serious damage of a wide variety of crops.

The most aggressive species of *Pythium* that cause important plant diseases is *P. aphanidermatum* (Parveen and Sharma, 2015). It is a soil as well as seed borne pathogen.

Pythium spp. and *Rhizoctonia solani* are probably the most abundant and widespread plant pathogenic fungal species in soil and they have a wide host range. Their wide host range also aids in the longevity of these fungi and fungus-like organisms (Pieczarka and Abawi, 1978, Coffua *et al*., 2016).

2.3. Importance of damping-off disease

Damping-off is a historical term coined during the early 19th century (Hartley and Pierce 1917). It is one of the oldest worldwide nursery problems (Tillotson 1917; Hartley 1921). Damping-off was considered "the most serious problem encountered in raising nursery seedlings," and consequently was one of the most focused research subject since the beginning of its description (Hartley and Pierce, 1917). 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. Damping-off involves non-germination, prevention of seedling emergence after germination, or the rotting and collapse of seedlings at the soil level (Gravel *et al*., 2005). It has been reported as one of the important disease in greenhouse and field bed of tomatoes, causing huge losses in nurseries (Shinde *et al.,* 2016).

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 seedlings (Kamala and Indira, 2011). These fungi are found in practically all soils and pose a large threat to plant propagation (Rajagopalan, 1961). *P. aphanidermatum* was a major species causing 75-80 per cent damping-off in tomato and chilli (Lamichhane *et al*., 2017). Manoranjitham *et al.* (2001) reported 60 per cent mortality of chilli seedlings both in nursery and main field due to damping-off caused by *Rhizoctonia solani* and *Pythium* spp.

Major lost in crop production especially in vegetables production is due to damping off disease in the nurseries where young seedlings are raised. Around 62 per cent mortality of seedlings were observed due to *Pythium aphanidermatum* (Ramamoorthy *et al*., 2002). *Pythium aphanidermatum*, *P. ultimum and Rhizoctonia solani* has been reported to be one of the causal agents of pre and post-emergence damping-off in tomatoes and it costs more than 50 per cent loss in nursery production (Gravel *et al*., 2005; Kumari *et al*., 2016).

2.4. Symptoms

Damping-off symptoms of tomato caused by *Pythium* sp. are of two distinct types as described by Alexander and his co-workers (1931). The one most commonly known is that in which the hypocotyl of the seedling, after emergence, becomes water soaked and discoloured at the surface of the soil. Such diseased plants fall over, quickly wither, and die. The other general symptom of damping-off is evidenced by a poor stand or stunting of the plants. In this case the hypocotyl is attacked before it emerges from the soil. The first symptoms of this attack are small, brown, water-soaked lesions which, under favourable conditions, affect the entire root system, frequently killing the seedling before it emerges. In case it does emerge, the hypocotyl and roots remain diseased, the plant is stunted, abnormally dark green, and the cotyledon leaves roll downward. This latter type of attack is frequently very destructive because such diseased plants, unnoticed by the grower, are transplanted and die later.

Also Alexander and his co-workers (1931) described that there are three general types of symptoms of the disease caused by *Rhizoctonia solani*. Two of these resemble very closely the symptoms caused by *Pythium* sp.; namely, the toppling over of seedlings and the general diseased condition of the hypocotyl and roots. The principal difference in detailed symptoms is a dry, shrivelled lesion instead of a water-soaked one. The third type is a deformation of the cotyledons. In all cases, seedlings stunted by either *R. solani* or *Pythium* sp. have an abnormally deep green colour.

Cerkauskas (2005) observed that affected tomato plants usually occur in patches in nursery beds or in low parts of sloped fields. In level fields, affected plants are generally found in scattered areas. Damping off may occur before and/or after emergence. In pre-emergence damping off, the seeds fail to emerge after sowing. They become soft, mushy, turn brown, and decompose as a consequence of seed infection. In post-emergence damping-off, the seedling emerges from the soil but dies shortly afterwards. The affected portions (roots, hypocotyls and perhaps the crown of the plant) are pale brown, soft, water soaked, and thinner than non-affected tissue. Infected stems collapse. Stunting of plants due to root rot or collar rot may also occur. Symptoms may vary with age and stage of development of the tomato plant. Infection by the pathogen(s) may occur much later after emergence; in this case, the infection is usually not lethal but plant growth and yield may be reduced.

Jiskani *et al.* (2007) reported the damping-off occurred on seedling stage. In early stage, plants became stunted with typical discolouration at roots and formed small lesions on roots near to soil level. The spots coalesced together and formed large areas. In severe infection, the entire root system rot and later the plants die.

Huang *et al.* (2011) observed the most common symptom of *Rhizoctonia* damping off is the failure of infected seeds to germinate. *R. solani* may invade the seed before it has germinated to cause the pre-emergent damping off, or it can kill very young seedlings soon after they emerge from the soil. Post-emergent damping off is that those seedlings which grow but have stem lesions at ground level. Stems of seedlings also become thin and tough ("wire-stem") resulting in reduced seedling vigor. Leaf spotting sometimes accompanies other symptoms, as does a grey mold growth on stems and leaves. Roots sometimes rot completely or back to just discoloured stumps. The seedling is most susceptible to disease in its early stages.

Deadman (2017) described symptoms of damping-off in seedlings as, a watery rot develops in the taproot and hypocotyl at or near the soil line, and plants frequently collapse. A slower decline may occur when seedling death is preceded by cotyledon and leaf chlorosis. Mature plants show symptoms of root and crown rot. Initially, feeder roots are destroyed. Soon after, brown lesions, 0.3–2.0 cm long, develop on lateral roots. Roots may have one to several lesions. Subsequent infections occur on the taproot or in the hypocotyl area. In severe infections, there is complete decay of the root cortex while the stele remains intact. As the severity and size of the lesions increase, the plant may show varying degrees of stress. The crown leaves often become chlorotic. Necrosis soon follows as the symptoms gradually move outward toward the tips. Fruit can be sunburned, and soluble solids are reduced. Sudden wilt is another symptom: healthy- appearing plants suddenly collapse during the heat of the day. Although some plants recover turgidity at night, wilting recurs the next day, and the plants die in 2–4 days. The onset and severity of sudden wilt vary from field to field, but the suddenness of collapse is directly proportional to the degree and speed of root infection.

2.5. The Bio-control Agent: Native *Trichoderma*

2.5.1. Isolation of *Trichoderma* spp.

Trichoderma species were distributed worldwide and found to occur in natural habitats (Ha, 2010). Cigdem and Merish (2003) from Turkey collected thirty-one soil samples from different agricultural fields and forests in Eskisehir and isolated

Trichoderma by inoculating on PDA (potato dextrose agar), MEA (Malt extract agar), rose Bengal agar and oat flour agar and incubated at 28°C for 5 days. Johnson and co-workers in 1987 isolated *Trichoderma* species from soils of Tennesse and Alaska on a selective medium at 25 degree celcius and found that cold tolerant species (those isolated at 10 or 12 degree celcius) were *T. pseudokoningii* and *T. harzianum* from Tennesse and *T. viride* from Alaska. Also Scarselletti and Faull in 1994 from China, isolated *Trichoderma* spp. from collected soil by plated 1 ml of suspension onto PDA (Potato Dextrose Agar) medium and reported *Trichoderma* was found from north to south ecosystem of China, represent the wide range of *Trichoderma* distribution. Vijayan *et al.* (2016) isolated *Trichoderma species* from disease suppressive cardamom soils using *Trichoderma* selective medium and identified as *T. harzianum* (T2, T4, T12) and *T. viride* (T1, T6, T7, T8, T13, T14). Rahman *et al.* (2014) isolated *Trichoderma species* from different habitats of Bangladesh and was quantified and characterised and identified as *T. harzianum* (IMI-392432, 392433, 392434); *T. pseudokoningii* (IMI-392431) and *T. virens* (IMI-392430).

Many workers isolated *Trichoderma* from around the world and observed that *Trichoderma* are more abundant in rhizospheric soil (Babu and Pallavi, 2013). Ngadin and his co-workers (2015) isolated 65 *Trichoderma* isolates from the rhizosphere soils of healthy rice plants collected from Kedah, Kelantan and Terengganu, Malaysia with serial dilution technique on *Trichoderma* Medium E agar (TME). Joshi *et al.* (2010) reported 62 isolates of *Trichoderma* spp. isolated from different rhizospheric soil samples collected from different places located in Western Himalayas region. Out of these only two species were found *i.e. T. harzianum* and *T. viride* effective against *P. aphanidermatum* and *Fusarium oxysporum* f. sp. *udum*. Anitha and Das (2011) collected soil from rhizosphere soil of organic farming fields of major vegetable growing tracts of Kerala and isolated *Trichoderma* spp. on Rose Bengal media and PDA (Potato Dextrose Agar). Initially the mycelia were found to be whitish. At maturity the fungal colonies was seen as dark green coloured colony. By staining using lacto phenol cotton blue, the fungal growth was identified as *Trichoderma* spp.

In 2005, 135 antagonist fungi *Trichoderma* spp. were isolated by Druzhinina and his co-workers from different substrates *viz*, cattle faeces, pasture soil, decayed agarics, feather, decayed bark, decayed vegetables, decayed timber, sheep faeces, defoliation, timber scraps, decayed sundries, decayed pine needle, decayed peach stone, decayed apricot stone, decayed paper, herbaceous plant and soil (loam, sandy soil, forest soil and paddy field soil) and found that *Trichoderma* live in

numerous types of soils and often found colonizing roots. *Trichoderma* was observed to be able to proliferate in healthy roots environment (Druzhinina *et al*., 2005).

2.5.2. Morphological Characteristics

The genus *Trichoderma* was classified as an imperfect fungus as it has no known sexual stage and it belongs to the class Deuteromycetes (Gams and Bisset, 1998). *Trichoderma* conidia are hyaline or, more usually, green, smooth-walled or roughened (Domsch *et al*., 1980). Their chlamydospores are hyaline in the mycelium of older cultures. The colony of *Trichoderma* was wooly and compact. It was white in colour; as the conidia formed scattered blue-green or yellow-green patches became visible (Sutton *et al*., 1998). These patches may sometimes formed concentric rings. They could be more readily visible on potato dextrose agar compared to other media. The genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophore with lageniform phialides and hyaline or green conidia born in slimy heads (Bissett, 1984). Some species produce a characteristic sweet or coconut odour (Aneja, 2003). Petrini (1986) worked on taxonomy of endophytes of aerial plant tissues and found that *Trichoderma* spp. rarely occurred on living plants and has not been found as endophyte of living plants. Rini and Sulochana (2007) reported from Kerela that white mycelia growth of *T. harzianum* was observed on sorghum grains on the third day of incubation and it covered the entire surface of the substrate with profuse green sporulation in 6 days. Again from Allahabad a report was mentioned that *T. viride* in solid and liquid media in different substrate produced hyaline colony, which gradually changed to yellowish green colour in later and further within 24 hours the mycelium spread over the surface of the substrates which become dark in colour due to abundant sporulation (Sobita and Anamika, 2011)

Rifai (1969) distinguished nine *Trichoderma* species *i.e*., *T. aureoviride, T. hamatum, T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. piluliferum, T. polysporum, T. pseudokoningii*, and *T. viride.* on by conidiophore branching patterns and conidium morphology. Zhang *et al*. (2005) from China identified 64 *Trichoderma* isolates on the basis of morphological and cultural characteristics and found 1 isolate as *T. virens*, 4 isolates as *T. asperellum,* 26 *isolates as T. harzianum*, 5 isolates as *T. longibrachiatum*, 13 isolates as *T. atroviride*, 1 isolate as *T. citrinoviride*, 3 isolates as *T. velutinum*, 4 isolates as *T. asperellum*, 5 isolates as *T. koningii*, 2 isolates as *T. cerinum*, 1 isolate as *T. sinensis* and 2 isolates as *T. viride*. Prameela *et al.* (2012) from India identified *Trichoderma* isolates on the basis of morphological and cultural

characteristics and found 11 isolates as *T. virens*, 15 isolates as *T. asperellum,14 isolates as T. harzianum* and 32 isolates as *T. longibrachiatum*.

Shaiesta *et al.* (2012) reported from Srinagar that various microfungi associated with *Pleurotus* spp. and found *Trichoderma* spp. to be the most predominant. Forty nine isolates of *Trichoderma* spp. were identified and classified into three species on the basis of cultural and morphological characteristics as *T. harzianum*, *T. viride* and *T. pseudokoningii*. *T. harzianum* was found to be predominant species followed by *T. viride* and *T. pseudokoningii*. All the three species were distinct from each other in the characteristics such as mycelium growth rate, colony appearance, shape of conidia and conidiophores and branching pattern of phialides.

Chennappa *et al.* (2017) collected rhizosphere soil from Hyderabad Karnataka region and *Trichoderma* cultures were isolated by serial dilution spread plate method. *Trichoderma* colonies were appeared white in beginning, varying in culture from dark green to light green or yellowish green in colour with advancement of age among the isolates. Apprised and flat growth was observed whereas fluffy growth was observed in few *Trichoderma* isolates. Few isolates showed smooth and irregular margins whereas some isolates showed smooth and uniform margins.

Sekhar *et al.* (2017) identified ten isolates of *Trichoderma* spp. upto species level based on colony colour, morphology included maximum radial growth. Microscopic observation was done by using Labomed LX 400 microscope. Species-level identification of *Trichoderma* isolates was done based on the formation of chlamydospores, conidiophores and phialides characters, shape of conidia as the main characters to identify the species. The identified strains are *Trichoderma viride* (GRT-1, GRT-6 and GRT-9), *Trichoderma koningii* (GRT-2, GRT-5 and GRT-8), *Trichoderma* spp (GRT-3), *Trichoderma reeseii* (GRT-4), *Trichoderma harizanum* (GRT-7), *Trichoderma aureoviride* (GRT-10). Maximum radial growth were recorded in isolates GRT-3, GRT-4 and GRT-9 at 5th day after inoculation (90.00mm) at growth rate of 30.00 mm/day and least radial growth rate was observed in case of GRT-7 (73.00) with growth rate of 24.33 mm/day.

2.5.3. Molecular Characterisation

The use of morphological and cultural characters alone to differentiate individuals within the genus *Trichoderma* to a level that is most informative has proved difficult due to a lack of reliable characters. So, molecular techniques were assessed for their ability to differentiate between isolates of the genus *Trichoderma*. Using the

universal primers (ITS 1 and 4) consistent results have been obtained when sequence analyses of 18S rRNA gene fragment and strains (Shahid *et al*., 2014). Amplified rDNA fragment of approximately 600 bp by ITS-PCR in *Trichoderma* has helped to detect identity of the *Trichoderma* isolates (Kannangara *et. al*., 2017). 64 *Trichoderma* isolates were verified at the species level by the oligonucleotide barcode program TrichO Key v.1.0 and the custom BLAST server Tricho BLAST, using sequences of the ITS1 and 2 region of the rRNA cluster and from the longest intron of the *tef1* (translation elongation factor 1-a) gene (Zhang *et al.,* 2005). Eleven known species (*Trichoderma asperellum T. koningii, T. atroviride, T. viride, T. velutinum, T. cerinum, T. virens, T. harzianum, T. sinensis, T. citrinoviride, T. longibrachiatum*) and two putative new species (*T.* spp. *C1, and T.* spp. *C2*), distinguished from known species by phylogenetic analysis, were identified (Venkateswarlu *et al*., 2008). Kredics *et al*. (2012) sequenced the ITS region of *Trichoderma* isolates for species identification and categorized six species including, *T. asperellum*, *T. virens, T. atroviride, T. brevicompactum, T. koningiopsis, Hypocrealixii* P based on the molecular analysis,.

Singh *et al*. (2014) employed PCR-Random amplified polymorphic DNA amplification to identified isolated *Trichoderma* upto species level and RAPD-PCR profiles showed genetic diversity among the isolates with the formation of six clusters. The average similarity percent based on amplified RAPD primers was 61.84 %. Dendrogram was constructed using UPGMA method based on similarity coefficient. The dendrogram showed that there are three major groups A, B and C, group. A group further divided into two sub-groups A_1 and A_2. Sub-group A_1 consisting all the strains of *T. harzianum* (*T. harzianum* 1, *T. harzianum* 2, *T. harzianum* 3). Subgroup A_2 having *T. atroviridi* and *T. viride* and showing 96 % similarity with *T. harzianum* and *T. virens* were found in group B with 94 % similarity to each other. Phylogenetic analysis was carried out to know the virulency of bioagents. On the basis of RAPD-UPGMA clustal analysis, it was confirmed that *T. harzianum* 1 is the most virulent bioagent.

Mahadevaswami *et al*. (2017) verified sixteen *Trichoderma* isolates with ITS primers *viz*., ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and amplified product was in the range of 600 to 650 bp length as compared to 100bp DNA ladder. With NCBI, BLAST conformity of the isolates was obtained which revealed that all the isolates belonged to *Trichoderma* spp. of which six were *T. harzianum*, four were *T. virens*, three were *T. viride*, two were *T. piluliferum* and *T. asperellum*. The sixteen rDNA sequences were deposited in the NCBI GenBank, Mary land, USA database under the accession no KT153650-KT153665.

Wu *et al*. (2017) in their attempt to identify a novel fungus, *Trichoderma asperellum* GDFS1009, the result of the PCR amplification of the ITS (KF367522) showed with a product size of 642 bp. NCBI BLAST followed by phylogenetic analysis of ITS sequences revealed that this novel strain shares the highest homology with *T. asperellum*, followed by *T. harzianum*, *T. longibrachiatum*, *T. hamatum*, *T. reesei*, and *T. virens.* Together with the morphological identification, they determined the strain to be *T. asperellum* and named it GDFS1009.

2.5.4. Antimicrobial Traits of *Trichoderma* spp.

2.5.4.1. Antifungal antibiotics and peptaibols

Antibiosis is one of the widespread strategies used by *Trichoderma* spp. against plant fungal pathogens, the mechanism of which, however, remains poorly understood (Viterbo *et al*., 2005). Antibiotics are defined as molecules of microbial origin having low molecular weight, chemically heterogeneous and at low concentrations are harmful to other microorganisms (Thomashow *et al*., 1997). *Trichoderma* spp. produce a variety of antibiotics, such as gliovirin, gliotoxin, viridin, pyrones and peptaibols, against fungal phytopathogens including *Rhizoctonia solani, Pythium* spp*, Phytopthora* spp*, Fusarium oxysporum* (Dennis and Webster, 1971a,b; Claydon *et al*., 1987; Harman *et al*., 2004, Shi *et al*., 2012; Vinale *et al*., 2014; Yu *et al*., 2017). Scarselletti and Faull (1994) studied the compound 6-pentyl-α-pyrone [6-p-p] produced by *Trichoderma harzianum* IMI 288012 in plate tests against *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici* and found that the addition of 0·3 mg ml^{-1} 6-p-p to agar medium caused a 69·6 per cent reduction of growth of *Rhizoctonia solani* and 31·7 per cent reduction of growth of *Fusarium oxysporum* f. sp. *lycopersici* after 2 days. A strong relationship was found between the production of the pyrone by *T. harzianum* and the antagonistic ability of this fungus *in vitro*.

Peptaibols are a large family of antibiotic peptides from soil fungi, including *Trichoderma* and related genera such as *Emericelopsis* and *Gliocladium* (Daniel and Filho, 2007). To date, 317 peptaibols have been reported, and among them more than 190 are synthesized by *Trichoderma*. *Trichoderma pseudokoningii* SMF2 produces three major peptaibols, trichokonin VI (TK VI), trichokonin VII and trichokonin VIII and have been shown to exhibit broad-spectrum antimicrobial activity against Gram-positive bacteria and filamentous fungi, including plant fungal pathogens (Huang *et al*., 1995a,b; Chen *et al*., 2009). Shi and his co-workers (2012) reported that trichokonin VI (TK VI) induced extensive apoptotic programmed cell death in cells of *F. oxysporum*.

2.5.4.2. Cell wall degrading enzymes

Lysis of the prey's cell wall is one of the key steps during mycoparasitism. Most phytopathogenic fungi have cell wall that contain chitin as a structural backbone and β-1, 3-glucan as affirming material (Gruber and Seidl-Seiboth, 2012). Chitinase and 1,3-β-D-glucanase have been found to be directly involved in the mycoparasitism interaction between *Trichoderma* and its hosts (Harman *et al*., 2004; Geraldine *et al*., 2013; Troian *et al.,* 2014). Upon induction with cell walls from *Rhizoctonia solani*, Aires and his co-workers (2012) observed that *Trichoderma asperullum* produces two extracellular 1,3-β-glucanase. Barbara *et al*. (2011) reported that endochitinase gene (ech42) coding for the endochitinases was produced by the biocontrol agent *Trichoderma* and responsible for mycoparasitism against test fungus (*R. solani*, *Fusarium oxysporum, Pythium* spp. and *Phytopthora solani*). Endochitinase gene specific primer sequence for ech42 gene used to validate the efficacy of bioagents were: 5'-CTTGTAGTCCCAAATACCGTTCTCCCA-3' (ech42F) and 5'-GCAAACGCCGTCTACTTCACCAACTGG-3' (ech42R). The ech42 specific primers pair amplified a corresponding fragment of 1.5 kb in all *Trichoderma* spp. Thus, the presence of the ech42 gene was found to be correlated with the biocontrol activity of *Trichoderma* species. Suparno *et al.* (2016) reported the present of antibiosis compound β-1, 3 glucanase and Chitinase which are produced by *Trichoderma* isolates that are able to inhibits growth of *Rhizoctonia solani* 73.08 per cent.

Cohen-Kupiec *et al*. (1999) had made an efforts to clone the corresponding genes for chitinases and β-1,3-glucanases which are considered the most effective enzymes in the degradation of fungal cell walls (composed mainly of chitin and laminarin) and studied their role in mycoparasitism . They found that the expression of the corresponding gene *lam1.3* in growth with laminarin but not with glucose. The PCR reaction employed was RT–PCR, using specific primers for the 5' end of the glucanase gene (5'-ATGGGGTTTATACGCTCCGC), and a primer constructed according to a sequence at the 3' end of the gene (5'-AATATTGTGAACCAGCACCAC), using the Titan@ One Tube RT–PCR System (Boehringer). In the reaction, 1 mg mRNA samples extracted from laminarin grown cultures were used. The nucleotide sequence has been submitted to EMBL databank and assigned accession number AJ002397.

2.5.4.3. HCN production

Microbial production of hydrogen cyanide (HCN) which a toxic chemical has been reported as an important antifungal trait for soil borne fungi pathogens

management (Ramette *et al.*, 2003). Manwar *et al.* (2011) reported hydrogen cyanide is effective in blocking the cytochrome oxidase pathway and which is highly toxic to microorganisms at picomolar concentrations. HCN cyanide can acts as a general metabolic inhibitor to avoid predation or competition without harming the host plant (Ajilogba *et al.*, 2013). The production of HCN by *Trichoderma* is believed to play a part in the suppression of pathogens. Various workers reported the production of HCN by *Trichoderma* (Noori and Saud, 2012; Dixit *et al.*, 2015; Ng *et al.*, 2015; Parameswari *et al.*, 2015; Rajendraprasad *et al.*, 2017).

2.5.4.4. Ammonia production

Ammonia is present in soil, water and air, and it is an important source of nitrogen for plants. Nitrogen promotes plant growth and improves fruit and seed production, resulting in a higher yield (Howitt and Udvardi, 2000; Harman, 2000). It's also essential for photosynthesis, which is the process in which plants convert light energy into chemical energy (Stulen *et al.*, 1998). Nitrate is one of the most important sources of nitrogen, not only for plant development but also for plant–microbe interactions (Dordas, 2008). Nitrate can also be incorporated in the soil biosystem either by degrading organic matter by the microorganisms or fixing atmospheric Nitrogen (Manoli *et al.*, 2014). *Trichoderma* sp. is one of the important rhizosphere microorganisms that can colonize at the outer epidermal layers of the roots and are found to increase nitrogen utilization efficiency in plants (Harman, 2000; Singh *et al.*, 2014). Seed treatment of the crops with *Trichoderma* has also increased yields (Harman, 2006). The ability of *Trichoderma* to elevate nitrogen utilization capacity in plants are reported by several workers (Sherameti *et al.*, 2005; Thakkar and Saraf, 2015; Singh *et al.*, 2018).

2.5.5 Competition for niche and nutrients

Successful colonization of a given habitat by any organism is crucially dependent on its potential to defend its ecological niche and to thrive and prosper despite competition for nutrients, space, and light (Schuster and Schmoll, 2010). Many fungi and especially those of the genus *Trichoderma* are found to be able to compete for space, nutrients and light (Vinale *et al.*, 2008; Ng *et al.*, 2015). Inhibition of fungal spore germination was observed when microorganisms compete for carbon nutrient (Kamilova *et al.*, 2005). There is also competition for trace of elements like iron, copper, zinc manganese, etc (Cawoy *et al.*, 2011). Siderophores are low molecular iron chelating compounds produced by fungi and bacteria under iron stress condition. Several species of *Trichoderma* produced siderophores as reported by various

workers (Qi and Zhao, 2013; Zhao *et al.*, 2014; Dixit *et al.*, 2015). Ghosh amd his co-workers (2017) reported that *T. harzianum* produced a maximum percentage of siderophore (85 %), followed by *T. viride* (65.50 %), *T. asperellum* (60.27 %) and *T. longibrachiatum* (45.50 %). Lehner *et al.* (2013) developed a novel screening approach for the detection of siderophores using liquid chromatography coupled to high-resolution tandem mass spectrometry to study the production of extracellular siderophores of 10 wild-type *Trichoderma* strains and reported that on average *Trichoderma* spp. produced 12 to 14 siderophores, with 6 common to all species tested. The highest number (15) of siderophores were detected in *Trichoderma harzianum*.

Ability to solubilize zinc, manganese, potassium, iron and copper not only protects the plant from phytopathogenic microorganisms but also promote plant growth directly by increasing the availability of iron, zinc, manganese and copper in the soil surrounding the roots or indirectly by competitively inhibiting the growth of other soil microorganisms by acquiring these elements under stress condition (Fgaier and Eberl, 2011). Many researchers reported the ability of *Trichoderma* to solubilised zinc, potassium and make it available for the plants (Schuster and Schmoll, 2010; Naher *et al.*, 2014; Thakkar and Saraf, 2015).

2.5.6. Phosphorus (P) solubilization

Phosphorus (P) is next to Nitrogen (N) as the most limiting macronutrient to plant growth (Nautiyal, 1999; Balemi and Negisho, 2012), making up about 0.2 per cent of a plant's dry weight (Schachtman *et al.*, 1998). The concentration of soluble P in soil ranges from 0.05 to 10 ppm (4) and in soil, more than 80 per cent of P becomes immobile and unavailable for plant uptake because of adsorption, precipitation or conversion to organic form (Holford, 1997). P-solubilization ability of the micro-organisms is considered to be one of the most important traits associated with plant P nutrition.

Many investigators reported that *Trichoderma* reside in the rhizosphere of plants and play an important role in solubilization of bound phosphates, making them available to plants (Saravanakumar *et al.,* 2012; Promwee *et al.,* 2014; Chagas *et al.,* 2015; Thakkar and Saraf, 2015).

The first report of the ability of a *Trichoderma* strain to solubilize insoluble or sparingly soluble minerals was reported by Altomare and his co-workers in 1999. They found that *Trichoderma harzianum* Rifai 1295-22 (T-22) was able to solubilize MnO_2, metallic zinc, and rock phosphate (mostly calcium phosphate) in a

liquid sucrose yeast extract medium. This activity explained, at least partially, the ability of T-22 to increase plant growth. Solubilization of metal oxides by *Trichoderma* involves both chelation and reduction. Both of these mechanisms also play a role in biocontrol of plant pathogens, and they may be part of a multiple-component action exerted by T-22 to achieve effective biocontrol under a variety of environmental conditions (Altomare *et al*., 1999).

Fourteen strains of *Trichoderma* spp. isolated from the forest tree rhizospheres of pinus, deodar, bamboo, guava and oak were tested for their *in-vitro* P-solubilizing potential using National Botanical Research Institute Phosphate (NBRIP) broth containing tricalcium phosphate (TCP) as the sole P source and results showed that all the cultures were found to solubilize TCP but with varying potential. The isolate DRT-1 showed maximum amount of soluble phosphate (404.07 µg/ml). Under glasshouse conditions, growth parameters including shoot length, root length, fresh and dry weight of shoot as well as roots, in P deficient soil containing only bound phosphate (TCP) were found to be increased in *Trichoderma* spp. inoculation chickpea (*Cicer arietinum*). Shoot weight was increased by 23 per cent and 33 per cent by inoculation with the isolate DRT-1 in the soil amended with 100 and 200 mg TCP per kg soil, respectively, after 60 days of sowing (Kapri and Tewari, 2010).

Franca *et al.* (2017) reported that among 16 *Trichoderma* spp. isolates obtained from soils of organic tomato growing areas tested *in vitro*, together with a commercial product (Trichodermil®), two of isolates identified as *Trichoderma atroviride* and *Trichoderma asperellum* showed the ability for phosphate solubilisation with *T. asperellum* recorded higher soluble phosphate concentrations (9.01 µg/L), and kept values above 10 µg/L up to the sixth day (144 hours) of incubation.

2.5.7 Plant growth promotion

The agricultural soil has lots of beneficial microorganisms (*Pseudomonas*, *Bacillus*, *Arbuscular mycorrhiza*, *Trichoderma* spp., etc.) which are non pathogenic and enhance the plant growth and development by one or more mechanisms like production of phytohormones (auxin, cytokinin and gibberellins), lowering of ethylene levels, solubilisation of minerals like Phosphorus, manganese and zinc (Singh *et al*., 2014; Bhattacharyya and Jha, 2017).

Promotion of root growth of the cucurbitaceous crops by several strains of *Trichoderma* spp. isolated from rhizosphere soils and rhizoplane of agricultural crops such as bitter gourd, loofah and cucumber in Taiwan was observed when compared with untreated plants. *Trichoderma* spp. significantly increased seedling

height by 26 to 61 per cent, root exploration by 85 to 209 per cent, leaf area by 27 to 38 per cent, and root dry weight by 38 to 62 per cent for 15 days after sowing seeds of bitter gourd. Similarly, these *Trichoderma* strains also promoted seedling growth of loofah and cucumber (Chaur-Tsuen and Chien-Yih, 2002).

Rice plants treated with *Trichoderma* 7 days before inoculation of pathogen showed an increase in biometric parameters and also infection rate was decreased in treated plants as compared to the control (Anitha and Das, 2011).

Sodimalla *et al.* (2012) evaluated the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth-promoting inoculants and found that these biofilms exhibited enhanced antifungal activity, ammonia, indole acetic acid (IAA) and siderophore production, as compared to the other treatments. *Trichoderma*–*Azotobacter* biofilm recorded the highest nitrogenase activity and 1-aminocyclopropane-1carboxylic (ACC) deaminase activity. All the cultures used were found to be IAA producers, with values ranging from 0.013 to 0.082 μg/ml and biofilms showed higher IAA production compared with the single cultures. The synergism in terms of the plant growth promoting (PGP) traits in the biofilms revealed their promise as superior PGP inoculants.

A potential IAA-producing isolate of *Trichoderma* (T61) identified by Ng *et al.* (2015) was suggested to be developed as plant growth promoter as it produced higher IAA concentration (93.75 μg/mg) as compared to those reported by Kotasthane *et al.* (2015) with only 30.08 μg/mg and Salas-Marina *et al.* (2011) with 27 μg/mg.

Pawar *et al.* (2015) carried out lab experiment to evaluate the zinc solubilizing ability of different microorganisms using zinc oxide, zinc carbonate and zinc phosphate in both plate and broth media assays. Results indicated that the, by plate assay *Trichoderma viride* formed significantly highest colony diameter (2.33 cm) and halozone diameter (4.10 cm) with zinc carbonate amended media. Also in Broth culture assay, maximum zinc solubilization (458 mg/lit) was observed with the *Trichoderma viride* in zinc carbonate amended media compared to control (140.0 mg/lit). In field condition, plots treated with this *Trichoderma viride* showed increased plant growth and productivity.

In South Kalimantan after application of *Trichoderma* isolates in rice field, the content of Nitrogen, Phosphorous, Potassium in soils and soil pH increased and also contributed to the plant growth, which was represented by number of growing tiller per clump and plant height (about 12.2 cm) (Luchman *et al.*, 2016)

Studies conducted by Franca *et al.* (2017) observed that the *Trichoderma* “F” and “G” isolates had a higher IAA production after 48 hours, with 42 µg L-1 and 34 µg L-1 of IAA concentration, respectively. Application in cherry tomato with these isolates showed increase in the leaf area and dry mass of leaves and roots as compared to the untreated control.

Faba bean treated with *Trichoderma hamatum* showed highest improvement in the length of shoot (7 per cent) and root (29 per cent) followed by *T. harzainum* with an increase of 6 per cent (shoot length) and 24 per cent (root length) over control. Significant effect of *T. viride*, *T. virens*, *T. koningii* and *T. atroviride* was recorded only on root length (5-18 per cent) over control. *T. hamatum* was found to improve the fresh and dry weight of shoot (63 per cent and 48 per cent) and root (53 per cent and 54 per cent), respectively over control in non-infested soil (Kumari *et al.*, 2017).

Rajendraprasad *et al.* (2017) studied the influence of bio-control agents alone and in combination on the growth parameters of tomato such as shoot length, root length, total length, fresh and dry weight when inoculated with *Pythium debaryanum* and found that the combination of fungal and bacterial treatment T6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7) recorded high shoot and root length of 33.0, 6.96 cm, respectively in *Pythium* inoculated plants while T5 (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) recorded 29.0 and 6.43 cm, respectively. The total length also increased significantly over control in the combination of fungal and bacterial treatment T6 and T5 recorded 39.96, 35.43 cm respectively. The fresh and dry weight of tomato seedlings also increased in the biocontrol treatments T6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7) with 3.80 and 0.40 g, respectively.

2.5.8 Rhizosphere competence of *Trichoderma* spp.

Rhizosphere competence (RC) is defined as the ability of an organism to colonise, grow and develop in the rhizosphere soil. Microbes that can establish and proliferate in the rhizosphere as the seed germinates and the roots develop stand a good chance of forming a long-term relationship with the plant (Brimecombe *et al.*, 2001). Root colonising microbes may cause plant disease (phytopathogenic) (Hawes, 1990) or may confer beneficial effects (Gregory, 2006), such as mycorrhiza and plant growth promoting rhizobacteria (PGPR). Cripps-Guazzone (2014) reported that the 22 isolates of 9 of the most common *Trichoderma* species (*T. harzianum*, *T. virens*, *T.*

viride, *T. viridescens*, *T. crassum*, *T. hamatum*, *T. atroviride*, *T. asperellum*, *T. asperelloides*) used for biological *control* of sweetcorn in a non-sterile Wakanui silt-loam soil showed 82 per cent of the *Trichoderma* isolates had populations that were significantly higher than the control indicating that rhizosphere competence was widespread within the selected species. Also the least and most rhizosphere competent isolates belong to the same species, indicating that RC was not species specific.

Application of *Trichoderma* spp. enriched vermi compost accelerated plant growth and grain yield of rice and also availability of micronutrients *i.e.* S, B, Cu, Zn, Mn and Fe content increased; where maximum microbial population and *Trichoderma* population were found (83.91 x 10^{-1} and 7.11 x 10^{-1}) (Yuvraj, 2017).

Several investigators reported that the *Trichoderma* strain possesses highly rhizosphere competent, *i.e.*, able to colonize and grow on roots as they develop (Ahmad and Baker, 1988; Sathiyaseelan *et al.*, 2009; Prabha *et al.*, 2015; Rao *et al.*, 2016).

2.6 Antagonistic Effects of *Trichoderma* spp. against *Rhizoctonia solani* and *Pythium spp*

Many researchers reported the antagonistic effect of *Trichoderma* spp. against *Rhizoctonia solani* and *Pythium* spp. like dual inoculation of *Trichoderma harzianum* and *Pythium aphanidermatum* significantly inhibited the growth of *P. aphanidermatum* by 83 per cent (Elad *et al.*, 1984). Sivan and Elad (1985) studied the effects of new isolate of *T. harzianum* against *Pythium aphanidermatum* and documented *Trichoderma* as effective biological control agents of plant diseases caused by soilborne fungi. Studies on *in-vitro* efficacy of *Trichoderma* spp. against soil borne pathogens revealed maximum inhibition of mycelial growth of *T. harzianum* against *Rhizoctonia solani, Pythium debaryanum*, *Sclerotinia minor* and *Fusarium oxysporum* f. sp. *pisi*, whereas *T. viride* showed maximum inhibition in the mycelia growth of *R. solani* and *Pythium debaryanum* (Kapoor, 2008; Muthukumar *et al.*, 2011). Suppression of growth of *R. solani* using biocontrol agents (BCA) like *Trichoderma* spp. and *Pseudomonas fluorescens* on co-culturing was reported by Anitha and Das (2011).

Ten *Trichoderma* isolates collected from different agro-ecological zone of West Bengal were evaluated for antagonistic activity against *Rhizoctonia solani.* Of the ten isolates two isolates *T. viride* from Bishnupur and *T. roseum* of Alipurduar were

most prominent showing superior antagonistic effects and fast growth against *R. solani*. On co-culturing these two antagonists inhibited the mycelia growth and reduced the abundance and size of sclerotial bodies irrespective of inoculation periods (Jash and Pan, 2004).

Different inhibitory effects of *T. harzianum*, *T. viride* and *T. spirale* against mycelial growth of *Rhizoctonia solani* and *F. oxysporum* f. sp. *phaseoli* of bean in laboratory tests were reported by Sallam and his co-workers (2008). They further reported that under greenhouse and field conditions, soil treatment with a powder formulation of *Trichoderma* spp. two weeks before planting or at the time of planting not only significantly reduced the incidence of both diseases but also enhanced the bean yield in comparison to infested control.

Evaluation of 13 isolates of biocontrol fungi and 4 bacterial strains against damping-off fungus, results showed that among the fungal species *T. harzianum* (T8) *and Gliocladium virens* were the most effective in inhibiting damping off fungus mycelial growth 74.82 per cent and 73.33 per cent, respectively (Malhotra *et al*., 2011).

Mishra *et al.* (2011) studied several isolates of *T. viride* for antagonistic effect against fungal pathogens such as *Rhizoctonia solani, S. rolfsii*, *Macrophomina phaseolina*, *Alternaria alternate*, *F. solani* and *Collectotrichum capsici* of moong bean and reported that *T. viride* isolate Tr8 showed 70 per cent, 68.2 per cent, 70 per cent, 73.3 per cent, 69.3 per cent and 70.1 per cent growth inhibition against *R. solani*, *S. rolfsi*, *M. phaseolina*, *A. alternate*, *F. solani* and *C. capsici*, respectively. The cell free culture filtratre of *T. viride* Tr8 showed 61.5 per cent, 58.32 per cent, 63.45 per cent and 62.62 per cent radial growth at 10 per cent concentration against *R. solani*, *S. rolfsi*, *M. phaseolina* and *C. capsici*, respectively while at 20 per cent concentration 100 per cent mycelia growth inhibition in all the pathogens was observed.

On-coculturing of seven known native BCA isolates against *P. aphanidermatum*, the least growth of the pathogen was recorded in *T. harzianum* (16.86 mm) which was significantly superior over the rest. Next best in order of merit was *P. fluorescens* (20.79 mm) which was followed by *T. fasciculatum* (21.07 mm) (Rakholiya *et al*., 2016). The antagonism of *Trichoderma* has been widely explored and arrived at conclusion that this fungus protects the plant roots from diseases and pathogens attack (Sukoso *et al*., 2016).

Waghunde *et al.* (2016) in his study on *in-vitro* efficacy of bioagent and organic amendment against damping-off of tomato pathogen (*Pythium*

aphanidermatum) indicated that *Trichoderma harzianum*, *Pseudomonas fluorescens* and *T. fasciculatum* exhibited strong antagonistic activity against *P. aphanidermatum* as compared to other antagonists tested and hence considered as potential antagonists against *P. aphanidermatum. T. virens* and *B. subtilis* have showed poor growth inhibition of the pathogen. This suggests that biological control of damping-off tomato using *T. harzianum*, *T. fasciculatum* and *P. fluorescens* were found very useful in South Gujarat area.

Under field conditions in South Kalimantan, *Trichoderma* spp. isolates from tidal swamp field suspected as *Trichoderma harzianum* were able to decrease plant disease intensity caused by *Rhizoctonia solani* about 7.4 per cent (Suparno *et al.*, 2016). Reduction in disease incidence up to 52 to 69 per cent in the field against damping off (*Rhizoctonia solani* and *Pythium* spp.) and root rot diseases of cardamom by an exotic isolate of *T. harzianum* and a native isolate of *T. viride* has been reported by Vijayan *et al.* (2016).

Volatile and non-volatile compounds produced by *T. harzianum*, *G. virens* and *T. aureoviride* suppressed the growth and sclerotia formation of *R. solani*. Also *T. harzianum* and *T. aeroviride* were most aggressive in inhibiting the mycelial growth of *R. solani* whereas *T. koningii* and *T. longibrachiatum* significantly reduced the formation of sclerotia of *R. solani* (Amaresh *et al.,* 2017).

Naik *et al.* (2017) tested dual culture assay against R*hizoctonia solani* and results revealed that per cent inhibition of growth of the pathogen ranged from 61.10- 89.01 per cent. The results further indicated that among all the *Trichoderma* isolates tested, Tri-9 was significantly superior over the other isolates and showed 89.01 per cent inhibition of growth of *R. solani*. The other isolates Tri-24, Tri-29, Tri-10 and Tri-28 showed 88.9, 88.60, 86.7 and 86.6 per cent inhibition, respectively.

In vitro evaluation of biocontrol agents against damping off disease caused by *Pythium debaryanum* on tomato revealved that all the isolates of *Trichoderma* showed antagonistic effect against *P. debaryanum* and significantly reduced the growth. The per cent inhibition of *P. debaryanum* ranged from 80.03 to 24.47 per cent. Maximum per cent inhibition of *P. debaryanum* was observed by *T. harzianum*-7 (80.03) followed by *T. viride*-6 (79.93) whereas it was minimum in case of *T. viride*-2 (24.47). Out of 24 native isolates of *Trichoderma*, isolate 6 recorded more than 70 per cent of inhibition of *P. debaryanum* (Vidyasagar *et al.,* 2017).

2.7. Seed Bio-priming

Seed priming with living beneficial organism is termed as biopriming that involves the application of seed with selected fungal antagonists to protect seed from soil and seed borne pathogens. Primed seeds exhibit faster rate of germination, more uniform emergence, greater tolerance to environmental stresses, and reduced dormancy in many species; also ensures rapid, uniform and high establishment of crops; and hence improves harvest quality and yield (Khan, 1992; Rahman *et al.*, 2014). Many workers reported that seed biopriming with biocontrol agents like *Trichoderma* and *Psuedomonas* improved the seedling germination, vigour, emergence and crop productivity and also reduce disease incidence (Farooq *et al.*, 2005; Kavitha, 2011; Mariselvam, 2012; Karthika and Vanangamudi, 2013; Ananthi *et al.*, 2017).

Application of conidia of seven *Trichoderma* strains on cucumber and radish seeds as a simple methyl cellulose coating or through an industrial film coating process and sown the seeds in a peat-based soil artificially infested by *Rhizoctonia solani* or *Pythium ultimum* revealed that four strains control damping off effectively both in cucumber and radish (Cliquet and Scheffer, 1996).

Maize seeds treated with some antagonistic bacteria and fungi including *T. virens* and *T. viride* showed uniform germination and also reduction in damping-off (*Pythium* sp.) disease incidence was observed (Mao *et al.*, 1998).

Velazhahan *et al.* (2006) treated tomato seeds with *Trichoderma* formulations and planted in sterilized vermiculite to study the colonization efficiency of *Trichoderma*-prapagules in rhizosphere and found that seed treatment with *Trichoderma* formulations yielded in reduced incidence of damping-off disease (up to 74 %) and thereby enhanced the healthy plant stands. All formulations were effective in the control of damping-off disease as compared to the untreated control. Seed treatment with *Trichoderma* formulations also significantly increased the plant biomass, when compared to control.

Biopriming of chilli, tomato and brinjal with seven isolates of *Trichoderma* using both mycelial and conidial, and one bacterial antagonist, *P. fluorescens* resulted in significant enhancements in the seed germination, vigour index and estimated biomass. The highest germination of seed was obtained when crop seeds were primed with mycelia form of inoculum of *T. harzianum* AN-5 and WB-1. Vigour index was higher in tomato (963), chilli (915) and brinjal (867) following seed priming with *T. viride* AN-5 or *T. viride* AN-3 (Bhagat and Pan, 2010).

Seed treatment of Jubilee tomato with *T. harzianum* strains T22 exposed to biotic stress (seed and seedling disease caused by *P. ultimum*) and abiotic stresses (osmotic, salinity, chilling, or heat stress) showed that under stress conditions *T. harzianum* strain T22 treated seeds germinated consistently faster and more uniformly than untreated seeds (Mastouri *et al.*, 2010).

Tomato plant applied with *T. harzianum* and *P. fluorescens* by seed bio-priming significantly increased seed germination (22-48 per cent) and reduced the days required for germination (2.0-2.5 days). The combination treatment of *P. fluorescens*, *T. harzianum* and *Glomus intraradices* also increased yield by 20 per cent (Srivastava *et al.*, 2010).

In pea, seed biopriming with *T. viride* showed highest vigour index (3888) whereas seed biopriming with *T. harzianum* showed highest biomass of root (313 mg) and shoot (2874 mg) (Pan and Das, 2011). 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.

Uma Devi *et al.* (2017) treated tomato seeds with *Trichoderma harzianum* and the results of experiments showed that the per cent germination of tomato seeds in all the treatments was significantly higher (39.58 to 57.08 %) as against 26.25 per cent in the inoculated control and also showed effectiveness in reducing the damping off incidence compared to control.

2.8. Efficacy of talc-based *Trichoderma* formulation

Development of efficient delivery systems for the application of appropriate micro-organisms to the targeted niche is an important component of biocontrol technology (Lewis *et al.*, 1998). Several formulations of *Trichoderma* are available like dusts, alginate pellets, kaolin, starch, cellulose granules and extruded granules (Hebbar *et al.*, 1997; Lewis and Fravel 1996; Lewis *et al.*, 1998).

Talc is a clay mineral composed of hydrated magnesium silicate with the chemical formula $Mg_3Si_4O_{10}(OH)_2$. It is the softest mineral on earth and does not generate a chemical reaction when ingested or used on the skin. People have taken advantage of its natural smoothness, safety and adsorbency since ancient Egyptian times (http://geology.com/minerals/talc.shtml).

Many workers used talc as a carrier for application of biocontrol agents (Mukhopadhyay, 1994; Singh *et al.*, 2000; Nakkeeran *et al.*, 2002; Warrior *et al.*,

2002). Soil and seed application of talc-based *Trichoderma harzianum* on pigeonpea wilt caused by *Fusarium udum* under field conditions recorded reduction of disease incidence in pigeon pea and also a significant increase in rhizosphere population of *T. harzianum* up to 45 days (Prasad *et al.*, 2002).

Jayaraj *et al.* (2006) developed a carrier based formulation of *T. harzianum* strain M1 for control of damping-off of tomato caused by *P. aphanidermatum*. Seven different formulations (talc, lignite, lignite + fly ash-based powder formulation, wettable powder, bentonite paste, polyethylene glycol-paste and gelatin-glycerin-gel) were developed for seed treatment. Shelf life of the formulations was evaluated under storage at 24˚C up to nine months and there was a gradual decrease in the population of propagules along with storage period. After six months of storage, the population of propagules reduced to approximately 50 per cent. The population of propagules was optimum in all the formulations up to three months of storage.

Ramanujam *et al.* (2010) applied talc based *Trichoderma* formulation and found that it is effective against a large number of soil-borne plant pathogenic fungi and also it has suppressive effects on some root nematodes without adversely affecting beneficial microbes like *Rhizobium* and capable of promoting growth of certain crops.

Shahid *et al.* (2013) studied the shelf-life of *Trichoderma viride* in talc and charcoal based formulations. Potato dextrose broth talc based formulation was prepared by adding three different volumes of biomass of *T. viride* along with medium @ 30, 40 and 50 ml/g talc. The initial mean CFU of *T. viride* at 30, 40 and 50 ml/100 g talc on 0 day was 227.2×10^9, 256.00×10^9 and 291.03×10^9 CFU/g sample, respectively. It gradually declined and at 120 days of storage the population came down to 70.33×10^9, 80.67×10^9 and 96.67×10^9 CFU/g which in terms of reduction in viability were 69.04, 68.48 and 66.78 per cent, respectively.

Field evaluation of talc formulation of *Trichoderma* for the management of *Fusarium* wilt in watermelon revealed that the wilt incidence in biocontrol treated plots was less than 8 per cent (5.04 to 7.78 %), whereas in control it was 26.31 per cent. The number of fruits from an average 32 m^2 biocontrol treated plots ranged from 48.50 to 50.90 and it was only 35.35 in the control plot. Per cent reduction of wilt in biocontrol treated plots was 70-80 per cent and increase in number of fruits was 27-30 per cent based on consolidated data of all the five trials (Ramesh and Singh, 2017).

Rajendraprasad *et al.* (2017) evualated biological control of tomato damping off caused by *Pythium debaryanum* and found that the disease was reduced by the application of talc based formulation of *Trichoderma viride* and *Pseudomonas fluorescence* in nursery beds before sowing. The lowest incidence (45 %) of pre emergence damping off was recorded in T5 (seed treatment with *Trichoderma harzianum* 7 + soil application with *Pseudomonas fluorescence*-3). Also among the bio-control treatments the lowest (47.92 and 52.08 %) post emergence damping off incidence was recorded in T5 (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) at 30 and 50 DAS.

2.9. Pot culture experiment

Application of potting mixture with BCA like *Trichoderma* spp., *Bacillus* and *Psuedomonas fluorescens* have proved to be beneficial to the crop as well as helps in reduction of diseases (Jogani, 2014). Treatment of tomato seed and potting mixture with *T. harzianum* and *T. hamatum* showed that naturally infested potting mixture with *P. aphanidermatum* (control), result in high damping off percentage (81%, 30 days after planting) whereas in potting mixture treated with *T. harzianum* and *T. hamatum* (20 % by volume) or as seed coating increased the number of healthy seedlings by 63-76 per cent and 56-58 per cent, respectively (Chet *et al.*, 1984). The pot culture experiment conducted in greenhouse showed that the inoculation of *Trichoderma* strains in the growing media was an effective treatment to control *Phytophthora parasitica* in pepper seedlings (Raut *et al.*, 2017).

Papavizas *et al.* (1982) reported the *T. harzianum* and *G. virens* reduced the damping off of snap bean caused by *Pythium* spp. and *Rhizoctonia solani* under greenhouse conditions. Results of two years pot studies indicated significant disease reduction in *Pythium* spp. and *Rhizoctonia solani* infested pots treated with *T. harzianum* (T7) and *G. virens* (T2) as compared to *Pythium* spp. and *Rhizoctonia solani* infested control (P = 0.05).

The application of *T. viride* and *P. fluorescens* effectively checked the pre-emergence and post-emergence damping off of tomato caused by *P. aphanidermatum* under pot culture experiments (Manoranitham *et al.*, 2000).

Under greenhouse conditions seed treatment with *Trichoderma* formulations reduced the incidence of damping-off disease of tomato up to 74 per cent and also enhanced the plant biomass as compared to the untreated plants (Radhakrishnan *et al.*, 2006).

Five antagonist fungi *viz*., *T. viride, T. harzianum, Chaetomium globosum, Aspergillus terreus and Penicillium cyclopium* were tested against *F. oxysporum* f. sp. *lycopersici* under pot conditions and tomato seed coated with *T. viride, T. harzianum* and *C. globosum* showed higher seed germination 74, 72 and 68 per cent and disease control 71.5, 69.0 and 63 per cent over control (Mehra, 2006)

Khalid *et al.* (2010) raised tomato seedlings in pot trial using bio-primed seeds (var. Pant Tomato-3) with T_{35} (*Trichoderma* spp) and found that the disease severity of damping off was reduced significantly by 74 per cent as compared to the non-treated pots.

Kamala and Indira (2011) conducted experiment in controlled environmental conditions (greenhouse) and found that soil treatments with *Trichoderma* isolates (T73, T80 and T105) significantly reduced the pre- and post-emergence damping-off disease incidence under artificial infection with *P. aphanidermatum* in greenhouse conditions. The damping-off disease incidence at the pre-emergence caused by combined application of *P. aphanidermatum* and *Trichoderma* spp. was in the range between 9–22 per cent. After 30 days of plantation, the growth patterns were observed and recorded. At this stage, T105 gave the highest reduction to disease incidence by 82.86 per cent, followed by T73 (63.81 %), and T80 (22.0 %). At post-emergence stage, the disease incidence ranges from 5.8 to 8.2 per cent. T105 gave the highest growth reduction of 90.72 per cent to disease incidence followed by T73 (88.00 %) and T80 (86.88 %), respectively. The survival bean plants were in the range of 78.5–90.1 per cent as compared to 42.4 per cent healthy bean plants in the control treatment. T105 gave 90.1 per cent healthy plants, followed by T73 (87.2 %), and T80 (78.5 %), respectively. From this data, it is clear that the indigenous T105 isolates have a good potentiality in controlling the damping-off disease of beans caused by *P. aphanidermatum*.

Studies on the efficacy of the different *T. harizanum* inoculum at green house level by pot culture against damping off of chilli seedlings showed that there was no disease developed in where the plants were treated with both pathogen and *T. harizanum* and the plants that received the biocontrol agent had a good vigour. Also observed that sugarcane baggase and talcum powder formulated *T. harizanum* can be optional not only for the control of damping off disease but also to augment the growth and yield regulation in chilli plant. *T. harizanum* treated plants all the flowers yielded fruits. These results showed that *T. harizanum* has protective effect on pathogens and increased the height of the plants (Subash *et al.*, 2014).

Soil application of *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *T. virens*, *T. atroviride* and *T. koningii* against charcoal rot of faba bean (*Vicia faba* L.) caused by *Macrophomina phaseolina* under pot conditions showed that inoculation with *M. phaseolina* resulted in a significant decline in the plant growth (18-23 %), biomass production (15-25 %), and root nodulation (15-20 %) whereas treatments with *Trichoderma* spp. reduced root rot severity (36-43 %) and subsequently improved the plant growth (7-13 %), seed germination (10-15 %) and nodulation (20-25 %) of infected faba bean plant. Among the biocontrol agents used, *T. harzianum* and *T. hamatum* proved to be most efficient in reducing the disease severity (40-43 %), and also improved the plant growth (9-63 %) and root nodulation (85-90 %) (Kumari *et al.*, 2017).

In vivo biocontrol efficacy of *T. asperellum* MSST under pot trials against tomato wilt showed increase in the vegetative parameters in the plants treated with respect to the untreated plant 60 DAS. There was 50 and 83.7 per cent increase in the shoot length and root length in the plants treated with *T. asperellum* MSST as compare to the control plants (Patel and Sharaf, 2017).

Ahsan *et al.* (2018) reported soil application of maize grain based culture of *T. harzianum* at 5, 10, 15 and 20g per pot showed significant reduction in mortality of chickpea seedlings caused by *Sclerotium rolfsii.* Maximum control of collar rot (53.33 %) was recorded in treatment where *T. harzianum* was applied @ 20 g per pot.

Chapter – 3
Materials and Methods

The present investigation was carried out at the Plant Pathology laboratory, School of Crop Protection (SCP), College of Post-Graduate studies in Agricultural Sciences, Central Agricultural University (CAU) Imphal, Umiam, Meghalaya with an aim to study the bio-efficacy of *Trichoderma* formulation in tomato nurseries against damping off caused by *Pythium* sp. and *Rhizoctonia solani.* The materials used and methodologies followed during the course of the investigation are described below in detail.

3.1. Geographical location

College of Post-Graduate studies, CAU (Imphal), Umiam, Meghalaya, India is situated between 25°41'01.9 North latitude and 91°54'46.24 East longitude and at an elevation of 1010 m above the mean sea level (MSL).

3.2. Isolation of fungal pathogens (*Pythium* sp. and *R. solani*)

Pythium sp. and *R. solani* Kuhn. were isolated from naturally infected tomato, showing damping off symptoms (soft rot and wire stem symptom for *Pythium* sp. and *R. solani*, respectively). The diseased samples collected from farmers' fields were cut into small bits along with diseased parts, surface sterilized with 1 per cent sodium hypochlorite solution, followed by serial washing (3 times) in sterile distilled water (SDW). Drained out excess water and then plated on Potato Dextrose Agar (PDA) medium and incubated at 28±1°C for 10 days. Purification of the cultures was carried out by following the hyphal tip method. The isolated fungi were identified on the basis of morphology and microscopic observation (Gaigole *et al.* 2011).

3.3. Pathogenecity test

Pathogenecity tests for both the fungal pathogens (*Pythium* sp. and *R. solani* Kuhn) were conducted by soil inoculation of pathogen (Weideman and Wehner, 1993). Mycelial discs (5mm diameter) on PDA were cut out from the actively growing edge of *Pythium* sp. and *R.solani* Kuhn. and used to inoculate in 50g of rice grain. Prior to the inoculation, the rice grains were dipped in water for 48 hours and autoclaved. Drained out excess water and put in autoclavable polypropylene (PP) bags for autoclaving. The inoculated sterilized grains in the PP bags were incubated

for 21 days at 28±1°C and used to inoculate the soil. The soils for pot mixture were autoclaved and inoculated by mixing 20g of the inoculated rice grain with 1 kg soil and put into the pots containing sterilized soil. Pots without the inoculum served as control. Six to eight seeds of tomato were sown per pot and 10 pots were kept for each treatment until symptoms developed. The associated pathogens were re-isolated and cultural and morphological characters were compared with original cultures of the pathogen used for inoculation. The pathogenicity test was carried out in SCP, CPGS, CAU, Umiam, Meghalaya.

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

Soil samples from root rhizosphere, coal mines, jhum fallows, manure compost and jhum 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 (90 mm in dia) were incubated at 28±1°C 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.

3.5. Maintenance of damping off of pathogen and *Trichoderma* isolates

The mycelial disc (5 mm) of isolates which showed positive result in pathogenicity test was taken with the help of cork borer, which was then transferred into sterilized distilled water stored in screw caped vials. These vials were kept at 4°C for short term storage. For long term storage fungal mycelial disc was kept in PDA slant with 20 % glycerol and in a mineral oil at -20°C in the refrigerator.

3.6. Morphological characterization

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 but for size measurements KOH and water was used as the mounting fluid.

3.7. Screening of isolated *Trichoderma* for functional attributes *in vitro*

3.7.1. Rapid screening of *Trichoderma* isolates

Isolates tentatively identified as *Trichoderma* were further exposed to rapid screening of *Trichoderma* isolates against *Pythium* sp. 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 $2/3^{rd}$ 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.

3.7.2. *In vitro* antagonistic potentials of isolates of *Trichoderma*

The potential *Trichoderma* isolates screened by rapid screening method were further evaluated for their antagonistic potential *in vitro* against *Pythium* sp. and *R. solani* Kuhn. through dual culture assay (Ramanathan *et al.*, 2013).

Dual culture assay: For mycelial growth inhibition of test plant pathogens by the *Trichoderma* sp., both pathogens (*Pythium* sp., *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* sp. and the pathogen was taken till the growth of the

pathogen fully covered in the control plate. The experiment was replicated three times. The suppression effect of all *Trichoderma* sp. isolates were evaluated in terms of Percentage Inhibition in Radial Growth (PIRG) of *Pythium* sp. and *R. solani* based on the following formula (Gaigole *et al.*, 2011).

$$PIRG = \frac{R_1 - R_2}{R_1} \times 100$$

Where,

R_1 = Radial growth of *Pythium* sp. and *R. solani* in the absence of the antagonist in the respective plate (control)

R_2 = Radial growth of *Pythium* sp. and *R. solani* in the presence of the *Trichoderma* isolates (treatment)

The experiment was conducted with three replicates per treatments. The Radial growth and PIRG were analyzed by one-way analysis of variance (ANOVA), completely randomized block design (CRD). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level. Square root and arc sine transformed values were done using WASP 2.0 software available online (www.ccari.res.in>wasp2.0)

3.7.3. Screening for antimicrobial traits of *Trichoderma* isolates

Detection of the genes that encode for the production of antibiotics by PCR using antibiotic gene-specific primers was done. Genomic DNA from each *Trichoderma* isolates was extracted by Fungal Genomic DNA Purification Kit (HiPurA™) by following the manufacturers' protocol. The DNA purity and quantity was checked using a Nano-drop® 2000 spectrophotometer (Thermo Scientific, USA) at 260 nm. Different antibiotic gene specific primers used in the present investigation to detect presence of antibiotic biosynthetic genes in *Trichoderma* isolates were as follows:

3.7.3.1. Detection of β-1,6-Glucanase (*Tvbgn3*)

Specific Primers used for detection of *Tvbgn3* gene were: Forward primer- 5'-ATTACAGGCGAGTGGAGCAT- 3' and Reverse primer 5'-GCGTTCGTTGGGATGTAGTT-3'. PCR was carried out with genomic DNA of bioagents in 25 µl reaction volume containing 25 ng genomic DNA, 0.4 µl (5 pmol) primer, 1.5 µl dNTPs (25 mM), 3 µl of 10x assay buffer with $MgCl_2$ (15 mM), 0.5 µl (3 U/ µl) of Taq DNA polymerase. PCR amplification of *Tvbgn3* fragments comprised of 94°C for 2 min, 30 cycles (each cycle, 30 s at 94°C, 20 s at 51.8°C, and 20 s at 72°C) then a final extension step of 72°C for 5 min. PCR product (5 µl) was mixed with

loading dye (2 µl) and then loaded in 1.5 % Agarose gel with 0.1% ethidium bromide for examination with horizontal electrophoresis (Djonovic *et al.,* 2006).

3.7.3.2. Detection of Trichodiene synthase (*tri5*)

Specific Primers used for detection of *tri*5 gene were: Forward Primer triF 5'-GGCATGTGTGTTTACTCTTGG-3' and Reverse primer triR 5'-ACCATCCAGTTCTCCATCTG -3'. About 50 ng of genomic DNA was used as a template in 25 µl PCR reaction mixture containing 1.5 mM $MgCl_2$ PCR buffer, dNTPs at 200 µM each, primer at 0.3 or 0.5 µM and 2.5 Units of Hot-Master Taq polymerase (Eppendorf, Hamburg, Germany).They were used with the following PCR conditions: 94°C for 5 min, 35 cycles of 94°C for 30 s, 52.1°C for 50s, 72°C for 50 s then a final extension step of 72°C for 5 min. The PCR negative control reaction mixture contained no DNA. PCR product (5 µl) was mixed with loading dye (2 µl) and then loaded in 1.5% Agarose gel with 0.1% ethidium bromide for examination with horizontal electrophoresis (Gallo *et al*., 2004).

3.7.3.3. Detection of Serine protease (*ser*)

Specific Primers used for detection of *ser* gene were: serF 5'-AGCTTTGCTCCCGGCTGTCC-3' and serR 5'-CCAACAACGTGAGGTGTAGCCATG - 3'. 25 µl PCR reaction contained 10 mM Tris-HCl pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP, 10 pmol of each primer, 10 ng of genomic DNA and 1.25 U *Taq* DNA polymerase. Amplifications consisted of 5 min at 94°C, followed by 30 cycles of 30s at 94°C, 30s at the annealing temperature 58.5°C and 1 min at 72°C, followed by 7 min at 72°C. PCR product (5 µl) was mixed with loading dye (2 µl) and then loaded in 1 % Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis (Vieira *et al.,* 2013).

3.7.3.4. Detection of Endochitinase (*ech42*)

Endochitinase gene specific primer sequence for *ech42* gene was used to validate the efficacy of bioagents. PCR was carried out with genomic DNA of bioagents in 25 µl reaction volume containing 25 ng genomic DNA, 0.4 µl (5 pmol) primer, 1.5 µl dNTPs (25 mM), 3 µl of 10x assay buffer with $MgCl_2$ (15 mM), 0.5 ul (3 U/ µl) of Taq DNA polymerase. The PCR cycles were as follows: 5 min at 94°C, followed by 30 cycles of 1 min at 95°C, 2 min at the annealing temperature 61.25°C and 2 min at 72°C, followed by 7 min at 72°C. Primers were: ech42F 5' - CTTGTAGTCCCAAATACCGTTCTCCCA-3' and ech42R 5'-GCAAACGCCGTCTAC TTCACCAACTGG -3'. PCR product (5 µl) was mixed with loading dye (2 µl) and then loaded in 1 % Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis (Singh *et al.,* 2014).

3.7.4. Chitinolytic Enzyme Assay

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

Preparation of colloidal chitin: 5.0 g of chitin was added to 60 ml of conc. HCl (hydrochloric acid) 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 per cent 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. The principle behind the formation of coloured zone is that the media is supplemented with a pH indicator dye bromocresol purple which transformed the yellow colour of the media (in acidic, pH 4.7) into purple colour due to increase in pH. The pH increased because of the utilization of chitin by the *Trichoderma* and its breakdown into product N-acetyl glucosamine. Colour intensity and diameter of the purple coloured zone were taken as the criteria to determine the chitinase activity after 3 days of incubation.

The experiment was conducted with three replicates per treatments. The diameter of the purple coloured zone were analyzed by one-way analysis of variance (ANOVA), completely randomized block design (CRD). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level. Square root and arc sine transformed values were done using WASP 2.0 software available online (www.ccari.res.in>wasp2.0).

3.7.5. Siderophores production test

The ability of *Trichoderma* sp. 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 HCl). 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* sp. 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.

3.7.6. HCN production

Production of HCN was detected by inoculated different isolates of *Trichoderma* sp. 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).

3.8. Screening for plant growth promoting attributes of *Trichoderma* isolates

3.8.1. 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* sp. 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).

3.8.2. 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* sp. 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 were 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).

3.8.3. 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 medium after 4-7 days of incubation was considered as positive result. The experiment was conducted with three replicates per treatment.

3.8.4. Ammonia production

Trichoderma isolates were 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.

3.9. Cultural characterictics and Anamorphic characteristics

Based on above mentioned antagonism potential and plant growth promoting traits, potential isolates were screened and their cultural and anamorphic characteristics were studied.

3.9.1. Cultural characteristics of *Trichoderma* isolates

The cultural characteristics of different isolates of *Trichoderma* were studied in potato dextrose agar (PDA). Mycelial discs (5mm) of young growing culture of respective isolates of *Trichoderma* were inoculated (centrally) in the petriplates containing the solidified media and incubated at 28±1°C for one week. Three plates were taken for each and the growth patterns of isolates of *Trichoderma* were observed daily and all the distinguishing characters were recorded. The experiment was replicated three times. The characters recorded were colony, growth rate, presence of pigments, hyphae colour and presence of any distinguishing odour.

3.9.2. Anamorphic characteristics of *Trichoderma* isolates

Anamorphic characteristics *viz*., conidiophores length and width, phialides length and width, conidia length and width were studied for the potent *Trchoderma* isolates by growing them in potato dextrose agar (PDA) medium. For anamorphic characteristics, Binocular microscope at 40x was used with the help of biowizard image analysis and measurement was done at 100x. At least 10 observations were randomly recorded while measuring the morphological characteristics of *Trichoderma* sp.

3.10. Phylogenetic analysis for Identification of Potent *Trichoderma* sp.

The phylogenetic analysis of the potent bio-agent was carried out by PCR amplification of ITS region using universal Primers ITS1 (TCTGTAGGT GAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (Shahid *et al*., 2014).

PCR amplification of ITS region of *Trichoderma* isolates

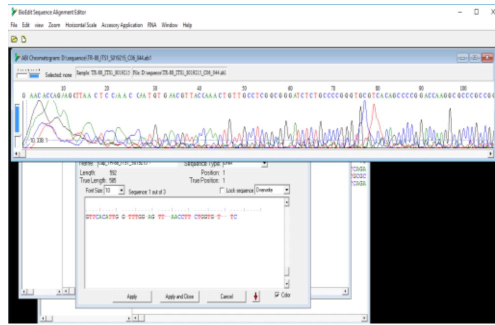
Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. PCR was performed in a total volume of 100 µl, containing 78 µl deionized water, 10 µl 10X Taqpol buffer, 1 µl of 1U Taq polymerase enzyme, 6 µl 2 mM dNTPs, 1.5 µl of 100 mM

reverse and forward primers and 1 µl of 50 ng template DNA. PCR was programmed with an initial denaturing at 94°C for 2 min. followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 1 min and extension at 72°C for 1 min and the final extension at 72°C for 10 min in a Primus 96 advanced gradient Thermocycler. PCR product (20 µl) was mixed with loading dye (8 µl) containing 0.25 % bromophenol blue, 40 % w/v sucrose in water and then loaded in 2 % Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis.

Amplified products were sent for sequencing to Xcelris laboratory, Gujarat Ahmedabad (genomics.corp@xcelrislabs.com) and the sequences obtained were trimmed using BioEdit Sequence Alignment Editor, version 7.2.6 (Hall, 1999). Forward and reverse for each of the isolates were aligned to obtain one concensus sequence. The sequence obtained for each of the isolate was confirmed using BLASTN 2.8.1+ (Zhang *et al*., 2000) function available at NCBI database against a non-redundant nucleotide (nr/nt) database using *Trichoderma* (taxid: 5543) as reference and MegaBLAST for highly similar sequence. After confirmation that the sequences obtained show hits to *Trichoderma* genus, multiple alignment was performed using ClustalW Multiple alignment (Thompson *et al*., 1994) with following option;

- /MATRIX= :BLOSUM, PAM, ID or filename
- /GAPOPEN=f :Gap opening penalty
- /GAPEXT=f :Gap extension penalty
- /ENDGAPS :No end gap separation pen.
- /GAPDIST=n :Gap separation pen. range
- /NORGAP :Residue specific gaps off
- /NOHGAP :Hydrophilic gaps off
- /HGAPRESIDUES= :List hydrophilic res.
- /MAXDIV=n :% ident. for delay
- /TYPE= :PROTEIN or DNA
- /TRANSITIONS :Transitions NOT weighted.

The phylogenetic analysis was done by using MEGA software (MEGA 5.2) (https://www.megasoftware.net) and the phylogenic tree was constructed using neighbor joining method available in MEGA 5.2 software with 1000 bootstrapping value.



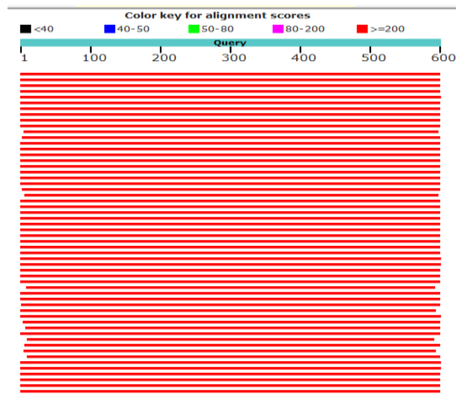


Fig 1: Flow chart of phylogenic analysis of potent *Trichoderma* isolates

3.11. To study the Plant growth promotion & Biocontrol potential of screened *Trichoderma* sp. against *Pythium* sp. and *Rhizoctonia solani* Kuhn.

3.11.1. Seed germination test

The potential antagonistic and plant growth-promoting activity of *Trichoderma* sp. were further confirmed by seed germination test and tomato seedling vigor index before evaluated under pot experiment.

The biopriming of seeds of tomato was done with the potential 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):

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

The experiment was conducted with three replicates per treatments. The Germination rate were analyzed by one-way analysis of variance (ANOVA), completely randomized block design (CRD). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level. Square root and arc sine transformed values were done using WASP 2.0 software available online (www.ccari.res.in>wasp2.0).

3.11.2. Tomato seedlings vigor index

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):

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

The experiment was conducted with three replicates per treatments. The Vigour index of seedlings were analyzed by one-way analysis of variance (ANOVA), completely randomized block design (CRD). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level. Square root and arc sine transformed values were done using WASP 2.0 software available online (www.ccari.res.in>wasp2.0).

3.11.3. *In vivo* efficacy of *Trichoderma* isolates

3.11.3.1. Mass production of *Pythium* sp. and *R. solani*

The pathogens *Pythium* sp. was cultured as described by Jayaraj *et al.* (2006) on broken maize-sand medium (broken maize 75.0 g; sand 205 g; tap water 720.0 ml) and *R. solani* Kuhn on rice bran – sand mixture (Ngullie and Daiho, 2013) 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.

3.11.3.2. 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). 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 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.

3.11.3.3. Pot culture experiment to evaluate potent *Trichoderma* isolates

In vivo efficacy of potent isolates of *Trichoderma* were evaluated against *Pythium* sp. *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, soil were inoculated with *Pythium* sp. 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) (1×10^8cfu/g)/kg seed
T_2	-	Soil treatment with *Trichoderma* @ 25g (talc powder) (1×10^8cfu/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:

Percentage of disease incidence (pre-emergence)

$$= \frac{\text{Number of seeds infected} \times 100}{\text{Total number of seeds sown}}$$

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 (Omokhua, 2011):

Biological control efficacy

$$= \frac{\text{disease incidence of control} - \text{disease incidence of treatment group}}{\text{disease incidence of control}} \times 100$$

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.

The experiment was conducted with five replicates per treatments. The Percentage of disease incidence (pre-emergence and post-emergence) and Biological control efficacy were conducted by using the statistical design randomized block design (RBD) and were analyzed by two-way analysis of variance (2-way Factor

ANOVA). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level.

3.11.4. 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* sp. was estimated by soil dilution plate technique (Dhingra and Sinclair, 1995).

The experiment was conducted with three replicates per treatments. The Rhizosphere colonization was analyzed by one-way analysis of variance (ANOVA). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level. Square root and arc sine transformed values were done using WASP 2.0 software available online (www.ccari.res.in>wasp2.0).

3.12. To study the 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 were 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. Other observations recorded were:

- Viability of antagonist for a duration of three months
- Effect of substrate on some functional characters like production of siderophore, HCN *etc.* by the antagonist

The experiment was conducted with three replicates per treatment. The viability of antagonists were analyzed by one-way analysis of variance (ANOVA). The significant difference if any, among the treatments were compared by using critical difference (CD) at p=0.05 significance level. Square root and arc sine transformed

values were done using WASP 2.0 software available online (www.ccari.res.in>wasp2.0).

3.13. Composition of different culture media, Reagents and Buffer

1. Potato Dextrose Agar (PDA)

Potato	200 g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

2. Malt Extract Agar (MEA)

Malt extract	30 g
Mycological peptone	5 g
Agar agar	15 g
Distilled water	1000 ml

3. Potato Dextrose Broth (PDB)

Potato	200 g
Dextrose	20 g
Distilled water	1000 ml

4. Chitinase Detection Medium

Colloidal chitin	4.5 g
Magnesium sulphate	0.3 g
Ammonium sulphate	3.0 g
Potassium dihydrogen phosphate	2.0 g
Citric acid monohydrate	1.0 g
Agar agar	15 g
Bromocresol purple	0.15 g
Tween-80	200 ul
Distilled water	1000ml
pH	4.7

5. **Chrome Azurol S (CAS) Blue Agar**

C.A.S (chrome Azurol S)	60.5 mg
Iron (III) solution (1 mM $FeCl_3.6H_2O$, 10 mM HCl)	10 ml
H.D.T.M.A. (Hexadecyl tri methyl-ammonium bromide)	72.9 mg
Agar agar	15 g
Pipes	30.24 g
NaOH	12 g
Distilled water	1000 ml

6. **HCN production**

Glycine	4.4gm/ml
Picric acid	0.5%
Sodium carbonate	2%
Whatsman no.1 filter paper	

7. **Indole-3-acetic acid (IAA) estimation test**

Tryptophan	2 ml/100 ml
Salkowski reagent (2% 0.5M $FeCl_3$ in 35% perchloric acid)	2 ml

8. **Pikovskaya's broth medium (PKV)**

Yeast extract	0.5 g
Dextrose	10 g
Calcium phosphate	5 g
Ammonium sulphate	0.5 g
Potassium chloride	0.2 g
Magnesium sulphate	0.1 g
Manganese sulphate	0.0001 g
Ferrous sulphate	0.0001 g
Distilled water	1000 ml

9. **Phosphate (P) solubilization**

Tricalcium phosphate	5 g/l
Ammonium molybdate	500 µl

Chlorostannous acid	13 µl
Whatman No.	42

10. DF (Difco) salts minimal medium

Potassium dihydrogen phosphate	4 g
Disodium hydrogen phosphate	6 g
Magnesium sulfate heptahydrate	0.2 g
Ferrous sulfate heptahydrate	0.1 g
Boric acid	10 ug
Manganese(II) sulfate	10 ug
Zinc sulphate	70 ug
Copper(II) sulfate	50 ug
Molybdenum (VI) oxide	10 ug
Glucose	2 g
Gluconic acid	2 g
Citric acid	2 g
Agar agar	12 g
Ammonium sulphate	0.002 g
Distilled water	1000 ml

11. Nessler's reagent

Mercuric chloride	10 g
Potassium iodide	7 g
Sodium hydroxide	16 g
Distilled water	100 ml

12. TBE buffer (10X)

Tris Base	121.1 g
Boric Acid	61.8 g
EDTA (disodium salt)	7.4 g
Distilled water	1000 ml

13. TBE buffer (1X)

TBE (10X)	100 ml
Distilled water	1000 ml

14. TBE buffer (0.5X)

TBE (10X)	50 ml
Distilled water	1000 ml

15. Ethidium bromide

Ethidium bromide	(10 mg/ml) 10 ul
Distilled water	200 ml

Chapter - 4

Experimental Results

T*richoderma* isolates collected from different habitats of all 11 districts of Meghalaya were identified, screened for their antagonism potentials against damping-off pathogens (P*ythium* sp. and *Rhizoctonia solani* kuhn.) during the present study and storage life of potential isolates were also studied. Results of the present findings are presented here under this chapter.

4.1. Isolation of Fungal Pathogens (*Pythium* sp. and *R. solani*)

Diseased samples collected from farmers' fields were brought to laboratory and isolations were done. With repeated isolations, *Pythium* sp. and *R. solani* Kuhn were consistently found with the infected seedlings of tomato.

4.1.1 Identification of *Pythium* sp.

On the 7th day of incubation, cornmeal agar (CMA) have a cottony aerial mycelium and with heavy aerial mycelia growth on the medium of potato dextrose agar (PDA). The mycelium was irregularly branched, coenocytic at initial stage and filamentous with full granular protoplasm. In older cultures, the mycelium was filamentous, hyaline and aseptate and found producing globose sporangia with smooth and spherical oospores. Based on the morphology characters, cultural characters and growth habit following the identification key (Alexopaulos *et al.*, 1996), the fungus was identified as *Pythium* sp by (Fig 1a).

4.1.2 Identification of *Rhizoctonia solani Kuhn*.

Fungus was fast growing, colonies dull white in colour initially and later became brown on potato dextrose agar (PDA). It was observed to produce light brown mycelium, with septate hyphae measuring around 5-14 µm width. It was observed branching at the right angles to the hyphal cell. Hyphae showed constriction at the point where branching was there. Also a barrel shaped sclerotia was observed with length of 12-18 µm. The individual sclerotium is less than 2.2 µm in diameter, with round shape and brown in colour. Based on the above diagnostic features and in comparison with the old cultures available in the Laboratory and following the identification key (Alexopaulos *et al.*, 1996), the pathogen was identified *R. solani* (Fig 1b). Observation was taken from 4th to 7th day of incubation.

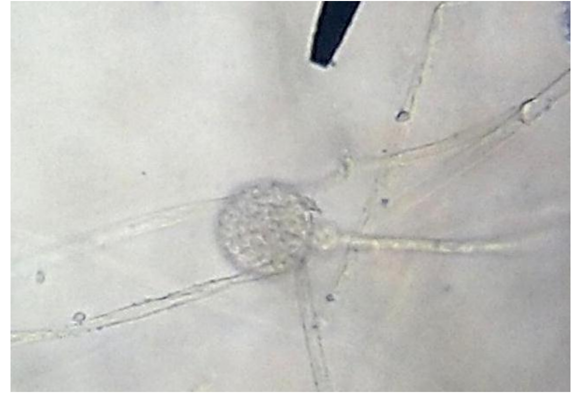


Fig. 1a: Culture of *Pythium* sp in petriplate and under microscope

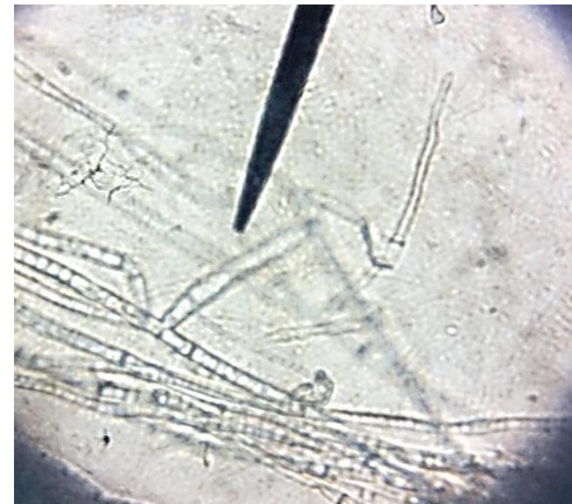


Fig. 1b: Culture of *Rhizoctonia solani* in petriplate and under microscope

4.2. Pathogenecity Test

4.2.1. Pathogenecity test of *Pythium* sp.

Pathogenecity test of *Pythium* species was carried out as described in 3.3. The symptoms observed were similar to the symptoms seen in the infected plants taken from the farmers' fields. The damping-off occurred on seedling stage after 10 days to 15 days of inoculation. In early stage, plants became stunted with typical discoloration at roots and formed small water soaked lesions on roots near to soil level. Brown spots coalesced together to formed large areas and profuse white mycelial growth was seen on collar region of the seedlings. In severe infection, the entire root found rotted and plants toppled over whereas the control plants did not exhibit any of these symptoms. The disease pathogen was isolated again from the diseased seedlings and fungus obtained resembled the original culture in all aspects.

4.2.2. Pathogenecity test of *Rhizoctonia solani Kuhn*

Pathogenicity test was done at seedling stage of tomato by soil inoculation method as mentioned in 3.3. The percent seed germination was poor in inoculated pots compared to control. Infected seedlings were stunted with shrivelled and dry lesion on the root just below the soil level and the cotyledons were deformed. The pathogen was re-isolated from the diseased plants and compared with original isolate and confirmed as *Rhizoctonia solani*.

4.3. Collection and isolation of *Trichoderma* from Different Locations of Meghalaya

Isolation of *Trichoderma* was done by dilution plate method as described in 3.4. Altogether 180 soil samples were collected from crop rhizosphere, coal mines, lime stone, pig manure, compost manure, jhum areas and forest of different locations of 11 districts of Meghalaya and results are shown in Table 1 along with details of host and location of soil samples collected from were presented. It was observed that 34 soil samples were collected from West Jaintia Hills, 33 samples from Ri Bhoi, 31 samples from East Khasi Hills, 24 from East Jaintia Hills, 18 from West Khasi Hills, 11 from East Garo Hills , 8 from South West Khasi Hills, 7 from South West Garo Hills, 6 from West Garo Hills, 5 from North Garo Hills and 3 from South Garo Hills (Fig 2).

Numbers of soil samples collected habitat wise are given in Table 2. From crop rhizosphere 161 soil samples, coal mine 5 samples, jhum areas 4 samples,

forest 3 samples, compost manure 3 samples, pig manure 2 samples and lime stone 2 samples.

Table 1. Host and location from where the soil samples were collected for isolation of *Trichoderma*

Sl. no.	Isolates	Host	Locations
1	TR1	Ginger	Mawkyndeng (West Jaintia Hills)
2	TR2	Sweet Potato	Mawkyndeng (West Jaintia Hills)
3	TR3	Cabbage	Mawkyndeng (West Jaintia Hills)
4	TR4	Pummelo	Mawkyndeng (West Jaintia Hills)
5	TR5	Turmeric	Raliang (West Jaintia Hills)
6	TR6	Chilli	Raliang (West Jaintia Hills)
7	TR7	Citrus	Raliang (West Jaintia Hills)
8	TR8	Peach	Shangpung (West Jaintia Hills)
9	TR9	Garden Pea	Shangpung (West Jaintia Hills)
10	TR10	Guava	Shangpung (West Jaintia Hills)
11	TR11	Onion	Shangpung (West Jaintia Hills)
12	TR12	Mustard	Laskein (West Jaintia Hills)
13	TR13	Bean	Laskein (West Jaintia Hills)
14	TR14	Papaya	Byndihati (East Jaintia Hills)
15	TR15	Coalmine	Byndihati (East Jaintia Hills)
16	TR16	Colocasia	Byndihati (East Jaintia Hills)
17	TR17	Coalmine	Byndihati (East Jaintia Hills)
18	TR18	Brinjal	Byndihati (East Jaintia Hills)
19	TR19	Turnip	Khliehriat (East Jaintia Hills)
20	TR20	Cauliflower	Khliehriat (East Jaintia Hills)
21	TR21	Mango	Khliehriat (East Jaintia Hills)
22	TR22	Bean	Khliehriat (East Jaintia Hills)
23	TR23	Leek	Khliehriat (East Jaintia Hills)
24	TR24	Tomato	Sutnga (East Jaintia Hills)
25	TR25	Bittergourd	Sutnga (East Jaintia Hills)
26	TR26	Marigold	Sutnga (East Jaintia Hills)
27	TR27	Ginger	Resubelpara (North Garo Hills)
28	TR28	Tomato	Resubelpara (North Garo Hills)
29	TR29	Onion	Resubelpara (North Garo Hills)
30	TR30	Brinjal	Daram (North Garo Hills)
31	TR31	Cowpea	Daram (North Garo Hills)
32	TR32	Chilli	Tura (West Garo Hills)
33	TR33	Carnation	Tura (West Garo Hills)
34	TR34	Raddish	Tura (West Garo Hills)
35	TR35	Colocasia	Rongram (West Garo Hills)
36	TR36	Turmeric	Rongram (West Garo Hills)
37	TR37	Black berry	Rongram (West Garo Hills)
38	TR38	Coalmine	Dkhiah (East Jaintia Hills)
39	TR39	Ginger	Dkhiah (East Jaintia Hills)
40	TR40	Garlic	Ampati (South West Garo Hills)

41	TR41	Chilli	Ampati (South West Garo Hills)
42	TR42	Cauliflower	Ampati (South West Garo Hills)
43	TR43	Raddish	Ampati (South West Garo Hills)
44	TR44	Mustard	Ampati (South West Garo Hills)
45	TR45	Garden Pea	Betasing (South West Garo Hills)
46	TR46	Pumpkin	Betasing (South West Garo Hills)
47	TR47	Citrus	Williamnagar (East Garo Hills)
48	TR48	Pear	Williamnagar (East Garo Hills)
49	TR49	Ginger	Williamnagar (East Garo Hills)
50	TR50	Jhum Rice	Williamnagar (East Garo Hills)
51	TR51	Turmeric	Rongjeng (East Garo Hills)
52	TR52	Cabbage	Rongjeng (East Garo Hills)
53	TR53	Brinjal	Rongjeng (East Garo Hills)
54	TR54	Chilli	Rongjeng (East Garo Hills)
55	TR55	Tomato	Mawkyndeng (West Jaintia Hills)
56	TR56	Turmeric	Mawkyndeng (West Jaintia Hills)
57	TR57	Citrus	Sumer (Ri Bhoi)
58	TR58	Jackfruit	Songsak (East Garo Hills)
59	TR59	Cabbage	Songsak (East Garo Hills)
60	TR60	Chilli	Songsak (East Garo Hills)
61	TR61	Mustard	Sumer (Ri Bhoi)
62	TR62	Bean	Sumer (Ri Bhoi)
63	TR63	Bittergourd	Sumer (Ri Bhoi)
64	TR64	Potato	Mawkyndeng (West Jaintia Hills)
65	TR65	Cauliflower	Mawkyndeng (West Jaintia Hills)
66	TR66	Brinjal	Mawkyndeng (West Jaintia Hills)
67	TR67	Pig Manure	Sumer (Ri Bhoi)
68	TR68	Rice	Sumer (Ri Bhoi)
69	TR69	Jhum Paddy	Baghmara (South Garo Hills)
70	TR70	Banana	Baghmara (South Garo Hills)
71	TR71	Turmeric	Baghmara (South Garo Hills)
72	TR72	Compost Manure	Mawkynrew (East khasi Hills)
73	TR73	French Bean	Mawkynrew (East khasi Hills)
74	TR74	Forest	Mawsmai (East khasi Hills)
75	TR75	Lime Stone	Mawsmai (East khasi Hills)
76	TR76	Cauliflower	Sohra (East khasi Hills)
77	TR77	Bean	Sohra (East khasi Hills)
78	TR78	Brinjal	Sohra (East khasi Hills)
79	TR79	Maize	Pynursla (East khasi Hills)
80	TR80	Sesamum	Pynursla (East khasi Hills)
81	TR81	Turmeric	Pynursla (East khasi Hills)
82	TR82	Tomato	Pynursla (East khasi Hills)
83	TR83	Pumpkin	Laitkor (East khasi Hills)
84	TR84	Paddy	Laitkor (East khasi Hills)
85	TR85	Lime Stone	Mawsmai (East khasi Hills)
86	TR86	Mustard	Laitkor (East khasi Hills)

87	TR87	Jhum Paddy	Sumer (Ri Bhoi)
88	TR88	Forest	Mawphlang (East khasi Hills)
89	TR89	Paddy	Mawphlang (East khasi Hills)
90	TR90	Bean	Mawryngkneng (East khasi Hills)
91	TR91	Compost Manure	Mawryngkneng (East khasi Hills)
92	TR92	Turmeric	Mawryngkneng (East khasi Hills)
93	TR93	Onion	Mawryngkneng (East khasi Hills)
94	TR94	Paddy	Umiam (Ri Bhoi)
95	TR95	Tomato	Umiam (Ri Bhoi)
96	TR96	Garlic	Umiam (Ri Bhoi)
97	TR97	Beet Root	Umiam (Ri Bhoi)
98	TR98	Coriander	Umiam (Ri Bhoi)
99	TR99	Lettuce	Umiam (Ri Bhoi)
100	TR100	Soyabean	Umiam (Ri Bhoi)
101	TR101	Mustard	Umiam (Ri Bhoi)
102	TR102	Carrot	Umiam (Ri Bhoi)
103	TR103	Maize	Umiam (Ri Bhoi)
104	TR104	Peach	Umiam (Ri Bhoi)
105	TR105	Garden Pea	Umiam (Ri Bhoi)
106	TR106	Tomato	Umiam (Ri Bhoi)
107	TR107	Bean	Umiam (Ri Bhoi)
108	TR108	Citrus	Umiam (Ri Bhoi)
109	TR109	Forest	Mawphlang (East khasi Hills)
110	TR110	Maize	Mawphlang (East khasi Hills)
111	TR111	Mustard	Mawkyndeng (West Jaintia Hills)
112	TR112	Tomato	Mawkyndeng (West Jaintia Hills)
113	TR113	Jhum Paddy	Mawkyndeng (West Jaintia Hills)
114	TR114	Turmeric	Mawkyndeng (West Jaintia Hills)
115	TR115	French bean	Mawkyndeng (West Jaintia Hills)
116	TR116	Brinjal	Mawkyndeng (West Jaintia Hills)
117	TR117	Cauliflower	Mawkyndeng (West Jaintia Hills)
118	TR118	Potato	Mawkyrwat (South West Khasi)
119	TR119	Onion	Mawkyrwat (South West Khasi)
120	TR120	Bean	Mawkyrwat (South West Khasi)
121	TR121	Ginger	Mawkyrwat (South West Khasi)
122	TR122	Tomato	Mawkyrwat (South West Khasi)
123	TR123	Cabbage	Mawkyrwat (South West Khasi)
124	TR124	Mustard	Mawkyrwat (South West Khasi)
125	TR125	Garlic	Mawkyrwat (South West Khasi)
126	TR126	Onion	Nongpoh (Ri Bhoi)
127	TR127	Mustard	Nongpoh (Ri Bhoi)
128	TR128	Marigold	Nongpoh (Ri Bhoi)
129	TR129	Chilli	Umsning (Ri Bhoi)
130	TR130	Bean	Umsning (Ri Bhoi)
131	TR131	Pea	Umsning (Ri Bhoi)
132	TR132	Chilli	Umsning (Ri Bhoi)

133	TR133	Bean	Umsning (Ri Bhoi)
134	TR134	Pumpkin	Nongstoin (West Khasi Hills)
135	TR135	Pig Manure	Nongstoin (West Khasi Hills)
136	T136	Tomato	Nongstoin (West Khasi Hills)
137	TR137	Soyabean	Nongstoin (West Khasi Hills)
138	TR138	Tomato	Nongstoin (West Khasi Hills)
139	TR139	Paddy	Nongstoin (West Khasi Hills)
140	TR140	Pine Tree	Nongstoin (West Khasi Hills)
141	TR141	Tomato	Nongstoin (West Khasi Hills)
142	TR142	Ginger	Mawsynram (West Khasi Hills)
143	TR143	Sweet Potato	Mawsynram (West Khasi Hills)
144	TR144	Celery	Mawsynram (West Khasi Hills)
145	TR145	Carrot	Mawsynram (West Khasi Hills)
146	TR146	Maize	Mawsynram (West Khasi Hills)
147	TR147	Coriander	Mairang (West Khasi Hills)
148	TR148	Compost Manure	Mairang (West Khasi Hills)
149	TR149	Cauliflower	Mairang (West Khasi Hills)
150	TR150	Beet root	Mairang (West Khasi Hills)
151	TR151	Groundnut	Mairang (West Khasi Hills)
152	TR152	Mustard	Upper Shillong (East Khasi Hills)
153	TR153	Banana	Upper Shillong (East Khasi Hills)
154	TR154	Citrus	Upper Shillong (East Khasi Hills)
155	TR155	Tomato	Upper Shillong (East Khasi Hills)
156	TR156	Bean	Upper Shillong (East Khasi Hills)
157	TR157	Pumpkin	Sumer (Ri Bhoi)
158	TR158	Pea	Sumer (Ri Bhoi)
159	TR159	Cauliflower	Sumer (Ri Bhoi)
160	TR160	Carrot	Laitumkhrah (East Khasi Hills)
161	TR161	Chilli	Laitumkhrah (East Khasi Hills)
162	TR162	Pea	Laitumkhrah (East Khasi Hills)
163	TR163	Brinjal	Phramer (West Jaintia Hills)
164	TR164	Bean	Phramer (West Jaintia Hills)
165	TR165	Coriander	Phramer (West Jaintia Hills)
166	TR166	Turmeric	Phramer (West Jaintia Hills)
167	TR167	Celery	Saipung (East Jaintia Hills)
168	TR168	Chilli	Saipung (East Jaintia Hills)
169	TR169	Bitter Brinjal	Saipung (East Jaintia Hills)
170	TR170	Pea	Saipung (East Jaintia Hills)
171	TR171	Tomato	Byndihati (East Jaintia Hills)
172	TR172	Sweet Potato	Byndihati (East Jaintia Hills)
173	TR173	Citrus	Byndihati (East Jaintia Hills)
174	TR174	Marigold	Mawkyndeng (West Jaintia Hills)
175	TR175	Pine Tree	Mawkyndeng (West Jaintia Hills)
176	TR176	Carrot	Mawkyndeng (West Jaintia Hills)
177	TR177	Chilli	Mawkyndeng (West Jaintia Hills)
178	TR178	Coal Mine	Byndihati (East Jaintia Hills)

179	TR179	Coal Mine	Byndihati (East Jaintia Hills)
180	TR180	Paddy	Mawkyndeng (West Jaintia Hills)

Table 2. Number of soil sample collected and their habitats

Sl. No.	Habitat	Total Number
1.	Crop Rhizosphere	161
2.	Forest	3
3.	Compost Manure	3
4.	Pig Manure	2
5.	Coal mine	5
6.	Lime stone	2
7.	Jhum Areas	4
Total		**180**

4.4. Morphological characterization

Soil samples collected from different locations of Meghalaya were checked for their morphological characters. 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 (Fig 3). The selected *Trichoderma* isolates were inoculated on Malt Extract Agar (MEA) medium to observe conidiophores, phialides and conidia. Based on taxonomic keys provided by Rifai (1969) and Bisset (1992), it is evident that altogether ninety seven (97) *Trichoderma* isolates were isolated from 180 soil samples collected (Table 3). Maximum *Trichoderma* isolates were obtained from East Khasi Hills (21 isolates), followed by West Jaintia Hills and Ri Bhoi (17 isolates each); East Jaintia Hills (10 isolates); West Khasi Hills and South West Garo Hills (6 isolates each); South West Khasi hills (5 isolates); North Garo hills, East Garo hills and West Garo Hills (4 isolates each) and least from South Garo Hills (3 isolates). Out of the 7 habitats collected, maximum were obtained from crop rhizosphere (87), followed by forest (3), jhum areas (3), compost manure (2), pig manure (1) and limestone (1). Although 5 samples were collected from coal mines but no isolate was obtained from coalmine.

Table 3: List of the *Trichoderma* isolates obtained from different districts of Meghalaya, India on the basis of morphological observation

Sl. No.	District	Habitat	*Trichoderma* Isolates
1.	West Jaintia Hills	Crop rhizosphere	TR1, TR2, TR3, TR5, TR6, TR9, TR10, TR11, TR12, TR13, TR55, TR64, TR66, TR112, TR114, TR116, TR174
2.	East Jaintia Hills	Crop rhizosphere	TR14, TR16, TR18, TR20, TR22, TR24, TR26, TR39, TR171, TR172
3.	East khasi Hills	Crop rhizosphere	TR73, TR76, TR77, TR78, TR80, TR81, TR82, TR83, TR84, TR86, TR89, TR90, TR92, TR93, TR155
		Forest	TR74, TR88, TR109
		Compost Manure	TR72, TR91
		Limestone	TR75
4.	West Khasi Hills	Crop rhizosphere	TR136, TR138, TR140, TR141, TR142, TR143
5.	South West Khasi	Crop rhizosphere	TR118, TR119, TR120, TR122, TR123
6.	Ri Bhoi	Crop rhizosphere	TR61, TR94, TR95, TR96, TR97, TR98, TR99, TR100, TR101, TR102, TR103, TR105, TR106, TR107, TR108
		Jhum area	TR87
		Pig Manure	TR67
7.	East Garo Hills	Jhum area	TR50
		Crop rhizosphere	TR51, TR59, TR60
8.	West Garo Hills	Crop rhizosphere	TR32, TR33, TR35, TR36
9.	North Garo Hills	Crop rhizosphere	TR27, TR28, TR29, TR30
10.	South Garo Hills	Crop rhizosphere	TR70, TR71
		Jhum area	TR69
11.	South West Garo	Crop rhizosphere	TR40, TR41, TR42, TR43, TR44, TR46

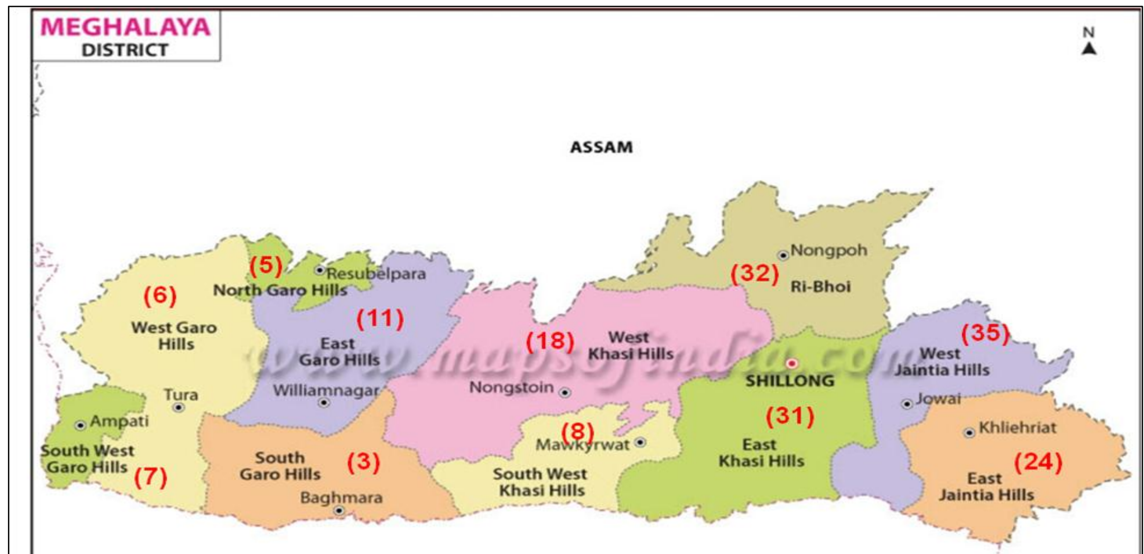


Fig. 2: Schematic representation of number of soil samples collected from 11 districts of Meghalaya

Fig. 3: Isolates showing the lime green to greenish colour sporulation

4.5. Screening of isolated *Trichoderma* for functional attributes *in vitro*

4.5.1. Rapid screening of *Trichoderma* isolates

Rapid screening of *Trichoderma* isolates against *Pythium* sp. and *R. solani* Kuhn. by dual culture assay on potato dextrose agar (PDA) medium were studied and presented in Table 4 and Fig 4. The results showed that 77 isolates attained S_4 stage (the pathogen and antagonist locked at the point of contact) for both the pathogens, whereas 7 isolates showed S_3 stage (the antagonized colonized half of the growth of pathogen i.e. 50 % overgrowth) for *R. solani* Kuhn. and 4 isolates for *Pythium* sp., 13 isolates showed S_2 stage (the antagonist overgrew at least 2/3rd growth of the pathogen *i.e*. 75 % growth) for *R. solani* Kuhn. and 16 isolates for *Pythium* sp. The observation was made from 4-7days after inoculation based on modified Bell's scales (Bell *et al.,* 1982).

4.5.2 *In vitro* antagonistic potential of the isolates of *Trichoderma*

In vitro antagonistic potential of those 20 isolates of *Trichoderma* sp. *i.e*., TR12, TR24, TR36, TR40, TR55, TR64, TR66, TR74, TR78, TR82, TR87, TR88, TR106, TR109, TR112, TR116, TR122, TR136, TR143 and TR171 which showed S_2 and S_3 stage based on modified Bell's scale were evaluated against *Pythium* sp. and *R. solani* Kuhn. through dual culture assay (Table 5 and Fig 5).

4.5.2.1. Dual culture assay against *Pythium* sp

The dual culture testagainst *Trichoderma* sp. and *Pythium* sp revealved that TR55 had the smallest diameter of radial growth with only 0.97 cm followed by TR122 (1.00 cm), TR66 (1.07 cm), TR136 (1.10 cm), TR143 (1.47 cm), TR88 (1.57 cm), TR112 and TR171 (1.63 cm), TR106 (1.70 cm), TR78 and TR116 (1.83 cm), TR82 (1.93 cm), TR64 (1.97 cm), TR74 and TR12 (2 cm), TR40 (2.23 cm), TR109 (2.33 cm), TR87 (2.37 cm), TR24 (2.43 cm) and largest radial growth in TR36 (2.80 cm). From table 5, it is evident that all 20 *Trichoderma* isolates tested against *Pythium* sp. were significantly superior over control with highest inhibition percent recorded in TR55 (89.26 %), followed by TR122 (88.89 %), TR66 (88.15 %), TR136 (87.78 %), TR143 (83.70 %), TR88 (82.59 %), TR112 and TR171 (81.85 %), TR106 (81.11 %), TR78 and TR116 (79.63 %), TR82 (78.52 %), TR64 (78.15%), TR74 and TR12 (77.78 %), TR40 (75.19 %),TR109 (74.07 %),TR87 (73.70 %), TR24 (72.96 %) and the least was observed in TR36 (with 68.89 %).

Table 4. Rapid screening of different *Trichoderma* isolates against *Pythium* sp. and *R. solani* Kuhn. based on modified Bell's scale (Bell *et al.*, 1982)

Sl. no.	*Trichoderma* isolates	Bell's scale	Sl.no.	*Trichoderma* isolates	Bell's scale	Sl. no	*Trichoderma* isolates	Bell's scale
1.	TR12	S2**	**2.**	TR24	S3*/S2#	**3.**	TR36	S3**
4.	TR40	S2**	**5.**	TR55	S2**	**6.**	TR64	S2**
7.	TR66	S2**	**8.**	TR74	S2**	**9.**	TR78	S2*/S3#
10.	TR82	S2**	**11.**	TR87	S3*/S2#	**12.**	TR88	S2**
13.	TR106	S2*/S3#	**14.**	TR109	S3*/S2#	**15.**	TR112	S2*/S3#
16.	TR116	S2*/S3#	**17.**	TR122	S2**	**18.**	TR136	S2**
19.	TR143	S2*/S3#	**20.**	TR171	S2*/S3#	**21.**	TR2	S4**
22.	TR5	S4**	**23.**	TR6	S4**	**24.**	TR18	S4**
25.	TR20	S4**	**26.**	TR22	S4**	**27.**	TR26	S4**
28.	TR27	S4**	**29.**	TR32	S4**	**30.**	TR33	S4**
31.	TR35	S4**	**32.**	TR39	S4**	**33.**	TR44	S4**
34.	TR50	S4**	**35.**	TR59	S4**	**36.**	TR61	S4**
37.	TR67	S4**	**38.**	TR69	S4**	**39.**	TR70	S4**
40.	TR72	S4**	**41.**	TR73	S4**	**42.**	TR75	S4**
43.	TR76	S4**	**44.**	TR77	S4**	**45.**	TR80	S4**
46.	TR81	S4**	**47.**	TR83	S4**	**48.**	TR84	S4**
49.	TR86	S4**	**50.**	TR91	S4**	**51.**	TR94	S4**
52.	TR96	S4**	**53.**	TR98	S4**	**54.**	TR100	S4**
55.	TR101	S4**	**56.**	TR103	S4**	**57.**	TR114	S4**
58.	TR118	S4**	**59.**	TR120	S4**	**60.**	TR123	S4**
61.	TR138	S4**	**62.**	TR140	S4**	**63.**	TR155	S4**
64.	TR1	S4**	**65.**	TR3	S4**	**66.**	TR9	S4**
67.	TR10	S4**	**68.**	TR11	S4**	**69.**	TR13	S4**
70.	TR14	S4**	**71.**	TR28	S4**	**72.**	TR29	S4**
73.	TR30	S4**	**74.**	TR41	S4**	**75.**	TR42	S4**
76.	TR43	S4**	**77.**	TR46	S4**	**78.**	TR51	S4**
79.	TR60	S4**	**80.**	TR71	S4**	**81.**	TR174	S4**
82.	TR89	S4**	**83.**	TR90	S4**	**84.**	TR92	S4**
85.	TR93	S4**	**86.**	TR95	S4**	**87.**	TR97	S4**
88.	TR99	S4**	**89.**	TR16	S4**	**90.**	TR102	S4**
91.	TR105	S4**	**92.**	TR107	S4**	**93.**	TR108	S4**
94.	TR119	S4**	**95.**	TR141	S4**	**96.**	TR142	S4**
97.	TR172	S4**						

N.B: Mean of the 3 (three) replications: * for *Pythium* sp.; # for *Rhizoctonia solani* and ** both

Table 5. *In vitro* efficacy of *Trichoderma* sp. against *Pythium* sp. and *R. solani* Kuhn. through the dual culture assay.

Sl. N0.	*Trichoderma* isolates	Growth (cm)		Per cent inhibition over control	
		***Pythium* sp**	***R. solani***	***Pythium* sp**	***R. solani***
1.	TR12	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.	TR24	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.	TR36	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} r(59.39)
4.	TR40	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.	TR55	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.	TR64	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.	TR66	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.	TR74	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.	TR78	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.	TR82	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.	TR87	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.	TR88	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.	TR106	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.	TR109	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.	TR112	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.	TR116	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.	TR122	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.	TR136	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.	TR143	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.	TR171	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

N.B: Mean of the 3 (three) replications

The figures in parentheses are values of square root transformed for growth and values ofarc sine transformed forper cent inhibition over control.

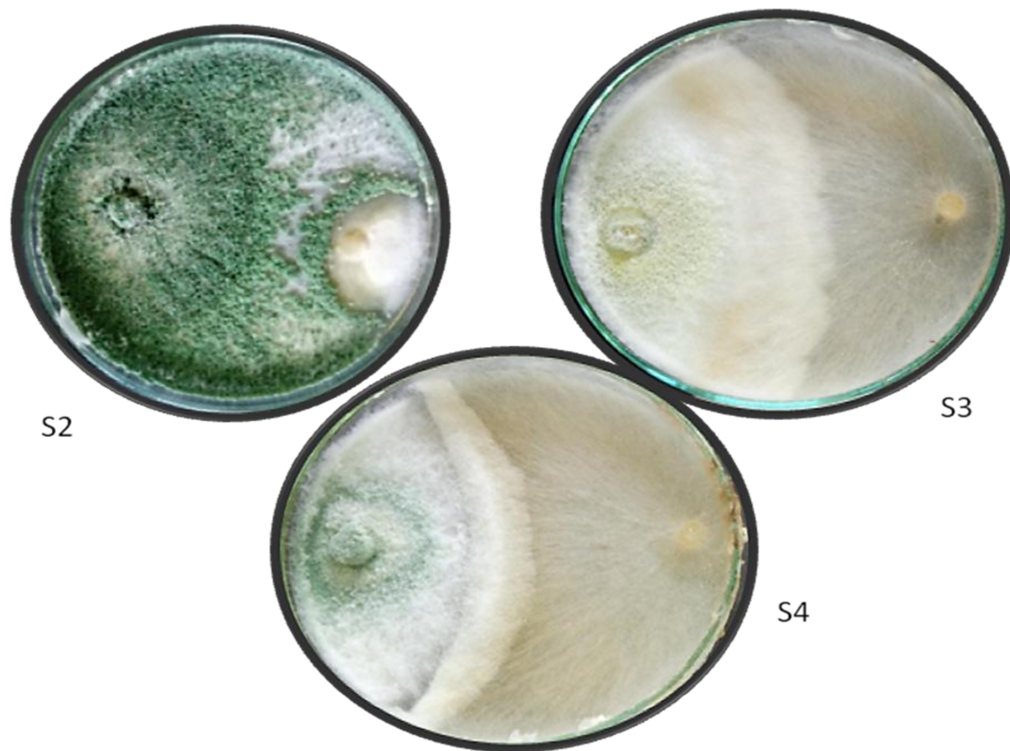


Fig 4: **Rapid screening of** ***Trichoderma*** **sp. following Modified Bell's scale**.

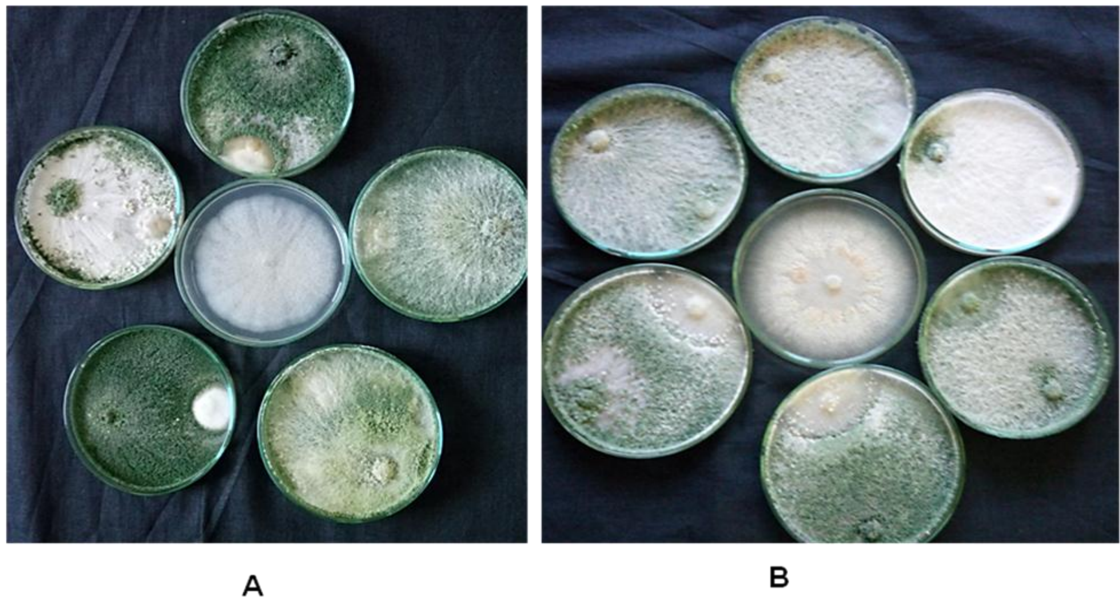


Fig. 5: Dual culture assay of (A) ***Pythium*** **sp. (B)** ***R. solani*** **against** ***Trchoderma*** **sp.**

4.5.2.2. Dual culture assay against *Rizoctonia solani* kuhn

Against *R. solani* Kuhn. the smallest diameter of radial growth with only 1.13 cm was seen in TR55 followed by TR122 (1.22 cm), TR66 (1.43 cm), TR88 (1.53 cm), TR74 (1.73 cm), TR136 (1.82 cm), TR12 (1.9 cm), TR64 (1.97 cm), TR24 and TR40 (2.07 cm), TR109 (2.12 cm), TR87 (2.18 cm), TR82 (2.23 cm), TR106 (2.30 cm), TR36 (2.33 cm), TR116 (2.35 cm), TR143 (2.37 cm), TR78 (2.4 cm), TR112 (2.63 cm) and largest radial growth in TR171 (2.7 cm). Maximum inhibition percent was recorded in TR55 (87.41 %), followed by TR122 (86.48 %), TR66 (84.07 %), TR88 (82.96 %), TR74 (80.74 %), TR136 (79.81 %), TR12 (78.89 %), TR64 (78.15 %), TR24 and TR40 (77.04 %), TR109 (76.48 %), TR87 (75.74 %), TR82 (75.19 %), TR106 (77.44 %), TR36 (74.07 %), TR116 (73.89 %), TR143 (73.70 %), TR78 (73.33 %), TR112 (70.74 %) and least TR171 (70.00 %). All isolates showed effective in inhibiting the radial growth of the pathogens with more than 70 per cent of inhibition over control.

4.5.3. Screening for antimicrobial traits of *Trichoderma* isolates

Detection of the genes that encode for the production of antibiotics by PCR using gene-specific primers was done for the 20 isolates of *Trichoderma* sp. *i.e.*, TR12, TR24, TR36, TR40, TR55, TR64, TR66, TR74, TR78, TR82, TR87, TR88, TR106, TR109, TR112, TR116, TR122, TR136, TR143 and TR171 and the results presented in the Table 6 and Fig 6 (a,b,c,d).

4.5.3.1. Detection of β-1,6-Glucanase (*Tvbgn3*)

Polymerase chain reaction (PCR) based method as described in 3.7.3.1 was employed to detect presence of *Tvbgn3* gene involved in degradation of β -1,3-glucan which contribute to the efficient disorganization and further degradation of the host cell walls that occur during mycoparasitism. Out of the 20 isolates of *Trichoderma*, 12 isolates were amplified at the expected amplicon size of 200 base pair (bp). Presence of expected amplification (200 bp) is denoted with “+” and without expected amplification is denoted as “-" (Table 6 and Fig 6a).

4.5.3.2. Detection of Trichodiene synthase (*tri5*)

Ten *Trichoderma* isolates showed expected amplicon size at 500 bp which was obtained when one primer set tri5F and tri5R was used for detection of the presence of trichodiene synthase (*tri*5) gene involved in production of toxic metabolites trichothecenes. Presence of expected amplification (500 bp) is denoted with “+” and without expected amplification is denoted as “-" (Table 6 and Fig 6b).

Table 6. Detection of antibiotic biosynthetic genes in *Trichoderma* sp

Sl. No.	*Trichoderma* sp	β-1,6-Glucanase (*Tvbgn3*)	Trichodiene synthase (*tri5*)	Serine protease (*prb1*)	Endochitinase (*ech42*)
1.	TR12	+	-	-	+
2.	TR24	-	-	+	+
3.	TR36	-	-	+	+
4.	TR40	-	-	+	+
5.	TR55	+	+	+	+
6.	TR64	+	+	-	+
7.	TR66	+	+	+	+
8.	TR74	-	-	+	+
9.	TR78	-	-	+	+
10.	TR82	+	-	-	-
11.	TR87	+	+	-	+
12.	TR88	-	+	+	+
13.	TR106	+	-	+	+
14.	TR109	+	-	+	+
15.	TR112	+	+	-	+
16.	TR116	+	-	+	-
17.	TR122	+	+	+	+
18.	TR136	+	+	+	+
19.	TR143	-	+	-	+
20.	TR171	-	+	+	-

N.B: “+” indicates presence of the genes; “-“indicates absence of the genes

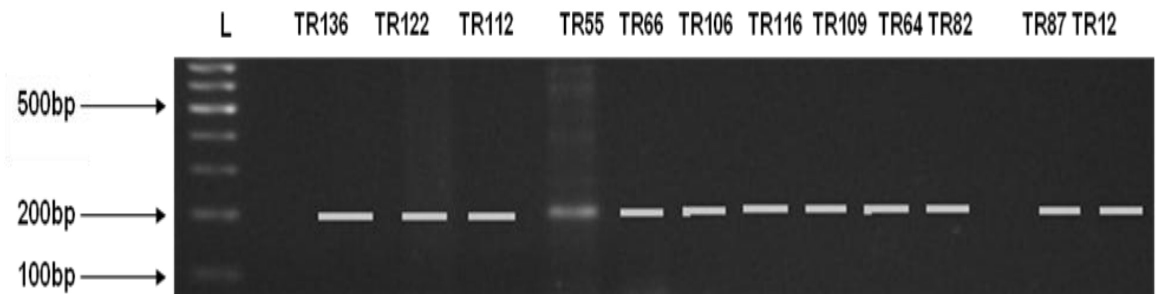


Fig. 6a: ***Trichoderma*** **Isolates showing amplification of β-1,6-Glucanase (200bp) using primer Tvbgn3F and Tvbgn3R**

L= 100bp ladder; Lane 1-12= ***Trichoderma*** **isolates**

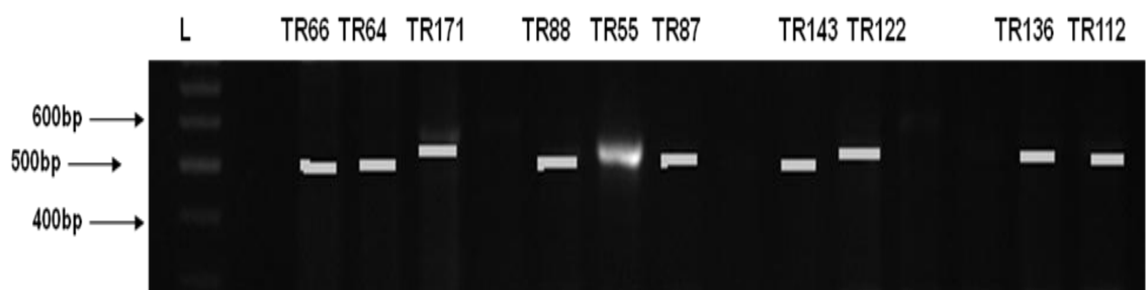


Fig. 6b: ***Trichoderma*** **isolates showing amplification of Trichodiene synthase (500bp) using primer tri5F and tri5R**

L= 100bp ladder; Lane 1-10= ***Trichoderma*** **isolates**

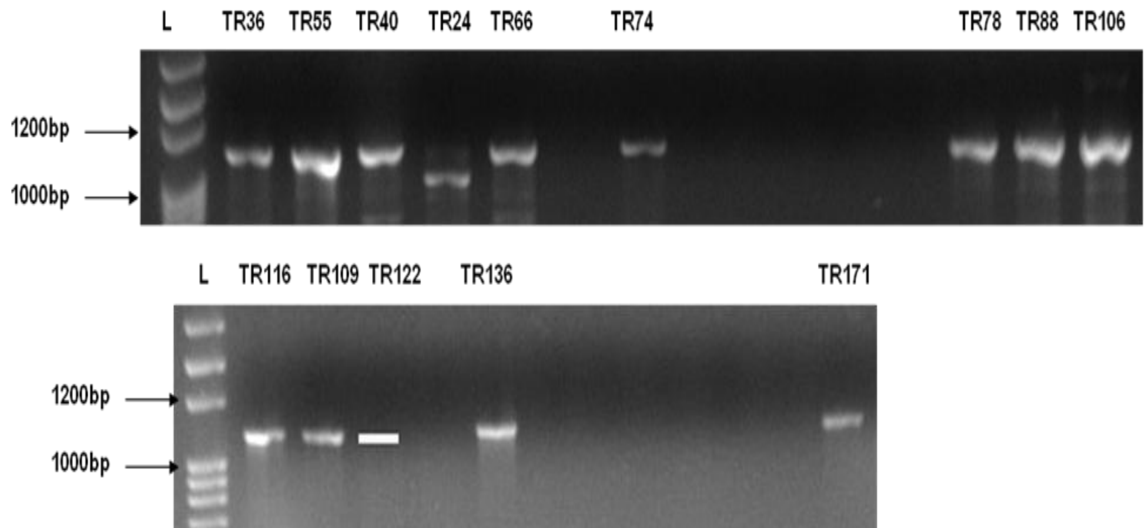


Fig. 6c: *Trichoderma* isolates showing amplification of Serine protease (800-1200bp) using primer serf and serR

L= 100bp ladder; Lane 1-14= *Trichoderma* isolates

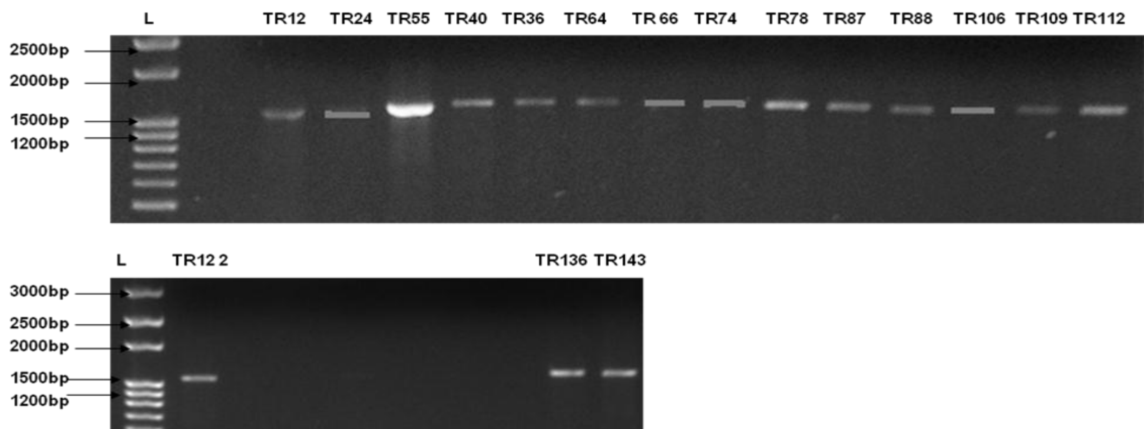


Fig. 6d: *Trichoderma* isolates showing amplification of Serine protease (1500bp) using primer ech42F and ech42R

L= 1kb ladder; Lane 1-17= *Trichoderma* isolates

4.5.3.3. Detection of Serine protease (*ser*)

Fourteen *Trichoderma* isolates showed expected amplicon size at 1100 bp which was obtained when one primer set serF and serR was used for detection of the presence of serine protease (*ser*) gene involved in degrading the protein components of the host cell wall as well as deactivation of the plant pathogens' enzymes. Presence of expected amplification (800-1200 bp) is denoted with "+" and without expected amplification is denoted as "-" (Table 6 and Fig 6c).

4.5.3.4. Detection of Endochitinase (*ech42*)

Seventeen *Trichoderma* isolates showed expected amplicon size at 1500 bp which was obtained when one primer set ech42F and ech42R was used for detection of the presence of endochitinase (ech42) gene involved in degradation of fungal cell wall chitin. Presence of expected amplification (1500 bp) is denoted with "+" and without expected amplification is denoted as "-" (Table 6 and Fig 6d).

4.5.4. Chitinolytic Enzyme Assay

The 20 *Trichoderma* isolates *i.e.*, TR12, TR24, TR36, TR40, TR55, TR64, TR66, TR74, TR78, TR82, TR87, TR88, TR106, TR109, TR112, TR116, TR122, TR136, TR143 and TR171 tested for chitinolytic activity on the medium used for chitin detection after 3 days of incubation. Colour intensity and diameter of the purple coloured zone were taken as the criteria to determine the chitinase activity and results are presented in table 7 and fig 7. For colour intensity, '+' indicates light purple, '++' dark purple and '+++' very dark purple. From the table, it is evident that 17 isolates showed chitinase activity with maximum diameter of 9 cm and purple colour intensity (+++) recorded in TR106 and TR136, followed by TR122 (8.23 cm and +++), TR12 (7.53 cm and +), TR24 (7.03 cm and +++), TR66 (6.97 cm and ++), TR55 (6.80 cm and ++), TR112 (5.47 cm and +), TR143 (4.9 cm and ++), TR87 (3.9 cm and ++), TR88 (3.9 cm and +), TR78 (3.27 cm and +), TR36 (3.43 cm and +), TR74 (2.5 cm and +), TR64 (2.17 cm and +++), TR40 (1.73 cm and ++) and least in TR109 (1.47 cm and +). In TR82, TR116 and TR171 there was no chitinase activity recorded.

4.5.5. Qualitative Assay for Siderophore Production

The ability of *Trichoderma* isolates to produce iron-binding compounds of siderophore-type was detected in solid medium by universal C.A.S assay. Color-changed from blue to purple or dark purplish- red (magenta) in the C.A.S.-blue agar, starting from the borderline between the two medias was noted as positive for the siderophore production and results showed that qualitative assay of 20 *Trichoderma*

Table 7. Chitinase production of *Trichoderma* isolates measured in diameter (cm)

Sl. No.	*Trichoderma* isolates	*Chitinase production (cm)	Purple Colour intensity
1.	TR12	7.53±0.13[c] (2.74)	+
2.	TR24	7.03±0.03[d] (2.65)	+++
3.	TR36	3.43±0.07[h] (1.85)	+
4.	TR40	1.73±0.07[k] (1.31)	++
5.	TR55	6.80±0.06[d] (2.60)	++
6.	TR64	2.17±0.03[j] (1.47)	+++
7.	TR66	6.97±0.09[d] (2.63)	++
8.	TR74	2.50±0.00[i] (1.58)	+
9.	TR78	3.27±0.28[h] (1.80)	+
10.	TR87	3.90±0.06[g] (1.97)	++
11.	TR88	3.90±0.06[g] (1.97)	+
12.	TR106	9.00±0.00[a] (3.00)	+++
13.	TR109	1.47±0.03[l] (1.21)	+
14.	TR112	5.47±0.09[e] (2.33)	+
15.	TR122	8.23±0.09[b] (2.86)	+++
16.	TR136	9.00±0.00[a] (3.00)	+++
17.	TR143	4.90±0.10[f] (2.21)	++
SE(m)		0.002	
CD (p=0.05)		0.091	

N.B: Mean of three replications; (+) indicates light color, (++) indicates dark color and (+++) indicates very dark color

Figures in parentheses are square root transformed values and values of arc sine transformed for purple color diameter.

Table 8. Qualitative assay of siderophore and HCN production by *Trichoderma* sp

Sl. No.	*Trichoderma* sp	Siderophore Production #	HCN Production **
1.	TR12	*++	-
2.	TR24	++	-
3.	TR36	+	-
4.	TR40	+++	-
5.	TR55	+++	++
6.	TR64	++	+
7.	TR66	+	++
8.	TR74	-	+
9.	TR78	++	-
10.	TR82	++	-
11.	TR87	++	+
12.	TR88	+	++
13.	TR106	++	+
14.	TR109	-	++
15.	TR112	+	++
16.	TR116	-	-
17.	TR122	++	++
18.	TR136	++	++
19.	TR143	-	+
20.	TR171	++	+

N.B: Mean of three replications

* Degree of activity (+ + + + + > + + + + > + + + > + + > +)

** Colour, ranged deep yellow (+) to orange (++) to orange brown or dark brown (+++)

Colour, ranged blue to purple (+) dark purplish (++) to red (magenta) (+ ++)

(-) indicates no production and (+) indicates production

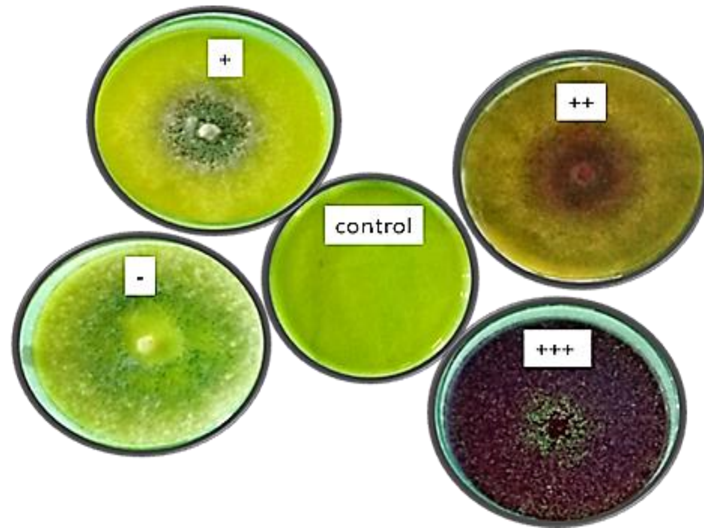


Fig. 7: Chitinase production test

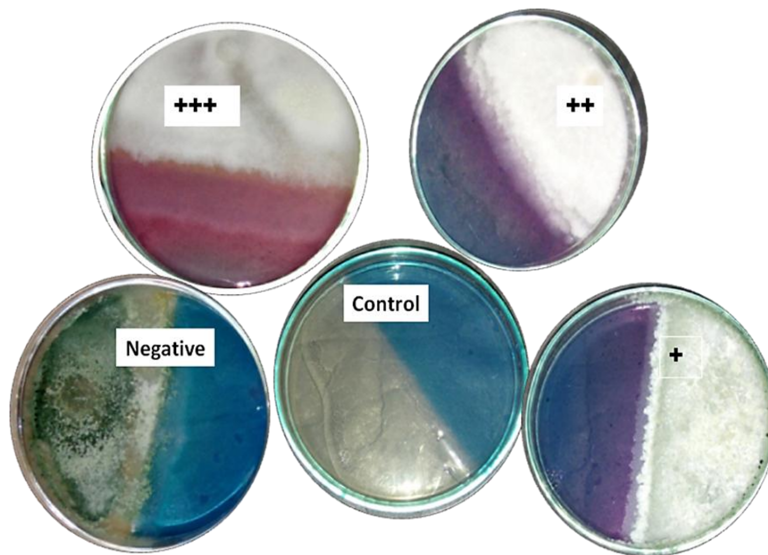


Fig. 8a: Siderophore production test

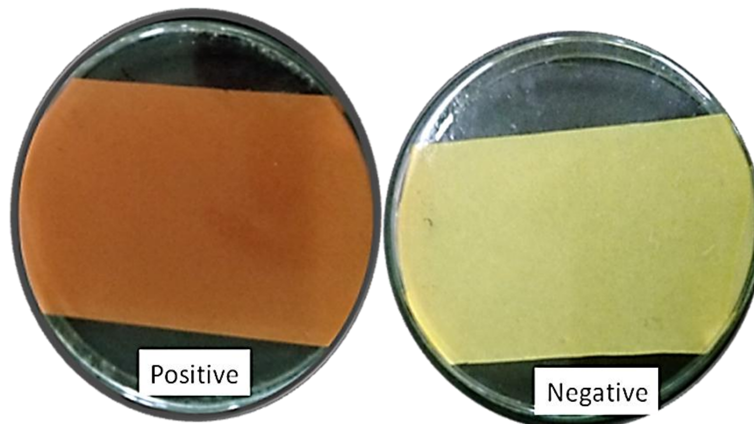


Fig. 8b: HCN production test

isolates revealed different response for their ability to produce siderophore production (Table 8 and Fig 8a). From the table, it is evidence that 2 isolates *viz*., TR55 and TR40 showed +++, whereas 10 isolates *viz*., TR12, TR24, TR64, TR78, TR82, TR87,TR106, TR122, TR136 and TR171 showed ++ and 3 isolates *viz*., TR36, TR66 and TR88 showed +. In TR74, TR109, TR116 and TR143 there were no colour change observed.

4.5.6. HCN production

HCN production was tested by amending PDA medium with 4.4 g/ml glycine and results are presented in table 8 and fig 8b. Scoring of negative (-) was given for absence of production and positive (+) for production, which was observed by colour change from yellow to deep yellow (+) to orange (++) to orange brown or dark brown (+++). The results showed that from all the 20 *Trichoderma* isolates screened, 13 isolates produced HCN, where 7 isolates *viz*., TR55, TR66, TR88, TR109, TR112, TR122 and TR136 showed '++' (orange colour), whereas 6 isolates TR64, TR74, TR87, TR106, TR143 and TR171 showed '+' (deep yellow colour) and rest of them showed negative *viz*., TR12, TR24, TR36, TR40, TR78, TR82 and TR116.

4.6. Screening for the ability of *Trichoderma* isolates to promote plant growth:

4.6.1. Indole-3-acetic acid (IAA) estimation test

In the present study, all the isolates estimated for IAA production were found to be IAA producers and results are presented in Table 9 and Fig 9a. From the table, it is evident that maximum IAA production with 4.96 µg/ml was produced by TR55, followed by TR88 (4.70 µg/ml), TR40 (3.22 µg/ml), TR36 (2.97 µg/ml), TR78 (2.51 µg/ml), TR74 (2.45 µg/ml), TR66 (2.14 µg/ml), TR112 (1.94 µg/ml), TR82 (1.53 µg/ml), TR12 (1.47 µg/ml) and TR87 (1.44). Other isolates *viz*., TR116, TR143, TR122, TR24, TR171 and TR136 produced very less amount of IAA with values 0.33, 0.58, 0.72, 0.84, 0.87 and 0.99 µg/ml respectively. TR106 and TR109 are statistically at par (0.48 µg/ml).

4.6.2. Phosphate (P) solubilization

Solubilization of P was tested quantitatively as described in 3.8.2 and results revealved that all the 20 isolates were able to solubilized Phosphorous (Table 9 and Fig 9b). Maximum solubilization of Phosphorous (P) was recorded in the isolate TR106 with value 0.98 µg/ml, followed by TR136 (0.79 µg/ml), TR66 (0.75 µg/ml), TR109 (0.74 µg/ml), TR55 (0.72 µg/ml), TR12 (0.68 µg/ml), TR143 (0.59 µg/ml), TR88 (0.58 µg/ml), TR36 (0.53 µg/ml), TR64 (0.41 µg/ml), TR112 (0.39 µg/ml), TR87 and

TR116 (0.32 µg/ml), TR171 (0.29 µg/ml), TR122 (0.25 µg/ml), TR78 (0.23 µg/ml), TR40 (0.22 µg/ml), TR24 (0.21 µg/ml) and the least solubilization were recorded in TR74 (0.09 µg/ml) and TR82 (0.03 µg/ml).

Table 9. IAA and phosphorous (P) production ability of *Trichoderma* sp

Sl. No.	*Trichoderma* sp	IAA produce (ug/ml)	Phosphorous solubilization (ug/ml)
1.	TR12	1.47	0.68
2.	TR24	0.84	0.21
3.	TR36	2.97	0.53
4.	TR40	3.22	0.22
5.	TR55	4.96	0.72
6.	TR64	1.81	0.41
7.	TR66	2.14	0.75
8.	TR74	2.45	0.09
9.	TR78	2.51	0.23
10.	TR82	1.53	0.03
11.	TR87	1.44	0.32
12.	TR88	4.70	0.58
13.	TR106	0.48	0.98
14.	TR109	0.48	0.74
15.	TR112	1.94	0.39
16.	TR116	0.33	0.32
17.	TR122	0.72	0.25
18.	TR136	0.99	0.79
19.	TR143	0.58	0.59
20.	TR171	0.87	0.29

N.B: Means of the 3 (three) replications

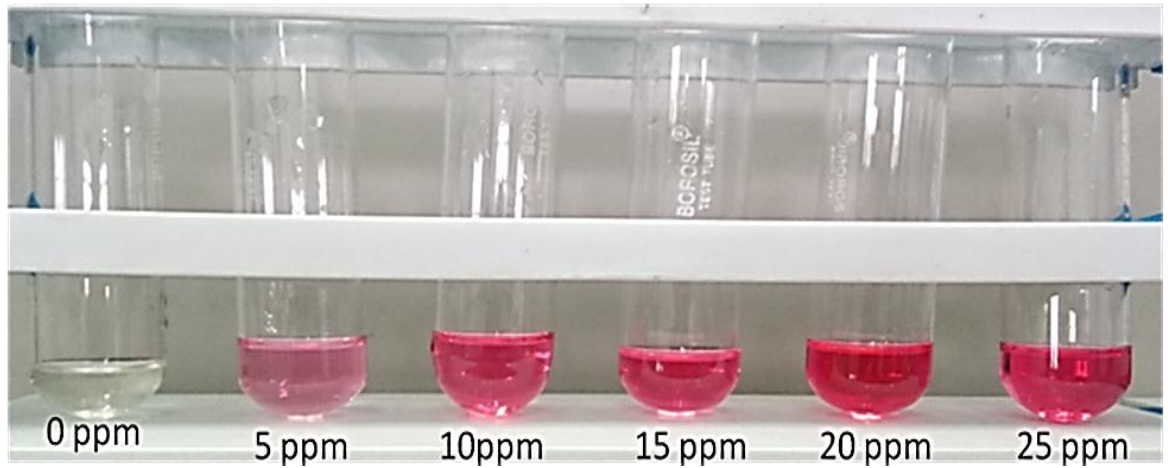


Fig. 9a.i. IAA production test

Fig. 9b.i.: Phosphorous solubilization test

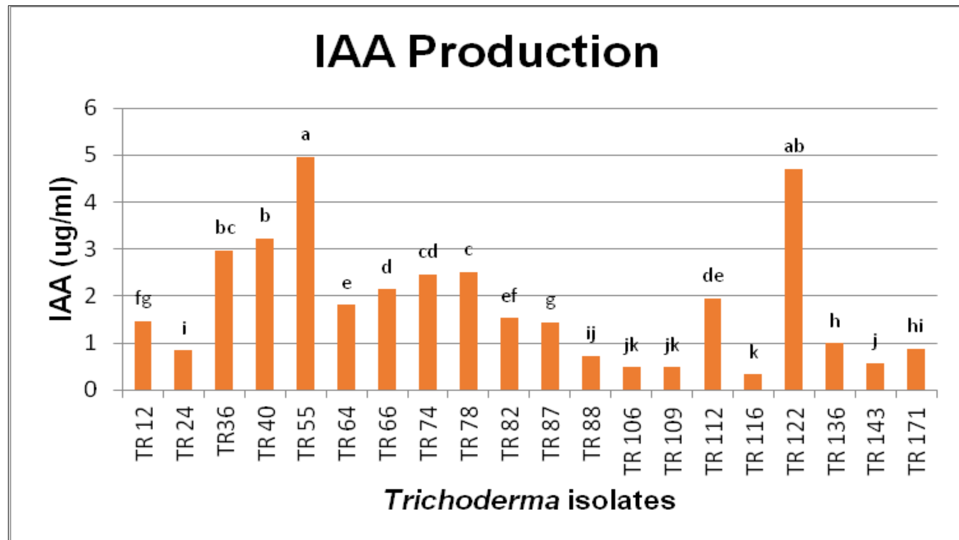


Fig. 9a.ii: Bar diagram showing IAA production test

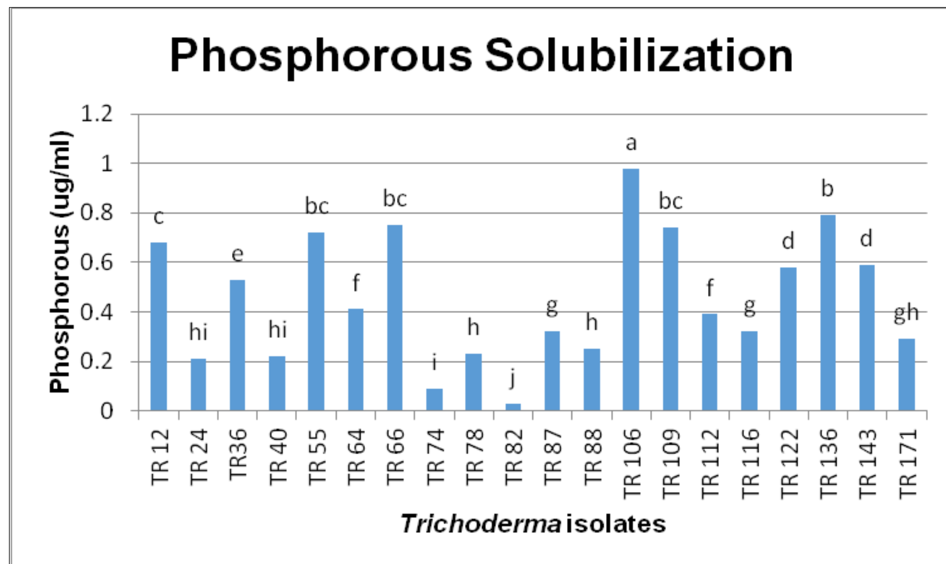


Fig. 9b.ii: Bar diagram showing phosphorous solubilization test

4.6.3. ACC deaminase production test

The ACC deaminase production by the *Trichoderma* isolates were screened qualitatively and as showed in Table 10 and Fig 10a, all screened isolates were able to grow on DF salts minimal medium after 4-7 days of incubation which was considered as positive result.

4.6.4. Ammonia Production

Trichoderma isolates were tested for the production of ammonia in peptone water and results are shown in Table 10 and Fig 10b. Scoring was given with

positive (+) when development of yellow to brown colour was observed and negative (-) when no colour changed. According to colour intensity, '+' was given for yellow colour, '++' for light brown and '+++' for dark brown colour. Out of 20 isolates, 16 showed positive for ammonia production and 4 isolates (TR12, TR36, TR78 and TR143) showed no colour development. From those 16 isolates, 4 isolates (TR55, TR64, TR122 and TR136) showed brown colour development (+++), 3 isolates (TR40, TR66 and TR116) showed light brown colour development and 9 isolates (TR24, TR74, TR82, TR87, TR88, TR106, TR109, TR112 and TR171) showed yellow colour development (+).

Table 10. Plant growth promoting traits of *Trichoderma* sp

Sl. No.	*Trichoderma* sp	Ammonia production #	ACC deaminase activity
1.	TR 12	-	+
2.	TR 24	+	+
3.	TR36	-	+
4.	TR40	++	+
5.	TR55	+++	+
6.	TR64	+++	+
7.	TR66	++	+
8.	TR74	+	+
9.	TR78	-	+
10.	TR82	+	+
11.	TR87	+	+
12.	TR88	+	+
13.	TR106	+	+
14.	TR109	+	+
15.	TR112	+	+
16.	TR116	++	+
17.	TR122	+++	+
18.	TR136	+++	+
19.	TR143	-	+
20.	TR171	+	+

N.B: Means of three replications

(-) indicates no production and (+) indicates production

Colour ranged, yellow (+) to light brown (++) to dark brown colour (+++)

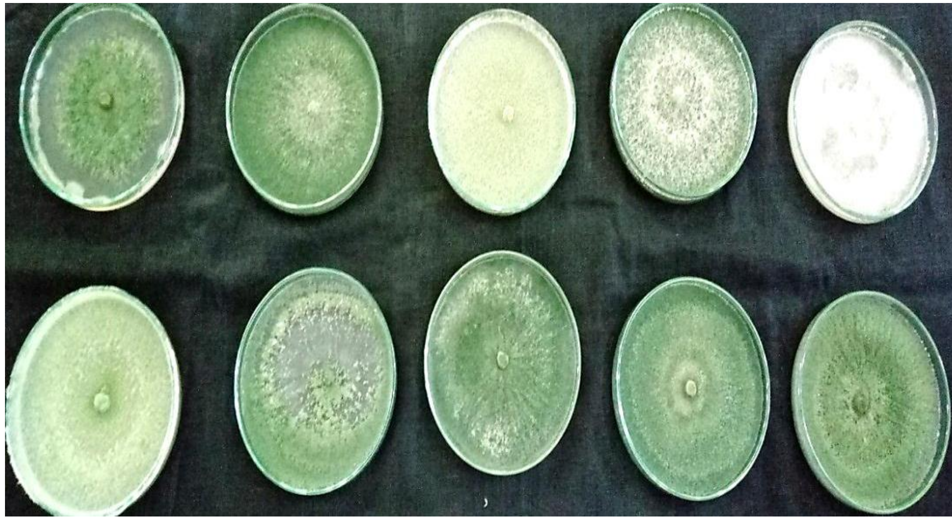


Fig.10a: ACC deaminase production by different isolates of *Trichoderma*

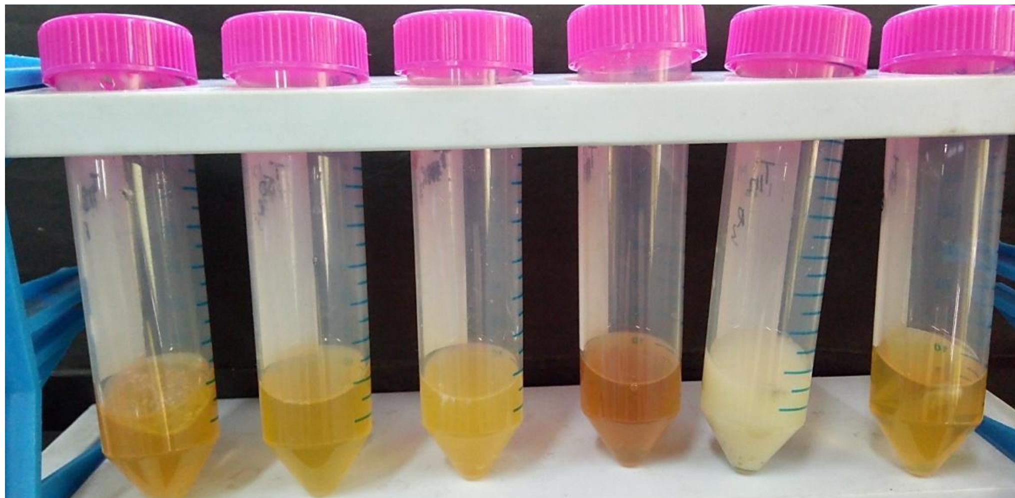


Fig. 10b: Ammonia production by different isolates of *Trichoderma*

Table 11: Score card of the 20 isolates of *Trichoderma*

***Trichoderma* sp**	**Chitinase production**	**Siderophore Production**	**HCN Production**	**Ammonia production**	**ACC deaminase activity**	**IAA produce (ug/ml)**	**Phosphorous production (ug/ml)**	**β-1,6-Glucanase (*Tvbgn3*)**	**Trichodiene synthase (*tri5*)**	**Serine protease (*prb1*)**	**Endochitinase (*ech42*)**	**Score**
TR12	✓	✓	-	-	✓	✓	✓	✓	-	-	✓	**7**
TR24	✓	✓	-	✓	✓	✓	✓	-	-	✓	✓	**8**
TR36	✓	✓	-	-	✓	✓	✓	-	-	✓	✓	**7**
TR40	✓	✓	-	✓	✓	✓	✓	-	-	✓	✓	**8**
TR55	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**11**
TR64	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	**10**
TR66	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**11**
TR74	✓	-	✓	✓	✓	✓	✓	-	-	✓	✓	**8**
TR78	✓	✓	-	-	✓	✓	✓	-	-	✓	✓	**7**
TR82	-	✓	-	✓	✓	✓	✓	✓	-	-	-	**6**
TR87	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	**10**
TR88	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	**10**
TR106	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	**10**
TR109	✓	-	✓	✓	✓	✓	✓	✓	-	✓	✓	**9**
TR112	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	**10**
TR116	-	-	-	✓	✓	✓	✓	✓	-	✓	-	**6**
TR122	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**11**
TR136	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**11**
TR143	✓	-	✓	-	✓	✓	✓	-	✓	-	✓	**7**
TR171	-	✓	✓	✓	✓	✓	✓	-	✓	✓	-	**8**

N.B: - indicates absence of the functional traits

✓ indicates presence of the functional traits

Based on the detection of antimicrobial and plant growth promoting traits in case of 20 *Trichoderma* isolates, 10 isolates which possessed 9 or 10 or 11 traits out of total 11 traits tested as shown in score card (Table 11) were further subjected for other studies like cultural characterization, anamorphic characterisation and phylogenic analysis.

4.7. Cultural and Anamorphic characteristics

4.7.1. Cultural characteristics of *Trichoderma* isolates

The cultural characteristics of those 10 isolates of *Trichoderma viz.*, TR55, TR64, TR66, TR87, TR88, TR106, TR109, TR112, TR122 and TR136were studied on PDA medium and results are presented in Table 12 and Fig 11. The hyphae of all the isolates were hyaline and no significant odour was observed. However, the growth rate of TR55 and TR122 were very fast on PDA with white pustules aggregated near the margin, whereas TR64 and TR88 growth rate were very fast but with dense light green colony. Again TR66 growth rate was very fast with compact white velvety pustules, whereas TR87 colony was dense light green velvety pustules. The growth of TR106 was fast with dark green pustules and colony was denser on periphery. The growth rate of TR109 and TR112 was fast but TR109 colony was light green powdery whereas in TR112 colony was compact and light green. The growth of TR136 was very fast with dense dark green colony.

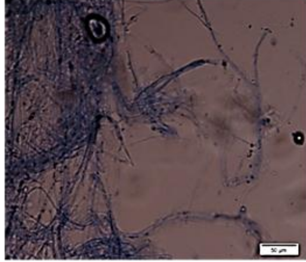
4.7.2. Anamorphic characteristics of *Trichoderma* isolates

Anamorphic characteristics *viz.*, conidiophores length and width, phialides length and width, conidia length and width were studied by growing them in PDA medium and results are presented in Table 13 and Fig 11. From the table, it is evident that TR55 conidiophores measured 4.7-31.6x3.2-4.2µm, conidia (3.0-4.4x2.0-2.2 µm) and phialides (3.4-5.8x 2.3-3.5 µm); TR64 conidiophores measured 4.8-27.1x2.4-4.7µm, conidia (2.7-3.4x2.1-2.6 µm) and phialides (3.7-7.4x2.6-3.7µm);TR66 conidiophores (5.7-33.1x3.2-4.6µm), conidia (3.1-5.2x1.9-2.7µm) and phialides (3.5-6.5x2.7-2.8µm); TR87 conidiophores (5.1-26.5x2.1-4.7µm), conidia (2.9-2.9x1.5-2.4 µm) and phialides (3.2-4.8x2.3-3.4µm);TR88 conidiophores (4.9-22.4x2.3-3.4µm), conidia (2.5-2.8x2.4-2.6 µm) and phialides (3.3-7.4x2.3-3.5µm);TR106 conidiophores (4.8-27.6x3.1-4.2µm), conidia (1.8-2.5x1.7-2.1µm) and phialides (3.0-6.8x2.3-2.6µm);TR109 conidiophores (4.8-28.7x4.5-5.5µm), conidia (2.2-3.3x1.4-2.9 µm) and phialides (3.8-7.6x2.1-3.0µm);TR112 conidiophores (6.8-37.8x3.1-3.7µm), conidia (2.3-3.6x1.6-2.5µm) and phialides (3.0-7.5x1.6-1.8µm);TR122 conidiophores (4.2-

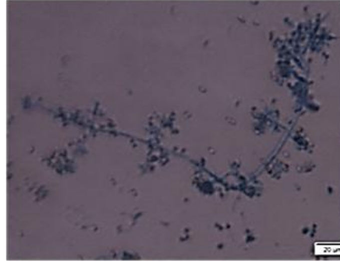
28.7x3.1-4.6µm), conidia (3.0-3.4x2.2-2.4µm) and phialides (3.5-5.4x2.6-3.4µm) and TR136 conidiophores (4.6-30.3x2.6-3.5µm), conidia (1.9-3.4x1.8-2.7 µm) and phialides (3.5-8.4x2.2-3.5µm).

Table 12. Cultural characteristics of potential *Trichoderma* isolates grown on PDA

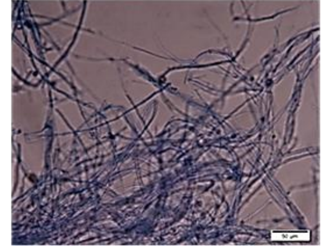
Sl. No.	*Trichoderma* isolates	Cultural characteristics				
		Colony	**Growth rate**	**Pigment**	**Hyphae**	**Odour**
1.	TR55	Aggregated near margin	Very Fast	White pustules	Hyaline	No characteristic smell
2.	TR64	Dense	Very Fast	Light green	Hyaline	No characteristic smell
3.	TR66	Compact	Very Fast	White velvety pustules	Hyaline	No characteristic smell
4.	TR87	Dense	Very Fast	Light green velvety pustules	Hyaline	No characteristic smell
5.	TR88	Dense	Very Fast	Light green	Hyaline	No characteristic smell
6.	TR106	More dense on periphery	Fast	Dark green	Hyaline	No characteristic smell
7.	TR109	Powdery colony	Fast	Light Green	Hyaline	No characteristic smell
8.	TR112	Compact	Fast	Light green	Hyaline	No characteristic smell
9.	TR122	Aggregated near margin	Very Fast	White	Hyaline	No characteristic smell
10.	TR136	Dense	Very Fast	Dark Green	Hyaline	No characteristic smell



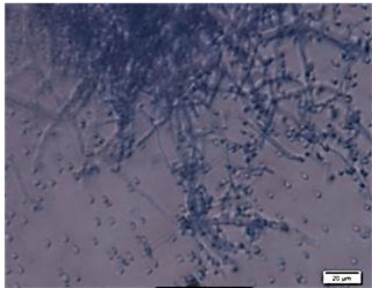
TR 55



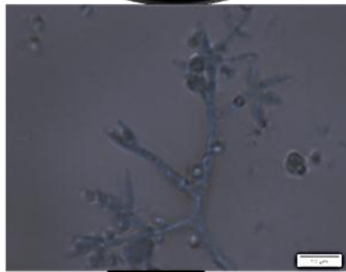
TR 64



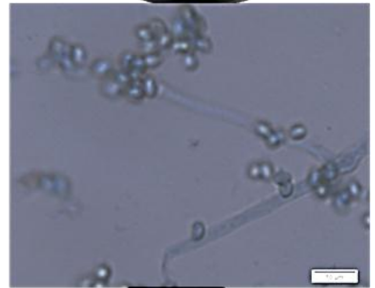
TR 66



TR 87



TR 88



TR 106

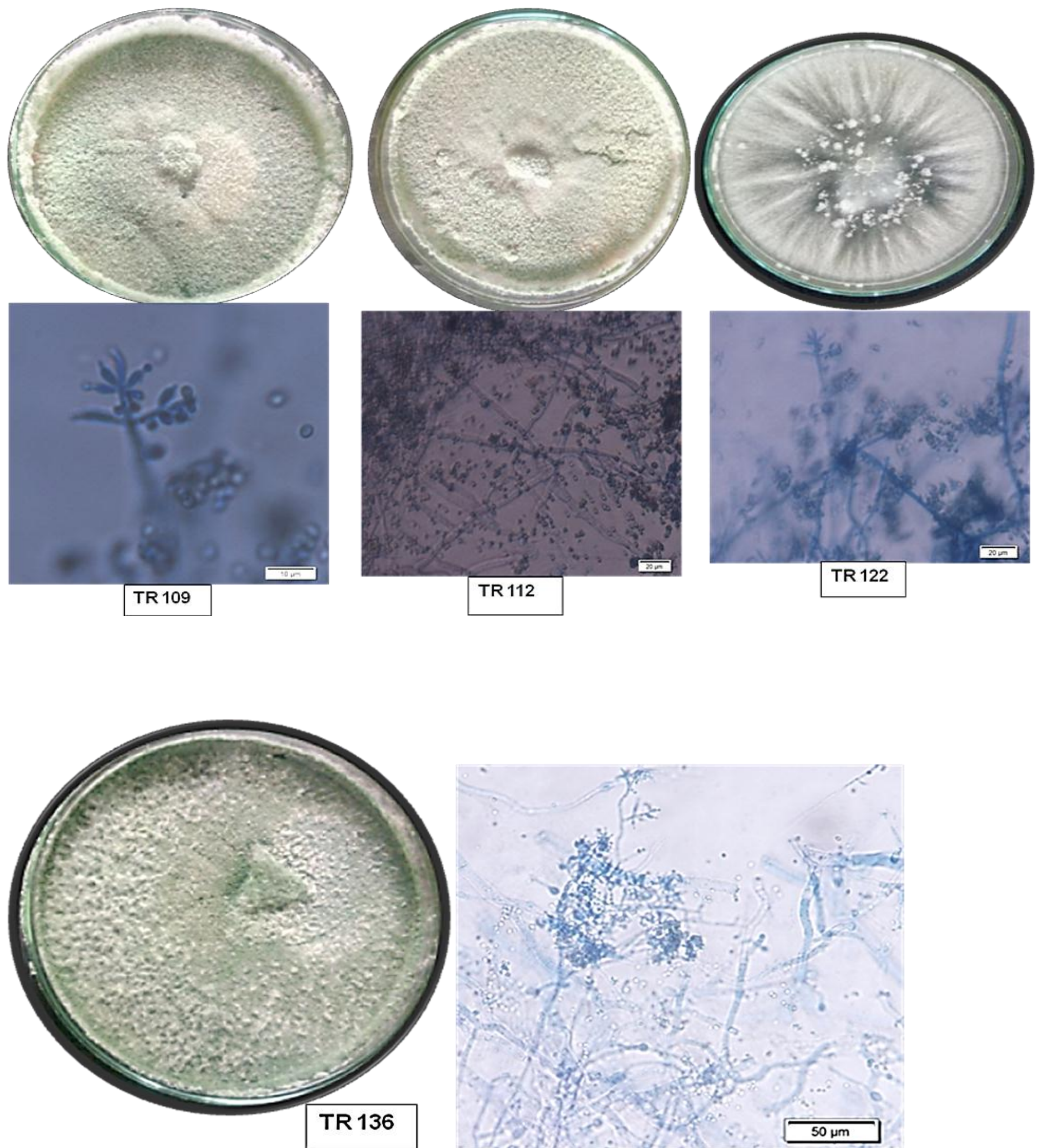


Fig. 11: Cultural and anamorphic characteristics of the 10 isolates of *Trichoderma* sp.

Table 13. Anamorphic characteristics of *Trichoderma* isolates

Sl.no.	*Trichoderma* isolates	Phialides (μm)	Conidia (μm)	Conidiophores (μm)
1.	TR55	3.4-5.8x 2.3-3.5	3.0-4.4x2.0-2.2	4.7-31.6x3.2-4.2
2.	TR64	3.7-7.4x2.6-3.7	2.7-3.4x2.1-2.6	4.8-27.1x2.4-4.7
3.	TR66	3.5-6.5x2.7-2.8	3.1-5.2x1.9-2.7	5.7-33.1x3.2-4.6
4.	TR87	3.2-4.8x2.3-3.4	2.9-2.9x1.5-2.4	5.1-26.5x2.1-4.7
5.	TR88	3.3-7.4x2.3-3.5	2.5-2.8x2.4-2.6	4.9-22.4x2.3-3.4
6.	TR106	3.0-6.8x2.3-2.6	1.8-2.5x1.7-2.1	4.8-27.6x3.1-4.2
7.	TR109	3.8-7.6x2.1-3.0	2.2-3.3x1.4-2.9	4.8-28.7x4.5-5.5
8.	TR112	3.0-7.5x1.6-1.8	2.3-3.6x1.6-2.5	6.8-37.8x3.1-3.7
9.	TR122	3.5-5.4x2.6-3.4	3.0-3.4x2.2-2.4	4.2-28.7x3.1-4.6
10.	TR136	3.5-8.4x2.2-3.5	1.9-3.4x1.8-2.7	4.6-30.3x2.6-3.5

N.B: Mean of 10 replications

4.8. Phylogenic analysis

The genotypic analysis of the potent bio-agent (10 isolates) was carried out by amplification of ITS region using universal primers ITS1 (TCTGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). All the 10*Trichoderma* isolates *viz*., TR55, TR64, TR66, TR87, TR88, TR106, TR109, TR112, TR122 and TR136 showed amplified product size of 600bp (Fig 12). The amplified products were outsourced for sequencing and identity was established as described in 3.10. Sequencing homology of 10 *Trichoderma* isolates (Table 14) revealed that the isolates belong to two major species i.e., *T. harzianum* (60 %) and *T. hamatum* (40 %) but of different strain. TR55 (from tomato rhizosphere, West Jaintia hills) showed 99 per cent identity to *T. hamatum* strain CEN693 (from soil of Strawberry crop, Brasil); TR64 (from potato rhizosphere, West Jaintia hills) showed 99 per cent identity to *T. harzianum* isolate ADB2 (from decaying wood, Sri Lanka); TR66 (from brinjal rhizosphere, West Jaintia hills) showed 99 per cent identity to *T. hamatum* strain US10g (from field soil, USA); TR87 (from jhum area, Ri Bhoi) showed 100 per cent identity to *T. hamatum* isolate HR5 (from root, U.P India); TR88 (from forest, East Khasi hills) showed 99 per cent identity to *T. harzianum* strain T6 (from rhizosphere soil, T.N India); TR106 (from tomato rhizosphere, Ri Bhoi) showed 99 per cent identity to *T. harzianum* strain GJS 04-70 (from *Capsicum annum* stem, Italy); TR109 (from forest, East Khasi hills) showed 99 per cent identity to *T. harzianum* strain

Table 14: Identification of potential strains of *Trichoderma* sp.

Isolate details			Homology details				Remarks (putative species designation)
Name	**Habitat**	**Contig Sequence lenght (bp)**	**E value**	**Identity (%)**	**Accession of closest homolog**	**Ecology of homolog**	
TR55	Tomato (West Jaintia Hills)	600	0.0	99	KC576720.1	Soil of Strawberry crop (Brasil)	*Trichoderma hamatum* strain CEN693
TR64	Potato (West Jaintia Hills)	593	0.0	99	MF671942.1	Decaying wood (Sri Lanka)	*Trichoderma harzianum* isolate ADB2
TR66	Brinjal (West Jaintia Hills)	554	0.0	99	KY552263.1	Field soil (USA)	*Trichoderma hamatum* strain US10g
TR87	Jhum area (Ri Bhoi)	586	0.0	100	KF018424.1	Root (U.P, India)	*Trichoderma hamatum* isolate HR5
TR88	Forest (East Khasi Hills)	541	0.0	99	MH333117.1	Rhizosphere soil (T.N, India)	*Trichoderma harzianum* strain T6
TR106	Tomato (Ri Bhoi)	592	0.0	99	FJ442674.1	*Capsicum annum* Stem (Italy)	*Trichoderma harzianum* strain GJS 04-70
TR109	Forest (East Khasi Hills)	601	0.0	99	KF313112.1	Pine root (South Korea)	*Trichoderma harzianum* strain SFCF20120803-29
TR112	Tomato (West Jaintia Hills)	581	0.0	99	MK036366.1	Contaminated soil (Italy)	*Trichoderma harzianum* culture MUT:3522
TR122	Tomato (South West Khasi Hills)	585	0.0	99	EU263999.1	Stem endophyte of *Theobroma gileri* (USA)	*Trichoderma hamatum* strain DIS 326F
TR136	Tomato (West Khasi Hills)	604	0.0	99	HG938369.1	Peat soil (Malaysia)	*Trichoderma harzianum*

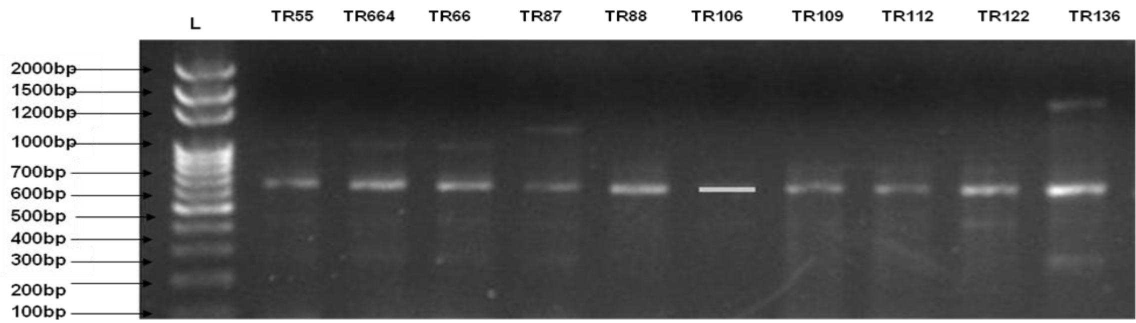


Fig. 12: *Trichoderma* Isolates showing amplification of Internal transcribed region(600bp) using primer ITS1 and ITS4

L= 100bp ladder; Lane 1-10= *Trichoderma* isolates

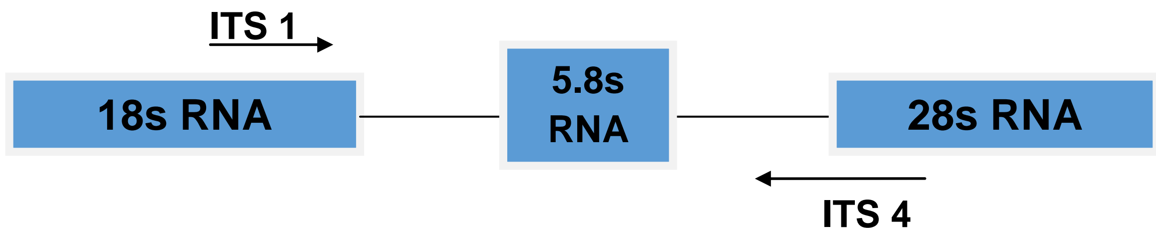


Fig 13: The position of primers for PCR based amplification of the region

Table 15: Position of ITS 1 and ITS 2 used for sequencing

18S rRNA	ITS 1	5.8S	ITS 2	28S RNA
<33	33 – 230	231-387	388-558	>559

position (bp)	64	92	95	97	101	102	103	105	123	125	143	145	147	149	158	159	164	165	166	177	178	179	181	185	186	187	189	191	192	200	201	202	203	204	205	206	207
TR 55	A	G	A	-	A	A	A	G	A	C	A	C	A	C	T	C	-	G	T	A	C	G	A	C	T	T	C	G	C	-	-	-	-	-	-	-	-
TR 64	A	A	T	T	C	G	C	G	C	A	C	A	C	A	T	T	T	A	C	G	T	T	T	-	-	-	T	A	-	C	T	T	C	T	C	G	G
TR 66	A	G	A	-	A	A	A	G	A	C	A	C	A	C	T	C	-	G	T	A	C	G	A	C	T	T	C	G	C	-	-	-	-	-	-	-	-
TR 87	A	G	A	-	A	A	A	G	A	C	A	C	A	C	T	C	-	G	T	A	C	G	A	C	T	T	C	G	C	-	-	-	-	-	-	-	-
TR 88	A	A	T	T	C	G	C	G	C	A	C	A	C	A	A	T	T	A	C	G	T	T	T	T	T	T	T	A	-	C	T	T	C	T	C	G	G
TR 106	A	A	T	T	C	G	C	G	C	A	C	A	C	A	A	T	T	A	C	G	T	T	T	T	T	T	T	A	-	C	T	T	C	T	C	G	G
TR 109	A	A	T	T	C	G	C	G	C	A	C	A	C	A	A	T	T	A	C	G	T	T	T	T	T	T	T	A	-	C	T	T	C	T	C	G	G
TR 112	A	A	T	T	C	G	C	G	C	A	C	A	C	A	A	T	T	A	C	G	T	T	T	T	T	T	T	A	-	C	T	T	C	T	C	G	G
TR 122	T	G	A	-	A	A	A	G	A	C	A	C	A	C	T	C	-	G	T	A	C	G	A	C	T	T	C	G	C	-	-	-	-	-	-	-	-
TR136	A	A	T	T	C	G	C	T	C	A	C	A	C	A	A	T	T	A	C	G	T	T	T	T	T	T	T	A	-	C	T	T	C	T	C	G	G

	208	209	210	211	212	213	214	215	216	217	219	220	221	222	225	226	227	420	438	439	441	442	444	445	446	447	448	449	450	451	454	455	460	462	464	523	544	545	550
TR 55	-	-	-	-	-	-	-	-	-	-	A	A	A	A	C	-	-	A	G	A	C	C	T	C	-	-	-	A	C	C	G	T	G	C	T	C	G	T	A
TR 64	C	G	C	C	T	C	T	C	G	T	G	G	C	G	T	C	G	G	C	C	T	G	C	T	C	T	T	G	G	C	T	G	T	T	C	T	A	G	T
TR 66	-	-	-	-	-	-	-	-	-	-	A	A	A	A	C	-	-	A	G	A	C	C	T	C	-	-	-	A	C	C	G	T	G	C	T	C	G	T	A
TR 87	-	-	-	-	-	-	-	-	-	-	A	A	A	A	C	-	-	A	G	A	C	C	T	C	-	-	-	A	C	C	G	T	G	C	T	C	G	T	A
TR 88	C	G	C	C	T	C	T	C	G	T	G	G	C	G	T	C	G	G	C	C	T	C	C	T	-	T	A	G	C	G	T	G	T	T	C	T	A	G	T
TR 106	C	G	C	C	T	C	T	C	G	T	G	G	C	G	T	C	G	G	C	C	T	C	C	T	-	T	A	G	C	G	T	G	T	T	C	T	A	G	T
TR 109	C	G	C	C	T	C	T	C	G	T	G	G	C	G	T	C	G	G	C	C	T	C	C	T	-	T	A	G	C	G	T	G	T	T	C	T	A	G	T
TR 112	C	G	C	C	T	C	T	C	G	T	G	G	C	G	T	C	G	G	C	C	T	C	C	T	-	T	A	G	C	G	T	G	T	T	C	T	A	G	T
TR 122	-	-	-	-	-	-	-	-	-	-	A	A	A	A	C	-	-	A	G	A	C	C	T	C	-	-	-	A	C	C	G	T	G	C	T	C	G	T	A
TR136	C	G	C	C	T	C	T	C	G	T	G	G	C	G	T	C	G	G	C	C	T	C	C	T	-	T	A	G	C	G	T	G	T	T	C	T	A	G	T

Fig 14: Showing SNP (Single Nucleotide Polymorphism)

(-) indicates In/del (Insertion/deletion)

SFCF20120803-29 (from pine root, South Korea); TR112 (from tomato rhizosphere, West Jaintia hills) showed 99 per cent identity to *T. harzianum* culture MUT:3522 (from contaminated soil, Italy); TR122 (from tomato rhizosphere, South West Khasi Hills) showed 99 per cent identity to *T. hamatum* strain DIS 326F (from Stem endophyte of *Theobromagileri*, USA) and TR136 (from tomato rhizosphere, West Jaintia hills) showed 99 per cent identity to *T. harzianum* (from peat soil, Malaysia).

The position of ITS1 and 2 used for sequencing of the 10 isolates were constructed and results shown in Table 15 and Fig 13. It is observed that 18S rRNA positioned <33bp, ITS1 (33-230bp), 5.8S (231-387bp), ITS2 (388-558bp) and 28S RNA (>559bp). SNP (Single Nucleotide Polymorphism) was seen in the position 64 to 227bp and 420 to 550bp which falls under ITS1 and ITS2 region, respectively (Fig 14). This suggested that the polymorphism among the isolates were in ITS1 and ITS2 region.

For phylogenetic analysis MEGA software (MEGA 5.2) was used and using neighbor joining method available in MEGA 5.2 software, the phylogenic tree was constructed with 1000 bootstrapping value. Clustering of the 10 isolates showed 2 main class (A and B). Class A showed sub-class 1A and 2A (where TR55 and TR122 falls and showed closeness of 96 %). Again sub-class 1A showed sub-class 1Aa and 1Ab with closeness of 13 %. TR66 falls under the sub-class 1Ab. Sub-class 1Aa showed sub-class $1Aa_1$ and $1Aa_2$, where TR87 and TR112 respectively fall with 65% closeness. Main class B showed sub-class 1B and 2B. Under sub-class 1B, TR88 falls with 23% closeness to sub-class 2B. Again sub-class 2B showed sub-class 2Ba and 2Bb. TR64 falls under sub-class 2Ba with 18% closeness to 2Bb. Sub-class 2Bb showed sub-class $2Bb_1$ and $2Bb_2$. Under sub-class $2Bb_1$TR136 falls and showed 37% closeness to sub-class $2Bb_2$ (where TR106 and TR109 under fall and showed 95% closeness).

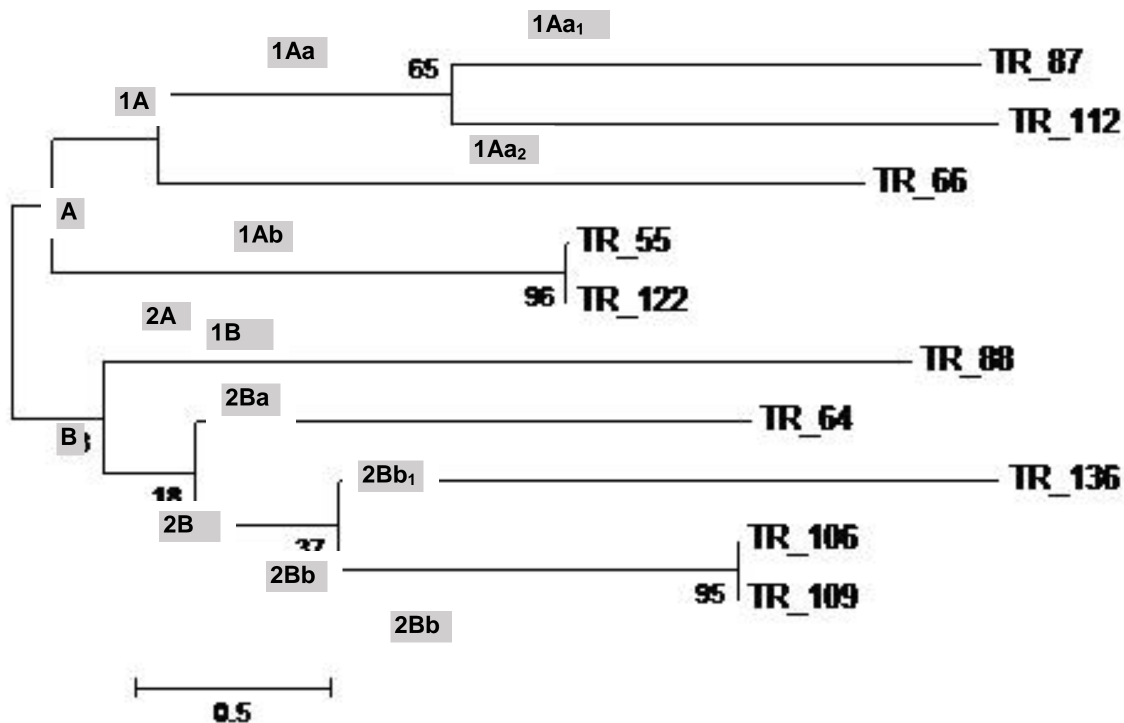


Fig 15: **Clustering of 10 *Trichoderma* isolates using neighbor joining method available in MEGA 5.2 software with 1000 bootstrapping value.**

From those 10 isolates, 4 isolates *viz.*, TR55, TR66, TR122 and TR136 which possessed 11 traits out of those 11 traits tested as shown in score card (Table 11) were selected for *in-vivo* studies.

4.9. To study the plant growth promotion & biocontrol potential of screened *Trichoderma* sp. against *Pythium* sp. and *Rhizoctonia solani* Kuhn.

4.9.1. Seed germination test

The biopriming of seeds of tomato (Hybrid-017) was done for potent isolates of *Trichoderma* sp. *viz.*, TR55, TR66, TR122 and TR136 and results are presented in Table 16 and Fig 16. Table 16 shown the highest root and shoot length was recorded the isolate in TR55 (14.15±0.29 cm and 10.9±0.56 cm, respectively) followed by TR122 (13.15±0.29 cm and 9.5±0.32 cm, respectively), TR66 (11.85±0.38 cm and 8.74±0.25 cm, respectively), TR136 (10.85±0.29 cm and 7.9±0.13 cm, respectively) and least root and shoot length was observed in control (6.85±0.35 cm and 4.85±0.24 cm, respectively). Again, TR55 showed the highest germination percentage 92 %, followed by TR122 (88 %), TR66 (86 %), TR136 (84 %) and least in control (62 %).

4.9.2. Tomato seedlings vigour index

The vigour index was recorded highest in TR55 (2306), followed by TR122 (1990.5), TR66 (1768.1), TR136 (1576.5) and least was observed in control (724). Significant difference in the vigour index was recorded among the isolates and it was also found significantly differ from control (Table 16).

Table 16. Biopriming 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.	TR55	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.	TR66	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.	TR122	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.	TR136	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

N.B: Means of three replications

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

4.9.3. Pot Culture Experiment To Evaluate Potent *Trichoderma* Isolates

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 evaluated. The effect of the different treatments on pre and post emergence damping off and their ability to reduce the damping off incidence caused by *Pythium* sp. and *R. solani* was studied under pot culture experiment and the results are presented below

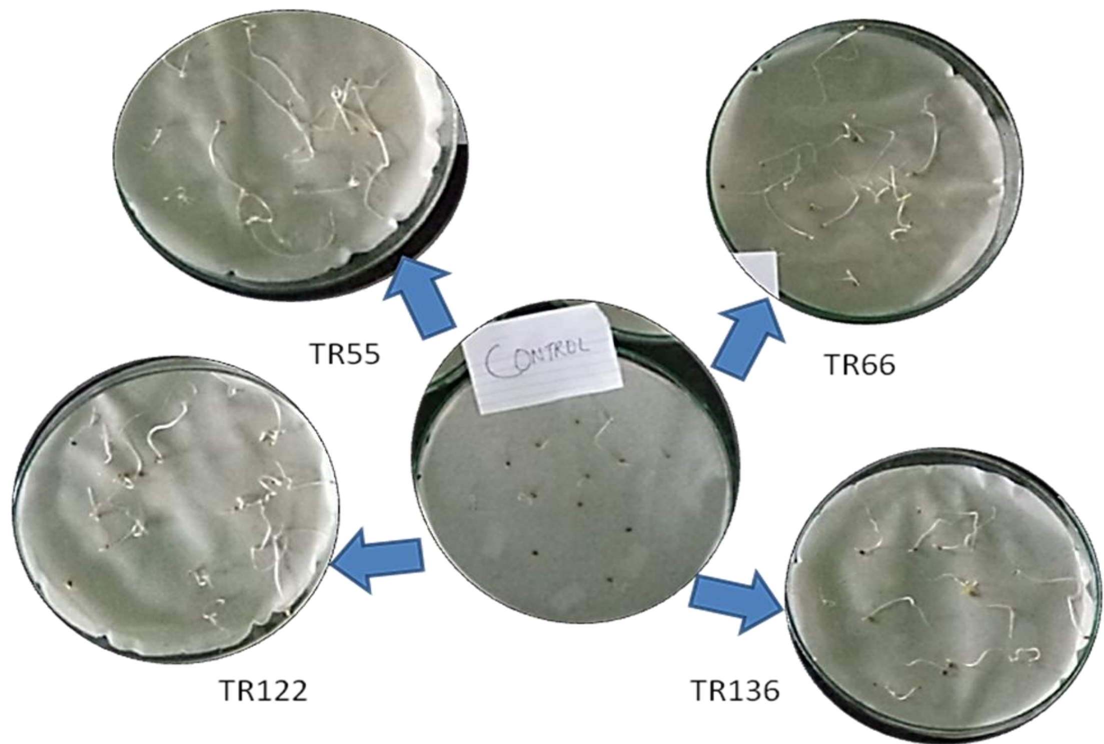


Fig. 16: Biopriming of tomato seed (Hybrid-017) with *Trichoderma* isolates

Fig. 17: Damping off caused by (A) Pythium and (B) *R. Solani*

4.9.3.1 Pre emergence damping off incidence caused by *Pythium* sp. and biological control efficacy of *Trichoderma* isolates against *Pythium* sp.

From Table17 and Fig 17, it is observed that all the treatments showed significant reduction of the disease incidence over control. The percent pre-emergence damping off incidence caused by *Pythium* sp. showed in the range of 20 to 50 per cent in the various treatments studied. The lowest pre emergence damping off disease incidence was obtained in the seed plus soil treatment with TR 55 with 20 per cent disease incidence (DI) when compared to control (62%).

Between the treatments, minimum percent damping off incidence was observed in the treatment T_3 (seed plus soil treatment) with the DI of 32 per cent. T_1 (Seed treatment) and T_2 (soil treatment) were statistically at par (46 %). Among the isolates, the lowest damping off incidence was observed in TR55 with DI 30.67 per cent, followed by TR122, TR66 and TR136 with DI of 33.33, 38.67 and 43.33 per cent, respectively. The maximum percentage of pre-emergence damping off incidence was seen in the untreated pot with 62 per cent DI.

Again from Table 17, it was observed that the biological control efficacy (BCE) was significantly different among the four isolates. The highest BCE was recorded in TR55 with BCE of 65.28 per cent, followed by TR122, TR66 and TR136 with BCE of 56.33, 50.31 and 35.15 per cent, respectively. The mode of application of *Trichoderma i.e.,* T_1 (Seed treatment), T_2 (soil treatment) and T_3 (seed + soil treatment) showed significantly superior over untreated control. The highest BCE was recorded in T_3 (with 43.22 % BCE), followed by T_2 (42.43 % BCE) and T_1 (38.59 % BCE).

4.9.3.2 Pre emergence damping off incidence caused by *R. solani* and biological control efficacy of *Trichoderma* isolates against *R. solani*

From Table 17, per cent pre-emergence damping off incidence caused by *R. solani* ranged between 28 to 48 per cent in various treatments with the lowest pre emergence damping off incidence attained in the treatment T_1 (seed treatment with TR55) and T_3 (seed + soil treatment with TR55) with incidence of 28 % when compared to control (64 %).

Among the isolates, minimum damping off incidence was observed in TR55 with DI 39.60 per cent, followed by TR122, TR66 and TR136 with DI of 34.00, 37.33 and 44.00 per cent, respectively. The highest pre-emergence damping off disease incidence observed in control (untreated pot) with 64 per cent DI. Between the treatments, minimum damping off incidence was recorded in the T_3 (seed plus soil

Table 17. Effect of *Trichoderma* isolates on incidence (%) and biological control efficacy of pre-emergence damping off disease of tomato under pot culture condition

Trichoderma isolates	Per cent of pre-emergence disease incidence (DI)								Biological control efficacy(BCE) over control							
	Pythium sp				*R. solani*				*Pythium* sp				*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
TR55	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
TR66	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
TR122	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
TR136	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- T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment

treatment) with DI of 39.60 per cent, followed by T_1 (Seed treatment) and T_2 (soil treatment) with 40.80 and 45.60 per cent DI, respectively.

The highest biological control efficacy (BCE) was achieved inTR55 with BCE of 57.14 %, followed by TR122, TR66 and TR136with BCE of 45.53 %, 35.41 % and 28.55 %, respectively. Among the treatments, the highest BCE was achieved in T_3 with BCE 44.12 %, followed by T_2 (33.29%) and T_1 (22.56 %).

4.9.3.3 Post-emergence damping-off incidence caused by *Pythium* sp. and biological control efficacy of *Trichoderma* isolates against *Pythium* sp.

Post emergence of the damping off disease percent incidence (DI) caused by *Pythium* sp. on the tomato crops treated with TR55, TR122, TR66 and TR136 was recorded at 15, 30 and 45 days after sowing (DAS). From table 18, it was observed that at 15, 30 and 45 DAS, all 4 *Trichoderma* isolates could inhibit the pathogen *Pythium* sp. upto 67.40 per cent (seed + soil treatment with TR55). The lowest post-emergence damping off incidence at 15, 30 and 45DAS was observed in TR55 (with 12.11 %, 26.26 % and 25.35 % DI, respectively), then followed by TR122 (with 14.46%, 28.12 % and 27.54 % DI, respectively), TR66 (16.12 %, 28.15 % and 32.47%, respectively), TR136 (19.38 %, 28.81 % and 35.77 % DI, respectively) and the highest disease incidence was seen in the 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 disease incidence was achieved in T_3 (16.24 %, 26.62 % and 32.38 % DI, respectively), followed by T_1 (17.74 %, 31.18 % and 33.80 % DI, respectively) and T_2 (18.45 %, 34.69 % and 36.26 % DI, respectively).

Among the isolates, highest biological control efficacy (BCE) was seen withTR55 (65.28 %), followed by TR122 (56.33 %), TR66 (50.31 %) and TR136 (33.15 %). Among the treatments, the highest BCE was achieved in T_3 with BCE 4 3.92 %, followed by T_1 (40.53 %) and T_2 (38.59 %).

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

Post emergence damping off incidence (DI) caused by *R. solani* on the tomato crops treated with TR55, TR122, TR66 and TR136was recorded at 15, 30 and 45 days after sowing (DAS). From Table 19, it was observed that at 15, 30 and 45 DAS, all 4*Trichoderma* isolates could inhibit the pathogen *R. solani* upto 68.27 per

Table 18. Effect of *Trichoderma* isolates on disease incidence (%) and their biological control efficacy against post-emergence damping off of tomato caused by *Pythium* sp. under pot culture experiment

Trichoderma isolates	Per cent (%) of disease incidence (post-emergence) *Pythium* sp												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
TR55	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
TR66	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
TR122	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
TR136	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, T_1 = Seed treatment, T_2 = Soil treatment and T_3 = Seed + Soil treatment

Table 19. Effect of *Trichoderma* isolates on disease incidence (%) and their biological control efficacy against post-emergence damping off of tomato caused by *R. solani* under pot culture experiment

Trichoderma isolates	Per cent 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
TR55	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
TR66	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
TR122	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
TR136	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

cent (seed + soil treatment with TR55). The lowest post-emergence damping off incidence at 15, 30 and 45 DAS was observed in TR55 (with 14.58 %, 14.29 % and 15.10 % DI, respectively), followed by TR122 (with 17.18 %, 18.69 % and 20.12 % DI, respectively), TR66 (20.34 %, 19.82 % and 23.22 %, respectively), TR136 (21.50 %, 24.51 % and 25.76 % 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 obtained 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).

Among the isolates, highest biological control efficacy (BCE) was achieved in TR55 (65.61 %), followed by TR122 (53.28 %), TR66 (46.21 %) and TR136 (26.45 %). Among the treatments, the highest BCE was seen in T_3 with BCE 41.56 %, followed by T_2 (38.58 %) and T_1 (34.80 %).

4.9.3.5 Effect of biocontrol agents on growth parameters of tomato "hybrid-017"

The influence of *Trichoderma* isolates on the growth parameters such as root length, plant height, number of leaves, number of flowers, fresh and dry weight and yields were studied in pots under pot culture and readings recorded are presented under Table 20 and 21.

1. Plant height (cm)

From Table 20, it was seen that plant height recorded at 45 DAS ranged between 92.20 cm to 127.20 cm when compared to control (90 cm). The highest plant height was observed in TR55 (107 cm), followed by TR122 (103.06 cm), TR66 (98.33 cm) and TR136 (96.60 cm). Among the treatments, maximum plant height was recorded in T_3 (109 cm), followed by T_2 (94.72 cm) and T_1 (93.28 cm).

2. Number of leaves/plant

The number of leaves recorded at 45 DAS ranged from 32 to 57.20 numbers, when compared to control with 30 numbers (Table 20). Maximum number of leaves/plant was recorded in TR55 (45), followed by TR122 (43.20), TR66 (36) and TR136 (34.46). Among the treatments, maximum number of leaves was achieved in T_3 (43.64), followed by T_2 (36.20) and T_1 (33.36).

Table 20. Plant growth promotion of tomato by *Trichoderma* isolates under pot culture experiment

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
TR55	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
TR66	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
TR122	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
TR136	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

	Plant height (cm)			Number of leaves/plant			Number of flowers/plant			Root length (cm)		
	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

Table 21. Yield, dry and fresh weight of tomato under pot culture experiment

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
TR55	370	410	572	450.67	61	64.16	81.42	68.86	1.92	1.83	3.00	2.25
TR66	328	340	432	366.67	51.86	48.22	68.80	56.29	1.52	1.65	1.91	1.69
TR122	354	428	514	432	54.30	68.70	78.52	67.17	1.53	1.62	2.14	1.76
TR136	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

	Fresh weight: Bioagents (A)	Fresh weight: Treatments (B)	Fresh weight: Interaction (AxB)	Dry weight: Bioagents (A)	Dry weight: Treatments (B)	Dry weight: Interaction (AxB)	Yield: Bioagents (A)	Yield: Treatments (B)	Yield: 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- $\mathbf{T_1}$ = Seed treatment, $\mathbf{T_2}$ = Soil treatment and $\mathbf{T_3}$ = Seed + Soil treatment

3. Number of flowers/plant

From table 20, it was seen that the number of flower recorded at 45 DAS ranged from 18 to 34.40 when compared to control (17). Maximum numbers was observed in TR55 (25.33), followed by TR122 (21.73), TR66 (20.53) and TR136 (20.06). Among the treatments, highest flower numbers was recorded in T_3 (25.08), followed by T_2 (19.20) and T_1 (18.52).

4. Root length (cm)

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) as shown in table 21. Maximum root length was recorded in TR55 (18.35 cm), followed by TR122 (16.67 cm), TR66 (16 cm) and TR136 (15.81 cm). Among the treatments, maximum root length was recorded in T_3 (17.42 cm), followed by T_2 (14.82 cm) and T_1 (14.46 cm).

5. Fresh weight/plant (g)

The fresh weight of tomato plants increased in all the treatments compared to control (300 g). The highest fresh weight was recorded in TR55 (450.67 g), followed by TR122 (432 g), TR66 (366.67 g) and TR136 (347.33 g). Among the treatments, maximum fresh weight was recorded in T_3 (438 g), followed by T_2 (364 g) and T_1 (336 g).

6. Dry weight/plant (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 TR55 (68.86 g), followed by TR122 (67.17 g), TR66 (56.29 g) and TR136 (55.34 g). Among the treatments, maximum dry weight was recorded in T_3 (67.94 g), followed by T_2 (58.04 g) and T_1 (54.60 g).

7. Yield/plant (kg)

The variation in the fruit yield of tomato plants due to different *Trichoderma* isolates and different treatments was found to be statistically significant. The yield increased in all the treatments compared to control (1.4 kg/plant). The highest yield was recorded in TR55 (2.25 kg/plant).), followed by TR122 (1.76 kg/plant), TR 136 (1.74 kg/plant) and TR66 (1.69 kg/plant). Among the treatments, maximum yield was recorded in T_3 (2.07 kg/plant), followed by T_2 (1.65 kg/plant) and T_1 (1.58 kg/plant).

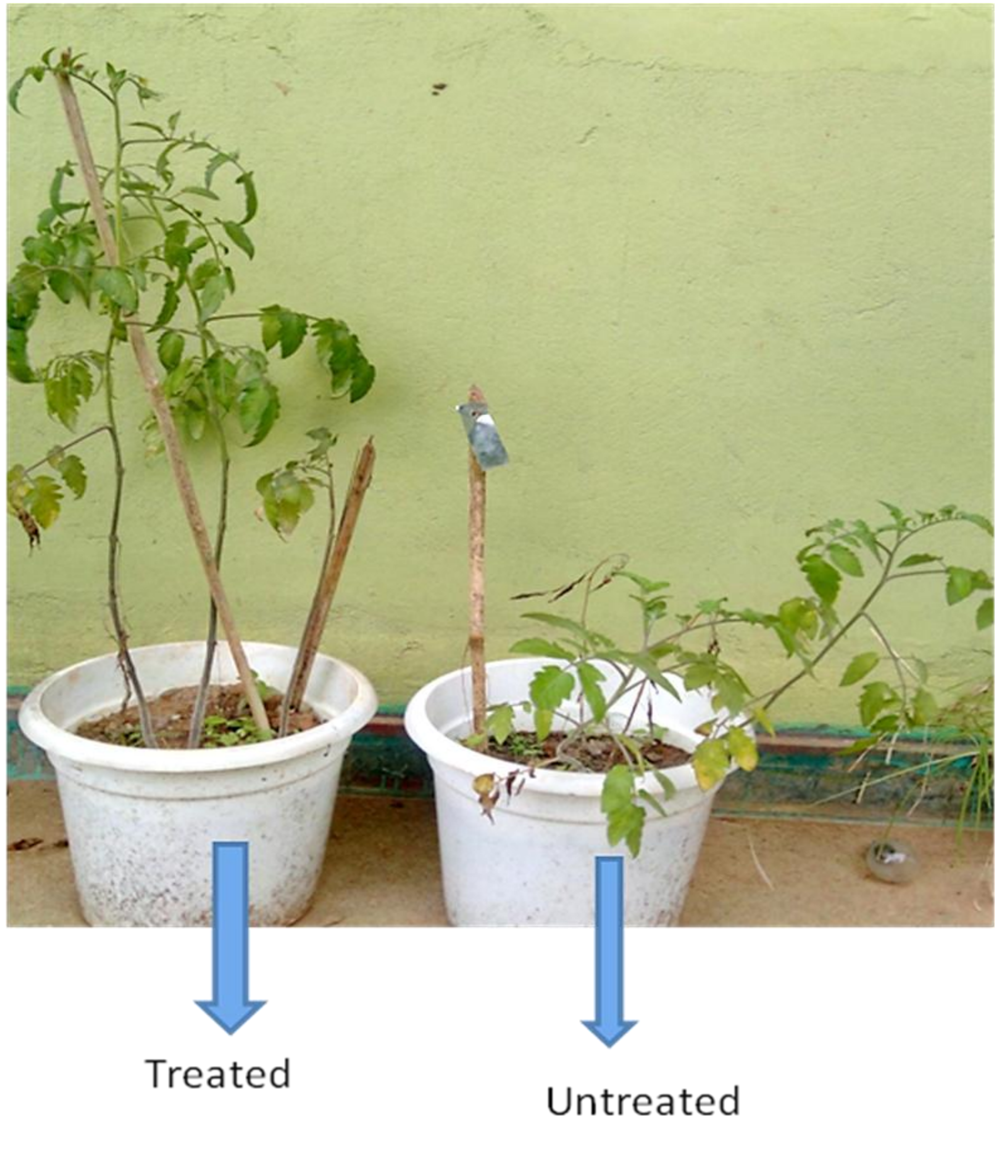


Fig. 18: Tomato plants treated with *Trichoderma* isolates

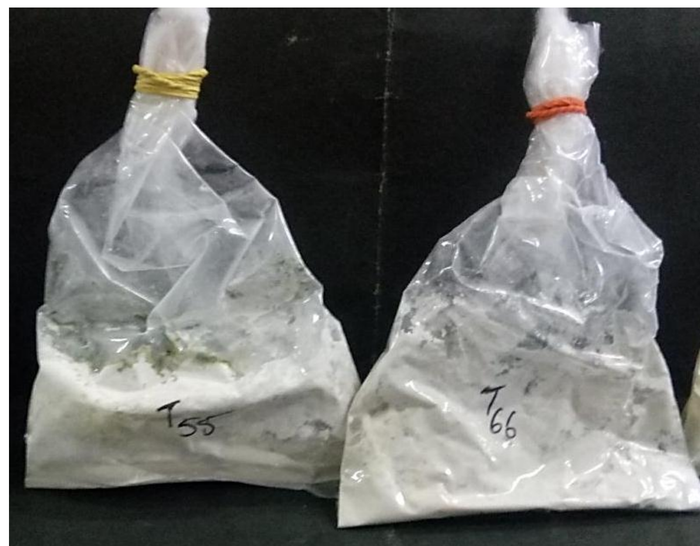


Fig. 19: Talc-based *Trichoderma* formulation

4.9.4. 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 22. From the Table, the mean population of *Trichoderma* isolates was observed to be increased rapidly up to 45 days and there after declined. At 15 days after sowing (DAS), maximum population was recorded in TR55 (0.78 x 10^6cfu/g) followed by TR122 (0.71 x 10^6cfu/g); TR66 (0.69 x 10^6cfu/g); and TR136 (0.64 x 10^6cfu/g). At 30 days after sowing (DAS), maximum population was recorded in TR55 (2.07 x 10^6cfu/g) followed by TR122 (1.92 x 10^6cfu/g); TR66 (1.87 x 10^6cfu/g); and TR136 (1.70 x 10^6cfu/g). At 45 days after sowing (DAS), maximum population was recorded in TR55 (3.73 x 10^6cfu/g) followed by TR122 (3.63 x 10^6cfu/g); TR66 (3.50 x 10^6cfu/g); and TR136 (3.37 x 10^6cfu/g). At 60 days after sowing (DAS), maximum population was recorded in TR55 (2.89 x 10^6cfu/g) followed by TR122 (2.72 x 10^6cfu/g); TR 66 (2.56 x 10^6cfu/g); and TR136 (2.46 x 10^6cfu/g).

Table 22. 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.	TR55	0.78^a (0.88)	2.07^a (1.44)	3.73^a (1.93)	2.89^a (1.70)
2.	TR66	0.69^b (0.83)	1.87^b (1.36)	3.50^c (1.87)	2.56^c (1.60)
3.	TR122	0.71^{ab} (0.84)	1.92^b (1.38)	3.63^b (1.90)	2.72^b (1.65)
4.	TR136	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

N.B: Means of the (3) three replications

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

4.10. To study the efficacy of talc-based *Trichoderma* formulation under *in vitro*

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

Table 23. Viability test of talc based *Trichoderma* formulation at different days of incubation by serial dilution technique

Sl. No.	*Trichoderma* isolates	Mean population (× 10^9cfu g-1) at different days of incubation (DOI)					
		15	30	45	60	75	90
1.	TR55	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.	TR66	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.	TR122	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.	TR136	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

N.B: Means of the three replications

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

Table 24. Effect of talc substrate on some functional characters of the potential antagonist *Trichoderma* isolates

Sl. No.	*Trichoderma* sp	Siderophore Production #	HCN Production **	Ammonia production ##	ACC deaminase activity
1.	TR55	*+++	++	+++	+
2.	TR66	+	++	++	+
3.	TR122	++	++	+++	+
4.	TR136	++	++	+++	+

Mean of three replications.

(-) indicates no production and (+) indicates production

* Degree of activity (+ + + + + > + + + + > + + + > + + > +)

** Colour, ranged deep yellow (+) to orange (++) to orange brown or dark brown (+++)

Colour, ranged blue (+) to purple or dark purplish (++) to red (magenta) (+ ++)

Colour ranged, yellow (+) to light brown (++) to dark brown colour (+++)

counted with the help of a colony counter after 24 hr of incubation for duration of three months at 15 days interval and observation recorded are presented in Table 23. It was observed that at 15 days after sowing (DAS), maximum population was recorded in TR 55 (5.41 x 10^9cfu/g) followed by TR122 (5.28 x 10^9cfu/g); TR66 (4.71 x 10^9cfu/g); and TR136 (4.39 x 10^9cfu/g). At 30 days after sowing (DAS), maximum population was recorded in TR55 (8.68 x 10^9cfu/g) followed by TR122 (7.67 x 10^9cfu/g); TR66 (7.07 x 10^9cfu/g); and TR136 (6.3 x 10^9cfu/g). At 45 days after sowing (DAS), maximum population was recorded in TR 55 (13.1 x 10^9cfu/g) followed by TR122 (11.44 x 10^9cfu/g); TR66 (10.85 x 10^9cfu/g); and TR136 (9.34 x 10^9cfu/g). At 60 days after sowing (DAS), maximum population was recorded in TR55 (9.29 x 10^9cfu/g) followed by TR122 (8.46 x 10^9cfu/g); TR66 (7.32 x 10^9cfu/g); and TR136 (6.97 x 10^9cfu/g). At75 days after sowing (DAS), maximum population was recorded in TR55 (6.52 x 10^9cfu/g) followed by TR122 (6.13 x 10^9cfu/g); TR66 (5.76 x 10^9cfu/g); and TR136 (5.46 x 10^9cfu/g). At 90 days after sowing (DAS), maximum population was recorded in TR55 (4.48 x 10^9cfu/g) followed by TR122 (4.23 x 10^9cfu/g); TR66 (3.73 x 10^9cfu/g); and TR136 (3.34 x 10^9cfu/g).

Effect of talc substrate on some functional characters like ACC deaminase activity, siderophore, HCN and ammonia production of TR55, TR66, TR122 and TR136 were studied and results are presented in Table 24. From the Table it was observed that the talc substrate did not effect the functional traits of tested isolates. All four isolates showed positive for ACC deaminase activity, siderophore, HCN and ammonia production.

Chapter-5

Discussion

Experimental findings of the present investigation was carried out to study the bio-efficacy of *Trichoderma* formulation against damping-off caused by P*ythium* sp. and *Rhizoctonia solani* kuhn. on tomato (S*olanum lycopersicum*) are being discussed in this chapter.

5.1. Isolation of Fungal Pathogens (*Pythium* sp. and *R. solani*)

Tomato damping off pathogen showed colourless filamentous mycelium with irregularly branched, coenocytic and aseptate hyphae under microscopic observation. The fungus was also found to produce globose sporangia, smooth and spherical oospores (Fig 1a). On cornmeal agar (CMA), the pathogen showed white cottony aerial mycelium and on PDA heavy aerial mycelia which are the characteristic of *Pythium* sp. (Alexopaulos *et al.*, 1996; Zappia *et al.*, 2014; Deadman, 2017). Hence, the pathogen isolated from damped-off tomato plant was confirmed as *Pythium* sp. Another pathogen showed dull white in the beginning which became brown later on potato dextrose agar (PDA). The fungus showed light brown mycelium, with septate hyphae measuring 5-14 µm in width and branched at right angles to the hyphal cell under microscopic observation. Hyphae showed constriction at the point where branching was there (Fig 1b). Similar cultural and morphological characters of the pathogen isolated in the present investigation were also observed by Gupta and Paul (2004), Gaigole *et al.* (2011) and Shinde (2016). So the pathogen was confirmed as *R. solani* Kuhn.

5.2. Pathogenecity Test

The pathogenicity for *Pythium* sp. and *R. solani* isolated from damped-off infested tomato nursery was successfully proved by soil inoculation method which confirmed the association of both the pathogens with the host plants. The pathogenic nature of both the pathogens producing damping-off disease symptoms in tomato “Hybrid-017” reported in this present investigation were in line with the findings of Jiskani *et al.* (2007), Huang *et al.* (2011) and Rajendraprasad (2014) who proved the the pathogenicity of *P. aphanidermatum* and *R. solani* Kuhn on tomato by soil inoculation method and they found 100 per cent damping off incidence in tomato.

5.3. Collection and isolation of *Trichoderma* from Different Locations of Meghalaya

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 habitats *i.e.*, crop rhizosphere, compost manure, pig manure, jhum areas and forest. The lime green to greenish colour sporulation with highly fluffy growth and sparse to compact colony after 7-10 days of incubation obtained from soil samples collected were identified as *Trichoderma* sp. based on Rifai (1969) and Bisset (1992). Several workers reported the predomination of *Trichoderma* in natural soils and decaying wood (Kredics *et al.*, 2012), plant materials and compost (Khandelwal *et al.*, 2012), jhum areas (Quimio, 2001). During the present study, maximum isolates (87) were obtained from crop rhizosphere which was similar to the findings of Kumar *et al.* (2012), Rai *et al.* (2016), Jaisani and Pandey (2017) who reported that the presence of organic humus matter in the soil largely determined the availability of promising antagonistic fungi.

5.4. Morphological characterization

Isolated fungal species were identified tentatively as *Trichoderma* sp. based on the study of their morphology characters (Rifai, 1969; Bisset, 1992) like; isolates which showed lime green to greenish colour sporulation with highly fluffy growth and sparse to compact colony after 7-10 days of incubation were selected (Fig 3). Under microscopic observation, it was observed that all selected isolates have main phialides and sub phialides which is 90° angle to main phialides and conidiophores contain spore on its tip. Therefore, during the present investigation, the morphological characteristics and microscopic observation of phialides and conidiophores initially confirmed those 97 isolates as *Trichoderma* species. Similar finding was reported by Domsch *et al.* (1980), Sutton *et al.* (1998), Rini and Sulochana (2007), Shaiesta *et al.* (2012) and Chennappa *et al.* (2017), who observed that *Trichoderma* colonies appeared white in beginning, with advancement of age among the isolates it varied from dark green to light green or yellowish green. Most of the isolates showed apprised and flat growth, whereas fluffy growth was observed in few *Trichoderma* isolates. Under microscopic observation features of mycelial of the isolates has main branch with sub branch at right angle to main branch and conidiospore was observed on the tip of the conidiophore (Rifai, 1969; Quimio, 2001; Prameela *et al.*, 2012; Chowdhury *et al.*, 2014; Sekhar *et al.*, 2017).

5.5. Screening of isolated *Trichoderma* for functional attributes *in vitro*

Via rapid screening of the 97 isolates, only 20 isolates *i.e.*, TR12, TR24, TR36, TR40, TR55, TR64, TR66, TR74, TR78, TR82, TR87, TR88, TR106, TR109, TR112, TR116, TR122, TR136, TR143 and TR171 were selected for screening of their functional attributes.

5.5.1. Dual culture assay against *Pythium* sp

The dual culture assay of the 20 isolates of *Trichoderma* sp. against *Pythium* sp. showed that most of the isolates inhibited the radial growth of the fungal pathogen ranged from 0.97 cm to 2.43 cm and the inhibition percentage ranged from 68.89 to 89.26 per cent. TR55 isolated from tomato rhizosphere was found to be the most effective isolate against *Pythium* sp., showing an inhibition up to an extent of 89.26 per cent followed by other isolates like TR66 (from brinjal rhizosphere) , TR122 (from tomato rhizosphere) and TR136 (from tomato rhizosphere) with inhibition percent of 88.89, 88.15 and 87.78 per cent, respectively. These isolates were found positive of HCN, chitinase, antibiotics (trichothecenes), secondary metabolites (serine protease) and enzymes production (endochitinase and β-1,6-Glucanase). So, the antagonism shown by the various isolates might be due to HCN, chitinase, antibiotic, enzymes and secondary metabolites production. The antagonism of *Trichoderma* sp. against *Pythium* sp. were widely reported (Schirmbock *et al*., 1994; Muthukumar *et al*., 2011; Waghunde *et al.,* 2016; Naik *et al.,* 2017; Rajendraprasad *et al.,* 2017) which supported the antagonism of *Trichoderma* sp. against *Pythium* sp. during the present investigation.

5.4.2. Dual culture assay against *Rhizoctonia solani* Kuhn.

Dual culture assay of 20 isolates of *Trichoderma* sp. against *R. solani* Kuhn. revealed that the radial growth of the fungal pathogen ranged from 1.13 cm to 2.7 cm and the inhibition percentage ranged between 70 to 87.41 per cent. TR55 isolated from tomato rhizosphere was found to be the most effective isolate against *R. solani*, showing an inhibition to the tune of 87.41 per cent followed by other isolates like TR122 (from tomato rhizosphere), TR66 (from brinjal rhizosphere) and TR88 (from forest) with inhibition of 86.48 , 84.07 and 82.96 per cent, respectively. These isolates were found positive to HCN, chitinase, antibiotics (trichothecenes), secondary metabolites (serine protease) and enzymes production (endochitinase and β-1,6-Glucanase) and their antagonism might be due to these traits. Sharma *et al*. (2014)

established that the antagonism of *Trichoderma* against *R. solani* was due to the production of cell wall degrading enzymes like chitinase and β-1,3-glucanase which degrades hyphae as well as digest the cell wall of fungal pathogen. The antagonism of *Trichoderma* sp. against *R. solani* were reported by several workers (Jash and Pan, 2004; Anitha and Das, 2011; Goud *et al*., 2015; Kotasthane 2015; Kumari *et al.,* 2016) which supported the antagonism of *Trichoderma* sp. against *R. solani* Kuhn. of the present investigation.

5.4.3. Screening for antimicrobial traits of *Trichoderma* isolates

Trichoderma species are known for ability to produce antifungal antibiotics (like gliovirin, gliotoxin, viridin, pyrones and peptaibols) and cell wall degrading enzymes (such as β-1,3 glucanase, Chitinase, etc) which are effective against several plant pathogens. In this investigation, the isolates were tested for detection of the presence of genes which are involved in the biosynthesis of β-1,6-Glucanase (*Tvbgn3*), Trichodiene synthase (*tri5*), Serine protease (*ser*) and Endochitinase (*ech42*) using specific primer Tvbgn3F-Tvbgn3R, tri5F- tri5R, serF-serR and ech42F-ech42R, respectively (Table 6). Out of the 20 isolates detected whether these genes presence or not, 12 isolates showed expected amplicon size for β-1,6-Glucanase (*Tvbgn3*), 10 isolates for Trichodiene synthase (*tri5*), 14 isolates for Serine protease (*ser*) and 17 isolates for Endochitinase (*ech42*). The findings are in accordance with the reports of Gallo *et al*. (2004), Djonovic *et al.* (2006), Barbara *et al*. (2011), Shi *et al*. (2012), Singh *et al.* (2014) and Fahmi and Ragaa (2016).

The production of lytic enzymes by *Trichoderma* species is known as one of the important mechanisms for biocontrol activity against phytopathogenic fungi. Out of the 20 *Trichoderma* isolates tested for chitinolytic activity on chitin detection medium, 17 showed positive for chitinase production. Involvement of chitinase in control of phytopathogens was reported by several workers (Harman *et al*., 2004; Harighi *et al*., 2007, Geraldine *et al.,* 2013; Asad *et al*., 2015, Thakkar and Saraf 2015) which support the present investigation.

Ability of *Trichoderma* sp. to produce siderophores under the condition where iron is less is believed to play a role in biological management of the pathogens. Screening of 20 *Trichoderma* isolates for siderophores production revealed 16 isolates possessed the ability for siderophore production as indicated by colour-changed from blue to purple or dark purplish- red (magenta) in the C.A.S.-blue agar (Fig 8b). Qi and Zhao (2013), Zhao *et al*. (2014), Dixit *et al*. (2015) and Ghosh *et al*. (2017) also

reported that several species of *Trichoderma* produced siderophores as observed in the present study.

The production of HCN by *Trichoderma* is believed to play a part in the suppression of pathogens. In the present investigation, 13 isolates were positive for HCN production as evidenced by the change in the colour of filter paper (Fig 8b). Rawat and Tewari (2011), Parameswari *et al.* (2015), Zhang *et al.* (2016) and Rajendraprasad *et al.* (2017) also reported the production of HCN by *Trichoderma*, which support the present findings.

5.5. Screening for the ability of *Trichoderma* isolates to promote plant growth

Trichoderma isolates promotes the plant growth directly or indirectly by one or more mechanisms like production of phytohormones (IAA, cytokinin and gibberellins), lowering of ethylene levels, production of ACC deaminase enzyme, nitrogen fixation, solubilisation of minerals like phosphorus, manganese and zinc (Singh *et al.*, 2014; Bhattacharyya and Jha, 2017).

In the present investigation, all 20 isolates estimated quantitatively for their IAA producing and Phosphorous solubilizing ability 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). Many workers reported that *Trichoderma* reside in the rhizosphere of plants and play an important role in solubilization of bound phosphates, making them available to plants (Saravanakumar *et al.,* 2012; Promwee *et al.,* 2014; Chagas *et al.,* 2015; Thakkar and Saraf, 2015). Aban and his co-workers (2017) also reported IAA production and phosphate solubilisation by *Trichoderma yunnanense* and *T. simmonsil* which is similar to the present findings.

Again in the present study, all 20 screened isolates grew on Difco (DF) salts minimal medium showing their ability to produce ACC deaminase whereas only 16 isolates were found positive for ammonia production indicated by development of yellow to brown colour in Nessler's reagent. Ammonia is present in soil, water and air, and it is an important source of nitrogen for plants. Harman (2000), Singh *et al.* (2014) and Singh *et al.* (2018) reported that *Trichoderma* sp. is a very important rhizosphere microorganisms that can colonize at the outer epidermal layers of the roots and the

nitrogen utilization efficiency in plants seem to be increasing. ACC deaminase production of *Trichoderma* sp was reported by several workers (Viterbo *et al*., 2010; Hermosa *et al*., 2012; Aban *et al*., 2017). Triveni *et al*. (2013) reported the IAA producing ability of *Trichoderma* inoculants, with values ranging from 0.013 to 0.082 µg/ml and observed increased in plant growth. The ability of *Trichoderma* sp. on the growth promotion *in-vitro* has been reported by several workers (Kamala and Indira, 2011; Srivastava *et al*., 2013; Rocha *et al*., 2016 and Rajendraprasad *et al*., 2017).

5.6. Cultural and anamorphic characteristics

Those 10 isolates *viz*., TR55, TR64, TR66, TR87, TR88, TR106, TR109, TR112, TR122 and TR136 which possessed 9 (TR109) or 10 (TR64, TR87, TR88, TR106 and TR112) or 11 (TR55, TR66, TR122 and TR136) traits out of those 11 traits tested as shown in score card (Table 11) were studied on PDA medium for their cultural and anamorphic characteristics and results (Table 12) showed that few isolates grew fast (TR106, TR109 and TR112) whereas few grew very fast (TR55, TR64, TR66, TR87, TR88, TR122 and TR136). Few isolates showed dense colony (TR64, TR87, TR88 and TR136), compact in few (TR66 and TR112) whereas powdery colony in TR109 and colony found aggregated near the margin in TR55, TR106 and TR122. White pustules pigment was observed in TR55, TR66 and TR122, light green in TR64, TR87, TR88, TR109 and TR112 whereas dark green pigment in TR106 and TR136. All isolates showed hyaline hyphae and no characteristics odour. Similarly Biswas (1999) and Rekha (2010) reported that mycelial growth of *Trichoderma* isolates did not show significant variations, colonies grew very fast and initially they were smooth surfaced and almost translucent, later became powdery or compactly tufted and pigmentation of the isolates were almost similar from light green to deep green or pure white coloration. The anamorphic characters *viz*., conidiophores length and width, phialides length and width, conidia length and width showed variation among the isolates studied in the present investigation. Size of phliades ranged from 3.0-8.4x1.6-3.7 µm, size of conidia from 1.8-5.2x1.4-2.7 µm and size of conidiophores from 4.2-37.8x2.1-5.5 µm. These findings are duly supported by earlier observations (Rifai, 1969; Domsch *et al.,* 1980, Bissett, 1991; Samuel, 1996; Samuel, 2006; Shaiesta *et al*., 2012 and Chennappa *et al*., 2017) where they characterized different species of *Trichoderma*.

5.7. Phylogenic analysis

Again those 10 isolates *viz*., TR55, TR64, TR66, TR87, TR88, TR106, TR109, TR112, TR122 and TR136 which possessed 9 or 10 or 11 traits out of those

11 traits tested as shown in score card (Table 11) were further confirmed as *Trichoderma* upto species level by PCR based method using a set of universal primer *viz*. ITS1 and ITS4 which amplify the internal transcribed region 1 and 2. The isolates (10) showed amplification at the expected size *i.e.*, 600bp (Fig 12). The amplified products were outsourced for sequencing and identity established (Table 14) based on the BLASTN 2.8.1+ software available in NCBI website showed hit to 2 species of *Trichoderma* but different strain. TR55, TR66, TR87 and TR122 showed 99-100 % identity to *Trichoderma hamatum* whereas TR64, TR88, TR106, TR109, TR112 and TR136 showed 99 % identity to *Trichoderma harzianum.* Based on MEGA 5.2 software clustering of the 10 isolates showed 2 main class (A and B). For phylogenetic analysis MEGA software (MEGA 5.2) was used and using neighbor joining method available in MEGA 5.2 software, the phylogenic tree was constructed with 1000 bootstrapping value. Clustering of the 10 isolates showed 2 main classes, class A and B (shown in Fig 15). 90 per cent of the isolates which fall under Class A belong to the same species *i.e*. *Trichoderma hamatum* (TR55, TR66, TR87 and TR122) with closeness ranging from 13-96 per cent whereas all of the isolates under Class B belong to *Trichoderma harzianum* (TR64, TR88, TR106, TR109 and TR136) with closeness ranging from 18-95 per cent. The findings are in accordance with the reports of Chakraborty *et al*. (2010) and Shahid *et al*. (2014) where similar universal primers set ITS1 and ITS4 was also used to characterized the *Trichoderma* isolates and 600 bp amplicon size was observed and also identified the *Trichoderma* species based on sequence homology. In the present study, TR55 and TR122 belonging to sub-class 2A showed 96 per cent closeness belong to same species *T. hamatum* whereas TR106 and TR109 belonging to sub-class $2Bb_2$ showed 95 per cent closeness belong to *T. harzianum*. This result was previously observed by other workers in the taxonomic studies on *Trichoderma* (Hermosa *et al.*, 2000; Maymon *et al.*, 2004; Kamala *et al*. 2015).

5.8. To study the plant growth promotion & biocontrol potential of screened *Trichoderma* sp. against *Pythium* sp*.* and *Rhizoctonia solani* Kuhn.

From those 10 isolates, 4 isolates *viz.*, TR55, TR66, TR122 and TR136 which possessed 11 traits (chitinase production, ammonia production, siderophore production, HCN production, ACC deaminase activity, IAA production, phosphorous solubilisation, trichodiene synthase, β-1,6-glucanase, serine protease and

endochitinase gene**)** out of those 11 traits tested as shown in score card (Table 11) were selected for *in-vivo* studies.

In the present study, the biopriming of seeds of tomato (Hybrid-017) done for potent isolates of *Trichoderma* sp. *viz*., TR55, TR66, TR122 and TR136 and results (Table 16) showed that they have significantly increased shoot and root length, the percentage of germination and also vigor index when compared to untreated control. TR55 (*T. hamatum* strain CEN693) recorded the highest root and shoot length, percentage of germination and also vigour index (14.15±0.29 and 10.9±0.56, 92 % and 2306, respectively), followed by other isolates like TR122, TR66 and TR136. Several researchers have reported the biological seed treatments for protection of seed and management of the pathogens causing diseases in seedling (Lifshitz *et al*., 1986; Lewis and Fravel, 1996; Mastouri *et al.,* 2010; Islam *et al.* 2011; Doni *et al*., 2014; Pandey, 2017). Reddy (2012) bio-primed sweet corn seeds with *Trichoderma harzianum* and reported that seed priming alone or in combination with low dosage of biological agents showed improvement in 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 the germination of seeds, the shoot length, the root length, seedling mean, dry matter production, vigour index in blackgram, chilli, cotton, sunflower, and tomato, which support the present finding.

Under pot experiment, the effect of the different treatments against pre and post emergence damping off and their ability to reduce the damping off incidence caused by *Pythium* sp. and *R. solani*, also effect on the growth parameters of the tomato variety Hybrid-017 was studied and the results (Table 17-21) showed that 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* sp. and *R.* solani). However, T_3 showed the best mode of application of *Trichoderma* for optimum management of pre and post-emergence damping off. TR55 (*T. hamatum* strain CEN693) showed the least pre and post emergence damping off disease incidence and highest biological control efficacy followed by TR122, TR66, TR136 and least in untreated control. The reason behind the most effectiveness of TR55 might be because of the ability of this isolate to compete for space, nutrients and light or by producing secondary metabolites, HCN, produce enzyme that degrade the cell wall, as it has showed under *in-vitro* experiment. A number of previous studies made by other researchers also support and reflect the same findings (Di -Pietro *et al*., 1993; Gravel *et al*., 2005; Shabir and Rubina, 2010; M-Uddin *et al*., 2011; Rakholiya *et al*., 2016). Thakur and Tripathi (2015) 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) reported pre and post emergence of damping off disease control in neem seedlings with application of *Trichoderma koningii* and *T. harzianum* either singly or with arbuscular mycorrhizal fungi. Uma Devi *et al.* (2017) 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 crops 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, the number of leaves per plant ranged 32 to 57.20, whereas the 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 weight as well as the dry weight of tomato plants was seen highest in TR55. The yield increased in all the treatments compared to control (1.4 kg) with maximum yield in TR55 (2.25 kg). This might be because of the IAA, ammonia, siderophore production, ACC deaminase activity and phosphorous solubilizing ability of TR55 as it showed positive for all these test *in-vitrocally*. The potential of *Trichoderma* sp. to enhance plant height, root length, fresh weight as well as dry weight and yield of the crop was also reported by several researchers (Gravel *et al.*, 2007; Kapri and Tewari, 2010; Tallapragada and Gudimi, 2011; Rehman *et al.,* 2012; Saravanakumar *et al.,* 2012; Borges *et al.*, 2015; ; Singh *et al.*, 2016). Altomare *et al.* (1999) reported that under glasshouse conditions, growth parameters including shoot length, root length, fresh weight as well as dry weight of shoot and roots, in P deficient soil containing only bound phosphate (TCP) were found to be increased in *Trichoderma* sp. inoculation chickpea (*Cicer arietinum*). Rajendraprasad *et al.* (2017) reported that the combination of *Trichoderma* isolates and potential bacterial treatment proved effective in increasing the shoot and root weight, fresh as well as dry weight and yield of tomato plants. Sodimalla *et al.* (2012) observed that *Trichoderma–Azotobacter* biofilm which recorded the highest nitrogenase activity and 1-aminocyclopropane-1carboxylic (ACC) deaminase increased growth parameters and also high yield was achieved when applied to the crop, which was similar to the present investigation.

5.9. Rhizosphere colonization

The rhizosphere competence of the potent isolates of *Trichoderma* sp. showed variation in the levels of colonization. It is observed from the result that the mean population of *Trichoderma* isolates was recorded to be increased rapidly up to 45 days and there after declined. Increase in the rate of colonization up to certain amount of time and the reduction in the population 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 before any other microorganisms, then they can provide a protective cover for root tips and hairs which otherwise vulnerable to attack by several plant pathogenic fungi. 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 (McLean *et al.,* 2005) Sathiyaseelan *et al.* (2009) reported that *Trichoderma viride* is highly rhizosphere competent and able to colonize and grow on roots as they develop. Prabha *et al.*, (2015) 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. In the present findings there were increased in the rhizosphere population of *Trichoderma* sp. with the advancement of crop age upto 45 DAS and thereafter the population declined marginally. Reduction in the population of *Trichoderma* after certain period may be due to the decrease in the amount of nutrients in the potting soil which was also reported by other workers like Aziz *et al.* (1997), Binitez *et al.* (2004) and Yang *et al.* (2011).

5.10. To study the efficacy of talc-based *Trichoderma* formulation under *in vitro*

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 recorded to be increasing rapidly upto 45 days and thereafter declined (Table 23). Maximum population was observed in TR55 (13.1 x 10^9cfu/g at 45 DAS). Also it is observed that the talc substrate did not hamper the functional attributes of the isolates (Table 24). Increase in the rate of population upto certain amount of time and the reduction in the population later may be the result of decrease in the availability of nutrient and moisture in the talc

substrates. These findings are in consistent with the findings of Das *et al.* (2006) 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) 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) reported that microbial count of *T. harzianum* and *Bacillus subtilis* were highest initially but a gradual decline was recorded with the increase in the storage time, which supported the present investigation.

Chapter - 6

Summary and Conclusion

Experimental findings of the investigation on bio-efficacy of native *Trichoderma* formulation against damping-off caused by *Pythium* sp. and *Rhizoctonia solani* on tomato (*Solanum lycopersicum*) are summarized below:

- From all 11 districts of Meghalaya, 180 soil samples were collected and *Trichoderma* isolates were found predominant in different habitats *viz.* crop rhizospheres, compost manure, pig manure, forest soil and jhum area soil.
- A total of 97 *Trichoderma* isolates were obtained on the basis of the morphology characters and maximum isolates were found associated with different crop rhizosphere (87).
- On the basis of modified Bell's scales, 20 *Trichoderma* isolates were screened to evaluate their antagonistic ability against *Pythium* sp. and *Rhizoctonia solani* by dual culture method.
- Dual culture assays of the 20 screened isolates against damping-off pathogens revealed that the 4 *Trichoderma* isolates *viz.* TR55, TR66, TR122 and TR136 were effective in inhibiting *Pythium* sp. with percent inhibition of 89.26, 88.15, 88.89 and 87.78 respectively, which were statistically similar, whereas only 2 isolates *viz.* TR55 and TR122 were found effective against *R. solani* with percent inhibition of 87.41 and 86.48, respectively, which were statistically similar. Isolate TR 55 recorded as the best antagonist against both the pathogens *i.e. Pythium* sp. and *Rhizoctonia solani.*
- All 20 screened isolates were found positive for ACC deaminase production and 17 positive for chitinase production with isolate TR 106 and TR 136 found best in chitinase production with purple colour zone of 9 cm in diameter. 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 4.96µg/ml and 0.03 to 0.98 µg/ml, respectively.

- Presence of antibiotic biosynthetic genes in the 20 screened isolates by using gene specific primers detected that 12 isolates were positive for β-1, 6-Glucanase (*Tvbgn3*), 10 isolates for Trichodiene synthase (*tri5*), 14 isolates for Serine protease (*ser*) and 17 isolates for Endochitinase (*ech42*). Only 4 isolates showed presence of all 4 antibiotic biosynthetic genes tested.
- Based on the detection of antimicrobial and plant growth promoting traits of the 20 *Trichoderma* isolates, 10 isolates which possessed 9 or 10 or 11 traits out of those 11 traits tested as shown in score card. Potential *Trichoderma* isolates were recorded for fast to very fast growth, no characteristic smell found and hyaline hyphae observed. Dense to compact mycelial growths were observed in all the isolates except for TR55, TR106 and TR122, where conidia found aggregated near the margin. Pigmentation of the hyphal growth was studied and found that isolates TR55, TR66 and TR122 produced white pustules pigment, light green observed in case of TR64, TR87, TR88, TR109 and TR112, whereas dark green pigment produced by isolates TR106 and TR136. Variation in anamorphic characters *viz*., size of phialides, conidia and conidiophores were seen among the 10 isolates**.**
- The phylogenic analysis of the 10 *Trichoderma* species based on the BLASTN 2.8.1+ software available in NCBI website showed hit to 2 species of *Trichoderma viz*., *T. hamatum* (TR55, TR66, TR87 and TR122) and *T. harzianum* (TR64, TR88, TR106, TR109 and TR136).
- Based on MEGA 5.2 software clustering of the 10 *Trichoderma* isolates showed 2 main class (A and B). Class A showed sub-class 1A and 2A. Again sub-class 1A showed sub-class 1Aa and 1Ab with closeness of 13 per cent. Sub-class 1Aa showed sub-class $1Aa_1$ and $1Aa_2$ with 65 per cent closeness. Main class B showed sub-class 1B and 2B with 23 per cent closeness. Again sub-class 2B showed sub-class 2Ba and 2Bb with 18 per cent closeness. Sub-class 2Bb showed sub-class $2Bb_1$ and $2Bb_2$ with 37 per cent closeness. TR 55 and TR122 belonging to sub-class 2A showed 96 per cent closeness belong to same species *T. hamatum* whereas TR106 and TR109 belonging to sub-class $2Bb_2$ showed 95 per cent closeness belong to *T. harzianum*.
- Four *Trichoderma* isolates (TR55, TR66, TR122 and TR136) which possessed 11 traits out of those 11 traits tested as shown in score card (Table 11) were finally selected for evaluation for their growth promoting ability and biological

control potential against *Pythium* sp. and *R. solani* under pot culture experiment.

- The biopriming of tomato seeds with 4 *Trichoderma* isolates showed considerable increase in germination percentage and vigour index over control. Highest germination percentage and vigour index was observed in TR55 (75.13 % and 47.99 % respectively), followed by TR122 (69.93 % and 44.61 %, respectively), TR66 (68.31 % and 42.04 %, respectively) and TR136 (66.68 % and 39.67 %, respectively) as compared to untreated control (51.97 % and 26.92 %, respectively). All isolates were statistically significant.
- *In vivo* efficacy of 4 potent isolates *viz*., TR55, TR66, TR122 and TR136 of *Trichoderma* against *Pythium* sp. and *R. solani* showed reduction of the disease incidences in all three treatments (seed application, soil application and seed and soil combined application) under pot culture experiment with TR 55 (*T. hamatum* strain CEN693, isolated from tomato rhizosphere, West Jaintia hills) showing highest biological control efficacy both in pre-emegence and post-emergence damping-off.
- Increased on the growth parameters such as root length, plant height, number of leaves, number of flowers, fresh as well as dry weight and yields of tomato "Hybri-017" was observed in all the treatments.
- Variation was observed in rhizosphere colonization of 4 potent isolates of *Trichoderma* sp. (TR55, TR66, TR122 and TR136) at different days after sowing/ inoculation in sterilised potting soils. Study revealed 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 TR55 (3.73×10^6)' followed by TR122 (3.63×10^6), TR66 (3.50×10^6) and TR136 (3.37×10^6).
- Viability of 4 potent *Trichoderma* isolates were checked in talc based formulation at different days (15, 30, 45, 60, 75 and 90 days) of incubation by serial dilution plate technique. *Trichoderma* isolates *viz*., TR55, TR66, TR122 and TR136 showed variation in the levels of colonization at different days of incubation in talc powder. It was observed that the rate of colonization increases up to 45 days after incubation and reduced at 60 days after incubation in all the treatments. Colonization was highest in TR55 (13.1×10^9)' followed by TR122 (11.44×10^9), TR 66 (10.85×10^9) and TR136 (9.34×10^9).

- The functional traits like siderophore production, HCN production, ammonia production and ACC deaminase activity of the four potent *Trichoderma* isolates (TR55, TR66, TR122 and TR136) were not hampered by talc substrate was observed during present investigation.
- Based on functional attributes, disease suppression, growth promotion, rhizosphere colonization and shelf life in talc formulation 3 native *Trichoderma* isolates TR55 (tomato, West Jaintia hills district), TR66 (brinjal, West Jaintia hills), TR122 (tomato, South West Khasi) and TR136 (tomato, West Khasi Hills) could be further evaluated under field condition against damping off of disease under Meghalaya condition and to develop *Trichoderma* formulation for tomato growers of the state.

Conclusion

T. hamatum and *T. harzianum* were identified as the dominant *Trichoderma* species associated with different habitats in Meghalaya. TR55, TR66, TR122 and TR136 were the potential isolates against the damping-off pathogens (*Pythium* sp. and *R. solani* Kuhn. of tomato under *in vitro* and pot culture experiments. All the four potent isolates remain viable for upto three months in talc formulation. These four potent isolates need further evaluation under field conditions at different location of Meghalaya to develop effective bio-formulation against damping off of tomato under Meghalaya conditions.

BIBLIOGRAPHY

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., Jiang, D. and Fu, Y. (2017). *Trichoderma* spp. as Antagonist of *Rhizoctonia solani*. *J. Plant Pathol. Microbiol.*, 8: 402.

Abdel-Fattah, G.M., Shabana, Y.M., Ismail, A.E. and Rashad, Y.M. (2007). *Trichoderma harzianum*: A biocontrol agent against *Bipolaris oryzae*. *Mycopathol.*, 164: 81-89.

Abd-El-Khair., Khalifa, H.R.Kh.M. and Karima, H.E.H. (2010). Effect of *Trichoderma* species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants. *J. Am. Sci.*, 6(9): 486-497.

Abdelzaher H. (2004). Occurrence of damping-off of wheat caused by *Pythium tokunaga* in El-Minia, Egypt and its possible control by *Gliocladium roseum* and *Trichoderma harzianum*. *Arch. Phytopathol. Plant Protect.*, 37: 147–159.

Agrios, G.N. (2005). *Plant Pathol.* Elsevier Academic Press, San Diego, CA. pp. 593–598.

Ahmad, J. and Baker, R. (1988). Rhizosphere competence of *Trichoderma harzianum*. *Can. J. Microbiol.*, 34: 229-234.

Ahsan, M.S., Kumar, M., Upadhyay, J.P., Hussain, M.A., Gupta, P.K. and Singh, A. (2018). Effect of different Doses of *Trichoderma harzianum* and Fungicides for the Management of Collar Rot of Chickpea Caused by *Sclerotium rolfsii*. *Int. J. Pure Appl. Biosci.*, 6(1): 1656-1660.

Aires, R.S., Steindorff, S.A., Ramada, S.M.H., Siqueira, S.J.L. and Ulhoa, C.J. (2012). Biochemical characterization of a 27 kDa 1,3-β-d-glucanase from *Trichoderma asperellum* induced by cell wall of *Rhizoctonia solani*. *Carbohydr. Polym.*, 87(2): 1219-1223.

Ajilogba, C., Babalola, O. and Ahmad, F. (2013). Antagonistic Effects of *Bacillus* Species in Biocontrol of Tomato *Fusarium* Wilt. *Ethno. Med.*, 7(3): 205-216.

Alexander, L.J., Young, H.C. and Kiger, C.M. (1931). The Causes and Control of Damping-off of Tomato Seedlings. *Bull. 496*, pp. 3-36.

Alexopaulos, C.J., Mims, C.W.C. and Blackwell, M. (1996). Introductory Mycology. John Willy and sons, Inc. New York, pp. 243-250.

Altomare, C., Novell, W.A., Bjorkman, T. and Harman, G.E. (1999). Solubilization of phosphates and micronutriens by the plant -growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Appl. Environ. Microbiol.*, 65: 2926-2933.

Alwathnani, A.H. (2012). Biological control of *Fusarium* wilt of tomato by antagonist fungi and cyanobacteria. *African J. Biotechnol.*, 11: 5.

Amaresh, Y.S., Chennappa, G., Naik, M.K., Kiran, R., Ashwathanarayan D.S., Patil, M.G., Mahadevaswami, Nair, D. and Shruthi, P. (2017). Morphological, Molecular Characterization of *Trichoderma* species Isolated from Different Rhizosphere Soils and Its Anti-Pathogenic Properties. *Imp. J. Interdiscip. Res.*, 3(4): 2454-1362.

Ananthi, M., Selvaraju, P. and Sundaralingam, K. (2017). Evaluation of seed biopriming with biocontrol agents and biopesticides spraying on pests and its effect on seed yield and quality in chilli. *J. Entomol. Zool. Stud.*, 5(4): 667-672.

Aneja, K.R. (2003). Experiments in microbiology, Plant pathology and Biotechnology. 4th edn. New Age International Publishers, India, pp. 13-19.

Anitha, A. and Das, M.A. (2011). Activation of rice plant growth against *Rhizoctonia solani* using *Pseudomonas fluorescens*, *Trichoderma* and Salicylic Acid. *Res. Biotechnol.*, 2(4): 07-12.

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

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

Askew, D. and Laing, M. (1994). The *in vitro* screening of 118 *Trichoderma* isolates for antagonism to *Rhizoctonia solani* and an evaluation of different environmental sites of *Trichoderma* as sources of aggressive strains. *Plant Soil*, 159: 277-281.

Aziz, N.H., El-Fouly, M.Z., El-Essawy, A.A. and Khalaf, M.A. (1997). Influence of bean seedling root exudates on the rhizosphere colonization by *Trichoderma lignorum* for the control of *Rhozoctonia solani*. *Bot. Bull. Acad. Sin.*, 38: 33-39.

Babu, K.N. and Pallavi, P.N. (2013). Isolation, identification and mass multiplication of *Trichoderma* an important bio-control agent. *Int. J. Pharm. Life Sci.*, 4(1): 2320-2323.

Bala, K., Robideau, G.P., Desaulniers, N., de Cock, A.W. and Levesque, C.A. (2010). Taxonomy, DNA barcoding and phylogeny of three new species of *Pythium* from Canada. *Persoonia*, 25: 22-31.

Balakrishnan, S., Gopalakrishnan, C., Kamalakannan, A., Kuppusamy, K.S. and Parthasarathy, S. (2017). Evaluation of antagonistic activity and plant growth promotion by paste formulation of *Trichoderma harzianum*. *J. Pharmacogn. Phytochem.*, 6(6): 355-360.

Balemi, T. and Negisho, L. (2012). Management of Soil Phosphorus and Plant Adaptation Mechanisms to Phosphorus Stress for Sustainable Crop Production: A Review. *J. Soil Sci. Plant Nutr.*, 12: 547-562.

Barbara, R., Enrique, I.L.. Robert, L.M and Alfredo. H.E. (2011). Identification of mycoparasitism-related genes in *Trichoderma atroviride*. *Appl. Environ. Microbiol.*, 70: 4361–4370.

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

Bhagat, S. and Pan, S. (2010). Cultural and phenotypic characterization of *Trichoderma* species from Andaman and Nicobar Islands. *J. Mycol. Plant Pathol.*, 40(1): 145-157.

Bhattacharyya, P.N. and Jha, D.K. (2017). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.*, 28(4): 1327-50.

Bhowmik, D., Kumar, K.S., Paswan, S. and Srivastava, S. (2012). Tomato-a natural medicine and its health benefits. *J. Pharmacogn. Phytochem.*, 1(1): 33-43.

Binitez, T., Rincon, A.M., Limon, M.C. and Codon, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbiol.*, 7(4): 249-260.

Bissett, J. (1984). A revision of the genus *Trichoderma* l*ongibrachiatum*. *Can. J. Bot.*, 62: 924-931.

Bissett, J. (1991). Exploration and Interaction of *Trichoderma* species. *Can. J. Bot.*, 69: 2373-2417.

Bissett, J. (1992). *Trichoderma atroviride. Can. J. Bot.*, 70(3): 639-641.

Biswas, K.K. (1999). Screening of isolates of *Trichoderma harzianum* Rifai for their relative biocontrol efficacy against *Fusarium oxysporum* f.sp. *udum* and *Rhizoctonia solani* Kuhn. *Ann. Plant Prot. Sci.*, 7(2): 125-130.

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

Brimecombe, M.J., De-Leij, F.A. and Lynch, J.M. (2001). The effect of root exudates on rhizosphere microbial populations. In: Pinton, R., Varanini, Z. and Nannipieri, P. (ed) The Rhizosphere: biochemistry and organic substances at the soil-plant interface. pp. 16.

Broders, K.D., Wallhead, M.W., Austin, G.D., Lipps, P.E., Paul, P.A., Mullen, R.W. and Dorrance, A.E. (2009). Association of soil chemical and physical properties with *Pythium* species diversity, community composition and disease incidence. *Phytopathol.*, 99: 957-967.

Cawoy, H., Bettiol, W., Fickers, P. and Ongena, M. (2011). Bacillus-Based Biological Control of Plant Diseases. doi: s10.5772/s17184.

Cerkauskas, R. (2005). Damping Off (*Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Rhizoctonia solani*, and other fungi). AVRDC–The World Vegetable Center. https://hortintl.cals.ncsu.edu/.

Chagas, L.F.B., Chagas Jr.A.F., Carvalho, M.R., Miller, L.O. and Colonia, B.S.O. (2015). Evaluation of the phosphate solubilization potential of *Trichoderma* strains (Trichoplus JCO) and effects on rice biomass. *J. Sci. Plant Nutr.,* 15:794-804.

Chakraborty, B.N., Chakraborty, U., Saha, A., Dey, P.L. and Sunar, K. (2010). Molecular Characterization of *Trichoderma viride* and *Trichoderma harzianum* Isolated from Soils of North Bengal Based on rDNA Markers and Analysis of Their PCR-RAPD Profiles. *Global J. Biotechnol. Biochem.,* 5(1): 55-61.

Chaur-Tsuen, L. and Chien-Yih, L. (2002). Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. *Plant Pathol. Bull.*, 11: 215-220.

Chaverri, P., Castlebury, L.A., Samuels, G.J. and Geiser, D.M. (2003). Multilocus phylogenetic structure of *Trichoderma harzianum*/*Hypocrea* lixii complex. *Mol. Phylogenet. Evol.*, 27: 302-313.

Chen, L.L., Liu, L.J., Shi, M., Song, X.Y., Zheng, C.Y., Chen, X.L. and Zhang, Y.Z. (2009). Characterization and gene cloning of a novel serine protease with nematicidal activity from *Trichoderma pseudokoningii* SMF2. *FEMS Microbiol. Lett.*, 299: 135-142.

Chennappa, G., Naik, M.K., Amaresh, Y.S., Ravikiran, Ashwathanarayan D.S., Patil, M.G., Swami, M.D., Nair, D. and Shruthi, P. (2017). Morphological, Molecular Characterization of *Trichoderma* species Isolated from Different Rhizosphere Soils and Its Anti-Pathogenic Properties. *Imp. J. Interdiscip. Res.*, 3(4): 2454-1362.

Chet, I. and Inbar, J. (1994). Biological control of fungal pathogens. *Appl. Biochem. Biotechnol.*, 48: 37-43.

Chet, I., Sivan, A. and Elad, Y. (1984). Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Phytopathol.*, 74: 498-501.

Chowdhury, A., Azam, M., Fakruddin, M., Hossain, M., Mahbub, K., Hoque, M. and Ahmed, M. (2014). Isolation and Characterization of Indigenous Cellulase Producing *Trichoderma* spp. from Bangladesh. *World Res. J. Biotechnol.,* 1: 32-35.

Cigdem, K. and Merish, K. (2003). Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. *Turk. J. Biol.*, 27: 247-256.

Claydon, N., Allan, M., Hanso, J.R. and Avent, A.G. (1987). Antifungal alkyl pyrones of *Trichoderma harzianum*. *Trans. Br. Mycol. Soc.*, 88: 503-513.

Cliquet, S. and Scheffer. R.J. (1996). Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* using *Trichoderma* spp. applied as industrial film coatings on seeds. *Eur. J. Plant Pathol.*, 102: 247-255.

Coffua, L.S., Veterano, S.T. and Clipman, S.J. (2016). Characterization of *Pythium* spp. associated with a symptomatic soybean in South Eastern Pennsylvania. *Plant Dis.* doi: s10.1094/PDIS-11-15-1355-RE.

Cohen-Kupiec, R., Broglie, K., Friesem, D., Broglie, R. and Chet, I. (1999). Molecular characterisation of a novel β-1,3-exoglucanase related to mycoparasitism of *Trichoderma harzianum*. *Genet.*, 226: 147-54.

Cripps-Guazzone, N. (2014). Rhizosphere competence of selected *Trichoderma* species. Ph.D. Thesis, Submitted to Lincoln University of New Zealand, Ellesmere Jct Rd, Lincoln 7647, New Zealand.

Daniel, J.F. and Filho, E.R. (2007). Peptaibols of *Trichoderma*. *Nat. Prod. Rep.*, 24: 1128-1141.

Das, B.C., Das, B.K., Dutta, P. and Sarmah, D.K. (2006). Bioformulation of *Trichoderma harzianum* Rifai for management of soybean stem-rot caused by *Rhizoctonia solani* Kuhn. *J. Biol. Control.*, 20(1): 57-64.

De Candolle, A. (1815). *Uredo rouille* des cereals In Forafran caise, famille des champigons. pp. 83.

De Meyer, G., Bigirimana, J., Elad, Y. and Hofte, M. (1998). Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *European J. Plant Pathol.,* 104: 279-286.

Deadman, M. (2017). *Pythium* damping off and root-rot. In: Keinath, A.P., Wintermantel, W.M. and Zitter T.A. (2nd ed) Compendium of Cucurbit Diseases and Pests. *American Phytopathol. Soc.*, pp. 48-50.

Dennis, C. and Webster, J. (1971a). Antagonistic properties of species groups of *Trichoderma*1. Production sof non-volatile antibiotics. *Trans. Br. Mycol. Soc.*, 57: 25-39. doi: s10.1016/s0007-1536(71)80077-3.

Dennis, C. and Webster, J. (1971b). Antagonistic properties of species groups of *Trichoderma*11. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.*, 57: 41-48 doi: s10.1016/s0007-11536(71)80078-5.

Dev, N. and Dawande, A.Y. (2012). Biocontrol of soil borne plant pathogen *Rhiozoctonia solani* using *Trichoderma* spp. and *Pseudomonas fluorescens*. *Asiatic J. Biotech. Res.*, 1: 39-44.

Dhingra, O.P. and Sinclair, J.B. (1995). Basic plant pathology methods, 2nd edn. CRC press, Bocca Raton, America, p. 21.

Di-Pietro, A., Lorito, M., Hayes, C.K., Broadway, R.M. and Harman, G.E. (1993). Endochitinase from *Gliocladium virens*: Isolation, characterization and synergistic antifungal activity in combination with gliotoxin. *Phytopathol.*, 83: 308-313.

Dixit, R., Singh, R.B. 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 Res.*, 38(3): 375-381.

Djonovic, S., Pozo, M.J. and Kenerley, C.M. (2006). *Tvbgn3*, a β-1,6-Glucanase from the Biocontrol Fungus *Trichoderma virens*, Is Involved in Mycoparasitism and Control of *Pythium ultimum*. *Appl. Environ. Microbiol.*, 72(12): 7661–7670.

Dominguesa, F.C., Queiroza, J.A., Cabralb, J.M.S. and Fonsecab, L.P. (2000). The influence of culture conditions on mycelial structure and cellulose production by *Trichoderma reesei* Rut-C 30 Enz. *Microb. Technol.*, 26: 394-401.

Domsch, K.H., Gams, W. and Anderson, T.H. (1980). Compendium of soil fungi. Academic Press, New York, pp. 859.

Doni, F., Anizan, I., Radziah, C.M.Z., Salman, A.H., Rodzihan, M.H. and Yusoff, W.M. (2014). Enhancement of Rice Seed Germination and Vigour by *Trichoderma* spp. *Res. J. Appl. Sci. Eng. Technol.*, 7(21): 4547-4552.

Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Verlag Sci.*, 28(1): 33-46.

Druzhinina, I., Kopchinskiy, A., Kubicek, C. (2006). The first 100 *Trichoderma* species characterized by molecular data. *Mycosci.*, 47: 55-64. doi: s10.1007/s10267-006-0279-7.

Druzhinina, I.S., Kopchinskiy, A.G., Komon, M., Bissett, J., Szakacs, G. and Kubicek, C.P. (2005). An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea. Fungal Genet. Biol.*, 42(10): 813-828.

Elad, Y., Barak, R. and Chet, I. (1984). Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum. Soil Biol .Biochem.*, 16: 381-386.

Elad, Y., Chet, I. and Henis, Y. (1982). Degradation of plant pathogenic fungi by *Trichoderma harzianum. Can. J. Microbiol.*, 28: 719-725.

Fahmi, A. and Ragaa, A.E. (2016). Identification of *Trichoderma* spp. by DNA Barcode and Screening for Cellulolytic Activity. *J. Microb. Biochem. Technol.*, 8: 1-9. doi: s10.4172/s1948-5948.1000286.

Farooq, M., Basra, S.M.A., Ahmad, N. and Hafeez, K. (2005). Thermal hardening: A new seed vigor enhancement tool in rice. *J. Integr. Plant Biol.*, 47: 187-193.

Fgaier, H. and Eberl, H.J. (2011). Antagonistic control of microbial pathogens under iron limitations by siderophore producing bacteria in a chemostat setup. *J. Theor. Biol.*, 273: 103-114.

Franca, V.C.D., Kupper, C.K., Rosa-Magri, M., Gomes, T. and Rossi, F. (2017). *Trichoderma* spp. Isolates with potential of phosphate solubilization and growth promotion in cherry tomato. *Pesqui. Agropecu. Trop.*, 47: 360-368.

Gaigole, A.H., Wagh G.N. and Khadse, A.C. (2011). Antifungal activity of *Trichoderma* species against soil borne pathogen. *Asiatic J. Biotechnol. Resour.*, 4: 461-465.

Gallo, A., Mule, G., Favilla, M. and Altomare, C. (2004). Isolation and characterisation of a trichodiene synthase homologous gene in *Trichoderma harzianum. Physiol. Molec. Plant Pathol.*, 65(1): 11−20.

Gams, W. and Bisset, J. (1998). Morphology and identification of *Trichoderma*. In: Harman, G.E. and Kubicek, C.P. (ed) *Trichoderma and Gliocladium*. Taylor, G.E. and Francis, London, pp. 3-43.

Geology.com. Talc: The Softest Mineral. http://geology.com/minerals/talc.shtml.

Geraldine, A., Alvares, C.L.F., Carvalho, C.D.D., Barbosa, A.E., Rodrigues, A., Ulhoa, C. and Lobo, J.M. (2013). Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by *Trichoderma* spp. *Biol. Control.*, 67: 308-316.

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

Ghosh, S.K., Banerjee, S. and Sengupta, C. (2017). Bioassay, characterization and estimation of siderophores from some important antagonistic fungi. *J. Biopest*, 10(2): 105-112.

Gonzalez, M., Pujol, M., Metraux, J.P., Gonzalez, V., Bolton, M. and Borras-Hidalgo, O. (2011). Tobacco leaf spot and root rot caused by *Rhizoctonia solani* Kuhn. *Mol. Plant Pathol.*, 12: 209-216. doi: s10.1111/j.1364-3703.2010.00664.x.

Goud, T.S., Raju, R.A., Karri, S. 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. doi: s10.5897/AJB2014.13904.

Gravel, V., Antoun, H. 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. Biochem.*, 39: 1968-1977.

Gravel, V., Martinez, C., Antoun, H. and Tweddell, R.J. (2005). Antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds in rockwool. *BioControl*, 50: 771–786.

Gregory, P.J. (2006). Roots and the biological environement. In: B.P. (ed) Plant Roots: Growth, activity and interactions with soil, 2nd edn, pp. 174-215.

Gruber, S. and Seidl-Seiboth, V. (2012). Self versus non-self: fungal cell wall degradation in *Trichoderma*. *Microbiol.*, 158: 26–34.

Gupta, M. and Dohroo, N.P. (2014). Shelf life study of formulations of fungal and bacterial antagonists as bioinoculants. *Agric. Sci. Digest*, 34(4): 281-284.

Gupta, V.K. and Paul, T.S. (2004). Fungi and Plant diseases. Kalyani publishers, New Delhi, pp. 25-47.

Guttierez, W.A., Shew, H.D. and Melton, T.A. (1997). Sources of inoculum and management of *Rhizoctonia solani* damping-off on tobacco transplants under greenhouse conditions. *Plant Dis.*, 81: 604-606.

Ha, T.N. (2010). Using *Trichoderma* Species for Biological Control of Plant Pathogens in Vietnam. *Int. J. Pharm. Life Sci.*, 16(1): 17-21.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, 41: 95-98.

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

Harman, G. (2006). Overview of Mechanisms and Uses of *Trichoderma* spp. *Phytopathol.*, 96: 190-194.

Harman, G.E. (2000). Myths and dogmas of biocontrol – changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis.*, 84: 377–393.

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

Hartley, C. (1921). Damping-off in forest nurseries. USDA Bureau of Plant Industry, Washington (DC). *Bull. 99*, pp. 21.

Hartley, C. and Pierce, R.G. (1917). The control of damping-off of coniferous seedlings. USDA *Bull. 453*, pp. 32.

Hawes, M.C. (1990). Living plant cells released from the root cap: a regulator of microbial populations in the rhizosphere. *Plant Soil.*, 129: 19-27.

Hebbar, K.P., Lewis, J.A., Poch, S.M. and Lumsden, R.D. (1997). Fermentation and formulation of mycoherbicidal strains of *Fusarium oxysporum*. (Abstr.). *Phytopathol.*, 86: 58.

Hermosa, M.R., Grondona, I., Iturriaga, E.A., Diaz-Minguez, J.M., Castro, C., Monte, E. and Garcia-Acha, I. (2000). Molecular Characterization and Identification of Biocontrol Isolates of *Trichoderma* spp. *Appl. Environ. Microbiol.*, 66(5): 1890–1898.

Hermosa, R., Viterbo, A., Chet, I. and Monte, E. (2012). Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiol.*, 158: 17–25. doi: s10.1099/mic.0.052274-0.

Hjeljord, L. and Tronsmo, A. (1998). *Trichoderma* and *Gliocladium* in biological control: an overview. In: Harman, G.E., Kubicek, C.P. (eds) *Trichoderma* and *Gliocladium*, Vol 2. Enzymes, Biological Control and Commercial Applications. Taylor and Francis, London, pp. 131-151.

Holford, I.C.R. (1997). Soil phosphorus: its measurement, and its uptake by plants. *Aust. J. Soil Res.*, 35: 227-239.

Howitt, S.M. and Udvardi, M.K. (2000). Structure, function and regulation of ammonium transporters in plants. *Biochem. Biophys. Acta.*, 1465(1-2): 152-170.

Huang, Q., Tezuka, Y., Hatanaka, Y., Kikuchi, T., Nishi, A. and Tubaki, K. (1995a). Studies on metabolites of mycoparasitic fungi. III. New sesquiterpene alcohol from *Trichoderma koningii. Chem. Pharm. Bull.*, 43: 1035-1038.

Huang, Q., Tezuka, Y., Kikuchi, T., Nishi, A., Tubaki, K. and Tanaka, K. (1995b). Studies on metabolites of mycoparasitic fungi. II. Metabolites of *Trichoderma koningii. Chem. Pharm. Bull.*, 43: 223-239.

Huang, X., Chen, L., Ran, W., Shen, Q. and Yang, X. (2011). *Trichoderma harzianum* strain SQR-T37 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off disease in cucumber seedlings mainly by the mycoparasitism. *Appl. Microbiol. Biotechnol.*, 91(3): 741-55. doi: s10.1007/s00253-011-3259-6.

Inbar, J., Abramsky, M., Cohen, D. and Chet, I. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Eur. J. Plant Pathol.*, 100(5): 337-346.

Industrial Minerals Association. "What is Talc." http://www.ima-na.org/?page=what_is_talc.

Islam, M.S., Rahman, M.A., Bulbul, S.H. and Alam, M.F. (2011). Effect of *Trichoderma* on seed germination and seedling parameters in chili. *Int. J. Expt. Agric.*, 2(1): 21-26.

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: s10.24838/ip.2017.v70.i2.71652.

Jash, S. and Pan, S. (2004). Evaluation of mutant isolates of *Trichoderma hardanum* against *Rhizoctonia solani* causing seedling blight of greengram (*Phaseolus radiatus*). *Indian J. Agric. Sci.*, 74: 190-193.

Jasim, B., Joseph, A. A., John, C.J., Mathew, J. 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: s10.1007/s13205-013-0143-3.

Jayaraj, J., Radhakrishnan, N.V. and Velazhahan, R. (2006). Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum. Arch. Phytopathol. Plant Prot.*, 39(1): 1–8.

Jegathambigai, V., Wijeratnam, R.S.W. and Wijesundera, R.L.C. (2007). Effect of *Trichoderma* spp. on *Sclerotium rolfsii*, the Causative Agent of Collar Rot on *Zamioculcas zamiifolia* and an on Farm Method to Mass Produce *Trichoderma* species. *Plant Pathol. J.*, 9(2): 47-55.

Jin, X. and Custis, D. (2011). Microencapsulating aerial conidia of *Trichoderma harzianum* through spray drying at elevated temperatures. *Biol. Control.*, 56: 202-208.

Jiskani, M.M., Pathan, M.A., Wagan, K.H., Imran, M. and Abro, H. (2007). Studies on the control of tomato damping-off disease caused by *Rhizoctonia solani* Kuhn. *Pak. J. Bot.*, 39(7): 2749-2754.

Jogani, V. (2014). Evaluation of different application methods of *Trichoderma harzianum* (Rifai) against *Fusarium* wilt of tomato. M.Sc Thesis, Submitted to the Navsari Agricultural University, Navsari. Gujarat, India.

Johnson, L.F., Bernard, E.C. and Peiyuan Q. (1987). Isolation of *Trichoderma* spp. at low temperatures from Tennessee and Alaska (USA) soils. *Plant Dis.*, 71: 137-140.

Joshi, B.B., Bhatt, R.P. and Bahukhandi, D. (2010). Antagonistic and plant growth activity of *Trichoderma* isolates of Western Himalayas. *J. Environ. Biol.*, 31(6): 921-928.

Kamala, T. and Indira, S. (2011). Evaluation of indigenous *Trichoderma* isolates from Manipur as biocontrol agent against *Pythium aphanidermatum* on common beans. *3Biotech*, 1(4): 217–225.

Kamala, T., Devi, I.S., Sharma, K.C. and Kennedy, K. (2015). Phylogeny and Taxonomical Investigation of *Trichoderma* spp. from Indian Region of Indo-Burma Biodiversity Hot Spot Region with Special Reference to Manipur. *BioMed. Res. Int.* 215: 1-21.

Kamilova, F., Validov, S., Azarova, T., Mulders, I. and Lugtenberg, B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environ. Microbiol.*, 7: 1809-1817.

Kannangara, S., Dharmarathna, R.M.G.C.S. and Jayarathna, D.L. (2017). Isolation, Identification and Characterization of *Trichoderma* Species as a Potential Biocontrol Agent against *Ceratocystis paradoxa*. *J. Agric. Sci.*, 12(1): 51-62.

Kapoor, A.S. (2008). Biocontrol potential of *Trichoderma* spp. against important soilborne diseases of vegetable crops. *Indian Phytopathol.*, 61(4): 492-498.

Kapri, A. and Tewari, L. (2010). Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp.. *Braz. J. Microbiol.*, 41: 787-795. doi: s10.1590/s1517-83822010005000001.

Karthika, C. and Vanangamudi, K. (2013). Biopriming of maize hybrid COH(M) 5 seed with liquid biofertilizers for enhanced germination and vigour. *Afr. J. Agric. Res.*, 8(25): 3310-3317.

Kavitha, S. (2011) Biopriming with biocontrol agents and liquid biofertilizers for rice seed cv. ADT 43. M.Sc. Thesis, Submitted to the Tamil Nadu Agricultural University, Coimbatore, India.

Kendrick, W.B. and Carmichael, J.W. (1973). Hyphomycetes, The Fungi, an advanced treatise. In: Ainsworth, G.C., Sparrow, F.K. and Sussman, A.S. (ed) A Taxonomic Review with Keys: ascomycetes and fungi imperfecti. Academic press, New York, 4: 323–509.

Khalid, A., Srivastava, R., Singh, U.S. and Sharma, A.K. (2010). Evaluation of arbuscular mycorrhizal fungus, *Pseudomonas fluorescens* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. *Biocontrol*, 53: 24-31.

Khan, A. A. (1992), Preplant physiological conditioning. *Hort. Rev.*, 13: 131-181.

Khandelwal, M., Datta, S., Mehta, J., Naruka, R., Makhijani, K., Sharma, G., Kumar, R. and Chandra, S. (2012). Isolation, characterization & biomass production of Trichoderma viride using various agro products- A biocontrol agent. *Adv. Appl. Sci. Res.*, 3(6): 3950-3955

Kotasthane, A., Agrawal, T., Kushwah, R. 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. *Eur.* J. *Plant Pathol.*, 141(3): 523-543.

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

Kuhls, K., Lieckfeldt, E., Samuels, G.J., Kovacs, W., Meyer, W., Petrini, O., Gams, W., Borner T., Kubicek, C.P. (1996) Molecular evidence that the asexual industrial fungus *Trichoderma reesei* is a clonal derivative of the ascomycete *Hypocrea jecorina*. *Proc. Nat. Acad. Sci.*, 93: 7755–7760.

Kulkarni, S. and Sagar, S.D. (2007). *Trichoderma* - A potential biofungicide of the millennium, Bulletin published by the Department of Plant Pathology, U.A.S., Dharwad, pp. 1-20.

Kullnig-Gradinger, C.M, Szakacs, G. and Kubicek, C.P. (2002). Phylogeny and evolution of the fungal genus *Trichoderma*: a multigene approach. *Mycol. Res.*, 106: 757–767.

Kumar, K., Amaresan, N., Bhagat, S., Madhuri, K. 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.

Kumar, S., Thakur, M. and Rani, A. (2014). *Trichoderma*: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. *Afr. J. Agric. Res.*, 9(53): 3838-3852.

Kumari, A., Kumar, R., Maurya, S. and Pandey, P.K. (2016). Antagonistic Potential of Different Isolates of *Trichoderma* against *Rhizoctonia solani*. *Eur. J. Exp. Biol.*, 6(2): 1-6.

Kumari, R., Khan, M.R., Bagri, G.K., Bagri, D.K. and Bagdi, D.L. (2017). Soil application of different species of *Trichoderma* for the management of charcoal rot of faba bean caused by *Macrophomina phaseolina*. *J. Pharmacogn. Phytochem.*, 6(6): 1483-1486.

Lamichhane, J.R., Durr, C., Schwanck, A.A., Robin, M.H., Sarthou, J.P., Cellier, V., Messean, A. and Aubertot, J.N. (2017). Integrated management of damping-off diseases. A review: Agronomy for Sustainable Development. *Springer EDP Sci.*, 37(2): 25.

Larson, R. 1987. Growing concerns. Pest control: How much is enough? *Mark. Lett.*, 2:5.

Le, H.T., Black, L.L. and Sikora, R.A. (2003). Evaluation of *Trichoderma* spp. for biocontrol of tomato sudden caused by *Pythium aphanidermatum* following flooding in tropical hot season. *Commun. Agric. Appl. Biol. Sci.*, 68: 463-474.

Leach, L.D. and Garher, R.H. (1970). Control of *Rhizoctonia*. In: Parmeter, J.R. (ed) Biology and Pathology of *Rhizoctonia solani*, 3rd edn. University of California Press, Berkeley, pp. 189-198.

Lehner, S.M., Atanasova, L., Neumann, N.K., Krska, R., Lemmens, M., Druzhinina, I.S. and Schuhmacher, R. (2013). Isotope-assisted screening for iron-containing metabolites reveals a high degree of diversity among known and unknown siderophores produced by *Trichoderma* spp. *Appl. Environ. Microbiol.*, 79(1): 18-31.

Lewis, J.A. and Fravel, D.R. (1996). Influence of Pyrax/biomass preparations of biocontrol fungi on snap bean damping-off in the field caused by *Sclerotium rolfsii* and on germination of sclerotia of the pathogen. *Plant Dis.*, 80: 655–659.

Lewis, J.A., Larkin, R.P., Rogers, D.L. (1998). A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in soil less mix. *Plant Dis.*, 82: 501–506.

Lieckfeldt, E., Kuhls, K., Muthumeenakshi, M. (1998). Molecular taxonomy of *Trichoderma* and *Gliocladium* and their teleomorphs. In: Kubicek, C.P. and Harman, G.E. (ed) *Trichoderma* and *Gliocladium* basic biology, taxonomy and genetics, 1st edn.. Taylor and Francis, London, pp. 35–74.

Lifshitz, R., Windhan, M.T. and Baker, R. (1986). Mechanism of biological control of preemergence damping-off of pea by seed treatment with *Trichoderma* spp. *Phytopathol.,* 76: 720-725.

Lorito, M., Hayes, C.K. and Di Pietro, A. (1993). Biolistic transformation of *Trichoderma harzianum* and *Gliocladium virens* using plasmid and genomic DNA. *Curr. Genet.*, 24(4): 349–356.

Lucas, G.B., Campbell, C.L. and Lucas, L.T. (1997). Introduction to plant disease identification and management. CBS Publisher Distributors, New Delhi, pp. 364.

Luchman, H., Suparno, Sukoso and Noor, A. (2016). *Trichoderma* spp. as Agent of Biological Control in Local Rice Diseases in Tidal Swamp Lands in South Kalimantan, Indonesia. *J. Agric. Vet. Sci.*, 9(1): 01-06.

Mahadevaswami, Chennappa, G., Naik, M.K., Amaresh, Y.S., Ravikiran, Ashwathanarayan D.S., Patil, M.G., Nair, D. and Shruthi, P. (2017). Morphological, Molecular Characterization of *Trichoderma* species Isolated from Different Rhizosphere Soils and Its Anti-Pathogenic Properties. *Imp. J. Interdiscip. Res.*, 3(4): 1-12

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

Malhotra, A., Agarwal, T. and Trivedi, P.C. (2011). *In vitro* efficacy of various fungal and bacterial antagonists against *Rhizoctonia solani*, causal agent of damping off disease in *Capsicum annuum*. *Int. J. Pharm. Biol. Sci.*, 3(2): 288-292.

Manganiello, G., Sacco, A., Ercolano, M.R., Vinale, F., Lanzuise, S., Pascale, A., Napolitano, M., Lombardi, N., Lorito, M. 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: s10.3389/fmicb.2018.01966.

Manoli, A., Begheldo, M., Genre, A., Lanfranco, L., Trevisan, S. and Quaggiotti, S. (2014). NO homeostasis is a key regulator of early nitrate perception and root elongation in maize. *J. Exp. Bot.*, 65: 185–200.

Manoranitham, S.K., Prakasam, V., Rajappan, K. and Amutha, G. (2000). Effect of two antagonists on damping off disease of tomato. *Indian Phytopathol.*, 53(4): 441-443.

Manoranjitham, S.K., Prakasam, V. and Rajappan, K. (2001). Biocontrol of damping off of tomato caused by *Pythium aphanidermatum*. *Indian Phytopath.*, 54(1): 59-61.

Manter, D.K. and Vivanco, J.M. (2007). Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *J. Microbiol. Methods*, 71: 7-14.

Manwar, A.V., Rakh, R.R., Raut, L.S. and Dalvi, S.M. (2011). Biological control of *Sclerotium rolfsii*, causing stem rot of groundnut by *Pseudomonas* cf. *monteilii*. *Recent Res. Sci. Technol.*, 3: 26-34.

Mao, W., Lumsden, R.D., Lewis, J.A. and Hebbar, P.K. (1998). Seed treatment using pre-infiltration and biocontrol agents to reduce damping-off corn caused by species of *Pythium* and *Fusarium*. *Plant Dis.*, 83: 294-299.

Mariselvam, D. (2012). Performance of bioprimed bhendi (cv. arka anamika) seeds with biocontrol agents and liquid biofertilizers under laboratory and field conditions. M.Sc. Thesis, Submitted to the Tamil Nadu Agricultural University, Coimbatore, India.

Mastouri, F., Bjorkman, T. and Harman, G.E. (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathol.*, 100: 1213–1221.

Maymon, M., Minz, D., Barbul, O., Zveibil, A., Elad, Y. and Freeman, S. (2004). Identification of *Trichoderma* Biocontrol Isolates to Clades According to ap-PCR and ITS Sequence *Anal. Phytoparasit.*, 32(4):370-375.

McLean, K.L., Swaminathan, J., Frampton, C.M., Hunt, J.S., Ridgeway, H.J. and Stewart, A. (2005). Effect of formulation on the rhizosphere competence and biocontrol ability of *Trichoderma atroviride*. *Plant Pathol.*, 54: 2212–2218.

MEGA software (MEGA 5.2) (https://www.megasoftware.net)

Mehra, R. (2006). Bio-control of *Fusarium* wilt of tomato using antagonistic microorganisms. *Plant Dis. Res.*, 21: 146-147.

Menzies, J.D. (1963). Survival of microbial plant pathogens in soil. *Bot. Rev.*, 29: 79-122.

Mishra, B., Mishra, R., Mishra, C.R., Tiwari, A., Singh Yadav, R. and Dikshit, A. (2011). Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. *Arch. Appl. Sci. Res.*, 3(2): 361-369.

Mohamed, M.H., Emad, G., El-Deeb, S. and Mostafa, H.M. (2015). Phenotypic diversity and molecular identification of the most prevalent anastomosis group of *Rhizoctonia solani* isolated from diseased faba bean plants. *Am. J. Life Sci.*, 3: 47-55.

Moussa, T.A.A. (2002). Studies on biological control of sugarbeet pathogen *Rhizoctonia solani* Kuhn. *J. Biol. Sci.*, 2: 800–804.

M-Uddin, M., Akther, N., Islam, M.T. and Farug, N.A. (2011). Effect of *Trichoderma harzanium* and some soil amendment against damping-off disease complex of potato and chilli. *Agric.*, 9(1-2): 106-116.

Mukherjee, A., Kumar, G., Maharshi, A., Patel, J., Singh, H. and Sarma, B. (2017). *Trichoderma*: A Potential Fungal Antagonist to Control Plant Diseases. *Annu. Tech. Issue*, pp. 206-218.

Mukherjee, A.K., Kumar, S.A., Kranthi, S. and Mukherjee, P.K. (2014). Biocontrol potential of three novel *Trichoderma* strains: Isolation, evaluation and formulation. *3Biotech*, 4(3): 275-281.

Mukhopadhyay, A.N. (1994). Biocontrol of soil borne fungal plant pathogens - current status, future prospects and potential limitations. *Indian Phytopathol.*, 47: 119- 126.

Muthukumar, A., Eswaran, A. and Sanjeevkumas, K. (2011). Exploitation of *Trichoderma* species on the growth of *Pythium aphanidermatum* in Chilli. *Braz. J. Microbiol.*, 42: 159-607.

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.

Naher, L., Rahman, M., Ali, M.A., Dey, T.K. and Islam, M. (2014). Evolution of disease and potential biocontrol activity of *Trichoderma* spp. against *Rhizoctonia solani* on potato. *Biosci. J.*, 30: 1108-1117.

Naik, M.K., Amaresh, Y.S., Kiran, R., Ashwathanarayan, D.S., Patil, M.G., Mahadeva, S., Nair, D. and Shruthi, P. (2017). Morphological, Molecular Characterization of *Trichoderma* species Isolated from Different Rhizosphere Soils and Its Anti-Pathogenic Properties. *Imp. J. Interdiscip. Res.*, 3(4): 1-12.

Nakkeeran, S., Krishnamoorthy, A.S., Ramamoorthy, V. and Renukadevi, P. (2002). Microbial inoculants in plant disease control. *J. Ecobiol.*, 14: 83-94.

Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening phosphorus solubilizing microorganisms. *FEMS Microbiol. Lett.*, 170: 2017-2021.

Ng, L.C., Ngadin, A., Azhari, M. and Zahari, N.A. (2015). Potential of *Trichoderma* spp. as Biological Control Agents against Bakanae Pathogen (*Fusarium fujikuroi*) in Rice. *Asian J. Plant Pathol.*, 9(2): 46-58.

Ngadin, A., Ng, L.C., Azhari, M. and Zahari, N.A. (2015). Potential of *Trichoderma* spp. as Biological Control Agents against Bakanae Pathogen (*Fusarium fujikuroi*) in Rice. *Asian J. Plant Pathol.*, 9(2): 46-58.

Ngullie, M. and Daiho, L. (2013). Efficacy of biocontrol agents in controlling *Rhizoctonia solani* on naga king chilli (*Capsicum chinense* Jacq.). *J. Exp. Biol. Agric. Sci.*, 1(3): 197-201.

Nongmaithem, N., Roy, A. and Bhattacharya, P.M. (2016). Screening of *Trichoderma* isolates for their potential of biosorption of nickel and cadmium. *Braz. J. Microbiol.,* 47(2): 305-313.

Noori, M.S. and Saud, H.M. (2012). Potential plant growth-promoting activity of *Pseudomonas* sp isolated from paddy soil in Malaysia as biocontrol agent. *J. Plant Pathol. Microbiol.* doi: s10.4172/2157-7471.1000120.

Omokhua, G.E. (2011). Pre and Post-Emergence Damping-Off of *Chrysophyllum albidum* in Port Harcourt. *Int. Multidiscip. J. Ethiopia.*, 5(6): 411-421.

Pan, S. and Bhagat, S. (2007). Antagonistic potential of *Trichoderma* spp. and *Gliocladium* spp. from West Bengal. *J. Mycol. Plant Pathol.*, 37: 235-239.

Pan, S. and Das, A. (2011). Control of cowpea (*Vigna sinensis*) root and collar rot (*Rhizoctonia solani*) with some organic formulations of *Trichoderma harzianum* under field condition. *J. Plant Prot. Sci.*, 3(2): 20-25.

Pandey, M. (2017). Effect of *Trichoderma* species on germination and root disease control in neem seedlings. *Pharma Innov. J.*, 6(11): 757-761.

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

Papavizas, G.C., Lewis, J.A. and Abd-El, T.H. (1982). Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. *Phytopathol.*, 72: 126-132.

Parameswari, K., Kishori, J., Hemalatha, M. (2015). Isolation, Screening and Molecular characterization of PGPR isolates, *Azatobacter* and *Trichoderma* and their beneficial activities. *J. Nat. Sci. Biol. Med.*, 6: 360-363.

Parmeter, J.R. and Whitney, H.S. (1970). Taxonomy and nomenclature of the imperfect state. In: Parmeter, J.R. (ed) Biology and Pathology of *Rhizoctonia solani*, 2nd edn.University of California Press, Berkeley, pp. 255.

Parveen, T. and Sharma, K. (2015). *Pythium* Diseases, Control and Management Strategies: A Review. *Int. J. Plant Anim. Environ. Sci.*, 5(1): 244-257.

Patel, S. and Sharaf, M. (2017). Biocontrol efficacy of *Trichoderma asperellum* MSST against tomato wilting by *Fusarium oxysporum* f. sp. *lycopersici. Arch. Phytopathol. Plant Prot.*, doi: s10.1080/03235408.2017.1287236.

Paul, B., Bala, K., Lassaad, B., Calmin, G., Sanchez, M.E. and Lefort, F. (2006). A new species of *Pythium* with ornamented oogonia: Morphology, taxonomy, internal transcribed spacer region of its ribosomal RNA, and its comparison with related species. *FEMS Microbiol. Lett.*, 254: 317-323.

Paulitz, T.C. (1997). Biological control of root rot pathogens in soilless and hydroponic systems. *Hortsci.*, 32: 193–196.

Pawar, A., Ismail, S., Mundhe, S. and Patil, V.D. (2015). Solubilization of insoluble zinc compounds by different microbial isolates *in-vitro* condition. *Int. J. Trop. Agric.*, 33: 865–869.

Peltier, G. L. (1916). Parasitic *Rhizoctonias* in America. Ill. *Agric. Exp. Stat. Bull.*, 189: 283-390.

Persoon, C.H. (1794). Dispositio methodica fungorum. Romer's neues. *Mag. Bot.*, 1: 81-128.

Petrini, O. (1986). Taxonomy of endophytes of aerial plant tissues. In: Fokkema, N.J. and Heuvel, J.V.D. (ed) Microbiology of the Phyllosphere. Cambridge University Press, UK, pp. 175-187.

Pieczarka, D.J. and Abawi, G.S. (1978). Influence of Soil Water Potential and Temperature on Severity of *Pythium* Root Rot of Snap Beans. *Phytopathol.*, 68: 766-772.

Prabha, T., King, S.E., Rajesh, K.V. and Senthil, K.R. (2015). Competence of *Trichoderma viride* as Biocontrol Agent against Soil Borne *Fusarium oxysporum* Wilt Disease on Onion Crop. *Eur. J. Environ. Ecol.*, 2(2): 72–77.

Prameela, D., Prabhakaran, N., Deeba, K., Pankaj, P. and Jyoti, L.B. (2012). Characterization of Indian native isolates of *Trichoderma* spp. and assessment of their bio-control efficiency against plant pathogens. *Afr. J. Biotechnol.*, 11(85): 15150-15160.

Prasad, R.D., Rangeshwaran, R., Hegde, S.V. and Anuroop, C.P. (2002). Effect of soil and seed application of *Trichoderma harzianum* on pigeonpea wilt caused by *Fusarium udum* under field conditions. *Crop Prot.*, 21: 293-297.

Promwee, A., Issarakraisila, M., Intana, W., Chamswarng, C. and Yenjit, P. (2014). Phosphate Solubilization and Growth Promotion of Rubber Tree (*Hevea brasiliensis* Muell. Arg.) by *Trichoderma* Strains. *J. Agric. Sci.*, pp. 6.

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.

Quimio, T.H. (2001). Workbook on tropical fungi. Collection, isolation and identification. The Mycological Society of the Phillippines, Inc. College, Laguna, Phillippines, pp. 259.

Radhakrishnan, N.V., Jayaraj, J. and Velazhahan, R. (2006). Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. *Arch. Phytopathol. Plant Prot.*, 39(1): 1-8.

Rahman, M.M., Ahammad, K.U. and Ahmed, M. (2014). Effect of seed priming on maize (Zea mays l.) Seedling emergence under different sowing dates. *Bangladesh J. Agril. Res.* 39(4): 693-707.

Rai, S., Kashyap, P.L., Kumar, S., Srivastava, A.K. and Ramteke, P.W. (2016). Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *SpringerPlus*, 5(1): 1939. doi: s10.1186/s40064-016-3657-4.

Raj, K. and Singh, T. (2014). *Trichoderma* species variety examination based on internal transcribed spacer. *Int. J. Mycol. Plant Pathol.*, 1(7): 59-64.

Rajagopalan, C.K.S. (1961). Studies on Phycomycetes in agricultural soils with special reference to Pythiaceae. Ph.D. Thesis, Submitted to University of Madras, India.

Rajendraprasad, M. (2014). Biological Control of Soilborne Fungal Diseases in Tomato (*Lycopersicon esculentum* Mill.). M.Sc. Thesis, Submitted to the Acharya N.G. Ranga Agricultural University, Hyderabad.

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

Rakholiya, K.B., Shinde M.S. and Waghunde, R.R. (2016). *In-Vitro* Efficacy of Bioagent and organic amendment against Damping-Off of Tomato Pathogen (*Pythium aphanidermatum*). *World J. Pharm. Life Sci.*, 2(6): 165-172.

Ramamoorthy, V., Raguchander, T. and Samiyappan, R. (2002). Enhancing resistant of tomato and hot pepper to *Pythium* disease by seed treatment with Fulorescent *Pseudomonas*. *Eur. J. Plant. Pathol.*, 108: 429-441.

Ramanathan, G., Saransundar, M. and Vinodhkumar, T. (2013). Evaluation of antifungal activity of metabolites from *Trichoderma* species against fungal phytopathogens. *Int. J. Sci. Innov. Discov.*, 3(5): 528-538.

Ramanujam, B., Sriram, S. and Palanna, K.B. (2010). Effect of chitin on the shelf life of *Trichoderma harzianum* in talc formulation. *Indian J. Agric. Sci.*, 80: 930-932.

Ramesh, R. and Singh, N.P. (2017). Field evaluation of talc formulation of *Trichoderma* for the management of *Fusarium* wilt in watermelon. *Indian Phytopathol.*, 70(1): 75-79.

Ramette, A., Moenne-Loccoz, Y. and Defago, G. (2003). Prevalence of fluorescent-*Pseudomonads* producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco root rot. *FEMS Microb. Ecol.*, 44: 35-43.

Rao, S.K.S., Tanvi, P.K., Dnyanobarao, G.S., Agrawal, T. and Kotasthane, A.S. (2016). Efficiency of Different *Trichoderma* Isolates on Plant Growth Promoting Activity in Rice (*Oryza sativa* L.). *Int. J. Bioresour. Stress Manag.*, 7(3): 489-500.

Raut, I., Oancea, F., Belen, A., Lopez, S.T., Calin, M., Constantinescu, A.D., Badea, D.M., Arsene, M.L., Vasilescu, G., Şesan, E.T. and Jecu, L. (2017). Evaluation of *Trichoderma* spp. as a biocontrol agent against *Phytophthora parasitica*. *Sci. Bull. Ser. Biotechnol. XXI*, pp. 179-182.

Rawat, R. and Tewari, L. (2011). Effect of abiotic stress on phosphate solubilization by biocontrol fungus *Trichoderma* spp. *Curr. Microbiol.*, 62(5): 1521-1526.

Reddy, P.P. (2012). Bio-priming of Seeds. In: Recent advances in crop protection. Springer, New Delhi. *Biomed. Life Sci.* doi: s10.1007/978-81-322-0723-8_6.

Rehman, S.U., Lawrence, R., Kumar, E.J. and Badri, Z.A. (2012). Comparative efficacy of *Trichoderma viride*, *T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani* Kuhn. *J. Biopest.*, 5(1): 23-27.

Rehner, S.A. and Samuels, G.J. (1995). Molecular systematics of the Hypocreales: A teleomorph gene phylogeny and the status of their anamorphs. *Can. J. Bot.*, 73(1): 816-823.

Rekha, D. (2010). Identification of potential indigenous isolates of *Trichoderma* spp. against major pathogens. M.Sc. Thesis, Submitted to the University of Agricultural Sciences, Raichur.

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

Rini, C.R. and Sulochana, K.K. (2007). Substrate evaluation for multiplication of *Trichoderma* spp. M.Sc. Thesis, Submitted to the Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram-695522, Kerala, India.

Robideau, G.P., De-Cock, A.W., Coffey, M.D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D.W., Desaulniers, N., Eggertson, Q.A., Gachon, C.M., Hu, C.H., Kupper, F.C., Rintoul, T.L., Sarhan, E., Verstappen, E.C., Zhang, Y., Bonants, P.J., Ristaino, J.B. and Levesque, C.A. (2011). DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Mol. Ecol. Resour.*, 11(6): 1002-1011.

Rocha, V.A.L., Maeda, R.N., Pereira, N.J., Kern, F.M., Elias, L. and Simister, R. (2016). Characterization of the cellulolytic secretome of *Trichoderma harzianum* during growth on sugarcane bagasse and analysis of the activity boosting effects of swollenin. *Biotechnol. Prog.*, 32(2): 327–36.

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

Rudresh, D.L., Shivaprakash, M.K. 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.

Sabalpara, A.N. (2014). Mass multiplication of biopesticides at farm level. *J. Mycol. Plant Pathol.*, 14(44):1-5.

Salas-Marina, M.A., Silva-Flores, M.A., Uresti-Rivera, E.E., Castro-Longoria, E., Herrera-Estrella, A. and Casas-Flores, S. (2011). Colonization of Arabidopsis roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur. J. Plant Pathol.*, 131: 15–26. doi: s10.1007/s10658-011-9782-6.

Sallam, M.N. Nashwa, K.A., Abo-Elyousr M. and Hassan, M.A.E. (2008). Evaluation of Trichoderma species as biocontrol agents for damping off and efficacy of suggested formula. *Egypt. J. Phytopathol.*, 36(1-2): 81-93.

Samuels, G.J. (1996). *Trichoderma*: A review of biology and systematics of the genus. *Mycol. Res.*, 100: 923-935.

Samuels, G.J. (2006). *Trichoderma*: Systematics, the Sexual State, and Ecology. *Phytopathol.*, 96: 195-206.

Samuels, G.J., Chaverri P., Farr, D.F. and Mccray, E.B. (2002). *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycol.*, 94: 146-170.

Saravanakumar, K., Shanmuga, A.V. and Kandasamy, K. (2012). Effect of *Trichoderma* on soil phosphate solubilization and growth improvementof *Avicennia marina*. *Aquat. Bot.*, pp. 104. doi: 10.1016/j.aquabot.2012.09.001.

Sathiyaseelan, K., Sivasakthivelan, P. and Lenin, G. (2009). Evaluation of Antagonastic Activity and Shelf Life Study of *Trichoderma viride*. *Bot. Res. Inst.*, 2(3): 195–197.

Scarselletti, R. and Faull, J.L. (1994). *In-vitro* activity of 6-pentyl-α-pyrone, a metabolite of *Trichoderma harzianum*, in the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*. *Mycol. Res.*, 98(10): 1207-1209.

Schachtman, D., Reid, J.R. and Ayling, M.S. (1998). Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol.*. 116: 447-453.

Schirmbock, M., Lorito, M., Wang, Y.L., Hayes, C.K., Arisan-Atlac, I., Scala, F., Harman, G.E. and Kubicek, C.P. (1994). Parallel formation and synergism of hydrolytic enzymes and peptide bond antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Appl. Environ. Microbiol.*, 60: 4364- 4370.

Schuster, A. and Schmoll, M. (2010). Biology and biotechnology of *Trichoderma*. *Appl. Microbiol. Biotechnol.*, 87(3): 787-799.

Sekhar, Y.C., Ahammed, S.K., Prasad T.N.V.K.V. and Devi, R.S.J. (2017). Evaluation of *Trichoderma* spp. Against *Sclerotium rolfsii* Sacc., Causing Stem Rot in Groundnut Crop. *Int. J. Pure Appl. Biosci.*, 5(5): 394-399.

Shabir, R. and Rubina, L. (2010). Biological control of damping-off disease of cabbage caused by *Rhizoctonia solani* Kuhn. *Appl. Biol. Res.*, 12: 38-41.

Shahid, M., Srivastava, M., Kumar, V., Singh, A., Sharma, A., Pandey, S., Rastogi, S., Pathak, N. and Srivastava, A.K. (2014). Phylogenetic diversity analysis of *Trichoderma* species based on internal transcribed spacer (ITS) marker. *Afr. J. Biotechnol.*, 13(3): 449-455.

Shahid, M., Srivastava, M., Sharma, A., Kumar, V., Pandey, S. and Singh, A. (2013). Morphological, Molecular Identification and SSR Marker Analysis of a Potential Strain of *Trichoderma*/*Hypocrea* for Production of a Bioformulation. *J. Plant Pathol. Microb.*, 4: 10.

Shaiesta, S., Sahera, N. and Sheikh, P.A. (2012). Cultural and morphological characterisation of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmir. *Res. J. Microbiol.*, 7: 139-144.

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

Sherameti, I., Shahollari, B., Venus, Y., Altschmied, L., Varma, A. and Oelmüller, R. (2005). The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan water dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *J. Biol. Chem.*, 280: 26241–26247.

Sherwood, R.T. (1970). Physiology of *Rhizoctonia solani*. In: Parameter, J.I.R. (ed) *Rhizoctonia solani*: Biology and Pathology, 2nd edn. University of California Press, London, pp. 69-92.

Shi, M., Chen, L., Wang, X.W., Zhang, T., Zhao, P.B., Song, X.Y., Caiyun, S., Chen, X.L., Cheng, Z. and Zhang, Y.Z. (2012). Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. *Microbiol.*, 158: 166-175. doi: 10.1099/mic.0.052670-0.

Shinde, M.S. (2016). Epidemiology and Management of Damping-off of Tomato (*Solanum esculentum* L.). M.Sc. Thesis, Submitted to the Navsari Agricultural University, Navsari, Gujarat.

Shinde, M.S., Rakholiya, K.B. and Waghunde, R.R. (2016). *In-Vitro* Efficacy of Bioagent and organic amendment against Damping-Off of Tomato Pathogen (*Pythium aphanidermatum*). *World J. Pharm. Life Sci.*, 2(6): 165-172.

Singh, A., Srivastava, S. and Singh, H. (2007). Effect of substrates on growth and shelf life of *Trichoderma harzianum* and its use in biocontrol of diseases. *Bioresour. Technol.*, 98: 470-473. doi: 10.1016/j.biortech.2006.01.002.

Singh, B.N., Dwivedi, P., Sarma, B.K., Singh, G.S. and Singh, H.B. (2018). *Trichoderma asperellum* T42 Reprograms Tobacco for Enhanced Nitrogen Utilization Efficiency and Plant Growth When Fed with N Nutrients. *Front. Plant Sci.*, 9: 163.

Singh, R.K., Kumar, P., Tiwari, N.N., Singh, S.P., Tiwari A.K., Vishwakarma, S.K., Singh, A. and Kumar, A. (2014). Role of Endochitinase Gene and Efficacy of *Trichoderma* against *Colletotrichum falcatum* Went. causing Red Rot Disease in Sugarcane. *Sugar Technol.*, 16(2):180-188.

Singh, R.S., Puneet, S., Jaspal, K.S.P. and Kaur, J. (2000). *Trichoderma* based biofungicide: progress and prospects. *Plant Dis. Res.*, 15: 155-161.

Singh, S., Singh, H., Singh, D. and Rakshit, A. (2014). *Trichoderma*-mediated enhancement of nutrient uptake and reduction in incidence of *Rhizoctonia solani* in tomato. *Egypt. J. Biol.*, 16. 29-38.

Singh, U.S., Kumar, J., Sachan, A. and Singh, P. (1995). Use of DNA-binding fluorochromes for the nuclear staining in fungi. In: Singh, R.P. and Singh, U.S. (ed) Molecular Methods in Plant Pathology. CRC, Lewis Publishers, Boca Raton, London, pp. 53–60.

Singh, V., Upadhyay, R., Sarma, B. and Singh, H. (2016). Seed bio-priming with *Trichoderma asperellum* effectively modulate plant growth promotion in pea. *Int. J.* of *Agric. Environ. Biotechnol.*, 9: 361. doi: s10.5958/2230-732X.2016.00047.4.

Sivan, A. and Elad, Y. (1985). Biological control of *Pythium aphanidermatum* by a new isolate of *Trichoderma harzianum*. *Phytopathol.*, 74: 498-501.

Sobita, S. and Anamika, S. (2011). Agro-based Waste Products as a Substrate for Mass Production of *Trichoderma* spp. *J. Agric. Sci.*, 3(4): 168-171.

Sodimalla, T., Prasanna, R., Shukla, L. and Saxena, A. (2012). Evaluating the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth-promoting inoculants. *Ann. Microbiol.*, pp. 63. doi: 10.1007/s13213-012-0573-x.

Srivastava, M.P., Tiwari, R. and Sharma N. (2013). Effect of different cultural variables on siderophores produced by *Trichoderma* spp. *Int. J. Adv. Res.*, 1(7): 1-6.

Srivastava, R., Khalid, A., Singh, U.S. and Sharma, A.K. (2010). Evaluation of arbuscular mycorrhizal fungus, *Pseudomonas fluorescens* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. *Biocontrol*, 53: 24-31.

Stulen I, Perez-Soba M, De Kok LJ, Van der Eerden L. (1998). Impact of gaseous nitrogen deposition on plant functioning. *New Phytologist.,* 139: 61–70.

Subash, N., Sundaram, M., Sasikumar, C. and Unnamalai, N. (2014). Mass cultivation of *Trichoderma harzianum* using agricultural waste as a substrate for the management of damping off disease and growth promotion in chilli plants (C*apsicum annuum* L.). *Int. J. Pharm. Pharma. Sci.*, 6: 188-192.

Sukoso, Suparno, Noor, A. and Luchman, H. (2016). *Trichoderma* spp. as Agent of Biological Control in Local Rice Diseases in Tidal Swamp Lands in South Kalimantan, Indonesia. *J. Agric. Vet. Sci.*, 9(1): 01-06.

Suparno, Sukoso, Noor, A. and Luchman, H. (2016). *Trichoderma* spp. as Agent of Biological Control in Local Rice Diseases in Tidal Swamp Lands in South Kalimantan, Indonesia. *J. Agric. Vet. Sci.*, 9(1): 04-09.

Sutton, D.A., Fothergill, A.W. and Rinaldi, M.G. (1998). Guide to Clinically Significant Fungi, 1st edn. Williams and Wilkins, Baltimore. pp. 32.

Tallapragada, P. and Gudimi, M. (2011). Phosphate solubility and biocontrol activity of *Trichoderma harzianum*. *Turk. J. Biol.*, pp. 35. doi: 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. *Agric. Sci.*, 6: 535-544.

Thomashow, L.S., Bonsall, R.F. and Weller, D.M. (1997). Antibiotic production by soil and rhizosphere microbes in situ, In: Hurst, C.J., Knudsen, G.R., McInerney M.J., Stetzenbach, L.D. and Walter, M.V. (ed) Manual of Environmental Microbiology. ASM Press, Washington DC, pp. 493-499.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22(22): 4673-4680.

Tillotson, C.R. (1917). Nursery practice on the national forests. *USDA Bull.*, 479: 86.

Triveni, S., Prasanna, R., Shukla, L. and Saxena, A.K. (2013). Evaluating the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth-promoting inoculants. *Ann. Microbiol.*, 63(3): 1147-1156.

Troian, R.F., Steindorff, A.S., Ramada, M.H.S., Arruda, W. and Ulhoa, C.J. (2014). Mycoparasitism studies of *Trichoderma harzianum* against *Sclerotinia sclerotiorum*: evaluation of antagonism and expression of cell wall-degrading enzymes genes. *Biotechnol. Lett.*, 36(10): 2095-2101.

Trow, A.H. (1901). Observations on the biology and cytology of *Pythium ultimum*. *Ann. Bot.*, 15: 269-312.

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

Van West, P., Appiah, A.A. and Gow. N.A.R. (2003). Advances in research on oomycete root pathogens. *Physiol.* Molecular *Plant Pathol.*, 62: 99-113.

Velazhahan, R., Jayaraj, J. and Radhakrishnan, N.V. (2006). Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. *Arch. Phytopathol. Plant Prot.*, 39(1): 1-8.

Venkateswarlu, R., Reddi Kumar, M., Eswar Reddy, N. and Sudhakar P. (2008). Molecular characterization of *Trichoderma* Spp. Used against the Fusarium wilt with PCR based RAPD and ITSPCR. *J. Mycol. Plant Pathol.*, 38(3): 559-563.

Vidhyasekaran, P. and Muthamilan, M. (1995). Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.*, 79(8): 782–786.

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

Vieira, P. M., Coelho, A. S., Steindorff, A. S., de Siqueira, S. J., Silva, R., & Ulhoa, C. J. (2013). Identification of differentially expressed genes from *Trichoderma harzianum* during growth on cell wall of Fusarium solani as a tool for biotechnological application. *BMC Genomics*, 14: 177. doi: 10.1186/1471-2164-14-177

Vijayan, A.K., Joseph T. and Thomas J. (2016). Use of *Trichoderma* as A Bioagent and Vermicompost as the Carrier for the Management of Rhizome Rot and Azhukal Diseases of Cardamom. Role of Biocontrol Agents for Disease Management in Sustainable Agriculture. pp. 392–398

Vinale, F., Manganiello, G., Nigro, M., Mazzei, P., Piccolo, A. and Pascale, A. (2014). A novel fungal metabolite with beneficial properties for agricultural applications. *Mol.*, 19: 9760–9772. doi: 10.3390/molecules19079760.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L. and Lorito, M. (2008). *Trichoderma*-plant-pathogen interactions. *Soil Biol. Biochem.*, 40: 1-10.

Viterbo, A., Harel, M., Horwitz, B.A., Chet, I. and Mukherjee, P.K. (2005). *Trichoderma* mitogen-activated protein kinase signaling is involved in induction of plant systemic resistance. *Appl. Environ. Microbiol.*, 71: 6241–6246.

Viterbo, A., Landau, U., Kim, S., Chernin, L. 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, R., Shelake, R. and Sabalpara, A.N. (2016). *Trichoderma*: A significant fungus for agriculture and environment. *Afr. J. Agric. Res.*, 11: 192-196.

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

Warrior, P., Kondru, K., Preeti, V. and Vasudevan, P. (2002). Formulation of biocontrol agents for pest and disease management. In: Gnanamanickam, S.S. (ed) Biological control of crop disease. New York Press, pp. 421-441.

Weideman, H. and Wehner, F.C. (1993). Green house evaluation of *Trichoderma harzianum* and *Fusarium oxysporum* for biological control of citrus root rot in soils naturally and artificially infected with *Phytophthora nicotianae. Phytophylactica*, 25: 101-105.

Wener, Z.H. (2017). Importance of the tomato. *Agri Support Online Vegetables Consultant*. http://www.agrisupportonline.com/Articles/importance_of_the_tomato.htm.

White, T.J., Burns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A. (ed) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp. 315-322.

Wilhite, S.E., Lumsden, R.D. and Straney, D.C. (1994). Mutitional analysis of gliotoxin production by the biocontrol fungus *Gliocladium virens* in relation to suppression of *Pythium* damping-off. *Phytopathol.*, 84: 816-821.

Wu, Q., Sun, R., Ni, M., Yu, J., Li, Y., Yu, C., Dou, K., Ren, J. and Chen, J. (2017). Identification of a novel fungus, *Trichoderma asperellum* GDFS1009 and comprehensive evaluation of its biocontrol efficacy. *Plos One*, 12(6): 1-20.

Yang, X., Chen, L., Yong, X. and Shen, Q. (2011). Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against *Fusarium* wilt of cucumbers. *Biol. Fertil. Soils*, 47: 239–248.

Yu, A., Basarova, A., Baskunov, B.P. and Medentsev, A.G. (2017). A low-molecular mass antimicrobial peptide from *Trichoderma* cf. *aureoviride* Rifai VKM F-4268D. *Microbiol.*, 86(2): 289-291.

Yuvraj (2017). Rhizosphere competence, plant growth promoting activities and disease controlling potential of *Trichoderma* spp. M.Sc. Thesis, Submitted to the Department of Plant Pathology College of Agriculture, Raipur, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh).

Zappia, R.E, Huberli, D., Hardy, G.E.S.J. and Bayliss, K.L. (2014). Fungi and oomycetes in open irrigation systems: knowledge gaps and biosecurity implications. *Plant Pathol.*, 63: 961-972. doi: 10.1111/ppa.12223

Zhang, C.L., Druzhinina, I.S., Kubicek, C.P. and Xu, T. (2005). *Trichoderma* biodiversity in China: evidence for a North to South distribution of species in East Asia. *Microbiol. Lett.*, 251(2): 251-257.

Zhang, S., Gan, Y. 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. *Front. Plant Sci.*, 7: 1405. doi: 10.3389/fpls.2016.01405.

Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.*, 7(1-2): 203-214.

Zhao, L., Wang, F., Zhang, Y. and Zhang, J. (2014). Involvement of *Trichoderma asperellum* strain T6 in regulating iron acquisition in plants. *J. Basic Microbiol.*, pp. 54. doi: 10.1002/jobm.201400148.

APPENDIX

Internal Transcribed Region (ITS) partial gene sequences (contig) of the isolated *Trichoderma* spp.

>TR 55

TTCTGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCA
ATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTAAA
AGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCC
CCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC
CCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACC
CCTCCGGGGGATCGGCGTTGGGGATCGGACCCCTCACCGGGTGCCGGCCCTG
AAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTC
GCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTCTGAAATGTT
GACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATAGCAATAAGCGGAG
G

>TR 64

CCTGCGCAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATGTGAACGTTAC
CAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCGCAGCCCCGGACC
AAGGCGCCCGCCGGAGGACCAACCAAAACTCTTTTTGTATACCCCCTCGCGGGT
TTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGAATCA
AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCT
CGAACCCCTCCGGGGGGTCGGCGTTGGGGATCGGCCCTGCCTCTTGGCGGTGG
CCGTCTCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTT
GCACACTCGCATCGGGAGCGCGGCGCGTCCACAGCCGTTAAACACCCAACTTCT
GAAATGTTGACCTCAGATCAGGTAGGAATACCCGCTGAACTTAAGCATA

>TR 66

TACAACTCCCAAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCAC
GCCCCGGGTGCGTAAAAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCA
AACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTC
AAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAC
GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCA
TTTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCAC
CGGGTGCCGGCCCTGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCA
GTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACC
CAACTTCTGAAATGTTGACCTCGGAT-CAGGTAGG-
AATACCCGCTGAACTTAAGCATAGCAATA

>TR 87

CTGTAGGGGGAACATGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCA
ATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTAAA
AGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCC

CCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC
CCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACC
CCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACCGGGTGCCGGCCCTG
AAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTC
GCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTCTGAAATGTT
GACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATAGCAATAAGGCGGG
AGG

>TR 88

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CAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGT
CGCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAACTCTTATTGTAT
ACCCCCTCGCGGGTTTTTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGG
CGTTTCGAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCC
GAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGGTCGGCGTTGGGGATCGGC
CCTCCCTTAGCGGGTGGCCGTCTCCGAAATACAGTGGCGGTCTCGCCGCAGCCT
CTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGCGCGGCGCGTCCACAGCC
GTTAAACACCCAACTTCTGAAATGTTGACCTCGGATACAGGTAGGGAATACCCTC
TGAACTTACGGGCATAGCAATAAGCGGAGGA

>TR 106

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ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAA
CCCTCGAACCCCTCCGGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGG
TGGCCGTCTCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAG
TTTGCACACTCGCATCGGGAGCGCGGCGCGTCCACAGCCGTTAAACACCCAACT
TCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAG

>TR 109

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CCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCGCAGCCCCGGAC
CAAGGCGCCCGCCGGAGGACCAACCAAAACTCTTATTGTATACCCCCTCGCGGG
TTTTTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA
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CACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAA
CCCTCGAACCCCTCCGGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGG
TGGCCGTCTCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAG
TTTGCACACTCGCATCGGGAGCGCGGCGCGTCCACAGCCGTTAAACACCCAACT
TCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATAGCA
AAA

>TR 112

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TCTCTGCCCCGGGTGCGTCGCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCA
ACCAAAACTCTTATTGTATACCCCCTCGCGGGTTTTTTTTTTATAATCTGAGCCTTC
TCGGCGCCTCTCGTAGGCGTTTCGAAAATGAATCAAAACTTTCAACAACGGATCT
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CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCT
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GCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGC
GCGGCGCGTCCACAGCCGTTAAACACCCAACTTCTGAAATGTTGACCTCGGAT-
CAGGTAGG-AATACCCGCTGAACTTAAGCATATCATTAA

>TR122

TTCTGTAGGGGGGCCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCC
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CCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACT
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AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
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CCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACCGGGTGCCGGCCCT
GAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACT
CGCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTCTGAAATGT
TGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATAGCAA-
AAGCGGAGGAA

>TR 136

GACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATGTGAACGTT
ACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCGCATCCCCGGAC
CAAGGCGCCCGCCGGAGGACCAACCAAAACTCTTATTGTATACCCCCTCGCGGG
TTTTTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA
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AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAA
CCCTCGAACCCCTCCGGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGG
TGGCCGTCTCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAG
TTTGCACACTCGCATCGGGAGCGCGGCGCGTCCACAGCCGTTAAACACCCAACT
TCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCA
TCAA

LIST OF PUBLICATIONS

- **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). *International Journal of Current Microbiology and Applied Sciences.* 8(02): 566-579. doi: https://doi.org/10.20546/ijcmas.2019.802.064

- **Markidahun Biam and Dipali Majumder (2019).** Biocontrol efficacy of *Trichoderma* isolates against tomato damping off caused by *Pythium* spp. and *Rhizoctonia solani* (Kuhn.). *International Journal of Chemical studies*. Volume 7, issue 3, May-June 2019. pp 81-89.

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