DEVELOPMENT OF MICROBIAL CONSORTIA FOR SUSTAINABLE MANAGEMENT OF LATE BLIGHT (*Phytophthora infestans* (Mont.) de Bary) IN TOMATO (*Solanum lycopersicum* L.)

Thesis

submitted to

NAGALAND UNIVERSITY

in partial fulfillment of requirements for the Degree

of

DOCTOR OF PHILOSOPHY

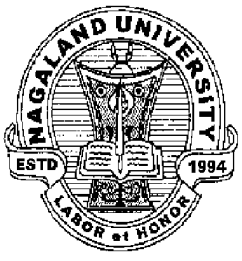
in

PLANT PATHOLOGY

by

RAGHUVEER SINGH

Admn. No. Ph-206/16 Regn. No. 851/2019



Department of Plant Pathology,
School of Agricultural Sciences and Rural Development,
Nagaland University, Medziphema Campus – 797106
Nagaland

2020

Affectionately
Dedicated
To My
Beloved Family
&
Friends

DECLARATION

I, Raghuveer Singh, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

This is being submitted to the Nagaland University for the degree of Doctor of Philosophy in Plant Pathology.

Date:
Place: Medziphema **(RAGHUVEER SINGH)**

……..
Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema-797 106, Nagaland

Dr. N. Tiameren Ao
Professor
Department of Plant Pathology

CERTIFICATE - I

This is to certify that the thesis entitled **"Development of microbial consortia for sustainable management of late blight (*Phytophthora infestans* (Mont.) de Bary) in tomato (*Solanum lycopersicum* L.)"** submitted to the Nagaland University in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Plant Pathology is the record of research work carried out by Mr. Raghuveer Singh, Registration No. 851/2019, under my personal supervision and guidance.

The results of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

Date:

Place: Medziphema

……
Dr N. Tiameren Ao
Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema-797 106, Nagaland

CERTIFICATE - II

VIVA VOCE ON THESIS OF DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY

This is to certify that the thesis entitled **"Development of microbial consortia for sustainable management of late blight (*Phytophthora infestans* (Mont.) de Bary) in tomato (*Solanum lycopersicum* L.)"** submitted by Mr. Raghuveer Singh, Admission No. Ph-206/16, Registration No. 851/2019 to the NAGALAND UNIVERSITY in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Plant Pathology has been examined by the Advisory Board and External examiner on …….....

The performance of the student has been found **Satisfactory/Unsatisfactory.**

Members	Signatures
1. Dr. N. Tiameren Ao (Supervisor)	…….
2. …… (External examiner)	…….
3. …… (Pro-Vice Chancellors' nominee)	…….
4. Dr. L. Daiho	…….
5. Dr. Susanta Banik	…….
6. Dr. Pankaj Neog	…….
7. Dr. S.P. Kanaujia	…….

Head
Department of Plant Pathology

Dean
School of Agricultural Sciences and Rural Development

ACKNOWLEDGEMENTS

All glory to Almighty, whose blessings is the success behind this thesis. Praise, Pride and Perfection belong to Almighty, so first of all I would like to express my deepest sense of gratitude to omniscient and omnipresent power of the Universe, The Almighty God.

I feel great contentment to express my sincere and profound sense of gratitude and indebtedness to my esteemed supervisor, ***Dr. N. Tiameren Ao****, Professor, Department of Plant Pathology, Nagaland University, SASRD for his valuable guidance, constructive suggestions, keen and sustained interest and incessant encouragement during the entire course of investigation and preparation of this manuscript.*

"Thank you sir, for all the freedom and having the confidence in my capabilities. I will treasure this learning experience for your expertise, perceptiveness and cracking-of-the-whip without which I would never have completed in time".

I extend my cordial thanks to express my indebtedness to ***Prof. (Dr.) L. Daiho,*** *Professor and Head, Department of Plant Pathology, Nagaland University, SASRD, for his active support and constant encouragement in the completing the research work and providing DBT project's Trichoderma isolates.*

My heartfelt gratitude also goes to ***Dr. Susanta Banik,*** *Assistant Professor, Department of Plant Pathology, Nagaland University, SASRD, for his innovative suggestions, excellent teaching of advanced courses and help with all the experimental analysis I had to endure.*

I have no words to express my deepest sense of gratitude towards my advisory committee members, ***Dr. Pankaj Neog,*** *Assistant Professor, Department of Entomology and* ***Dr. S.P. Kanaujia,*** *Associate Professor, Department of Horticulture, Nagaland University, SASRD for their encouragement and valuable suggestions in completing my research works.*

My heartfelt thanks and immense gratitude to ***Dr. Narola Pongener,*** *Assistant Professor, and* ***Dr. H. Meronbala Devi,*** *Assistant Professor and Department of Plant Pathology, NU, SASRD for their cooperation during the research and course work.*

I am also highly obliged and grateful to the ***Dean,*** *SASRD for rendering all necessary facilities and infrastructures to carry out the investigations.*

I wish to express heartfull thanks to my parent organization ***Indian Council of Agricultural Research (ICAR)*** *for providing opportunity to me for higher study.*

I have absolutely no words to express my sincere thanks to ***Dr. Susheel Kr. Sharma,*** *ARS, Scientist (Plant Pathology), ICAR, Manipur Centre, Lamphelpat and* ***Dr. Rajesha G.,*** *ARS, Scientist (Plant Pathology), ICAR, Nagaland Centre, Medziphema for their help rendered during my molecular and biochemical works.*

I am lucky to have brilliant and even dedicated galaxy of friends, ***Valenta, Mapuii, Leima, Nchinle, Priya, Rina, Badapmein, Meshenjee, Homseng, Kania, Allole, Imti, Emika, Nungshi, Dr. Jitendra Kr.*** *and* ***Dr. Rajesh A. Alone*** *for their crucial assistance throughout my course and research work.*

Words fail to express my deep gratitude from the depth of my heart for my loving and caring wife ***Ms. Saroj Kanwar,*** *my kids* ***Hitendra*** *and* ***Rudra,*** *my parents* ***Sh. Padam Singh*** *and* ***Mrs. Som Kanwar*** *who provided me relentless encouragement, pure love, unmatched affection that made this work to reach its fruitful destination.*

I shall fail in my duty, if I don't thanks the non teaching staff members of Department of Plant Pathology, NU, SASRD for their help and cooperation.

Date: 18th Oct., 2019 (**Raghuveer Singh**)

Place: Medziphema

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LIST OF ABBREVIATIONS AND SYMBOLS

@	:	at the rate of
%	:	per cent
/	:	per
&	:	and
a.i.	:	active ingredient
BOD	:	biological oxygen demand
bp	:	base pair
^{o}C	:	degree Celsius
CD (p=0.05)	:	critical difference at 5 per cent probability
cm	:	centimetre
cv.	:	cultivar
CFU/ml	:	colony forming unit per millilitre
CRD	:	completely randomized design
CV	:	co-efficient of variation
CMC	:	compatible microbial consortia
conc.	:	concentration
CTAB	:	cetyl trimethyl ammonium bromide
dNTP	:	deoxynucleotide triphosphates
DAS	:	days after sowing
DAT	:	days after transplanting
DBT	:	days before transplanting
DNA	:	deoxyribo nucleic acid
EC	:	emulsifiable concentrate
et al.	:	et alia (and others)
etc.	:	etcetera
EDTA	:	ethylene diamine tetra acetic acid

Fig	:	figure
FYM	:	farm yard manure
f.sp.	:	forma specialis
g	:	gram
ha^{-1}	:	per hectare
h	:	hour
hrs	:	hours
i.e.	:	that is
in vivo	:	in a living thing
in vitro	:	in laboratory
K	:	potassium
kg	:	kilogram
kg ha^{-1}	:	kilogram per hectare
kg^{-1}	:	per kilogram
L	:	liter
L^{-1}	:	per liter
m	:	meter
m^2	:	square meter
ml	:	millilitre
mg	:	milligram
min	:	minutes
mt	:	million tonnes
M	:	molar
N	:	nitrogen
NS	:	non significant
No.	:	number
O.D.	:	optical density
P	:	phosphorous
PCR	:	polymerase chain reaction

pH	:	potential of hydrogen
PDA	:	potato dextrose agar medium
PDI	:	per cent disease index
q	:	quintal
rpm	:	revolution per minute
R.H.	:	relative humidity
SEm (±)	:	standard error of mean
sp., spp.	:	species (singular and plural)
t ha^{-1}	:	tonnes per hectare
viz.	:	namely
wt.	:	weight

CHAPTER I

INTRODUCTION

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a solanaceous fruit vegetable originated in tropical America (Thompson and Kelly, 1957) having immense value in olericulture. In India it was introduced by Portuguese in early 18th century. It is grown both under green house as well as field conditions throughout the year. It is grown worldwide supplying in the fresh market as well as for processing purposes (Bhati and Kanaujia, 2014). It is one of the most important "protective foods" because of its exceptionally versatile food use and high nutritive value (Afroz *et al*., 2009; Saleem *et al*., 2009; Noureen *et al*., 2010).

The total global area under tomato is 4.73 m ha and production is to the tune of 163.96 m t. Among the tomato producing countries in the world, China is the largest producer accounted for about one quarter of the global output, followed by India and USA. The total vegetable area in India is 10.28 m ha with production of 175 m t and productivity as 17 t ha^{-1}. In India, tomato is grown in an area of 0.797 m ha with annual production of 20.71 m t and productivity of 24.36 t ha^{-1}. Major tomato producing states are Karnataka, Bihar, Himachal Pradesh, Odisha, Maharashtra, West Bengal, Uttar Pradesh, Tamil Nadu and Gujarat, among them Karnataka is the highest tomato producer state in India (Anon., 2017).

Tomato is a highly adaptive to warm season and can be grown successfully in plains as well as in hills. Cultivation of tomato in monsoon season is assuming a great importance in the north-eastern region in general and Nagaland in particular owing to its high prices of produce obtained from other parts of the country during this period (Babu, 2006).

Though tomato crop occupies a very important place among the vegetable crops grown in India, the average yield of this crop on farmers' fields is reasonably poor. One of the constraints for poor yield is the devastating effect of certain disease incidence. Tomato crop is affected by large number of diseases incited by viruses, fungi, oomycetes, namatodes and bacteria (Barone *et al*., 2007). Among the diseases, late blight of tomato caused by *P. infestans* (Mont.) de Bary is destructive and wide spread in nature. It is an economically important disease of tomato worldwide including India (Son *et al*., 2008). Worldwide losses were estimated to $170 billion annually and thus the pathogen was regarded as a threat to global food security (Latijnhouwers *et al.,* 2004; Wu *et al*., 2012). This disease can cause total destruction of all plant parts within a week or two when weather conditions are favourable (Agrios, 1997). And also could cause up to 100 % crop losses if not controlled (HCDA, 1996). Yield losses up to 79 % from late blight damage have been reported from India (Arora *et al*., 2014; Chowdappa *et al*., 2015).

Late blight disease in tomato was first reported from France by Payen in 1847 (Payen, 1847). Subsequently, it was reported from India by Butler in 1900 (Butler, 1903), Mexico (Ramirez, 1921), Puerto Rico (Matz, 1921), Bermuda (Russell, 1936), New Jersey (Small, 1936), Malaya (Thompson, 1936), USA (Lancashire and Counter, 1940), Indiana (Samson, 1947), Quebec (Lavalee, 1948), Kenya (Nattrass, 1950), South Africa (Wager, 1952), West Africa (Russell, 1954), Rhodesia (Riley, 1955), Argentina (Taboada and Montanola, 1959), Ghana (Nyako, 1972), Nigeria (Erinle and Quinn, 1980), Pakistan (Majid *et al*., 1992), Oceana and Australian countries (Sokhi *et al*., 1993).

Late blight disease can spread rapidly during cool and moist weather, killing tomato plants within a few days and causing total crop loss. Effects on tomato plant include reduced photosynthetic leaf area, extensive defoliation,

loss of fruits, plant death, reproductive capacity and loss of seeds by attacking whole parts of the plants (Scot, 2008). *P. infestans* sporulates most abundantly at relative humidity near 100 % and at temperatures between 16 to 22 ^{o}C (Hartman and Huang, 1995).

P. infestans can attack stems, leaves, petioles, fruits and seeds of tomato (Irzhansky and Cohen, 2006). It may be initiated in nursery and adult plants by air-borne sporangia or by oospores harbouring the seed and soil (Rubin and Cohen, 2004; Govers, 2005). Thick-walled oospores of *P. infestans* can survive harsh environmental conditions and are means for overwintering (Aylor, 1978). Oospores have been found to survive in the soil for 5-7 months at temperatures ranging between 0 to 20 °C (Pittis and Shattock, 1994). Furthermore, oospores remain capable of germinating after exposure to temperatures between 20 to 40 °C (Fay and Fry, 1997). It has a wider host range which includes *Capsicum annuum, Calibrachoa* spp., *Datura metel, D. stramonium, Nicotiana benthamiana, Solanum lycopersicum*, *S. tuberosum*, *S. melongena, S. sarrachoides, S. triflorum, S. dulcamara, S. sisymbriifolium, S. nigrum* and *S. dulcamara* (Flier *et al.,* 2003; Bectell *et al.,* 2006; Dandurand *et al.,* 2006; Lebecka, 2008).

Although, several management components *viz.,* cultural practices, biological agents, host resistance and chemicals are available, but fungicides and host resistance hold promise in managing the disease. Use of fungicides is costly and beyond the reach of most of the marginal and small farmers. Moreover, their extensive use may lead to environmental pollution (Ragunathan and Divakar, 1996) and less effective due to increasing resistance of the pathogen (Griffith *et al.,* 1992). Under such conditions, the most effective method is the biological control (Ellis *et al.,* 1999). In recent years, biocontrol gained importance as an alternative to fungicides for plant disease management. It is a good alternative for sustainable agriculture to overcome the

problems of public concern associated with fungicides and pathogens resistant to fungicides and to become eco-friendly (Akhtar and Siddiqui, 2008). BCAs are improving plant growth, easy to deliver and activate resistance mechanism in the host and increase yield and biomass production. These antagonists act through secretion of volatile toxic metabolites, mycoparasitism, mycolytic enzymes, antibiosis and through competition for nutrients and space. Some species of *Pseudomonas* and *Trichoderma* are among the major microorganisms that have shown efficacy in controlling a number of fungal diseases, including late blight of tomato (Siddiqui and Shaukat, 2003).

Emerging strategies for plant disease control involve biocontrol and integrated biocontrol by applying antagonistic microorganisms alone or in consortia. Single antagonistic strains or isolates often result in inconsistent disease control under field and for overcoming such inconsistent performance, mixture of two or more biological agents as biocontrol consortium, promises more efficient disease control (Harish *et al.,* 2008). Mixed BCAs have the potential to colonize more effectively the rhizosphere, to express more consistent beneficial traits under various soil conditions and to control a wide range of plant pathogens than singly used bioagents due to their ability to produce various lipopeptide antibiotics (Idris *et al.,* 2007). Microbial consortia are much more efficient than single strains or isolates of microorganisms with diverse metabolic capabilities (Yan *et al.,* 2002). Many of these PGPRs and BCAs are known to produce vitamins, amino acids and growth promoting substances like cytokinins, GA and IAA which help in better growth of crop plants (Raupach and Kloepper, 1998).

Hence looking into the aforesaid realities, the use of mixture of two or more biological agents, as biological control consortium, promises more efficient disease control. Also not much systematic research work has been

carried out on late blight of tomato under Nagaland condition. Hence, the present investigation entitled "Development of microbial consortia for sustainable management of late blight (*Phytophthora infestans* (Mont.) de Bary) in tomato (*Solanum lycopersicum* L.)" was undertaken with the following objectives:

1. To evaluate the antagonistic activity of biocontrol agents against *Phytophthora infestans* (Mont.) de Bary *in vitro*

2. To study the compatibility of biocontrol agents and test the efficacy of their combination against *Phytophthora infestans* (Mont.) de Bary *in vitro*

3. To evaluate the field efficacy of compatible microbial consortia against late blight of tomato

4. To evaluate the native tomato genotypes for their resistance to late blight of tomato under field condition

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The pertinent literature available on the following aspects of late blight of tomato is reviewed here as under:

2.1 History

Late blight, caused by the Oomycete *P. infestans* (Mont.) de Bary, is a devastating disease of tomato and also for potato. This disease is responsible for the Irish potato famine during 1845-46. The resulting from Irish famine, late blight was responsible for over one million Irish citizens' deaths and the emigration of at least 1.5 million Irish citizens (Large, 1940). The Irish potato famine led to intense investigation into the nature of plant disease and resulted in de Bary's confirmation of germ theory (Fry and Goodwin, 1997).

2.2 Geographical distribution

Late blight of tomato is one of the most destructive diseases of the crop and its occurrence worldwide. The disease was first recorded on tomato in France by Payen in 1847 (Payen, 1847) and in India by Butler in 1900 (Butler, 1903). Subsequently, it was reported from Mexico (Ramirez, 1921), Puerto Rico (Matz, 1921), Bermuda (Russell, 1936), New Jersey (Small, 1936), Malaya (Thompson, 1936), USA (Lancashire and Counter, 1940), Indiana (Samson, 1947), Quebec (Lavalee, 1948), Kenya (Nattrass, 1950), South Africa (Wager, 1952), West Africa (Russell, 1954), Rhodesia (Riley, 1955), Argentina (Taboada and Montanola, 1959), Ghana (Nyako, 1972), Nigeria (Erinle and Quinn, 1980), Pakistan (Majid *et al*., 1992), Oceana and Australian countries (Sokhi *et al*., 1993).

2.3 Taxonomy

The genus *Phytophthora* was first time described by Anton de Bary in 1876, with *P. infestans* being the type species (Zentmyer, 1983). *Phytophthora* genus belongs to the kingdom *Stramenopila,* phylum *Oomycota*, order *Peronsporales* and family *Pythiaceae* (Volk, 2001). There are 60 species within *Phytophthora*, including *P. capsici* which affects pepper, *P. cactorum* important pathogen of apple, the citrus pathogen *P. citrophthora* and *P. cinnamomi*, which affect many woody plants including conifers (Hawksworth *et al.,* 1995; Erwin and Ribeiro, 1996). Although originally it was classified as a fungus, omycetes have been reclassified in the kingdom *Straminopila*. Oomycetes are distinguishable from fungi based on metabolism (Pfyffer *et al*., 1990), zoospore motility (Zentmyer, 1983), rRNA sequence (Förster *et al.,* 1990; Cooke *et al*., 2000) and cell wall composition (Bartnicki-Garcia and Wang, 1983). Hence, members of *Phytophthora* can be distinguished from the fungi since they have the following characteristics, cell walls composed of glucans and cellulose instead of chitin, motile zoospores with tinsel and whiplash flagella, several sporangia produced on each sporangiophore and diploid vegetative cells (Zentmyer, 1983).

2.4 Pathogen life-cycle

In the absence of sexual spores, *P. infestans* is an obligate parasite and survives the winter on living host tissue such as infected potato tubers or field debris in warmer areas. The primary inoculum for late blight is air-borne sporangia from infected plants, mycelium and sporangia on infected tubers left in fields or gardens, infected volunteer plants and infected transplants (Agrios, 1997). In addition to overwintering in infected potato tubers, *P. infestans* can also survive on seeds from infected tomato fruits (Boyd, 1935).

Pittis and Shattock (1994) reported the sporangia can be transported over a large distance by wind in the season if moist and cool conditions prevail for few days. Sexual spores of *P. infestans,* oospores survives on debris or in the soil and serve as source of primary inoculum. Soil-borne sporangia can survive for a maximum of 77 days, oospores have been shown to survive for 8 months even in extreme temperatures.

2.5 Biology

P. infestans is the causal agent of tomato late blight disease. It is heterothallic in nature, it required two known mating types (A1 and A2) (Gallegly and Galindo, 1958). In Central Mexico both the mating type present. Mostly pathogen global population consisted of the A1 mating type during the 1980's. A2 mating type was discovered in the early 1980's. It had migrated to Europe and many other countries in the world from central Mexico (Fry *et al*., 1993).

2.6 Symptomatology

Turkensteen (1973) reported that the late blight affects all the above ground plant parts like leaves, stems and fruits. The symptoms also vary depending upon whether the pathogen is an aggressive or non-aggressive strain.

Stevenson (1991) observed that in addition to attacking foliage and stems, late blight also infects tomato fruit. Tomato fruits are susceptible until ripening. Symptoms appeared on fruits start as greasy dark spots, later these spots may increase in size to cover the entire fruit area. White mycelium and pathogen sporulation may be visible on fruit under favourable environmental conditions. Lesions of disease on fruit may be visible a ringed pattern, similar to buckeye rot, *P. parasitica* (Erwin and Ribeiro, 1996).

Agrios (1997) described non-aggressive strains form brown to black coloured lesions which expand slowly up to a rate of 1 mm per day. Often these lesions become angular in shape as the veins limit the expansion of the lesions. Under favourable weather, lesions may expand across smaller veins. Typically lesion development ceases in an early stage when lesions are less than 2-3 mm in diameter. The initial foliar symptoms of aggressive strains on tomato are small, irregular, water-soaked spots which are light to dark green in colour. Under favourable conditions, these spots develop into large brown necrotic regions with indefinite borders.

Thurston and Schultz (1997) recorded the lesions are often surrounded by a yellow green halo of chlorotic tissue. Sporangiophores and sporangia may be visible as a whitish downy ring 3-5 mm wide around the edge of the lesion, especially on the leaf underside.

Scot (2008) reported the lesions begin as water-soaked, indefinite that enlarge rapidly into black to brown lesions that cover entire areas of stems and petioles. During moist weather, lesions may be covered with a white to gray mouldy growth of the pathogen. Affected petioles and stems may eventually merged at the point of infection, leading to death of all distal parts of the plant.

2.7 Host-range

P. infestans generally considered to have limited host range and in agriculture, the two most important primary hosts being tomato and potato. The main secondary hosts include red pepper (*C. annuum* L.). There are also a number of wild hosts like hindu datura (*Datura metel*), jimson weed (*D. stramonium*), eggplants (*S. melongena*), black nightshade (*S. nigrum*), bittersweet (*S. dulcamara*) and petunia (Scot, 2008).

2.8 Environmental factors

Van der Plank (1968) mentioned that environment, pathogen and host are important factors in disease triangle and importance of environmental factors on disease progression is important. Environment factors have been recorded that a major component of the Irish potato famine during 1845-46, apart from the reliance on potato as a main food and the migration of the late blight pathogen into the other part of world, period during Irish potato famine was characterized by abnormally low temperatures and increased RH facilitating pathogen establishment (Schumann, 1991).

Duniway (1983) reported the late blight is highly responsive to weekly or even daily environmental changes. For late blight disease initiation favourable temperatures are required in between 15 to 20 °C and high RH (Watterson, 1986). Temperature for germination of zoospore is required in between 12 to 15 °C, if temperature is higher than 15 °C direct germ tube will produced (Agrios, 1997). Optimum temperatures 21 °C and RH of 100 % is required for mycelial growth of the pathogen (Alexopoulos, 1962).

Carlile and Watkinson (1994) mentioned sporangia can be dispersed by water splash or by wind. Primary factors are responsible for late blight spore dissemination is air movements and moisture. Survival of sexaual spores of *P. infestans,* Oospores can survive in harsh environmental conditions and they are sources for primary infection (Aylor, 1978). Oospores have been found to survive in the soil for 5-7 months at temperatures ranging in between 0 to 20 °C (Pittis and Shattock, 1994). Furthermore, oospores can germinate after exposure to temperatures between 20 to 40 °C (Fay and Fry, 1997).

2.9 *In vitro* antagonistic efficacy of biocontrol agents

Baker (1987) defined biological control as "the decrease of inoculum or the disease producing activity of a pathogen accomplished through one or more organisms including the host plant but excluding the man".

Naseby *et al.* (2000) recorded *P. fluorescens* inhibited *P. infestans* mycelial growth *in vitro.* The per cent inhibition was recorded 88 %. The clear inhibition zone was recorded in dual culture plate. This isolate was obtained from tomato rhizosphere.

Torres-Rubio *et al*. (2000) also reported *P. fluorescens* inhibited *P. infestans* mycelia growth (74 %). The per cent inhibition was recorded 74 %. The clear inhibition zone was recorded in dual plate. Bioagent was isolated from tomato rhizosphere.

Anith *et al*. (2002) tested anatagonistic activity of *P. fluorescens* against *P. infestans* by dual culture technique using potato dextrose agar (PDA) and carrot agar (CA) media and it was found that bioagent inhibit the growth of test pathogen in 5-7 days. The clear inhibition zone was recorded in dual culture plate.

Akhtar and Siddiqui (2008) reported that biocontrol is a best option for the management of plant diseases in sustainable agriculture. It overcomes the problems concern to fungicides and pathogens resistant to fungicides and it become eco-friendly.

Patel and Mukadam (2011) tested the antagonistic activities of three *Trichoderma* spp. *i.e. T. harzianum, T. viride* and *Trichoderma* sp. against *P. infestans*. They found that all three species of *Trichoderma* suppressed the mycelial growth of test pathogen in dual culture plate.

Zegeye *et al.* (2011) also tested the antagonistic activity of *P. fluorescens* and *T. viride* against *P. infestans.* They observed a radial growth

inhibition of 36.7 %. In case of inhibition of *P. infestans* by *P. fluorescens,* a radial growth inhibition was 88 %. A clear zone of inhibition of *P. infestans* was also noticed. These isolates were obtained from tomato rhizosphere. They observed a radial growth inhibition of 36.7 %. In case of inhibition of *P. infestans* by *P. fluorescens,* a radial growth inhibition was 88 %.

Kabir *et al.* (2013) also tested five isolates of bacteria (BI 06, BI 09, BI 11, BI 15 and BI 18) against *P. infestans.* These five isolates were inhibited *P. infestans* by more than 62.50 % *in vitro*. However, BI 18 was the most effective, inhibiting mycelial growth of the test pathogen by 78.75 %. The clear inhibition zone was recorded in dual culture plate. These five isolates were inhibited *P. infestans* by more than 62.50 % *in vitro*. These isolates were obtained from tomato rhizosphere.

Lamsal *et al.* (2013) evaluated seven isolates of bacteria *viz.,* AB 05, AB 10, AB 11, AB 12, AB 14, AB 15 and AB 17 against *P. infestans*. These isolates were isolated from rhizosphere of tomatoes in Korea. All 7 isolates inhibited the pathogen by more than 60 % *in vitro*. However, AB 15 was the most effective, inhibiting the pathogen by 80.75 %. Isolate AB 14 showed the least inhibition, with a 64.17 % reduction in mycelial growth of *P. infestans*. The antagonistic activity was higher in isolates AB 05, AB 11, AB 12, AB 15 and AB 17, with a more than 15 mm inhibition zone observed on dual culture plates. Isolate AB 14 showed the least inhibition, with a 64.17 % reduction in mycelial growth of *P. infestans*. These isolates were obtained from tomato rhizosphere.

Tomar *et al*. (2014) also evaluated the antagonistic properties of 95 isolates of bacteria against *P. infestans*. Out of 95 bacterial isolates, only five

isolates of *P. aeruginosa*-1 to 5, exhibited best antagonistic activities when tested in dual culture method. These isolates showed 62.22, 38.33, 46.42, 32.66 and 35.33 % inhibition, respectively. The clear inhibition zone was recorded in dual culture plate. These isolates were obtained from tomato rhizosphere.

Kumar *et al*. (2015) recorded the bio-efficacy of *Trichoderma* spp. against *P. infestans*. Eight species of *Trichoderma* inhibited the growth of test pathogen. The data revealed that *T. viride* showed the maximum inhibition percentage of mycelia growth was recorded as 80.83 % followed by *T. reesei* (80.33 %), *T. atroviride* (79.17 %), *T. longibrachiatum* (75.00 %), *T. harzianum* (72.92 %), *T. virens* (71.25 %), *T. asperellum* (68.75 %) and *T. koningii* (66.25 %). These isolates were obtained from tomato rhizosphere.

Kumar *et al.* (2015) evaluated *P. putida* OPf1 against *P. infestans*. The *P. putida* OPf1 significantly reduced mycelia growth of test pathogen by 72.90 %. The clear inhibition zone was recorded in dual culture plate.

Majaw *et al*. (2016) reported the antagonistic properties of known microbial bioagent strains against *P. infestans*. Strains of known bioagents such as *B. subtilis, P. fluorescens, T. viride* and *T. harzianum* were isolated from different agricultural practices in Meghalaya. These isolates were *in vitro* tested for antagonism against *P. infestans*. It was observed that *B. subtilis, P. fluorescens, T. harzianum* and *T. viride,* reduced the growth of *P. infestan*s by 45.55, 75.55, 55.55 and 62.22 %, respectively.

2.10 Mechanisms of biological control

Biological control of plant diseases is a result of many different types of interaction among microorganism and can occur through different following mechanisms.

2.10.1 Volatile metabolites

Many volatile metabolites have already been reported to be involved in microbial interactions. Soil microorganisms and plant associated microorganisms produced secondary metabolites, volatile compounds, they are believed to be volatile organic compounds (VOCs). VOCs are low molecular mass (<300 Da), low boiling point, small in size, odorous compounds (<C15), high vapour pressure and possess a lipophilic moiety. Volatile compounds have properties like evaporation and diffusion. Diffusion occurred in above ground and below ground through water- and gas-filled pores in rhizosphere and soil environments. Microbial volatile organic compounds (mVOCs) belong to different chemical classes including alcohols, alkenes, ketones, pyrazines, sulphides, benzenoids and terpenes (Vespermann *et al*., 2007).

Dennis and Webster (1971) reported that some isolates of *Trichoderma* produced volatile components which were inhibitory to the mycelia growth of other pathogenic fungi. *T. viride* has a characteristic odour, acetaldehyde was tentatively identified. These isolates were obtained from tomato rhizosphere.

Laha *et al.* (1996) also recorded that volatile cyanogenic metabolites produced by *P. fluorescens* suppressed the growth of *S. rolfsii* causing cotton wilt under *in vitro* condition. This isolate was obtained from tomato rhizosphere.

Kapri and Tewari (2010) recorded the effect of volatile metabolites produced by *T. viride, T. harzianum* and *T. longibrachiaum* on *S. rolfsii* with mycelial growth inhibition of 60.8, 58.8 and 58.4 %, respectively.

Manikandan *et al*. (2010) reported *P. aeruginosa* produces the toxic volatile compound (HCN) that reduced the growth of *F. oxysporium* and *Helminthosporium* sp. about 75 % and 25 %, respectively.

Dixit *et al.* (2015) evaluated 20 isolates of *Trichoderma* and 11 isolates of fluorescent *Pseudomonas* for volatile metabolites production against *S. sclerotiorum.* Among fluorescent *Pseudomonas* spp. isolates Pf12 (85.18 %), followed by Pf19 (85.18 %), Pf28 (85.18 %), Pf11 (82.96 %) and Pf20 (82.96 %) were observed to be better as compared to control. All isolates of *Trichoderma* spp. resulted with abnormal morphological characters *i.e.,* sparse mycelial growth and reduced number of sclerotia in test fungus. *T. pseudokoningii* TR17 and *T. longibrachiatum* TR2 derived volatile substances caused maximum inhibition of mycelia growth of test pathogen about 54 and 48 %. The production of sclerotia by *S. sclerotiorum* was also reduced in the volatile effect of *T. viride* (70.15 %). These isolates were obtained from tomato rhizosphere.

Vrieze *et al*. (2018) tested volatile metabolite of 39 isolates of *Pseudomonas* and 29 isolates of *Trichoderma* against *P. infestans***.** ***Among tested isolates,*** *Trichoderma* isolate OTPB3 (92.06 %) and *P. fluorescens* OTPB1 (88.1 %) exhibited maximum inhibition against *P. infestans*. They also reported that different strains of *Trichoderma* spp. produced 15 volatile compounds such as alcohols, benzene derivatives, normal saturated hydrocarbons (C7–C30), cyclopentane, cyclohexane, fatty acids, esters, sulfur containing compounds, simple pyrane, trichodermin, paracel icine, trichotoxin, gliotoxin, staldeid and viridian.

2.10.2 Ammonia production

Bhakthavatchalu *et al.* (2013) recorded that strongly exhibited ammonia production by isolate of *P. aeruginosa* FP6, which is usually used by plants as a source of nitrogen for their growth.

Dixit *et al.* (2015) evaluated 11 isolates of fluorescent *Pseudomonas* for ammonia production. All isolates showed positive result for ammonia production. Pf20, Pf28 and Pf27 showed high ammonia production turning yellow to dark brown colour development. These isolates were obtained from tomato rhizosphere.

Prasad *et al.* (2017) tested 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for ammonia production. These isolates were isolated from the rhizosphere of tomato. All the bioagents showed positive results to ammonia production except *T. harzianum*-6. These isolates were obtained from tomato rhizosphere.

Rai (2017) also tested 20 isolates of *Trichoderma* for ammonia production. Efficient ammonia production was recorded in all the isolates. Around 25 % of isolates were showed remarkably higher ammonia production and rests of them are moderate producer. These isolates were isolated from the rhizosphere of tomato.

Lalngaihawmi and Bhattacharyya (2019) studied ammonia production as a mode of action of *Trichoderma* spp. These isolates from banana rhizosphere and soil samples were collected from different regions of Assam, Mizoram, Meghalaya and Nagaland. The results revealed that all the *Trichoderma* spp. showed positive result for ammonia production by turning initial peptone water broth from yellow to brownish orange. It had also been observed that *T. reesei* (RMF-13) produced more amount of ammonia while *T. reesei* (RMF-25) and *T. harzianum* (RMF-28) produced mediocre amount of ammonia.

2.10.3 Indole acetic acid (IAA) production

Lynch (1985) described IAA is one of the most important physiologically active auxins. The rhizosphere PGPR isolated have ability to produce IAA as secondary metabolites due to rich supply of substrates. IAA

also helps increased number of root hairs, the production of longer roots and root laterals which are involved in nutrient uptake. IAA also promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting in plants.

El-Tarabilya *et al*. (1997) reported the IAA producing actinomycetes such as *Actinomadura, Micromonospora*, *Streptosporangium* and *Nocardia* found to increase dry weight of tomato, corn, cucumbers, carrot and sorghum.

Nimnoi and Pongslip (2009) reported that *Brassica oleracea* and *Raphanus sativus* enhanced shoot and root development by the isolates of IAA synthetic bacteria. It was more than five times when compared with control treatment.

Gangwar *et al*. (2012) evaluated 45 isolates of endophytic actinomycetes from surface sterilized root, stem and leaf tissues of rice. 17 (37.7 %) out of 45 isolates produced IAA and 11 of these were *Streptomyces* spp. These isolates were obtained from rice rhizosphere.

Bhakthavatchalu *et al.* (2013) tested the isolate *P. aeruginosa* FP6 for the production of IAA, it was found to be positive. They observed that significantly increased of IAA production in the medium have precursor, L-tryptophan *i.e.,* 80 µg/ml. IAA when compared to its absence (16 µg/ml). They also observed that IAA production was increasing up to 96 hrs when bacteria reached stationary phase of bacteria growth and then decreased slowly which may be due to release of IAA degrading enzymes. This isolate was obtained from tomato rhizosphere.

Dixit *et al.* (2015) evaluated 20 isolates of *Trichoderma* for IAA production. All *Trichoderma* spp. isolates elucidated positive results for IAA production. IAA production in *Trichoderma* spp. isolates and it ranged from 15.47 µg/ml to 39.60 µg/ml. Maximum IAA production was observed in isolates *viz.,* T9 (39.60 µg/ml) followed by T8 (37.73 µg/ml), T6 (34.40 µg/ml), T12 (31.60 µg/ml) and T10 (31.06 µg/ml) as compared to control treatment. These isolates were obtained from tomato rhizosphere.

Prasad *et al.* (2017) also evaluated 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for IAA production. These isolates were isolated from the rhizosphere of tomato. It was recorded with *Trichoderma* but maximum IAA production was observed with the bacterial isolates *B. subtilis*-5, *P. fluorescens*-2 and 6. Maximum IAA production was observed in isolates *viz.,* T9 (39.60 µg/ml) followed by T8 (37.73 µg/ml), T6 (34.40 µg/ml), T12 (31.60 µg/ml) and T10 (31.06 µg/ml) as compared to control treatment. All the bioagents showed positive results to IAA production except *T. harzianum*-6. These isolates were obtained from tomato rhizosphere.

Rai (2017) studied 20 isolates of *Trichoderma* for IAA production. All isolates were found positive for IAA production. Maximum IAA production was observed in isolates T9 (39.60 µg/ml) followed by T8 (37.73 µg/ml), T6 (34.40 µg/ml), T12 (31.60 µg/ml) and T10 (31.06 µg/ml) as compared to control treatment. These isolates were isolated from the rhizosphere of tomato.

Lalngaihawmi and Bhattacharyya (2019) also studied IAA production as a mode of action of *Trichoderma* spp. These isolates from banana rhizosphere and soil samples were collected from different regions of Assam, Mizoram, Meghalaya and Nagaland. The results revealed that all the potential *Trichoderma* spp. elucidated positive results for IAA production. Maximum IAA production was observed in *T. reesei* (RMF-25) with 13.38 µg ml^{-1}

followed by *T. harzianum* (RMF-28) and *T. reesei* (RMF-13) with 9.34 μg ml^{-1}, 6.32 μg ml^{-1}, respectively.

2.10.4 Phosphate solubility

Phosphorus (P) is second most important element after Nitrogen (N), it required for plant growth, about 0.2 % of a plant's dry weight made up of phosphorus. In soil the concentration of soluble phosporus from 0.05 to 10 ppm and more than 80 % of phosphorus have immobile and unavailable for plant uptake because of precipitation, adsorption or conversion to organic form. Many research workers has been recorded that a high proportion of phosphorus solubilizing microorganisms (PSMs) especially actinomycetes, fungi and bacteria available in the rhizosphere zone of plants and they play an important role in solubilization of bound phosphates and making them available to the plants for their growth (Kapri and Tewari, 2010).

Kapri and Tewari (2010) evaluated 14 isolates of *Trichoderma* spp. for phosphate solubilizing potential. These isolates were obtained from the forest tree rhizospheres of bamboo, deodar, pinus, oak and guava. These isolates were found positive in solubilizing TCP but invariably showed very good mycelial growth in NBRIP broth, with simultaneous disappearance of TCP within 72 hrs in most of the cases. Evaluation of tree rhizosphere isolates for phosphorus solubilizing potential was performed in National Botanical Research Institute Phosphate (NBRIP) broth containing TCP as the sole P source and they compared with a standard culture of *T. harzianum*.

Gangwar *et al*. (2012) also evaluated 45 isolates of endophytic actinomycetes from surface sterilized root, stem and leaf tissues of rice. Qualitative estimation of isolated actinomycetes showed that 20 isolates out of 45 isolates were able to solubilize phosphate. Diverse levels of phosphate solubilizing activities observed. These isolates were obtained from rice.

Bhakthavatchalu *et al.* (2013) observed that *P. aeruginosa* FP6 haved positive in estimation of phosphate test. They also recorded clear visible halos around the inoculated colonies of *P. aeruginosa* FP6 on Pikovskaya agar medium for 3 days. The *P. aeruginosa* FP6 observed maximum solubilization zone followed by *P. fluorescens*-1. All isolates did not show any phosphate solubilization zone. These isolates isolated from tomato rhizosphere.

Prasad *et al.* (2017) screened 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for phosphate solubilization test. These isolates were isolated from the rhizosphere of tomato. Among 12 bacterial isolates, 11 bacterial isolates showed solubilization zone except *P. fluorescens*-4. The *P. fluorescens*-2 observed maximum solubilization zone followed by *P. fluorescens*-1. All *Trichoderma* isolates did not show any phosphate solubilization zone.

Rai (2017) tested 20 isolates of *Trichoderma* for utilizing tri-calcium phosphate in both agar plate and broth assays. Around 90 % of isolates were found to possess efficient phosphate solubilizing activity while highest solubilization was observed by *T. harzianum* and *T. virens* and approximately 25 % of isolates were moderate solubilizer. These isolates were isolated from the rhizosphere of tomato.

Lalngaihawmi and Bhattacharyya (2019) studied phosphate solubilization as a mode of action of *Trichoderma* spp. These isolates from banana rhizosphere and soil samples were collected from different regions of Assam, Mizoram, Meghalaya and Nagaland. The result of the qualitative determination of phosphate solubilization for all the isolates of *Trichoderma* did not show any clear halo zone on Pikovskaya's Agar. These isolates isolated from banana rhizosphere.

2.10.5 Siderophore production

Scher and Bakker (1982) defined siderophores (sid = iron, phores = bearers) are low molecular weight (<1000 D) iron chelating compounds

produced by microorganisms under iron stress conditions. A large number of bacteria and fungi are known to produce siderophores under iron limiting conditions in the soil. Based on this mechanism, the siderophore producing organisms have been used in biological control of plant pathogens as well as for obtaining higher yield from crop plants.

Demanage *et al.* (1987) reported that fluorescent pseudomonds were positive in siderophore production and they also characterized the siderophores. They have tested Pf1 cultures of different ages and it exhibited yellow coloured halo around the bacterial streak.

Yeole *et al.* (2001) evaluated twelve fluorescent *Pseudomonas* isolates for siderophore production in different media. All isolates were positive but varied in the quantity of siderophores produced.

Manikandan *et al*. (2010) also reported siderophore production by *P. fluorescens* Pf1. They have tested Pf1 cultures of different ages and it exhibited yellow coloured halo around the bacterial streak.

Gangwar *et al*. (2012) also evaluated 45 isolates of endophytic actinomycetes from surface sterilized root, stem and leaf tissues of rice. They observed ability of 45 isolates of actinomycete to produce siderophores *in vitro*. Out of 45 isolates, 15 isolates were positive in siderophores production. Most of them were *Streptomyces* spp.

Bhakthavatchalu *et al.* (2013) recorded ability of *P. aeruginosa* FP6 to produce siderophore and they observed orange red color of the CAS agar from blue.

Dixit *et al.* (2015) also evaluated 20 isolates of *Trichoderma* spp. and 11 isolates of fluorescent *Pseudomonas* spp. for siderophore production in CAS solid medium. Among *Trichoderma* spp., isolates T5, T6, T8 and T15 positive production of pink halo colour was deduced in the medium. Among *Pseudomonas* spp., maximum siderophore production was observed in Pf12 followed by Pf27 and Pf28 except Pf25. These isolates obtained from tomato rhizosphere.

Ghosh *et al*. (2015) also reported siderophore production by BCAs and plant growth promoting microbes (PGPM). They have tested three fungi *viz., T. viride, T. harzianum* and *Candida famata* and three bacteria *viz., B. subtilis*, *B. megatericus* and *P. aeroginosa* for qualitative assay of siderophore production. All tested fungi and bacteria gave positive response in qualitative assay. Among tested isolates, maximum siderophore production was observed in *P. aeroginosa* followed by *T. harzianum* and *B. subtilis*.

Prasad *et al.* (2017) also evaluated 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for siderophores production. These isolates were isolated from the rhizosphere of tomato. All the bacterial isolates showed siderophore production in the plate assay of which the bacterial isolates of *B. subtilis*-4, *P. fluorescens*-1 showed prominent orange colour zone. The *T. harzianum*-2 and *T. viride*-9 showed lower production of siderophores.

Rai (2017) screened 20 isolates of *Trichoderma* for siderophore production. Around 25 % of isolates were showed remarkably higher siderophore production and rests of them are moderate producer. These isolates were isolated from the rhizosphere of tomato.

Lalngaihawmi and Bhattacharyya (2019) studied siderophore production as a mode of action of *Trichoderma* spp. These isolates from banana rhizosphere and soil samples were collected from different regions of Assam, Mizoram, Meghalaya and Nagaland. The results revealed that *T. reesei* (RMF25) and *T. reesei* (RMF-13) were able to secrete siderophore by the

production of yellow halo surrounding the growing *Trichoderma* spp. The observations revealed that *T. reesei* (RMF-25) secretes more amount of siderophore as compared to *T. reesei* (RMF-13) which produced mediocre amount however secretion of siderophore production was not observed by *T. harzianum* (RMF 28).

2.10.6 Hydrogen cyanide (HCN) production

Corbett (1974) described that the HCN first the disruption of the energy supply leading to inhibits the electron transport, leading to the death of the organisms. It inhibits natural receptors reversible mechanism of inhibition and proper functioning of enzymes.

Ramette *et al*. (2003) recorded production of HCN by many plant associated fluorescent pseudomonads, involved in biocontrol of root rot disease.

Manikandan *et al*. (2010) recorded the HCN production by *P. fluorescens* Pf1. They have tested Pf1 cultures of different ages for HCN production and they observed yellow colour of the filter paper changed in to dark brown.

Bhakthavatchalu *et al.* (2013) also reported that strong production of HCN by isolate *P. aerugonisa* FP6HCN. They recorded that HCN as an important antifungal trait to control root pathogenic fungi. They also observed colour change in filter paper from yellow to reddish-brown after 2-3 days of incubation at room temperature.

Dixit *et al.* (2015) evaluated 11 isolates of fluorescent *Pseudomonas* for HCN production. They found colour change from yellow to dark brown in

most of the isolates, except Pf4, Pf6, Pf25. While, Pf20, Pf27 and Pf28 were highly HCN producing isolates as compared to others. Maximum HCN production was recorded in Pf-4.

Prasad *et al.* (2017) screened 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for HCN production. These isolates were isolated from the rhizosphere of tomato. All the bioagents showed positive results for HCN production. Maximum HCN production was recorded with the *T. harzianum*-2, 5, 6, 11, *T. viride* isolates-2, 9, 10 and bacterial isolates of *B. subtilis*-2, *P. fluorescens*-2 and 3.

2.10.7 Mycoparasitism

McIntyre *et al*. (2004) described the mycoparasitism is antagonistic interaction between two species of fungi, where the direct attack of one fungus on another is known as mycoparasitism by several sequential events, including recognition, attack and subsequent penetration and killing of the host. *Trichoderma* genera may exert necrotrophic mycoparasitism and are considered as effective BCAs due to their antagonistic capacities against a broad range of phytopathogenic fungi. The most evident morphological changes in *Trichoderma* hyphae, such as coiling and development of appressorium-like penetration structures, serves to penetrate the host and contain high concentration of osmotic solutes such as glycerol.

Srivastava and Singh (2000) recorded the mycoparasitism interactions between *Rhizoctonia solani* and *A. flavus, T. viride, T. hamatum* and *T. harzianum.* They observed lysis of cell wall of pathogen by enzyme preparation of BCAs. Maximum lysis was observed in *T. harzianum* (86 %), followed by *T. viride* (45 %), *T. hamatum* (15 %) and *A. flavus* (6 %).

Kubicek *et al*. (2001) reported that mycoparasitism interaction is key antagonistic mechanisms in *Trichoderma* spp. They also reported that after host recognition, *Trichoderma* spp. confers to the host hyphae via penetrate the cell-wall by wide range of lytic enzymes and a versatile metabolites production.

Ramanujam *et al.* (2002) reported that mycoparasitism interactions of *T. viride* and *T. harzianum* on *P. infestans.* They observed that hyphae of *T. harzianum* coiled around the hyphae of the pathogen and establishment of contact, *T. harzianum* showed mycoparasitic interactions. Although the lysis of protoplasm of pathogen was uniformly recorded in the hyphae during the mycopasasitism interaction. They also observed that the hyphae of the pathogen showed lysis of protoplasm immediately after contact with *T. viride.*

Zegeye *et al.* (2011) recorded the mycoparasitism interactions between *T. viride* and the *P. infestans.* They also revealed that *T. viride* hyphae coiled around the hyphae of *P. infestans*. Similar mycoparasitic action of *Trichoderma* strains were also obseved in *P. cinnamomi* (Pugeg and Ian, 2006) and *P. capsici* (Ezziyyani *et al.,* 2007).

2.11 Molecular identification and phylogenetic analysis of *Trichoderma*

Trichoderma spp. are difficult to distinguish morphologically, so 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). With the beginning of molecular methods and identification tools, which are based on multiple genes sequence analysis, it is now possible to recognize every *Trichoderma* isolate and /or diagnose it as a putative new species (Kullnig-Gradinger *et al*., 2002). ITS region of 18S rRNA is one of the most consistent

targets to identify a *Trichoderma* strain at the species level, but this cannot differentiate all *Trichoderma* spp. (Rifai, 1969).

Kindermann *et al*. (1998) studied a first phylogenetic analysis of the whole genus, using sequence analysis of the ITS1 region of the 18S rRNA. Phylogenetic studies of 88 species showed that *Hypocrea* and *Trichoderma* form a single holomorph genus, within which two major clades can be distinguished.

Sun *et al.* (2012) accomplished a comprehensive study about the biodiversity of *Trichoderma* spp. in China. They identified 12 taxa of *Trichoderma* (*T. asperellum*, *T. atroviride, T. brevicompactum, T. citrinoviride, T. hamatum, T. koningiopsis, T. harzianum, T. reesei, T. spirale, T. stromaticum, T. vermipilum* and *T. virens*) by ITS bar coding.

Gherbawy *et al*. (2014) identified 90 isolates of *Trichoderma* (Teleomorph: *Hypocrea*) species and one isolate of *Gliocladium viride* (Tel. *Hypocrea lutea*). Regions of nuclear rDNA, containing 18S rRNA gene; ITS1, 5.8S rRNA gene and ITS2 and 28S rRNA gene were amplified to identify the collected isolates. The sequencing results indicated that 78 isolates of the population were identified as *T. harzianum* (Tel. *Hypocrea lixii*). Also, two isolates were identified as *T. longibrachiatum* (Tel. *H. orientalis*) and one isolate as *Gliocladium viride* (Tel. *H. lutea*). The remaining 10 isolates were postulated as three new species according to their phylogenic tree. *T. harzianum* isolates collected during this work showed high degrees of variability which supported that it is a "species complex".

Rai (2017) studied the molecular diversity and phylogenetic analysis of 20 isolates of antagonistic *Trichoderma* using ITS region of 18S rRNA. Comparison of oligonucleotide fragments of 18S rRNA sequences, which included the flanking ITS1 and ITS4 regions, with reference sequences from public databases, showed that they were very similar. The ITS1 (forward) and ITS4 (reverse) oligonucleotide pairs amplified a single DNA fragment of approximately 550 bp amplicon size in all *Trichoderma* isolates. These isolates were isolated from the rhizosphere of tomato.

2.12 Compatibility amongst microbial consortia

Siddiqui and Shaukat (2003) reported *in vitro* compatibility between *P. aeruginosa* and *Pochonia chlamydosporia* and between *Streptomyces rochei* and *T. harzianum*. These isolates were obtained from tomato rhizosphere.

Zegeye *et al.* (2011) also reported compatibility between *T. viride* and *P. fluorescens in vitro.* No clear inhibition zone was also observed between the tested bacterial and the fungal colonies.

Mishra *et al.* (2013) tested *in vitro* compatibility among 43 isolates of *Trichoderma* and 40 isolates of *P. fluorescens*. These isolates were isolated from non-rhizosphere and rhizosphere soils of peas, tomato, lentil, soybean, chickpea and rice. Some isolates of *Pseudomonas* inhibited mycelia growth of the *Trichoderma* isolates, but per cent inhibition varied widely (1.83 to 51.1 %) depending on the isolates of both the antagonists. Isolates PBAP-27, PBAP-17, PBAP-15, PBAP-10 of *P. fluorescens* and isolates PBAT-43, PBAT-38, PBAT-6 and PBAT-1 of *Trichoderma* exhibited no or very little antagonism against each other. Bacterial isolate PBAP-27 (*P. fluorescens*) and fungal isolate PBAT-43 (*T. harzianum*) and emerged as most compatible and efficient and therefore were used for development of mixed formulation.

Kumar *et al.* (2014) evaluated compatibility between *B. subtilis* OTPB1 and *T. harzianum* OTPB3 *in vitro*. When one loop of culture broth streaked on PDA, both *B. subtilis* and *T. harzianum* exhibited growth on PDA without any antagonistic activity after 72 hrs of incubation. These isolates were obtained from tomato rhizosphere.

Nath *et al.* (2016) recorded *in vitro* compatibility among *T. parareesei*, *P. fluorescens*, *B. subtilis* and *Azotobacter chroococcum* and their consortia. These isolates were obtained from tomato rhizosphere.

Harshita *et al*. (2018) also tested compatibility among the fungal (*T. harzianum*) and bacterial (*B. subtilis* and *P. fluorescens*) BCAs *in vitro.* Absence of inhibition zone indicated that the BCAs were compatible with each other. These isolates were obtained from tomato rhizosphere.

Lalngaihawmi and Bhattacharyya (2019) also tested *in vitro* compatibility among different isolates of *Trichoderma.* The experiment was carried out in all permutations and combination amongst the isolates of *Trichoderma*. The result of the experiment revealed that all the *Trichoderma* spp. were found to be compatible with each other in all combinations without inhibiting each other. These isolates were isolated from the rhizosphere of banana.

2.13 *In vitro* antagonistic efficacy of microbial consortia

Sundaramoorthy and Balabaskar (2013) tested indigenous consortia of *P. fluorescens* and *B. subtilis* against *F. oxysporum* f.sp. *lycopersici.* They have isolated 25 native bacterial antagonists from healthy tomato rhizosphere soil. They had tested singly or in consortia and the highest inhibition of test pathogen was recorded against consortia of Pf-5 + Bs-4 + Pf-7 (40 %) followed by Bs-4 + Pf-7 (39.89 %) and Pf-5 + Bs-4 (37.55 %). The results revealed that the combined application was found to efficient in inhibition the mycelial

growth of the test pathogen when compared to application of individual strains [Pf-5 (35.55 %), Pf-7 (33 %) and Bs-4 (32.22 %)] of the bacterial antagonists. These isolates were obtained from tomato rhizosphere.

Nath *et al.* (2016) evaluated the antagonistic potential of *T. parareesei*, *P. fluorescens*, *B. subtilis* and *Azotobacter chroococcum* against *R. solanacearum.* BCAs applied alone and combination, significantly suppreded test pathogen *in vitro* producing varying sizes of inhibition zones in TTC medium. They had tested *in vitro* singly or in consortia and the highest inhibition of test pathogen was observed against consortia of *B. subtilis* + *T. parareesei* + *P. fluorescens* + *A. chroococcum* (91.10 %) followed by *B. subtilis* + *T. parareesei* + *P. fluorescens* (81.10 %) and *A. chroococcum* + *P. fluorescens* + *B. subtilis* (68.14 %). These isolates were obtained from tomato rhizosphere.

Khan *et al.* (2018) also evaluated the efficacy of three compatible potential microbial bioagents, *viz. T. viride, B. thuringiensis* and *P. fluorescens* against *R. solanacearum in vitro.* BCAs applied alone and combination, significantly suppreded test pathogen *in vitro* producing different sizes of inhibition zones in TTC medium. Combination of three antagonists *B. thuringiensis* + *T. viride* + *P. fluorescens,* inhibition produced significantly highest (70.27 %) followed by combination of two bioagents *B. thuringiensis* + *T. viride* (63.83 %). This was followed by *P. fluorescens* + *T. viride* (59.84 %). These isolates were obtained from lettuce rhizosphere.

Vrieze *et al.* (2018) also evaluated nine strains of *Pseudomonas* and their consortia against *P. infestans.* These strains isolated from the rhizosphere (R) or phyllosphere (S) of potatoes. When inoculated singly, 3 strains (R47, R32 and S49) were capable to inhibit fully the mycelial growth of the pathogen, *P. infestans.* The per cent inhibition over control was calculated after 48, 72 and 96 hrs after inoculation. They had tested singly or in consortia and the highest inhibition of test pathogen was recorded against consortia of Pf-5 + Bs-4 + Pf-7 (40 %) followed by Bs-4 + Pf-7 (39.89 %) and Pf-5 + Bs-4 (37.55

%). The results revealed that the combined application was found to effectively inhibit the mycelial growth of the test pathogen when compared to application of individual strains [Pf-5 (35.55 %), Pf-7 (33 %) and Bs-4 (32.22 %)] of the bacterial antagonists. These isolates were obtained from potato rhizosphere.

Lalngaihawmi and Bhattacharyya (2019) also evaluated the antagonistic potential of the three promising *Trichoderma* spp. individually as well as in combinations against *F. o.* f.sp. *cubense.* The result revealed that the efficacy of all the treatments differed significantly with that of control at all the intervals. The per cent inhibition over control was calculated after 48, 72 and 96 hrs after inoculation. After 96 hrs of incubation, the per cent inhibition of radial growth of test pathogen *in vitro* was observed highest by the combination of the three *Trichoderma* spp. *viz*., *T. reesei* (RMF-25) + *T. reesei* (RMF-13) + *T. harzianum* (RMF- 28) with 69.18 per cent followed by the combination of *T. reesei* (RMF-25) + *T. harzianum* (RMF 28) with 66.86 per cent and combination of *T. reesei* (RMF-13) + *T. harzianum* (RMF 28) with 68.60 per cent. The per cent inhibition recorded by the rest of microbes either singly or in combination ranged from 65.12 per cent in case of *T. reesei* (RMF-13) alone to 68.02 per cent in case of combination of *T. reesei* (RMF-25) + *T. reesei* (RMF13). These isolates were isolated from the rhizosphere of banana.

2.14 *In vitro* and *in vivo* plant growth promoting efficiency of microbial consortia

Bioinoculants have the ability to produce plant growth promoting substances like Gibberellins, IAA, B-vitamins, cytokinins and antifungal substances, which favours better growth of crop plants. They helped the plant growth directly by either assisting in resource acquisition (essential minerals, phosphorus and nitrogen) or modulating plant growth hormone levels, or

indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of BCAs (Ahemad and Kibret, 2014).

Manoranjitham and Prakasam (1999) reported that seed treatment with *P. fluorescens* and *T. viride* for the management of pre and post emergence damping off of chilli. They also reported that increased the shoot and root length and dry matter production of chilli seedlings compared to control treatment.

Raj *et al*. (2004) revealed that due to *P. fluorescens* resulted in increase seed germination, seedling vigour index, leaf area, plant height, tillering capacity, seed weight yield and induces resistance against downy mildew of pearl millet at Mysore.

Zaidi and Singh (2004) carried out the experiment on seed bio-priming. They applied *T. harzianum* on the seeds of tomato, soybean and chickpea. They recorded that bioagent population, it enhanced on surface of treated seeds by almost 10000 folds in 48 hrs. Maximum germination of seeds was also high in treated seeds than non-bioprimed seeds. This isolate was obtained from tomato rhizosphere.

Raja *et al*. (2006) also evaluated *B. megaterium* var. *phosphaticum, Azospirillum lipoferum* and *P. fluorescens* Pf-1 as individual and microbial combination under hydroponic culture of rice for rice exudates and plant growth which increased the plant growth positively. These isolates also increased dry weight, fresh weight of rice.

Sharma *et al.* (2009) reported that cumin seed treated with *T. harzianum* enhanced seed germination while *T. viride* showed good shoot and root ratio in pot condition against cumin wilt. These isolates were obtained from cumin rhizosphere.

Kumar *et al.* (2010) also reported that consortial application as seed treatment of P. *fluorescens* and *T. harzianum,* resulted in significant enhanced seedlings growth (18.38 cm at 30 DAS) of sweet pepper.

Manikandan *et al*. (2010) also recorded plant growth promotion by liquid formulation of *P. fluorescens* Pf1. They observed that two day old culture of Pf1 significantly increased tomato seedling vigour index, including greater germination per cent (92 %), shoot length (6.2 cm) and root length (14.11 cm) with vigour index of 1868. This isolate was obtained from tomato rhizosphere.

Someshwar and Sitansu (2010) recorded that seeds of brinjal, tomato and chili stimulating the germination by treatment of bioagents *viz., T. harzianum* (AN-13) and *T. viride* (AN-10) which was equivalent to *T. harzianum* (WB-1) in inducing germination of the seeds. The maximum seed germination was obtained when seeds were bioprimed with mycelial form of inoculum of *T. harzianum* AN-13 and WB-1.

Nazir *et al.* (2011) reported that *T. viride* and *T. harzianum* showed significant reduction in the radial growth of *P. aphanidermatum* and *Thanatephorus cucumeris* and increased seed germination, plant height, leaf number and fresh weight of tomato and chilli compared to the control. These isolates were obtained from tomato rhizosphere.

Ananthi *et al*. (2013) standardized the biopriming of chilli seed with *T. viride* and *P. fluorescens* in order to improve seed germination and seedling vigour.

Bhakthavatchalu *et al.* (2013) also recorded a significant difference in cowpea seeds treated with *P. aeruginosa* FP6 and non-treated plants, showed stimulatory effects on all plant vegetative parameters. Maximum seed germination per cent was in treated seeds (92.0 %), which germinated earlier in comparison to control treatment (72.0 %) and good overall seed vigour index as compared to control.

Eutesari *et al.* (2013) reported that three fungal BCAs including *T. harzianum, T. virens, T. atroviride* and a bacterium; *P. fluorescens* were applied on soybean seed and their impact on seedling were evaluated under greenhouse conditions. Soybean seed germination, seedling growth and chlorophyll amount were investigated. There are improved seed factors such as germination rate and seedling growth indices including seedling length, root length and dry weight of root. Also they enhanced seedlings and leaf area per plant and total chlorophyll amount.

Kabir *et al.* (2013) also tested five isolates of PGPR, all of the bacterial isolates (BI 06, BI 09, BI 11, BI 15 and BI 18) were capable of increasing different growth parameters (fresh biomass, shoot/root length and dry matter) in comparison with control plants. BI 18 treated plants in particular showed the highest enhancement in fresh biomass with 36 and 39 % increments in the root and shoot biomass, respectively. However, isolate BI 10 showed the maximum shoot and root growth with 26 and 35 % increments, respectively.

Lamsal *et al.* (2013) evaluated efficacy of seven isolates of bacteria *viz.,* AB 05, AB 10, AB 11, AB 12, AB 14, AB 15 and AB 17 for PGPR on tomato. These isolates were isolated from rhizosphere of tomatoes in Korea. In a PGPR assay, all of the bacterial isolates were capable of increasing different growth parameters (dry matter, fresh biomass and shoot/root length) in comparison with control treatment. AB 17-treated plants in particular showed the maximum enhancement in fresh biomass with 27 and 32 % increments in the root and shoot biomass, respectively. However, isolate AB 10 showed the maximum shoot and root growth with 18 and 26 % increments, respectively.

Sandheep *et al.* (2013) also evaluated the efficiency of plant growth promoting activities of combined inoculation of rhizobacteria on vanilla plants. The heighest percentage of growth increase was recorded in the consortia of *P. fluorescens* with *T. harzianum* treatment followed by *P. fluorescens*, *T. harzianum*, *P. putida* and *T. virens*, respectively in decreasing order. Consortia of *T. harzianum* and *P. fluorescens* registered the maximum length of vine (82.88 cm), recorded the highest fresh weight of shoots (61.54 g plant^{-1}), highest number of leaves (26.67/plant), fresh weight of roots (4.46 g plant^{-1}) and dry weight of shoot (4.56 g plant^{-1}) where as the highest dry weight of roots (2.08 g plant^{-1}) were achieved with treatments of *P. fluorescens*.

Sudharani *et al*. (2014) also evaluated the plant growth promoting and disease suppressing abilities of BCAs and PGPRs under greenhouse conditions. The combination of *Azotobacter chroococcum* + *B. megaterium* + *P. fluorescens* + *B. subtilis* + *T. harzianum* showed enhanced cabbage seedling vigour, total biomass, least disease incidence and more biocontrol efficiency. These isolates were isolated from the rhizosphere of cabbage.

Kumar *et al.* (2015) developed a seed coating formulation of *B. subtilis* OTPB1 and *T. harzianum* OTPB3 and their consortia. The tomato seeds treated with a mixture of *T. harzianum* OTPB3 and *B. subtilis* OTPB1 or singly, exhibited increase in seedling growth parameters significantly compared to Dithane M-45 (0.2 %) and control treatment. The combination increased shoot and leaf area, root lengths, fresh weight of roots and shoots by 56.3, 40.9, 34.0, 56.9 and 50.2 % respectively as compared to the control treatment. The result also indicated that the microbial combination stimulated better growth than singly applied.

Singh *et al.* (2016) also tested bioprimed seeds of tomato with different spore doses of *T. asperellum* BHUT8. Bioprimed of different doses in ranging from 10^2 to 10^8 spores ml^{-1}. The effect of biopriming was seen on tomato seed germination and development. At the most effective spore dose, the increase in germination percentage was 5 % while increase in radicle length was 73.17 % over control. Higher spore dose **i.e**. 10^7–10^8 spores ml^{-1} reduced seed germination percentage and radicle growth compared to control. The most effective spore dose for increasing in seed germination and radicle length was found to be 10^3 spores ml^{-1}.

Khan *et al.* (2018) also tested three compatible microbial bioagents, *viz. T. viride, B. thuringiensis* and *P. fluorescens* for plant growth parameters in lettuce plant. There was significant increase in root, shoot and total biomass of lettuce plants in treatments where bioformulations of *T. viride* + *B. thuringiensis* + *P. fluorescens* were applied as combination of root treatment (2.0 %) and foliar treatment (1.0 %). The highest shoot length (34.00 cm), root length (27.75 cm), shoot dry weight (16.41 g), root dry weight (5.62 g), root fresh weight (27.12 g) was recorded when lettuce plants was treated with application of consortia of *T. viride* + *B. thuringiensis* + *P. fluorescens*.

Biam and Majumder (2019) also evaluated the four isolates of *Trichoderma* (*T. harzianum* strain CEN693 (TR 55), *T. harzianum* strain US10 (TR 66), *T. harzianum* strain DIS 326F (TR 122) and *T. harzianum* (TR 136) as biopriming of tomato seeds. They observed that considerable enhanced the germination percentage, shoot and root length and vigour index over control treatment, with maximumt germination percentage and vigour index recorded in TR 55 (75.13 and 47.99 % respectively). The maximum root (14.15±0.29 cm), shoot length (10.9±0.56 cm) and germination percentage 92 % were recorded in TR 55. The minimum was recorded in control (with 6.85±0.35 cm root length, 4.85±0.24 cm shoot length and 62 % germination percentage). The

vigour index was maximum in TR 55 (2306), followed by TR 122 (1990.5), TR 66 (1768.1), TR 136 (1576.5) and minimum was recorded in control treatment (724).

2.15 Field efficacy of microbial consortia

Application of BCAs or PGPR is considered as an important strategy in crop protection against wide range of plant pathogens. Several microbes have been studied extensively as BCAs against various plant pathogens and these also showed plant growth promotion activity (Singh *et al.,* 2003; Lucy *et al.,* 2004; Mathivanan *et al.,* 2005; Srinivasan, 2007). PGPR strains were also tested individually and in combinations (two/more strains) against multiple plant pathogens (Raupach and Kloepper, 1998; Yan *et al.,* 2002; Idris *et al.,* 2007; Harish *et al.,* 2008).

Mutitu *et al*. (2003) tested the culture filtrates of *Streptomyces* against late blight of tomatoes showed that they had positive activity against late blight of tomato.

Yigit and Dikilitas (2007) recorded that application of consortia of *P. fluorescens,* non-pathogenic *Fusarium* and *T. harzianum* control *Fusarium* wilt of tomato in greenhouse conditions.

Maketon *et al*. (2008) investigated on management of bacterial wilt (*R. solanacearum*) and damping-off (*P. aphanidermatum*) in tobacco by *B. subtilis* AP-01 and *T. harzianum* AP-001. They also reported that *B. subtilis* AP-01, *T. harzianum* AP-001 alone could not control the bacterial wilt and damping-off, but when applied as consortia, their controlling capabilities enhanced and similar to the chemical treatment.

Maiyappan *et al*. (2010) evaluated *Bacillus* sp., *Streptomyces* sp., *Azotobacter* sp. and *Frauteria* sp. as microbial combinations for plant growth

promoting efficacy and its antagonistic activities against *S. rolfsii*, *F. oxysporum* and *R. solani* in blackgram. High percentage of seed germination, maximum radical length and effective antagonistic activity of microbial consortia were observed under pot conditions. These isolates were obtained from blackgram rhizosphere.

Manikandan *et al*. (2010) conducted an experiment on liquid bioformulation of *P. fluorescens* Pf1 against *Fusarium* wilt of tomato under glass house and field conditions. These were comparable with the chemical treatment (17.25 % under glasshouse and 3.68 % under field). In case of untreated control, high incidence (62.5 % under glasshouse and 25.56 % under field) was recorded. Per cent reduction of wilt incidence over control was maximum in chemical control (72.40 % under glasshouse and 85.60 % under field) followed by seed treatment + seedling dip + soil drenching of liquid formulation of Pf1 (68.88 % under glass house and 81.18 % under field). Simultaneously, liquid formulation of Pf1 significantly enhanced the plant height (50.75 % under glasshouse conditions) and fruit yield (138.96 % under glasshouse and 53.67 % under field conditions) over untreated control. Among the different treatments combinations like seed treatment + seedling dip + soil drenching of liquid bioformulation of Pf1 significantly decreased the *Fusarium* wilt incidence (19.45 % under glasshouse and 4.81 % under field) compared to all other treatments.

Muthukumar *et al.* (2010) also recorded the biocontrol efficacy of *P. fluorescens* and *T. viride* against *P. aphanidermatum.* They enhanced the root and shoot length, yield and plant growth of chilli when compared to control treatment. Theses isolates from chilli rhizosphere.

Srivastava *et al*. (2010) reported that management of tomato wilt by application of a consortium of *P. fluorescens,* AM fungus and *T. harzianum*. They recorded significantly suppresed wilt disease in field and pots. They

applied as consortia of all three bioagents with cow dung compost. These isolates obtained from tomato rhizosphere.

Akrami *et al*. (2011) eveluated the bioefficacy of *Trichoderma* isolates alone and consortia against *F. oxysporum*. *T. asperellum* and *T. harzianum* isolates and consortia of BCAs were more effective than other treatments in controlling the disease. These isolates were obtained from tomato rhizosphere.

Hema and Selvaraj (2011) tested a consortium of *G. aggregatum, B. coagulans* and *T. harzianum*. They studied the effect of consortia on nutrition, growth and content of secondary metabolities of *Solanum viarum* seedlings under green house. They observed heighest plant weight and yield in the treatment of three combinations of *G. aggregatum* + *B. coagulans* + *T. harzainum* in Solanum *viarum* in a green house nursery. These isolates were obtained from *S. viarum* rhizosphere.

Rajasekar and Elango (2011) studied the effect of microbial consortium on *Withania somnifera.* They recorded significantly enhanced root length, plant height and alkaloid content in combination of *Azotobacter, Azospirillum, Pseudomonas* and *Bacillus.* They tested separately and consortia for two years. The consortia of above PGPR strains were obtained from *Withania somnifera.*

Jain *et al*. (2012) studied the potentiality of three rhizosphere microorganisms *B. subtilis* BHU100, *P. fluorescens* PJHU15 and *T. harzianum* TNHU27 in reduction of *Sclerotinia* rot in pea in combination mode. They recorded that increased level of defence responses in peas. They also found that microbial combination triggered the defence response in peas than the microbes alone and provided better protection against *Sclerotinia* rot.

Lamsal *et al.* (2013) also evaluated seven isolates of bacteria *viz.,* AB 05, AB 10, AB 11, AB 12, AB 14, AB 15 and AB 17 against late blight of tomato under greenhouse conditions. The results demonstrated that isolates AB 05, AB 11, AB 15 and AB 17 significantly inhibited *P. infestans*. Treatment with isolate AB 15 resulted in a 74.01 % suppression of late blight, whereas treatment with AB 05, AB 11, AB 12 and AB 17 resulted in a disease reduction of 62.0, 63.26, 65.24 and 61.47 %, respectively. Control plants without bacterial treatment but inoculated with *P. infestans* alone exhibited an up to 98.16 % disease severity, with the majority of plants being completely stunted or dead. Among the selected biological control agents in this study, isolate AB 17 and isolates AB 15 and AB 12 reduced the shoot and root dry weights by 28 and 26 %, respectively.

Kumar *et al.* (2015) also evaluated the efficacy of seed biopriming with *T. harzianum* OTPB3 and *B. subtilis* OTPB1 combination, *P. putida* OPf1 strain and Dithane M-45 as chemical check coupled with foliar sprays of *P. putida* OPf1 against late blight of tomato under pot culture conditions. The results demonstrated that isolates AB 05, AB 11, AB 15 and AB 17 significantly inhibited *P. infestans*. Treatment with isolate AB 15 resulted in a 74.01 % suppression of late blight, whereas treatment with AB 05, AB 11, AB 12 and AB 17 resulted in a disease reduction of 62.0, 63.26, 65.24 and 61.47 %, respectively. They applied consortia in the combination of *T. harzianum* OTPB3 + *B. subtilis* OTPB1 + *P. putida* OPf1 as seed biopriming and foliar spray. They recorded that significantly reduced late blight disease severity by 73.1 % compared to control treatment.

Sharma *et al.* (2015) tested consortia of *Bacillus* strains against *P. capsici* in tomato under net house conditions. Consortial application of four strains of Bacillus (S_{21} + S_{25} + B_6+ A_{10}) had significant effect in achieving biocontrol efficacy of 66.7 %. Combination of the 4 strains resulted in significant enhance in plant parameters including shoot and root length, fresh weight of plant and plant dry weight over control treatment.

Gupta (2016) also evaluated the efficacy of four bioagents, *viz., T. viride, T. harzianum*, *Penicillium viridcatum* and *Myrothecium verrucaria* for the management of potato late blight. For field experiment potato var. Kufri Chandramukhi used. Result of experiment was revealed that *T. viride* followed by *P. viridicatum* were most effective in controlling late blight (lesion area 3.48 and 3.66 cm^2 respectively as against 9.96 cm^2 in untreated control). *T. viride* and *P. viridicatum* were found better in both the methods (detached leaves and whole plants). Bioefficacy of *T. harzianum* was recorded at 24 hrs after bioagent application (lesion area 6.10 cm^2) and maximum at 72 hrs after bioagent application (lesion area 5.69 cm^2), when it evaluated through detached leaf method. Least antagonistic effect was visible at 24, 48 and 72 hrs after bioagent application (establishment time).

Nath *et al.* (2016) developed a biocontrol strategy for the management of bacterial wilt of tomato caused by *R. solanacearum* using 4 PGPM. Significantly maximumt reduction of bacterial wilt incidence (95.09 %) and maximum yield (1.692 kg $plant^{-1}$) of tomato was recorded in treatment comprising of *B. subtilis* + *T. parareesei* + *P. fluorescens* + *A. chroococcum*. These isolates were isolated from the rhizosphere of tomato.

Ouhaibi-Ben *et al.* (2016) tested three native tomato-associated rhizobacteria (*B. thuringiensis* B10, *B. subtilis* B2 and *Enterobacter cloacae* B16). They recorded maximum plants grown in *S. sclerotiorum* infected peat (38.36 - 80.95 %). It was challenged with the three-strain consortium were taller than control ones whereas height increment noted using single strains and fungicide was of about 32.35 -79.01 % and 29.62 -51.85 %, respectively. They also observed that the disease reduction potential of consortia as in ranged between 80.79 and 88.01 %. It was compared to pathogen-inoculated and control. Using the three-strain consortium, disease reduction potential was in ranged between 70.00 - 82.07 %. With the used of single strains, disease

reduction potential was in ranged between 32.13 - 58.97 %. These isolates obtained from tomato rhizosphere.

Khan *et al.* (2018) also evaluated the efficacy of three compatible potential microbial bioagents, *viz. T. viride, B. thuringiensis* and *P. fluorescens* against bacterial wilt of lettuce. The disease incidence caused by *R. solanacearum* in lettuce decreased significantly accompanied by significant increased in yield (g plant^{-1}) when plants were treated with individual as well as consortia of different bioagents. Lowest disease incidence was exhibited by the bioformulation of consortia of *T. viride* + *B. thuringiensis* + *P. fluorescens* (18.57 %) applied in nutrient solution as root treatment (2.0 %) and foliar spray (1.0 %) followed by the treatment with consortia of *T. viride* + *B. thuringiensis* (30.75 %). Plants treated with *B. thuringiensis* showed significantly higher disease incidence (73.14 %), however these were significantly lower as compared to control (77.58 %). The significant decrease of bacterial wilt incidence and increase in lettuce yield in two best treatments, *i.e*., consortia of *T. viride* + *B. thuringiensis* + *P. fluorescens* and *T. viride* + *B. thuringiensis* applied as combinations of root, foliar and water treatments. The yield of lettuce treated with consortia of different bioagents followed similar trend as recorded for disease incidence. Highest yield was recovered from plants treated with bioformulation consortia of *T.* viride + *B. thuringiensis* + *P. fluorescens* (242.50 g plant^{-1}) followed by plants treated with *T. viride* + *B. thuringensis* (219.25 g plant^{-1}). The highest disease incidence (77.58 %) and lowest yield (27.25 g plant^{-1}) of lettuce plant was recorded in the control treatment, where no bioagent was applied. These isolates were isolated from the rhizosphere of lettuce.

Vrieze *et al.* (2018) also evaluated nine strains of *Pseudomonas* and their consortia against late blight of potato. These strains isolated from the rhizosphere (R) or phyllosphere (S) of field-grown potatoes. They observed that single strain (S35) significantly reduced disease. They also observed that

one strain (R84) was inefficient in all potato varieties. Dual consortia offered best protection in terms of quantitative disease inhibition, while 7 offered protection on some but not all potato varieties. Among the 84 triple consortia, only 7 triple consortia were able to significantly reduce disease progression in Lady Claire. Among the dual consortia, 6 (out of 36 consortia) provided protection on all three cultivars, *i.e.,* R32/S34, R76/S49, R84/S35, R84/S49, S04/S49 and S19/S49. Triple consortia were able to reduce disease progression in at least two of the three varieties, 16 consortia were found to be efficient, among which 7 consortia contained strain S35 and six consortia contained strain S49, indicating putative synergistic effects of these two strains when applied in consortia with two additional other strains. These isolates isolated from the rhizosphere (R) or phyllosphere (S) of potatoes.

Biam and Majumder (2019) reported the influence of *Trichoderma* isolates (*T. harzianum* strain DIS 326F (TR 122), *T. harzianum* strain CEN693 (TR 55), *T. harzianum* strain US10 (TR 66) and *T. harzianum* (TR 136) on the growth parameters of tomato such as root length, plant height, numbers of leaves and yields under greenhouse conditions. It was noticed that the plant height observed after 45 DAS (92.20 cm to 127.20 cm), when compared to control (90 cm). Among the treatments, heighest plant height was recorded in T_3 (109 cm), followed by T_2 (94.72 cm) and T_1 (93.28 cm). They recorded root length of tomato plant was maximum after 45 DAS (15.04 cm to 22.80 cm), when compared to untreated pots (11 cm). They also found number of leaves heigest after 45 DAS ranged from 32 to 57.20, when compared to control with 30 numbers. Among the treatments, maximum yield was recorded in T_3 (2.07 kg), followed by T_2 (1.65 kg) and T_1 (1.58 kg). The maximum yield was recorded in TR 55 (2.25 kg), followed by TR 122 (1.76 kg), TR 136 (1.74 kg) and TR 66 (1.69 kg). Among the treatments, heighest root length was recorded in T_3 (17.42 cm), followed by T_2 (14.82 cm) and T_1 (14.46 cm). The yield enhanced in all the treatments compared to control (1.4 kg). The maximum

plant height was recorded in TR 55 (107 cm), followed by TR 122 (103.06 cm), TR 66 (98.33 cm) and TR 136 (96.60 cm). Heighest number of leaves/plant was recorded in TR 55 (45), followed by TR 122 (43.20), TR 66 (36) and TR 136 (34.46). Heighest root lenght was recorded in TR 55 (18.35 cm), followed by TR 122 (16.67 cm), TR 66 (16 cm) and TR 136 (15.81 cm).

2.16 Field evaluation of tomato genotypes

Various research workers evaluated different cultivars/hybrids against late blight to locate sources of resistance, if any. Cultivars with spherical, smooth fruit especially John Bear, Main Crop, Sunshine, Victory and Matchless (Bondartzeva, 1926), Burwood Prize, Danish Extra Early, Determinate Shipper, Adelaide Dwarf, Dobbies Holywood, Australian Earliana, Chemin Early Red, Dobbies Champion, Dwarf Earliest Red, Garden State, Marnana, San Maezano, Potentate, Primrose Gage, Ventura and Ventomold (Andrus, 1946) were reported to possess resistance to late blight of tomato.

Islam *et al*. (2001) screened 15 advanced lines of tomato against late blight under natural epiphytotic condition. They recorded maximum late blight disease severity in V-215 and V-52 and the minimum in V-378. They also observed that 2 lines moderate resistant (V-259 and V-426), two resistant (V-385 and V-187), two moderately susceptible (V-422 and V-282), four tolerant (V-138, V-378, V-258 and BARI-10), three were highly susceptible (V-201, V-330 and Manik) and two susceptible (V-215 and V-52), but none was found highly resistant.

Nazrul *et al*. (2001) also evaluated 15 advanced lines of tomato but none of them were resistant. While only two advanced lines (V-187 and V-385) were moderately resistant.

Khalid *et al*. (2012) evaluated 82 tomato genotypes against late blight using whole-plant and detached-leaf assays. None of the test germplasms were immune or highly resistant. Of the 82 germplasms only TMS-2 was resistant with severity index (2.4) in the detached leaf assay and under the whole plant assay (23.3 PDI). They recorded that disease symptoms started on LA1963 and L06049 in the form of small lesions on the bottom leaves after 72 hrs. It was followed by complete death of the plant within 5 to 7 days of inoculation with PDI 100. Among the evaluated germplasms, 40 were highly susceptible and 41 were susceptible under the detached leaf assay, while 63 were highly susceptible and 18 were susceptible under the whole plant assay. In the case of TMS-2, the pace of symptom development was slow and which appeared as minor lesions on the lower leaves after 5 days post inoculation and remained localized to a few older leaves on 0-20 % with 2-3 infection type range till after 15 days of inoculation with PDI 23.3. The overall results indicate that TMS-2 is an important source of resistance and it can be useful for the development of tomato hybrid cultivars resistant to late blight.

Dufera (2014) also evaluated 21 tomato genotypes against late blight. The genotypes Roma VF, CLN-2037I, CLN-2037H, CLN-2037E, Melkashola, CLN-2037H and Melkashola, showed resistance to late blight disease infection under natural condition, green house and detached-leaf evaluation, revealing their resistance performance to late blight disease.

Kumar *et al.* (2015) also screened 56 lines of tomato against late blight under natural condition. Almost all the lines exhibited resistant to highly resistant response. Eight crosses *viz.,* LBR-12 × EC-119197 (PDI 14.00), LBR-6 × 1-6-1-4 (PDI 18.00), LBR-10 × 8-2-1-2-5 (PDI 18.00), LBR-12 × 1-6-1-4 (PDI 18.00), LBR-13 × 1-6-1-4 (PDI 18.00), LBR-21 × 8-2-1-2-5 (PDI 18.00),

LBR-21 × 1-6-1-4 (PDI 18.00) and LBR-13 × EC-119197 (PDI 20.00) were found resistant under whole-plant assay.

Shivanand *et al.* (2015) also evaluated 22 tomato hybrids for their resistance against late blight but none of them found resistant. While the only one genotype (US 618) was moderately resistant.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

All the experiments related to research work entitled "**Development of microbial consortia for sustainable management of late blight (*Phytophthora infestans* (Mont.) de Bary) in tomato (*Solanum lycopersicum* L.)**" were carried out in the laboratory and experimental farm of the Department of Plant Pathology, School of Agricultural Science and Rural Development (SASRD), Nagaland University, Medziphema Campus, Nagaland during 2017-18 and 2018-19. The details of materials and methods used in investigation for recording various observations and analysis are described below:

3.1 General information

3.1.1 Location

The present experiment site is located in the foothills of Nagaland and situated at 25° 45′ 45′′ North latitude and 93° 51′ 45" East longitudes at an elevation of 310 m above MSL.

3.1.2 Climate

The site of experimental area enjoys a sub-humid tropical with high humidity and moderate temperature (12-32 °C), having moderate to high rainfall (2000-3000 mm) and R.H. of 70-80 %. The meteorological data recorded during the field experiments are presented in table 3.1.

Table 3.1 Meteorological data recorded during the field experiments (Sept., 2017- Jan., 2018 and Sept., 2018 – Jan., 2019)

Month	Temperature (˚C)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Max.	Min.	
2017-2018					
September, 2017	31.6	24.5	95	74	235.9
October, 2017	30.3	22.0	95	75	130.0
November, 2017	28.1	16.3	96	63	16.4
December, 2017	25.5	12.3	96	66	31.8
January, 2018	23.7	9.6	97	63	23.0
2018-2019					
September, 2018	33.6	23.9	94	67	115.7
October, 2018	29.9	20.1	96	67	64.0
November, 2018	28.2	14.1	97	54	13.3
December, 2018	24.6	11.0	96	56	50.0
January, 2019	24.8	8.1	95	46	00.0

Source: ICAR Research Complex for NEH Region, meteorological observatory, Medziphema, Nagaland.

3.2 General laboratory procedures

3.2.1 Apparatus and equipments

Laminar air flow cabinet, B.O.D. incubator, autoclave, hot air oven, pH meter, electronic balance, physical balance, micro wave oven, microscope with photo-micrographic attachment, digital camera, refrigerator, spirit lamp, haemocytometer, shakers, inoculation needle etc. were used in various experiments.

3.2.2 Glasswares, materials and chemicals

Throughout the experimental work Corning/Borosil made glasswares Petri plates, flasks, test tubes, pipette, beakers, funnels, glass slides, cover slips and measuring cylinders, etc. were used. Tarson's disposable Petri plates and micro tips were used for serial dilution. Polyethylene bags of different sizes, rubber bands, non absorbent cotton, tags, malt extract, dextrose, agar-agar and buffer solutions were used in various experiments.

3.2.3 Cleaning of glasswares

The glasswares were cleaned with 6 per cent chromic acid ($K_2Cr_2O_7$ 60 g mixed in 1000 ml of distilled water and 60 ml conc. sulphuric acid added with constant stirring), followed by washing with detergent powder and througly washing in running tap water and finally cleaned with distilled water and air dried in hot air oven before use.

3.2.4 Sterilization

The glasswares were sterilized in hot air oven at 180 °C for 20 min. Water and media were sterilized in an autoclave at 121 °C at 1.1 kg/cm^2 pressure (15 lb psi) for 20 min. The inoculation needle, inoculation loop, forceps, cork borer etc. were sterilized on flame of spirit lamp and cooled before use. The plant tissues were surface sterilized in 1.0 % sodium hypochlorite solution followed by three changes in sterile distilled water. All *in vitro* studies were conducted in aseptic condition under laminar air flow.

3.2.5 Preparation of culture medium

The following culture media were prepared in steel containers and distributed equally into 250 ml. conical flasks @ 100 ml/flask and autoclaved at 15 lb psi pressure for 20 min.

Potato dextrose agar medium (PDA) (HIMEDIA)

Potatoes 200 g	Dextrose 20 g
Agar 15 g	Distilled water 1000 ml

Trichoderma selective medium (HIMEDIA)

$MgSO_4$ 0.2 g	Rose bengal 0.15 g
K_2HPO_4 0.9 g	Glucose 3.0 g
NH_4NO_3 1.0 g	Agar 20.0 g
KCl 0.15 g	Distilled water 1000 ml

King's B medium (HIMEDIA)

Peptone 20 g	Glycerol 15 ml
K_2HPO_4 1.5 g	Agar 20 g
$MgSO_4.7H_2O$ 1.5 g	Distilled water 1000 ml

King's B broth medium

Peptone 20 g	Glycerol 15 ml
K_2HPO_4 1.5 g	$MgSO_4.7H_2O$ 1.5 g
Distilled water 1000 ml	

Rye-A agar medium (HIMEDIA)

Rye extracts 60 g	Agar 15 g
Sucrose 20 g	Distilled water 1000 ml

Rye-B agar medium (HIMEDIA)

Rye extracts 60 g	Agar 15 g
Sucrose 20 g	Distilled water 1000 ml
β-sitosterol 0.05g	

Chrome azurol sulfonate (CAS) agar* medium

Chrome azurol sulfonate 60.5 mg/50 ml distilled water

CTAB 72.9 mg/40 ml distilled water

King's B medium (HIMEDIA) 42.23 g (For *Pseudomonas* isolates)

PDA medium (HIMEDIA) 39 g (For *Trichoderma* isolates)

Distilled water 900 ml

pH 6.8±0.2

*Mix CAS solution (50 ml) and CTAB solution (40 ml) and then add it to 10 ml of 1mM $FeCl_3.6H_2O$ solution prepared in 10 mM HCl. Add the final solution (100 ml) to 900 ml of King's B / PDA medium.

3.3 Collection, isolation, pathogenicity test, characterization and identification of the pathogen

3.3.1 Collection of diseased specimens

Diseased specimens of late blight of tomato were collected from tomato field, Horticulture farm and backyard tomato cultivation in staff quarters, SASRD, Nagaland University, Medziphema campus showing typical symptoms of late blight. The diseased specimens were collected in butter paper bag and later kept in polythene bags and brought to the laboratory.

3.3.2 Isolation and purification of the pathogen

Standard tissue isolation procedure was followed to isolate the causal pathogen (Hollomon, 1965). The naturally infected tissues of tomato leaves (single lesions only) were cut into small pices with the help of sterilized blade. These pices were surface sterilized with 1.0 % sodium hypochlorite solution for 2 min. The bits were then washed thoroughly in sterile distilled water thrice to remove traces of sodium hypochlorite, if any, and then transferred aseptically to sterilized rye-A agar plates. They were incubated at 18 ±1 °C and checked on 24 hrs interval for growth of the fungus. Later the bit of fungal growth was transferred to rye-B agar slants. The pure culture of the fungus was obtained by hyphal tip culture under aseptic conditions. These isolates were used for pathogenicity test.

3.3.3 Pathogenicity test

In order to prove the Koch´s postulate of *P. infestans* isolated from the samples of tomato fruits and leaves showing characteristic symptoms of late blight, the surface of plant part to be inoculated was first sterilized with 70 % ethanol and then thoroughly washed with sterilized distilled water. The susceptible tomato cv. Pusa Ruby was grown under green shade net house conditions. Healthy tomato seedlings' leaves were inoculated by rye-B agar plugs technique (Loliam *et al*., 2012). The rye-B agar plugs (10 mm), taken from the edge of the young colony of pathogen (9 days old) and sterilized rye-B agar (without pathogen to serve as a control) were artificial inoculated on the leaves of seedlings (4 weeks old) at the rate of 6 agar plugs/seedling. Four seedlings were maintained for each treatment. For providing humidity to inoculated seedlings, they were covered by polyethylene bags for 4 days. The observations of inoculated seedlings for disease development were recorded. Re-isolation was made from artificially inoculated seedlings showing typical

symptoms of late blight symptoms. The pure culture obtained from inoculated seedlings. Re-isolation was transferred on rye-B agar slants for comparison with the original isolates and further studies.

3.3.4 Characterization and identification of the pathogen

Morphological characters of the pathogen were studied in pure culture on Rye-B agar medium as well as on host plant. Infected fruits were brought to the laboratory and sections from diseased portions were examined under microscope. The fungus growing on Rye-B agar medium was examined microscopically and morphological characters of mycelium, sporangiophores and sporangia were recorded. The isolated fungus was identified on the basis of morphological characters documented in taxonomic keys (Waterhouse, 1963).

3.4 Collection and isolation of native biocontrol agents

3.4.1 Collection of tomato rhizosphere soil samples

A field survey was undertaken for the collection of tomato rhizosphere soil samples from tomato growing areas *viz.,* Merema, Tsiesema, SASRD, campus and CIH polyhouse, Medziphema. Soil samples were taken from the rhizosphere of healthy tomato plants. For collection of the soil samples, the area around healthy tomato plants were dug upto a depth of about 10-15 cm. The soils were collected close to the root of the tomato plant and kept in polyethylene bags until it was brought to the laboratory for isolation. The individual sample was mixed thoroughly after air drying for further isolation.

3.4.2 Isolation of tomato rhizosphere microbes

The soil dilution plate technique (Waksman, 1927) was used for isolation of native rhizosphere microbial isolates. For the isolation of *Trichoderma,* the soil samples were serially diluted (10^{-4}) and plated on *Trichoderma* Selective Medium (TSM) (Elad and Chet, 1983). The fluorescent pseudomonads were isolated by plating the serially diluted (10^{-6}) on King's B medium (King *et.al.,* 1954). The plates were incubated at 28 °C for three days.

3.5 Maintenance of the cultures

The pure culture of the *P. infestans, Trichoderma* and *Pseudomonas* isolates were maintained throughout the period of investigation on the Petri plates and test tubes slants of Rye-B agar, PDA and King's B medium, respectively in BOD incubator. Sub-culturing was done periodically to maintain the purity of the cultures.

3.6 Evaluation of antagonists against *P. infestans*

3.6.1 *In vitro* antagonistic effect of *Trichoderma* isolates on radial growth of the pathogen

The antagonistic effect of *Trichoderma* isolates were evaluated against *P. infestans* by dual culture technique (Sivakumar *et al.,* 2000). A 10 mm diameter mycelial disc of pathogen (9 days old) was placed on one side of a Petri plate (90 mm diameter) containing rye-B agar medium (20 ml). Simultaneously, 10 mm diameter disc of *Trichoderma* isolates (9 days old) were placed 60 mm away from the pathogen on the dual plates, whereas sterile PDA disc was placed in the control plates. The radial growth of the pathogen was measured at 8 days after incubation at 18 ±1 °C.

Linear mycelial growth was recorded from the centre of the disc towards periphery of the Petri plate after the control plate was completely covered by growth of the test pathogen and inhibition of the growth of pathogen by BCAs over control was calculated (Vincent, 1927).

The per cent radial growth inhibition: $PI = \frac{C-T}{C} X\ 100.$

Where C = Radial growth of *P. infestans* (cm) in control plate

T = Radial growth of *P. infestans* (cm) in dual plate

PI = Per cent inhibition

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. Data were analysed statistically. The treatment combinations for the experiment were laid as follows:

T_0	- Control- *P. infestans* alone	T_{13}	- *P. infestans* + T-13
T_1	- *P. infestans* + T-1	T_{14}	- *P. infestans* + T-14
T_2	- *P. infestans* + T-2	T_{15}	- *P. infestans* + T-15
T_3	- *P. infestans* + T-3	T_{16}	- *P. infestans* + T-16
T_4	- *P. infestans* + T-4	T_{17}	- *P. infestans* + T-17
T_5	- *P. infestans* + T-5	T_{18}	- *P. infestans* + T-18
T_6	- *P. infestans* + T-6	T_{19}	- *P. infestans* + T-19
T_7	- *P. infestans* + T-7	T_{20}	- *P. infestans* + T-20
T_8	- *P. infestans* + T-8	T_{21}	- *P. infestans* + T-21
T_9	- *P. infestans* + T-9	T_{22}	- *P. infestans* + T-22
T_{10}	- *P. infestans* + T-10	T_{23}	- *P. infestans* + T-23
T_{11}	- *P. infestans* + T-11	T_{24}	- *P. infestans* + T-24
T_{12}	- *P. infestans* + T-12	T_{25}	- *P. infestans* + T-25

3.6.2 *In vitro* antagonistic effect of *Pseudomonas* isolates on radial growth of the pathogen

The antagonistic effect of *Pseudomonas* isolates were evaluated against *P. infestans* by slight modification of dual culture technique (Georgakopoulos *et al*., 2002). A 20 µl of an overnight culture (12 hrs old, 2 ml) of *Pseudomonas* isolates were poured in wells (5 mm diameter) at the centre of a Petri plate containing rye-B agar medium (20 ml). Simultaneously, 10 mm disc of pathogen (9 days old) was placed at either side of the bacterial culture. In control plates, 20 µl of sterile King's B broth was poured at the centre. The radial growth of the pathogen was measured at 8 days after incubation at 18 ±1 °C. Linear mycelial growth was recorded from the centre of the disc towards periphery of the Petri plate after the control plate was completely covered by growth of the test pathogen and inhibition of the growth of pathogen by BCAs over control was calculated (Vincent, 1927).

The per cent radial growth inhibition: $PI = \frac{C-T}{C} X\ 100.$

Where C = Radial growth of *P. infestans* (cm) in control plate

T = Radial growth of *P. infestans* (cm) in dual plate

PI = Per cent inhibition

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. Data were analysed statistically. The treatment combinations for the experiment were laid as follows:

T_0	- Control- *P. infestans* alone	T_5	- *P. infestans* + Pf-5
T_1	- *P. infestans* + Pf-1	T_6	- *P. infestans* + Pf-6
T_2	- *P. infestans* + Pf-2	T_7	- *P. infestans* + Pf-7
T_3	- *P. infestans* + Pf-3	T_8	- *P. infestans* + Pf-8
T_4	- *P. infestans* + Pf-4		

3.7 Investigation on the biocontrol mechanisms of BCAs

3.7.1 Volatile metabolites

The effects of volatile metabolites of BCAs were assessed against *P. infestans* by Dennis and Webster (1971) technique. The pathogen was inoculated (10 mm diameter disc) at the centre of a Petri plate containing rye-B agar medium (20 ml). After 3 hrs of incubation at 18±1 °C, the Petri plates were inverted on the actively grown three days old culture of *Trichoderma* and *Pseudomonas* isolates and sealed with parafilms under aseptic condition and incubated at 18±1 °C. Diameter mycelial growth was measured at 6 days after incubation when control plate was completely covered by growth of the test pathogen and inhibition of the growth of pathogen by volatile metabolites over control was calculated (Vincent, 1927).

The per cent growth inhibition: $PI = \frac{C-T}{C} X\ 100$.

Where C = Diameter growth of *P. infestans* (cm) in control plate
T = Diameter growth of *P. infestans* (cm) in treatment plate
PI = Per cent inhibition

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. Data were analysed statistically. The treatment combinations for the experiment were laid as follows:

For *Trichoderma* isolates

T_0	- Control- *P. infestans* alone	T_{13}	- *P. infestans* + T-13
T_1	- *P. infestans* + T-1	T_{14}	- *P. infestans* + T-14
T_2	- *P. infestans* + T-2	T_{15}	- *P. infestans* + T-15
T_3	- *P. infestans* + T-3	T_{16}	- *P. infestans* + T-16

T_4	- *P. infestans* + T-4	T_{17}	- *P. infestans* + T-17
T_5	- *P. infestans* + T-5	T_{18}	- *P. infestans* + T-18
T_6	- *P. infestans* + T-6	T_{19}	- *P. infestans* + T-19
T_7	- *P. infestans* + T-7	T_{20}	- *P. infestans* + T-20
T_8	- *P. infestans* + T-8	T_{21}	- *P. infestans* + T-21
T_9	- *P. infestans* + T-9	T_{22}	- *P. infestans* + T-22
T_{10}	- *P. infestans* + T-10	T_{23}	- *P. infestans* + T-23
T_{11}	- *P. infestans* + T-11	T_{24}	- *P. infestans* + T-24
T_{12}	- *P. infestans* + T-12	T_{25}	- *P. infestans* + T-25

For *Pseudomonas* isolates

T_0	- Control- *P. infestans* alone	T_5	- *P. infestans* + Pf-5
T_1	- *P. infestans* + Pf-1	T_6	- *P. infestans* + Pf-6
T_2	- *P. infestans* + Pf-2	T_7	- *P. infestans* + Pf-7
T_3	- *P. infestans* + Pf-3	T_8	- *P. infestans* + Pf-8
T_4	- *P. infestans* + Pf-4		

3.7.2 Ammonia production

Trichoderma and *Pseudomonas* isolates were tested for the production of ammonia in peptone water (Peptone 10 g, Sodium chloride 5 g and distilled water 1 L). Freshly grown cultures were inoculated (5 mm diameter mycelial disc of *Trichoderma* isolates and 100 µl of *Pseudomonas* isolates) in 10 ml peptone water in each test tube and incubated for 3 days at 36± 2°C. Nessler's reagent (0.5 ml) was added in each test tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

3.7.3 Indole-3- acetic acid (IAA) production

The production of IAA by *Trichoderma* and *Pseudomonas* isolates were determined by qualitative assay (Gordon and Weber, 1951). Ten millimeter mycelial discs of *Trichoderma* and 1 ml of *Pseudomonas* isolates were inoculated into 50 ml of yeast malt extract broth (Dextrose 10 g, Peptone 5 g, Malt extract 3 g, Yeast extract 3 g and Distilled water 1 L) containing 0.2 % L-tryptophan and incubated at 28 °C with shaking at 125 rpm for three days. Cultures were centrifuged at 11,000 rpm for 15 min. Two milliliter of the supernatant was mixed with 4 ml of the Salkowski reagent (0.5 M $FeCl_3$ 2 ml, 70 % perchloric acid 49 ml and distilled water 49 ml). Development of pink colour indicated IAA production.

3.7.4 Phosphate solubility

Phosphate solubilization test was conducted qualitatively by inoculating of *Trichoderma* and *Pseudomonas* (10 mm mycelial disc of *Trichoderma* isolates and loop full spot inoculation of *Pseudomonas* isolates) on National Botanical Research Institute's phosphate (NBRIP) agar medium (Glucose 10 g, $Ca_3 (PO_4)_2$ 5 g, $MgSO_4.7H_2O$ 0.25g, $MgCl_2.6H_2O$ 5 g, KCl 0.2 g, $(NH_4)_2 SO_4$ 0.1 g, agar 15 g and distilled water 1 L) according to Nautiyal (1999). The presence of halo clearing zone around fresh colony after incubating at 28 °C for 7 days was used as an indicator for positive P solubilization.

3.7.5 Siderophore production

A modified Chrome azurol sulfonate (CAS) agar medium test plate using the method of Milagres *et al.* (1999) to detect siderophore production. The medium contains CAS blue agar (Schwyn and Neilands, 1987) in Petri plates. The microbial isolates were inoculated (5 mm mycelium disc of *Trichoderma*

and loop full spot inoculation of *Pseudomonas* isolates) and incubated at 28 ºC for 3 days. The colonies with yellow/orange zones were considered as siderophore producing isolates.

3.7.6 Qualitative assay of Hydrogen cyanide (HCN) production by *Pseudomonas* isolates

HCN production was determined by modified protocol of Miller and Higgins (1970). *Pseudomonas* isolates were streaked onto tryptic soya agar medium (HIMEDIA, 45 g and distilled water 1 L). Filter paper (whatman No.1) strips were soaked in picric acid solution (Picric acid 2.5 g, Na_2CO_3 12.5 g and distilled water 1 L) and placed on the upper lid of each Petri plates. Petri plates were sealed with parafilm and observation was made for 5 days after incubation at 28 °C. Reactions were observed as strong (reddish brown), moderate (brown) and weak (yellow to light brown).

3.7.7 Mycoparasitism activity of native *Trichoderma* isolates

The method consist of inoculating antagonist in the same Petri plate containing PDA medium (20 ml) culture having three sterile cover slips in the center of the plate for check interaction between the hyphae. Three disc (10 mm) of each isolates of *Trichoderma* and pathogen were inoculated as opposite poles in the dual plate and incubated at a temperature of 18±1 °C for 10 days in the absence of light. After the incubation period, cover slips remove and superimposed on microscope slides with dye cotton blue to verify the interaction between the hyphae (Rodrigues, 2010). The presence or absence of coiling and other hyphal interactions were observed under compound microscope.

3.8 Identification of potential BCAs

3.8.1 Molecular identification and phylogenetic analysis of potential *Trichoderma* isolates

3.8.1.1 Fungal growth condition

Four mycelial disc (5 mm diameter) from six days old cultures were transferred to 100 ml of potato dextrose broth (PDB) medium (HIMEDIA) and incubated at 28 °C for six days. The mycelium was filtered through a sterile whatman filter paper-42, washed twice with sterile distilled water, drained on filter paper and ground using a mortar and pestle in liquid nitrogen.

3.8.1.2 Extraction of DNA

The genomic DNA of *Trichoderma* isolates (T-11 and T-14) were extracted from 200 mg of ground mycelia using commercial DNA isolation kit (GCC Biotech). The quality and concentration of the genomic DNA was assessed using a spectrophotometer (Shimadzu UV-160), which measured the UV absorbance at 260 and 280 nm and computed the 260/280 absorbance ratio. DNA resuspended in 50 µl of TE buffer and concentration of the genomic DNA was quantified by use of ethidium bromide fluorescens.

3.8.1.3 PCR amplification

The universal primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS6 (5'-GAAGGTGAAGTCGTAACAAGG-3') was used for amplifying and sequencing the *Trichoderma* 18S rRNA ITS region (White *et al*., 1990). Amplification reaction was prepared in a total volume of 50 µl containing 4 µl of 5 x Gitschier buffer, 2.5 DNA polymerase (5 U μl^{-1}) (Banglore Genie,

India). 50 pmol each of forward (ITS6) and reverse (ITS4) primers and 2.5 µl of 50 ng DNA template.

Thermal cycling (G Storm GS4, Somerset, UK) consisted of a 2 min initial denaturation at 95 ^{0}C, followed by 40 cycles of elongation (denaturation at 94 ^{0}C for 1 min, annealing at 55 ^{0}C, for 1 min and extension at 72 ^{0}C for 1 min) and ending with a final extension at 72 ^{0}C for 10 min.

3.8.1.4 Agarose gel electrophoresis of PCR products

A 1-kb ladder (Bangalore Genei, India) was used as a molecular size standard marker. The PCR products were separated by electrophoresis (at 75 V cm^{-1} for 50 min) on 1.5 per cent agarose gel with 1x Tris acetate EDTA buffer. The gels were then stained with ethidium bromide (0.5 µg ml^{-1}) before pouring. The ethidium bromide stained gel was viewed and image captured using gel documentation system (Bio-Rad, Philadelphia, PA, USA).

3.8.1.5 Sequencing and data analysis

PCR products of 18S rRNA gene of T-11 and T-14 isolates obtained through amplification with specific primer were freeze dried in a lyophilizer (CHRIST ALPHA I-2LD) and sent for custom sequencing using same upstream and downstream primers used for the amplification of 18S rRNA gene (Eurofins Genomics India, Pvt. Ltd., Bengaluru, India).

3.8.1.6 Gene phylogenetic analysis of *Trichoderma* spp.

For species identification, ITS4 and ITS6 gene sequences were submitted to BLAST (Basic local alignment search tool) interface in NCBI (http://blast.ncbi.nlm.nih.gov). All positions containing gaps and missing data were eliminated from the dataset. Phylogenetic analysis was performed in

MEGA 5.2 (Tamura *et al*., 2004). The nucleotide sequences of ITS 18S rRNA gene were deposited in NCBI GenBank.

3.8.2 Identification of potential *Pseudomonas* isolates

The identity of the fluorescent pseudomonads was confirmed with Gram's staining technique, colony morphology, fluorescens and cell shape (Garrity *et al.*, 2005).

3.9 *In vitro* testing of compatibility amongst microbial consortia

The compatibility of *Trichoderma* and *Pseudomonas* consortia were evaluated by dual culture plate method (Siddiqui and Shaukat, 2003). An overnight culture (12 hrs old, 2 ml) of Pf-2 and Pf-3 were streaked on one side of the Petri plates (90 mm diameter) containing King's B agar medium (20 ml). The other side of the Petri plates were inoculated with 10 mm diameter disc of T-11 and T-14 (9 days old). The plates were incubated at 25 °C.

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. The treatment combinations for the experiment were laid as follows:

T_1	- Control-I, Pf-2 alone	T_9	- Pf-2+ T-14
T_2	- Control-II, Pf-3 alone	T_{10}	- T-11 + T-14
T_3	- Control-III, T-11 alone	T_{11}	- Pf-2 + Pf-3 + T-11
T_4	- Control-IV, T-14 alone	T_{12}	- Pf-2 + Pf-3 + T-14
T_5	- Pf-2 + Pf-3	T_{13}	- Pf-3 + T-11 + T-14
T_6	- Pf-3 + T-11	T_{14}	- Pf-2 + T-11 + T-14
T_7	- Pf-3 + T-14	T_{15}	- Pf-2 + Pf-3 + T-11 + T-14
T_8	- Pf-2+ T-11		

3.10 *In vitro* antagonistic efficacy of compatible microbial consortia (CMC) against *P. infestans*

The bioassay technique was used for the testing of compatible microbial consortia against *P. infestans.* A 10 mm diameter mycelial disc of pathogen (9 days old) was placed at centre of Petri plate (90 mm diameter) containing rye-B agar medium (20 ml). Simultaneously, 10 mm diameter disc of T-11 and T-14 isolates (9 days old) and 20 µl of an overnight culture (12 hrs old, 2 ml) of Pf-2 and Pf-3 isolates were poured in wells (5 mm diameter) at different corner of Petri plate; whereas sterile PDA disc and 20 µl of sterile King's B broth was placed/poured in the control plates. The radial growth of the pathogen was measured at 5 days after incubation at 18±1 °C. Linear mycelial growth was recorded from the centre of the disc towards periphery of the Petri plate when mycelium of test pathogen touched any BCAs and inhibition of the growth of pathogen by BCAs over control was calculated (Vincent, 1927).

The per cent radial growth inhibition: $PI = \frac{C-T}{C} X\ 100.$

Where C = Radial growth of *P. infestans* (cm) in control plate

T = Radial growth of *P. infestans* (cm) in dual plate

PI = Per cent inhibition

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. Data were analysed statistically. The treatment combinations for the experiment were laid as follows:

T_0	- Control, *P. infestans* alone	T_6	- T-11 + T-14
T_1	- Pf-2 + Pf-3	T_7	- Pf-2 + Pf-3 + T-11
T_2	- Pf-3 + T-11	T_8	- Pf-2 + Pf-3 + T-14
T_3	- Pf-3 + T-14	T_9	- Pf-3 + T-11 + T-14
T_4	- Pf-2+ T-11	T_{10}	- Pf-2 + T-11 + T-14
T_5	- Pf-2+ T-14	T_{11}	- Pf-2 + Pf-3 + T-11 + T-14

3.11 Preparation of liquid compatible microbial consortia (CMC)

A 250 ml suspension of each selected *T. asperellum* isolates (T-11 and T-14) was prepared from 9 days old cultured PDA medium plates. The plates were rinsed with sterile distilled water and the mycelia were carefully scraped off the agar with a bent glass rod. This suspension was filtered through filter paper (Whatman No.1) to separate the spores from the mycelia. The concentration was adjusted to 3.7×10^8 spores/ml (Dubos, 1987) with the help of haemocytometer.

A 250 ml of each selected *P. fluorescens* isolates (Pf-2 and Pf-3) cell suspension was prepared by inoculating the strain into King's B broth followed by shaking for 48 hrs (150 rpm) at room temperature. The bacterial suspension was roughly adjusted optically at 1 x 10^9 cfu/ml (O.D. 600= 1) (Mulya *et al.,* 1996). Liquid consortium was prepared by mixing equal volume (1:1:1 and 1:1:1:1) of each selected isolate just before use (Srinivasan and Mathivanan, 2009).

3.12 *In vitro* effect of compatible microbial consortia (CMC) on tomato seedlings

The healthy seeds of tomato cv. Pusa Ruby were selected for experimental purpose. The seeds were obtained from local market. Tomato seeds were surface sterilized with 1.0 % sodium hypochlorite for 2 min for all treatments followed by three rinsed with sterile distilled water.

3.12.1 Wet seed treatment

The surface sterilized seeds were soaked with liquid formulations of consortia [@ 1.0 % or 10 µl/ 1 g seeds; 10 µl formulation of CMC added in

990 µl of water/1 g seed (400 tomato seeds)] and shade dried in laminar air flow for 5 hrs (Srinivasan and Mathivanan, 2009).

3.12.2 For chemical control treatment

The surface sterilized seeds were treated with captan 50 % WP (seed dressing @ 0.3 % or 3 mg/1 g seed) (Srinivasan and Mathivanan, 2009).

3.12.3 For control treatment

The surface sterilized seeds were soaked in sterile distilled water (@1 ml/1 g seed) and shade dried in laminar air flow for 5 hrs (Srinivasan and Mathivanan, 2009).

3.12.4 Details of experiment

- Experimental design: Complete Randomised Design (CRD)
- No. of treatment: 4
  (T_1- CMC 1, T_2 - CMC 2, T_3 - Chemical control and T_4 - Control)
- No. of replication: 6
- No. of seeds/treatment: 120 seeds (20 seeds/replication)
- No. of seeds/ Petri plate: 10 seeds
- Method: Standard filter paper method (three layered moistened filter papers in Petri plates) (ISTA, 1993).

3.12.5 Observation and recording procedures

3.12.5.1 Per cent germination at 10 DAS

Per cent germination was calculated using the following formula –

$$\text{Per cent germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \text{ X } 100$$

3.12.5.2 Seedlings shoot length and root length (cm) at 10 DAS

The root and shoot length of individual seedlings (10 seedlings/replication) were measured. The shoot length was recorded from collar to the tip of the seedling with the help of a scale and the average shoot length was expressed in cm. The root length recorded from collar to the tip of primary root with the help of a scale and the average root length was expressed in cm.

3.12.5.3 Seedling vigour index (SVI) at 10 DAS

The vigour index of seedlings was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in number by using the below formula.

SVI = Germination (%) x [Mean shoot length (cm) + Mean root length (cm)].

3.12.5.4 Fresh weight (mg) of seedling shoot and root at 10 DAS

The fresh weight (mg) of root and shoot of individual seedlings (10 seedlings/replication) were measured.

3.12.5.5 Dry weight (mg) of seedling shoot and root at 10 DAS

The dry weight of root and shoot of individual seedlings (10 seedlings /replication) were measured after oven drying at 60° C (when constant weight obtained) for 24 hrs. The weight of shoot and root was observed and average dry weight of seedlings was calculated and was expressed in mg.

3.12.5.6 Per cent increase of plant growth promotion over control at 10 DAS

$$\text{Per cent increase} = \frac{\text{Treatment value} - \text{Control value}}{\text{Control value}} \text{ x } 100$$

3.13 *In vivo* effect of compatible microbial consortia (CMC) on tomato seedlings

3.13.1 Details of experiment

- Experimental design: Complete Randomised Design (CRD)
- No. of treatment: 4
  (T_1- CMC 1, T_2 - CMC 2, T_3 - Chemical control and T_4 - Control)
- No. of replication: 6
- No. of experiment: twice (2017-18 and 2018-19)
- No. of seeds/treatment: 400
- Method: Tomato seeds were sown in pre sterilized nursery beds with 2 per cent formalin.

3.13.2 Observation and recording procedures

3.13.2.1 Per cent germination at 10 DAS

Per cent germination was calculated using the following formula –

$$\text{Per cent germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \text{ X } 100$$

3.13.2.2 Seedlings shoot length and root length (cm) at 20 DAS and 30 DAS

The root and shoot length of individual seedlings (10 seedlings/replication) were measured. The shoot length was recorded from collar to the tip of the seedling with the help of a scale and the average shoot

length was expressed in cm. The root length recorded from collar region to the tip of primary root with the help of a scale and the average root length was expressed in cm.

3.13.2.3 Seedling vigour index (SVI) at 20 DAS and 30 DAS

The vigour index of seedlings was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in number by using the below formula.

SVI = Germination (%) x [Mean shoot length (cm) + Mean root length (cm)].

3.13.2.4 Fresh weight (mg) of seedling shoot and root at 20 DAS and 30 DAS

The fresh weight (mg) of root and shoot of individual seedlings (10 seedlings/replication) were measured.

3.13.2.5 Dry weight (mg) of seedling shoot and root at 10 DAS and 30 DAS

The dry weight of root and shoot of individual seedlings (10 seedlings /replication) were measured after oven drying at 60° C (when constant weight obtained) for 24 hrs. The weight of shoot and root was observed and average dry weight of seedlings was calculated and was expressed in mg.

3.13.2.6 Per cent increase of plant growth promotion over control at 20 DAS and 30 DAS

$$\text{Per cent increase} = \frac{\text{Treatment value} - \text{Control value}}{\text{Control value}} \text{ x } 100$$

3.14 Field evaluation of compatible microbial consortia (CMC) against late blight of tomato

3.14.1 Details of experiment and layout

- Experimental design: Randomized block design (RBD)
- No. of treatment: 4
- No. of replication: 6
- Crop and variety: Tomato and Pusa Ruby
- Crop season: September-January (Favorable season for late blight)
- Plot size: 1.8 m x 1.8 m (raised plot)
- Spacing: 60 cm x 45 cm
- No. plants / replication: 12
- Date of sowing: 10/09/2017 and 10/09/2018
- Date of transplanting: 08/10/2017 and 08/10/2018
- Pattern of sowing: Line sowing in nursery
  (Depth, 1 cm; spacing of row to row, 8 cm and seed to seed, 1 cm)
- No. of experiment: twice (2017-18 and 2018-19)

3.14.2 Preparation of field experimental plots

The field was ploughed once and was brought to fine tilth by repeated harrowing. Then layout (Fig 3.1) was made and the plots were prepared as per the specifications and well rotten FYM was applied at 10 DBT. All the cultural operations were followed as per the package of practices. The experimental plots were kept free of weeds by regular hand weeding.

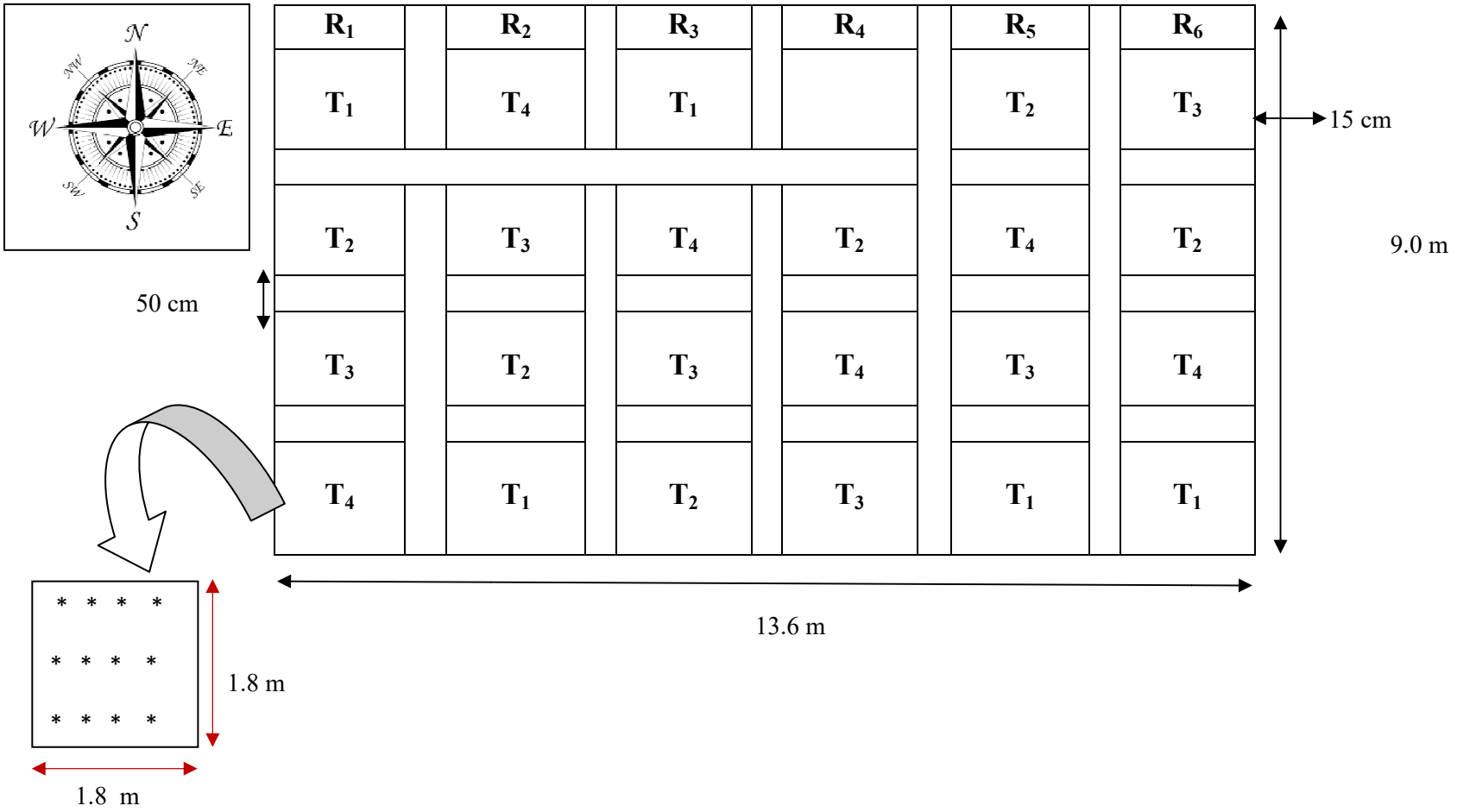


Fig 3.1 Field layout of the experiment no.-I in Randomized Block Design

3.14.3 Treatment combination

T_1: CMC-1 (Seed treatment + Soil application + Foliar sprays at 15, 30 and 45 DAT)

T_2: CMC-2 (Seed treatment + Soil application + Foliar sprays at 15, 30 and 45 DAT)

T_3: Chemical control (Seed treatment + Foliar sprays at 15, 30 and 45 DAT)

T_4: Control (Sterile distilled water)

3.14.4 Applications of treatment

3.14.4.1 Wet seed treatment

For T_1 and T_2, the surface sterilized seeds were soaked with liquid formulations of consortia [@ 1.0 % or 10 µl/ 1 g seeds; 10 µl formulation of CMC added in 990 µl of water/1 g seed (400 tomato seeds)] and shade dried in laminar air flow for 5 hrs (Srinivasan and Mathivanan, 2009).

3.14.4.2 For chemical control treatment

The surface sterilized seeds were treated with captan 50 % WP (seed dressing @ 0.3 % or 3 mg/1 g seed).

3.14.4.3 For control treatment

The surface sterilized seeds were soaked in sterile distilled water (@1 ml/1 g seed)) and shade dried in laminar air flow for 5 hrs.

3.14.4.4 Soil application

For T_1 and T_2 FYM inoculation (@ 1.0 % of CMC, 5 ml/500 g of FYM/plant) at 10 days before transplanting (Srinivasan and Mathivanan, 2011).

For chemical control and control treatment only applied FYM (5 ml of sterile distilled water/500 g of FYM/plant).

3.14.4.5 Foliar spray

For T_1 and $T_{2,}$ (three foliar sprays @ 1.0 % of CMC or 1.5 ml/150 ml of water per plot (12 plants) at 15, 30 and 45 DAT) (Srinivasan *et al*., 2009).

For chemical control (three foliar sprays of Ridomil MZ-72 % WP @ 0.25 % or 0.375 g /150 ml of water per plot (12 plants) at 15, 30 and 45 DAT).

For control (three foliar sprays of sterile distilled water @ 150 ml /plot).

3.14.5 Observation and recording procedures

Five plants in each plot were selected randomly for recording the observations and were duly tagged.

3.14.5.1 Plant height (cm)

Plant height of the selected (5 plants per replication) plants was recorded from the base of plant to the terminal growing point of the main stem. The mean plant height was expressed in cm.

3.14.5.2 Number of leaves per plant

The leaves which were fully opened, matured and were not senescent were counted for each plant and observed as number of leaves per plant (5 selected plants/replication).

3.14.5.3 Number of branches per plant

The number of branches per plant (5 selected plants per replication) were counted and recorded.

3.14.5.4 Number of fruits per plant

The numbers of fruits harvested from five earlier randomly selected plants at different pickings were counted from which total number of fruits per plant was calculated.

3.14.5.5 Fresh weight of fruit (g fruit^{-1})

Fresh weight of randomly selected 30 fruits from each replication was recorded and divided by number of fruits to get the mean weight of the fruit.

3.14.5.6 Marketable fruit yield (g plant^{-1})

The yield of ripened marketable fruits harvested at different dates from sampling plants was computed and their average per plant was noted in g.

3.14.5.7 Calculated marketable fruit yield (t ha^{-1})

The actual mean yield per plant in replication was converted in t ha^{-1}.

3.14.5.8 Root length (cm)

The root length was measured with the help of a scale from uprooted tagged plants. The root length recorded from collar region to the tip of primary root with the help of a scale and the average root length was expressed in cm.

3.14.5.9 Disease severity

The disease severity was evaluated visually on fruits, leaves and stems of all plants of each replication following scale 0-5 (Irzhansky and Cohen, 2006) when total late blight infestation had occurred in the control plot under natural epiphytotic condition. The severity grades were converted into percentage disease index (PDI) for analysis (Wheeler, 1969).

PDI = (Sum of numerical ratings / No. of plant scored x maximum score in scale) x 100.

Rating	Description
0	No visible symptoms apparent.
1	A few minute lesions to about 10 % of the total leaf area is blighted and usually confined to the 2 bottom leaves.
2	Leaves on about 25 % of the total plant area are infected.
3	Leaves on about 50 % of the total plant area are infected.
4	Leaves on about 75 % of the total plant area are infected.
5	Leaves on whole plant are blighted and plant is dead.

3.14.5.10 Mortality per cent

Observations for mortality per cent of plants in each replication were observed when total late blight infestation had occurred in the control plot under natural epiphytotic condition.

Mortality per cent = Number of diseased dead plants/total number of plants x 100.

3.14.5.11 Per cent reduction over control

$$\text{Disease reduction} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \text{ x } 100$$

3.14.5.12 Per cent increase over control

$$\text{Per cent increase (\%)} = \frac{\text{Treatment value} - \text{Control value}}{\text{Control value}} \text{ x } 100$$

3.15 Field evaluation of native tomato genotypes for their resistance to late blight under natural epiphytotic condition

3.15.1 Collection of tomato genotypes

Ripened fruits of native tomato genotypes were collected from different tomato growing areas of Nagaland, Manipur and Arunachal Pradesh. Known susceptible hybrid (Arka Rakshak, F_1) and susceptible variety (Pusa Ruby) seeds were also collected from reliable and reputable sources.

3.15.2 Experiment details and layout

- Experimental design: Randomized block design (RBD)
- No. of genotypes: 22
- No. of replication: 3
- Crop season: September-January (Favorable season for late blight)
- Plot size: 1.95 m x 2.0 m (raised plot)
- Spacing: 65 cm x 50 cm
- No. plants / replication: 12
- Date of sowing: 10/09/2017 and 10/09/2018

- Date of transplanting: 08/10/2017 and 08/10/2018
- Pattern of sowing: Line sowing in nursery
  (Depth, 1 cm; spacing of row to row, 8 cm and seed to seed, 1 cm)
- No. of experiment: twice (2017-18 and 2018-19)

3.15.3 Preparation of field experimental plots

The land was ploughed once and was brought to fine tilth by repeated harrowing. Then layout (Fig 3.2) was made and the plots were prepared as per the specifications and well rotten FYM was applied at 10 DBT. All the cultural operations were followed as per the package of practices. The experimental plots were kept free of weeds by regular hand weeding.

3.15.4 Observation and recording procedures

3.15.4.1 Fruit yield (g plant^{-1})

The yield of ripened fruits harvested at different dates from all plants were computed and their average per plant was noted in g.

3.15.4.2 Calculated fruit yield (t ha^{-1})

The actual mean yield per plant in replication was converted in t ha^{-1}.

3.15.4.3 Disease severity

The disease severity was evaluated visually on fruits, leaves and stems of all plants of each replication following scale 0-5 (Irzhansky and Cohen, 2006) when total late blight infestation had occurred in the control plot under natural epiphytotic condition. The severity grades were converted into percentage disease index (PDI) for analysis (Wheeler, 1969).

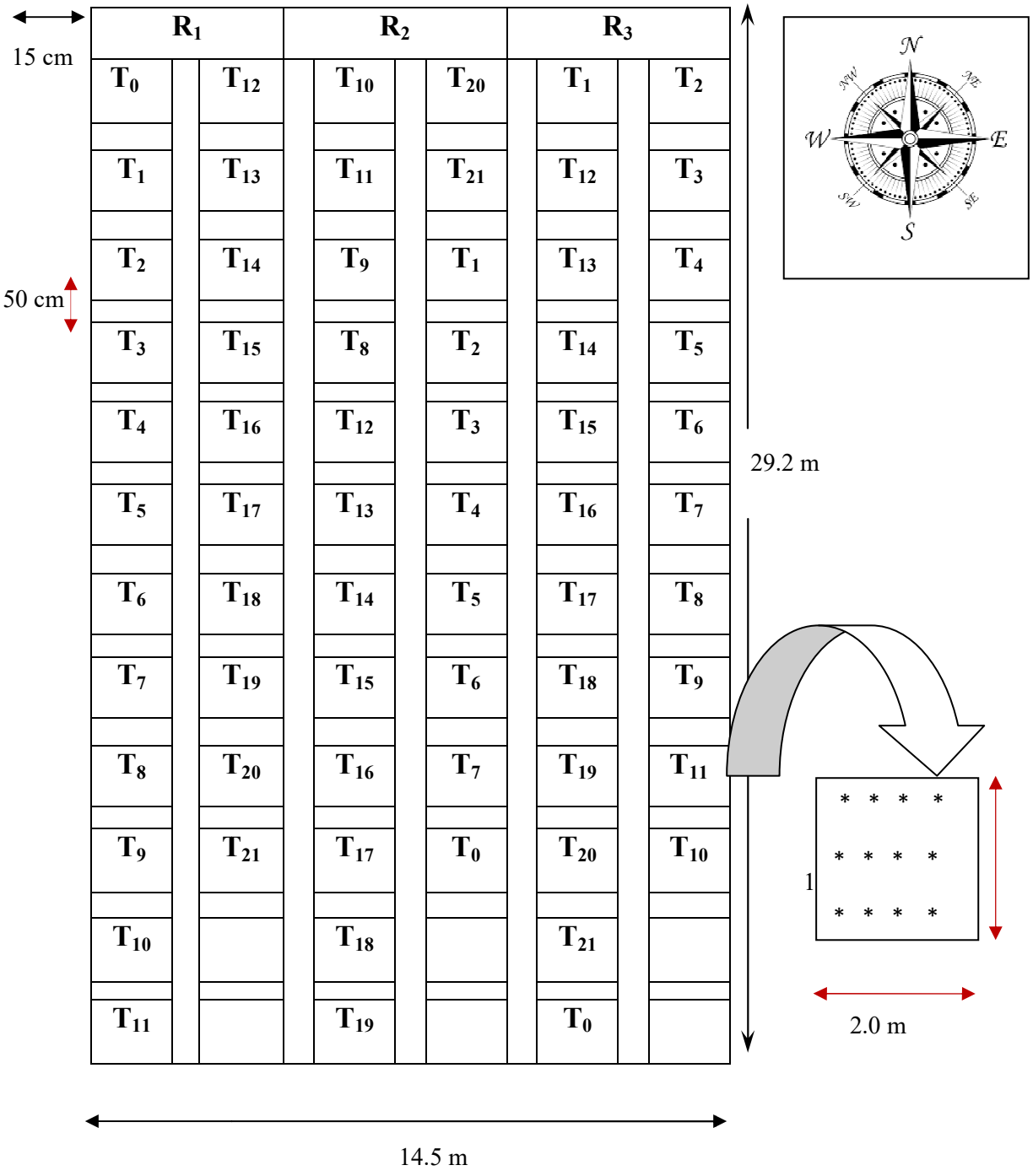


Fig 3.2 Field layout of the experiment no.-II in Randomized Block Design

PDI = (Sum of numerical rating / No. of plant scored x maximum score in scale) x 100.

Disease severity was assessed by the following rating scale (Irzhansky and Cohen, 2006).

Rating	PDI	Description	Reaction
0	0	No visible symptoms apparent.	Immune
1	0.01-10	A few minute lesions to about 10 % of the total leaf area is blighted and usually confined to the 2 bottom leaves.	Highly resistant
2	10.01-25	Leaves on about 25 % of the total plant area are infected.	Resistant
3	25.01-40	Leaves on about 50 % of the total plant area are infected.	Tolerant
4	41.01-60	Leaves on about 75 % of the total plant area are infected.	Susceptible
5	> 60.01	Leaves on whole plant are blighted and plant is dead.	Highly Susceptible

3.16 Statistical analysis and interpretation

The data recorded were subjected to statistical analysis wherever required. The differences exhibited by treatments in various experiments were tested for their significance by employing Completely Randomized Design (CRD) or Randomized Block Design (RBD) as per the details given by Panse and Sukhatme (1967). The percentage values were converted to arc sine values wherever required. Results of the measurements were subjected to analysis of variance (ANOVA) by Least Significant Difference (LSD) using WASP 2.0 (WebAgrilStatPackage) software.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Observations and results of the present investigation, "**Development of microbial consortia for sustainable management of late blight (*Phytophthora infestans* (Mont.) de Bary) in tomato (*Solanum lycopersicum* L.)**" were analyzed statistically to assess the efficacy of the treatments applied. The findings thus obtained are presented in this chapter with appropriate headings supported by suitable tables and illustrations. The results obtained are also discussed in the light of the findings of earlier research works.

4.1 Symptomatology

The disease was found to be prevalent during cool and wet period. The symptoms appeared on all the above ground parts like leaves, stems, branches and fruits. Tomato plants of all the growth stages were attacked by the pathogen and symptoms commonly observed on leaves and fruits are described as under:

4.1.1 On leaves

Typical initial foliar symptoms of late blight of tomato were recorded on lower leaves as small, irregular, light to dark green and water soaked spots. Under favourable weather conditions, these spots develop into large brown necrotic lesions with indefinite borders (Plate 1a). On the ventral surface of the leaves, a fluffy, white downy growth of the fungus appeared around the necrotic tissues. The affected leaves soon turned brown in colour, shriveled and later dried off.

4.1.2 On petioles and stems

Symptoms also appeared on petioles and stems as brown streaks (Plate 1b). In moist weather, disease progressed very rapidly and infects the whole foliage.

4.1.3 On fruits

On tomato fruits, the symptoms appeared only on green fruits as a dark olivaceous, greasy spots which gradually covers the entire fruit (Plate 1c). Initially, the tissue of the affected fruit remain firm with varying depths of discoloured tissue below the skin but when soft rot appears after the blight attack, fruit disintegration rapidly.

The symptoms of the late blight of tomato as observed on leaves, petioles, stems and fruits are in conformity with those described by Turkensteen (1973), Stevenson (1991), Agrios (1997), Thurston and Schultz (1997) and Scot (2008).

4.2 Collection, isolation, pathogenicity test, characterization and identification of the pathogen

4.2.1 Collection of diseased specimens

Diseased specimens of late blight of tomato were collected from tomato field, Horticulture farm and backyard tomato cultivation in staff quarters, SASRD, Nagaland University, Medziphema campus showing typical symptoms of late blight disease (Plate 1).

(a) Typical initial foliar symptoms appeared as small, irregular and water soaked spots

(b) Symptoms appeared on petioles and stems as brown streaks

(c) On tomato fruits, the symptoms appeared as a dark olivaceous and greasy spots

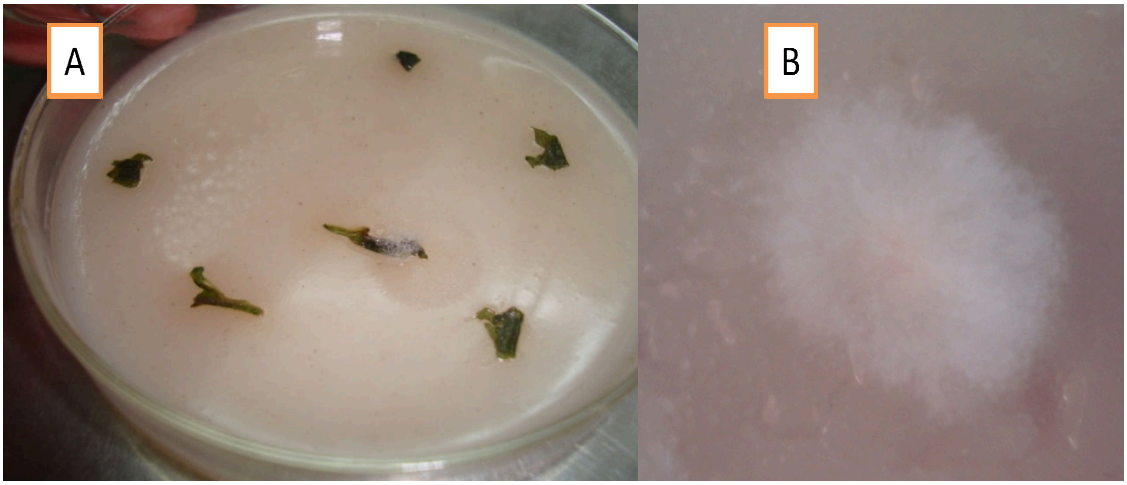
Plate 1 Typical symptoms of late blight of tomato on a) leaves, b) petioles and stems and c) fruits

4.2.2 Isolation and purification of the pathogen

The causal pathogen from naturally infected tissues of tomato leaves (single lesions only) were isolated (Plate 2A) by tissue isolation technique. The typical pathogen colonies developed within 72 hrs (Plate 2B). The pure culture of the fungus was obtained by hyphal tip isolation technique (Plate 2E) and each isolate thus obtained was coded. These isolates were then used for pathogenicity test. Hollomon (1965) also isolated and purified the causal pathogen of late blight from tomato using similar methodology.

4.2.3 Pathogenicity test

The isolates obtained were subjected to pathogenicity test (Plate 3). For this purpose, isolated pathogen was inoculated on susceptible tomato cv. Pusa Ruby by rye-B agar plugs technique. The observations on disease development were recorded, the results indicated that the isolated pathogen was capable of causing cent per cent infection by rye-B agar plugs inoculation method. After 5 days of inoculation (Plate 3E), infected leaves showed typical signs and symptoms of late blight. The pathogen was re-isolated from the same infected leaves and when compared they were observed to be akin to the original pathogen, whereas no symptoms were observed on un-inoculated seedlings (Plate 3G). The most virulent isolate was selected for further characterization studies. The results of the present findings are in agreement with the findings of earlier workers (Chanthini *et al.,* 2012; Loliam *et al.,* 2012; Patel, 2015; Shinde, 2016).

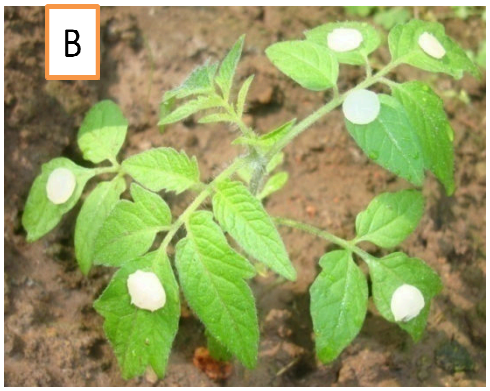


(A) Single lesion tissues inoculated on rye-A agar medium (B) Close view of initial growth of the pathogen (72 hrs old)

(C) Bit of fungal growth transferred to rye-B agar slants

(D) Close view of re-inoculated from slants (E) Hyphal tip pure culture of the pathogen

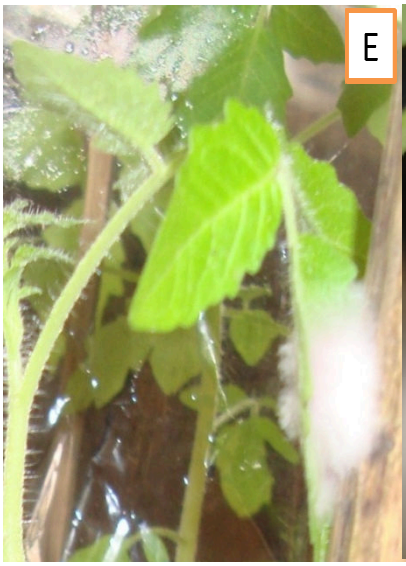
Plate 2 Different procedure for isolation and purification of the pathogen

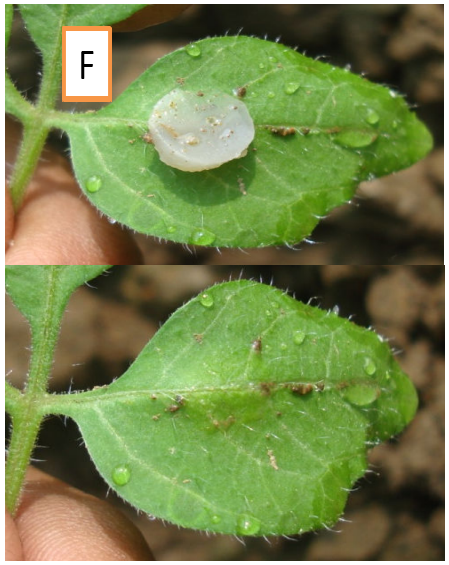


(A) Before inoculation the pathogen

(B) After inoculation of rye-B agar plugs with

(C) Rye-B agar plugs without the pathogen (control) (D) Inoculated seedling inside polyethylene bag





(E) Typical symptoms and signs, observed after 5 days of inoculation

(F) Rye-B agar blocks after 5 days of inoculation in control

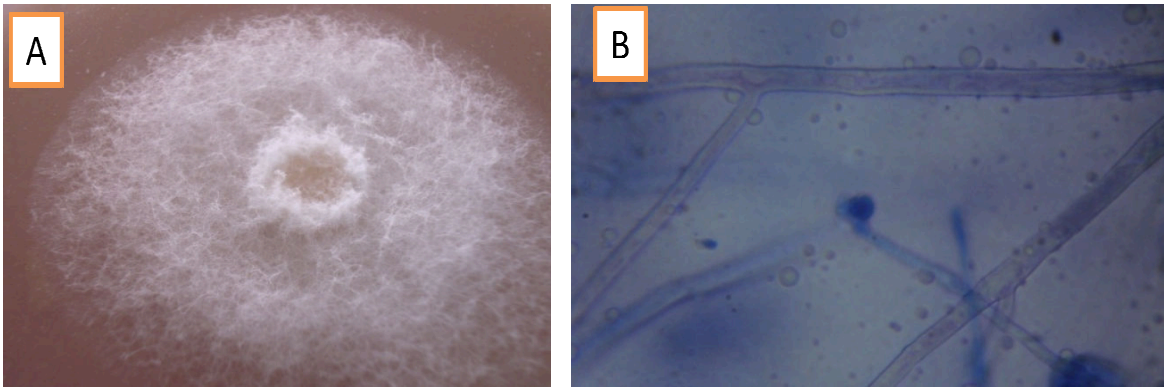
(G) Apparently healthy leaf in control

Plate 3 Pathogenicity test

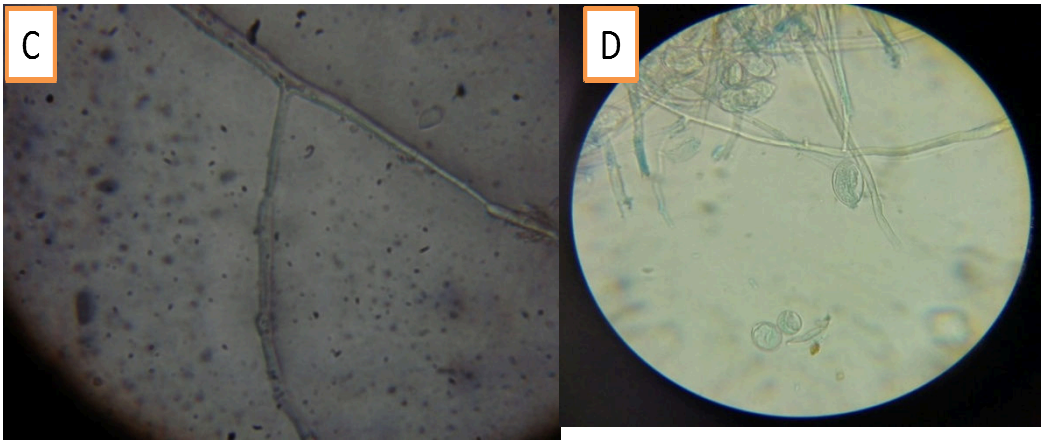
4.2.4 Characterization and identification of the pathogen

The pure culture isolates obtained from the diseased specimens (leaves, fruits and stems) were identified as *P. infestans* based on macroscopic and microscopic characters of the isolates. The morphological characteristics of pathogen were fluffy cottony mycelium with slightly striated pattern (Plate 4A), slow growth rate on the rye-B agar medium. Microscopic observation revealed that the fungal hyphae were hyaline, coenocytic, moderately thick hyphae and profusely branched (Plate 4B). Sporangiophores were sympodial, branched compound, small swelling at the base of each branch (Plate 4C). This type of sporangia were comparatively more frequently observed on the tomato plants than in culture while sporangia were lateral or terminal (Plate 4E), ellipsoid, limoniform or ovoid (Plate 4F), semipapillate, pedicelless and deciduous. Chlamydospores of the pathogen were also observed from diseased specimens (Plate 4G).

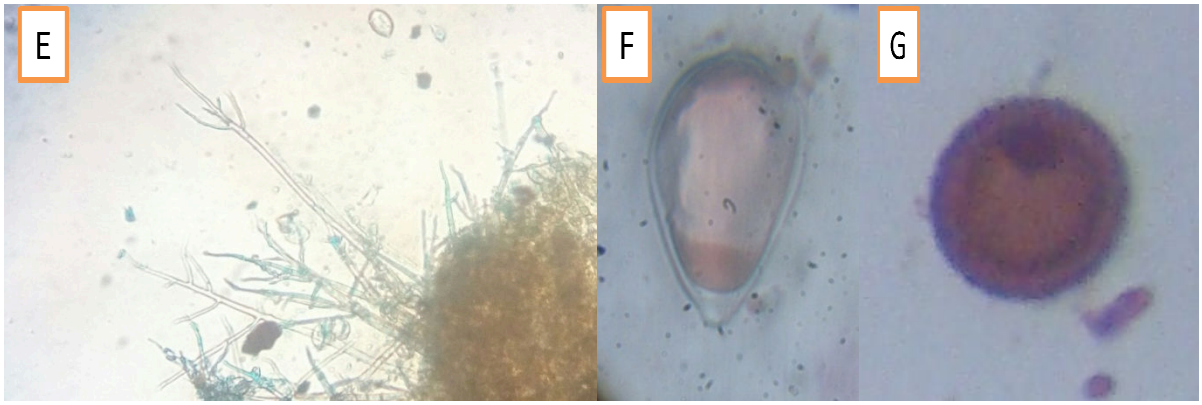
The pathogen was identified as *P. infestans* based on the nature of disease observed, cultural and morphological characters seen under the microscope. These characters were further compared with the characters reported in manual (Waterhouse, 1963) and description given by Singh and Srivastava (1953), Agrios (1997), Zentmyer (1983), Nethravathi (2001) and Shinde (2016) and it was found that the present observations corroborates with their descriptions and findings.



(A) Fluffy cottony mycelium with slightly striated pattern (B) Microscopic view of hyaline, profusely branched, coenocytic, moderately thick hyphae under 45 x



(C) Sympodial with nodal swellings of sporangiophore under 45 x
(D) Microscopic view of sporangiophore with sporangium under 45 x



(E) Microscopic view of branching pattern of sporangiophore under 45 x
(F) Microscopic view of a hyaline, limoniform, an apical papilla on sporangium under100 x
(G) Microscopic view of chlamydospore under 45 x

Plate 4 Characterization of the pathogen

4.3 Collection and isolation of native biocontrol agents

A field survey was undertaken for the collection of tomato rhizosphere soil samples from tomato growing areas *viz.,* Merema, Tsiesema, SASRD, campus and CIH polyhouse, Medziphema (Table 4.1). Soil samples were taken from the rhizosphere of healthy tomato plants (Plate 5A). The individual sample was mixed thoroughly after air drying. Sixteen isolates (8 isolates of *Pseudomonas* and 8 isolates of *Trichoderma*) were isolated from tomato rhizosphere (Plate 6 and 7) by soil dilution plate technique (Waksman, 1927). For the isolation of *Trichoderma* the soil samples were serially diluted (10^{-4}) and plated on *Trichoderma* selective medium (Plate 5B). The fluorescent pseudomonads were isolated by plating the serially diluted (10^{-6}) on King's B medium (Plate 5C). Seventeen isolates of *Trichoderma* were also obtained from the department of Plant Pathology, SASRD, Nagaland University, Medziphema.

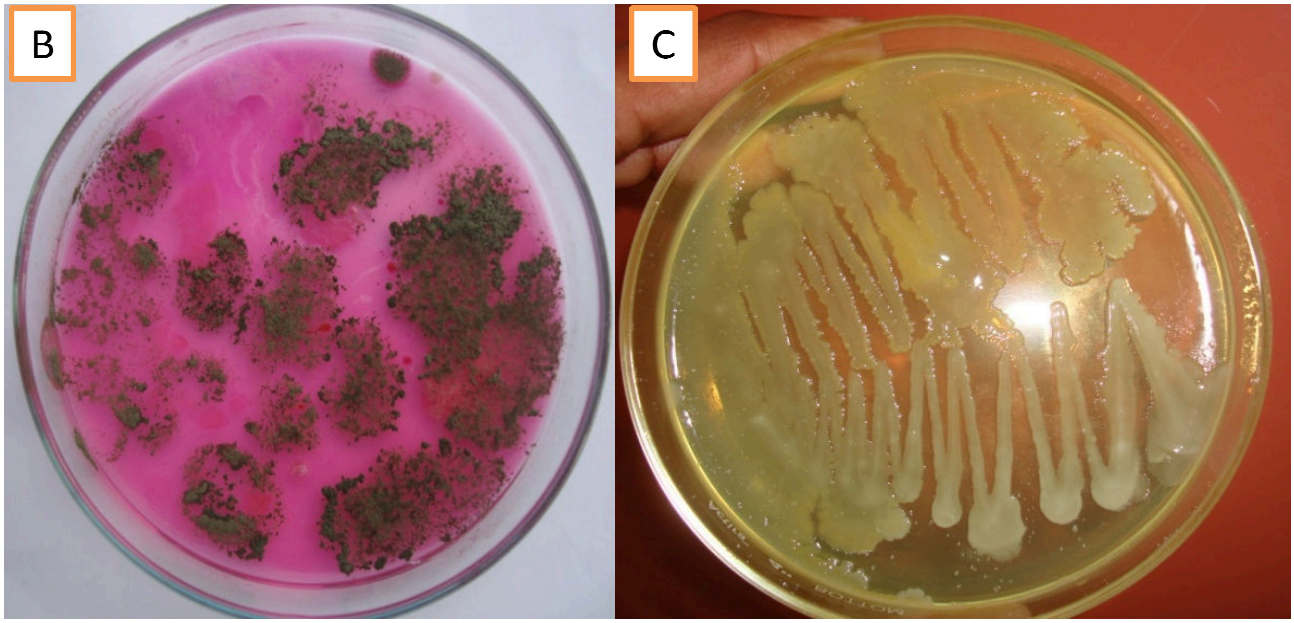
Similarly soil sampling and soil dilution plate technique were used by Waksman (1927), Naseby *et al.* (2000), Torres-Rubio *et al*. (2000), Rai (2017) and Lalngaihawmi and Bhattacharyya (2019) for the isolation of rhizosphere microbes.

Table 4.1 Native biocontrol agents (BCAs) and their collection locations

S.No.	Isolate code	Isolation from	Location	District	State
(A) Native *Pseudomonas* isolates					
01	Pf-1	Tomato Rhizosphere	Polyhouse (Sample-I), CIH, Medziphema	Dimapur	Nagaland
02	Pf-2	Tomato Rhizosphere	Tomato field (Sample-I), Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
03	Pf-3	Tomato rhizosphere	Farmers' field (Sample-I), Merema	Kohima	Nagaland
04	Pf-4	Tomato rhizosphere	Farmers' field (Sample-I), Tsiesema	Kohima	Nagaland
05	Pf-5	Tomato rhizosphere	Polyhouse (Sample-II), CIH, Medziphema	Dimapur	Nagaland
06	Pf-6	Tomato rhizosphere	Tomato field (Sample-II), Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
07	Pf-7	Tomato rhizosphere	Farmers' field (Sample-II), Merema	Kohima	Nagaland
08	Pf-8	Tomato Rhizosphere	Farmers' field (Sample-II), Tsiesema	Kohima	Nagaland
(B) Native *Trichoderma* isolates					
09	T-1 (*T. harzianum*)	Virgin forest	DBT project site, Dziilakie	Dimapur	Nagaland
10	T-2 (*T. virens*)	Virgin forest	DBT project site, Dziilakie	Dimapur	Nagaland
11	T-3 (*T. asperellum*)	Virgin forest	DBT project site, Dziilakie	Dimapur	Nagaland
12	T-4	Tomato rhizosphere	Polyhouse (Sample-I), CIH, Medziphema	Dimapur	Nagaland
13	T-5	Tomato rhizosphere	Tomato field (Sample-I), Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
14	T-6	Tomato rhizosphere	Farmers' field (Sample-I), Merema	Kohima	Nagaland
15	T-7	Tomato rhizosphere	Farmers' field (Sample-I), Tsiesema	Kohima	Nagaland
16	T-8	Tomato rhizosphere	Polyhouse (Sample-II), CIH, Medziphema	Dimapur	Nagaland
17	T-9	Tomato rhizosphere	Tomato field (Sample-II), Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
18	T-10	Tomato rhizosphere	Farmers' field (Sample-II), Merema	Kohima	Nagaland

19	T-11 (*T. asperellum*)	Tomato rhizosphere	Farmers' field (Sample-II), Tsiesema	Kohima	Nagaland
20	T-12	Rice rhizosphere	Upland Rice field-I, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
21	T-13	Rice rhizosphere	Upland Rice field-II, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
22	T-14 (*T. asperellum*)	Rice rhizosphere	Upland Rice field-III, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
23	T-15	Rice rhizosphere	Upland Rice field-IV, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
24	T-16	Rice rhizosphere	Upland Rice field-V, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
25	T-17	Soils	Fallow land, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
26	T-18	Soils	Upland Rice field-I, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
27	T-19	Soils	Black gram field, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
28	T-20	Soils	Cauliflower field, Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
29	T-21	Soils	Fallow land, Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
30	T-22	Soils	Upland Rice field-II, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
31	T-23	Soils	Upland Rice field-III, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
32	T-24	Soils	Upland Rice field-IV, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland
33	T-25	Soils	Soybean field, Agronomy farm, SASRD, Medziphema	Dimapur	Nagaland

(A) Different steps for soil sampling



(B) *Trichoderma* colonies on TSM medium (C) *Pseudomonas* colonies on King's B medium

Plate 5 Collection of tomato rhizosphere soil samples and isolation of tomato rhizosphere microbes

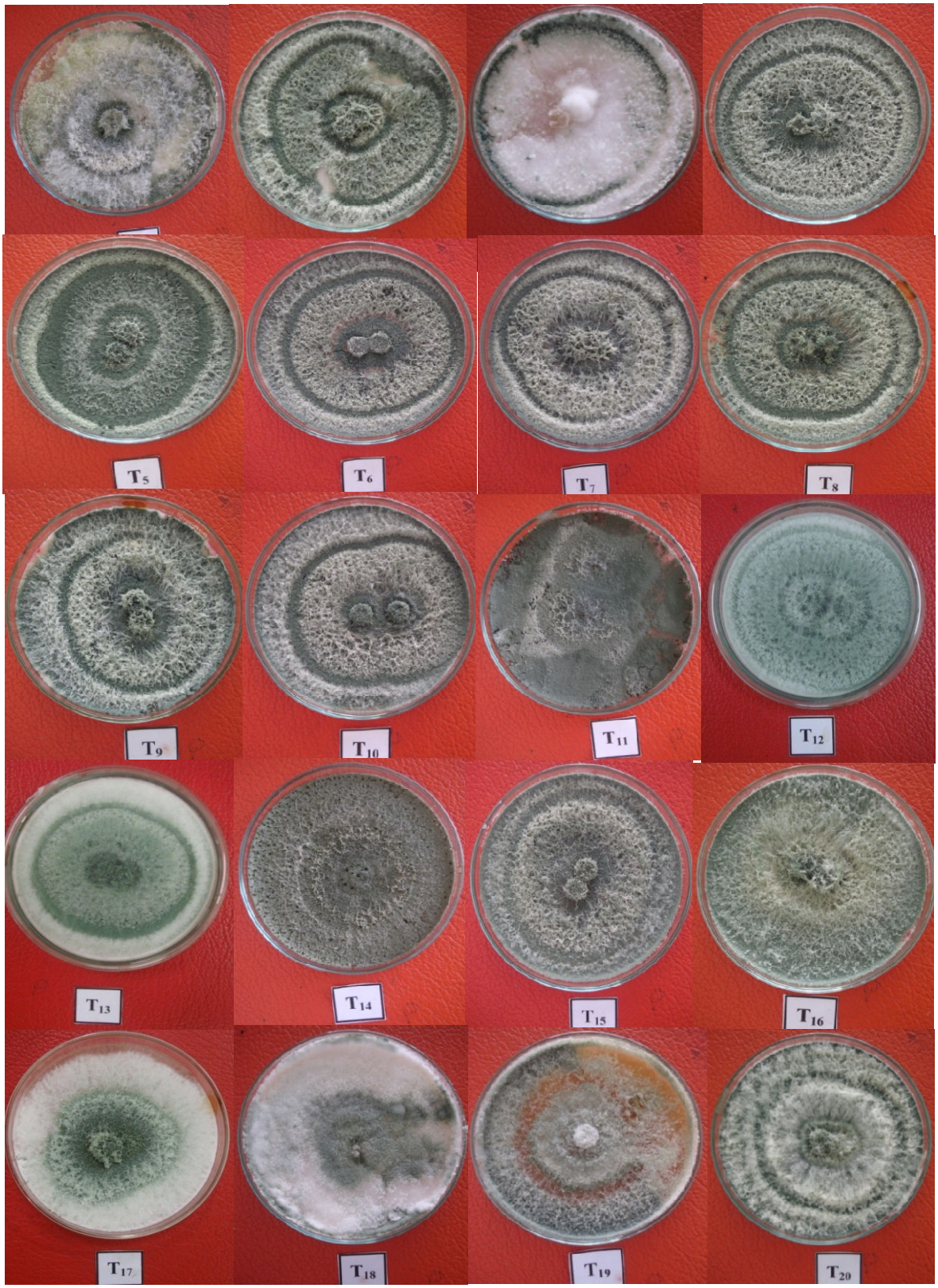
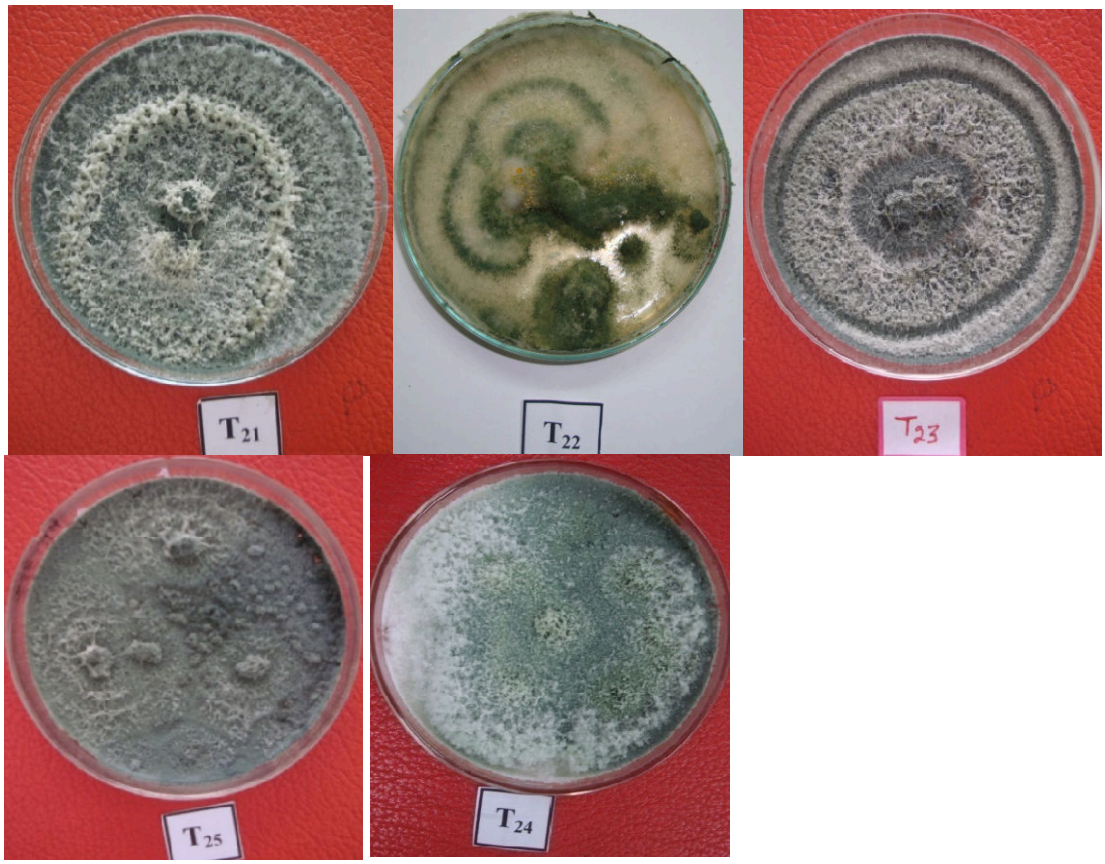
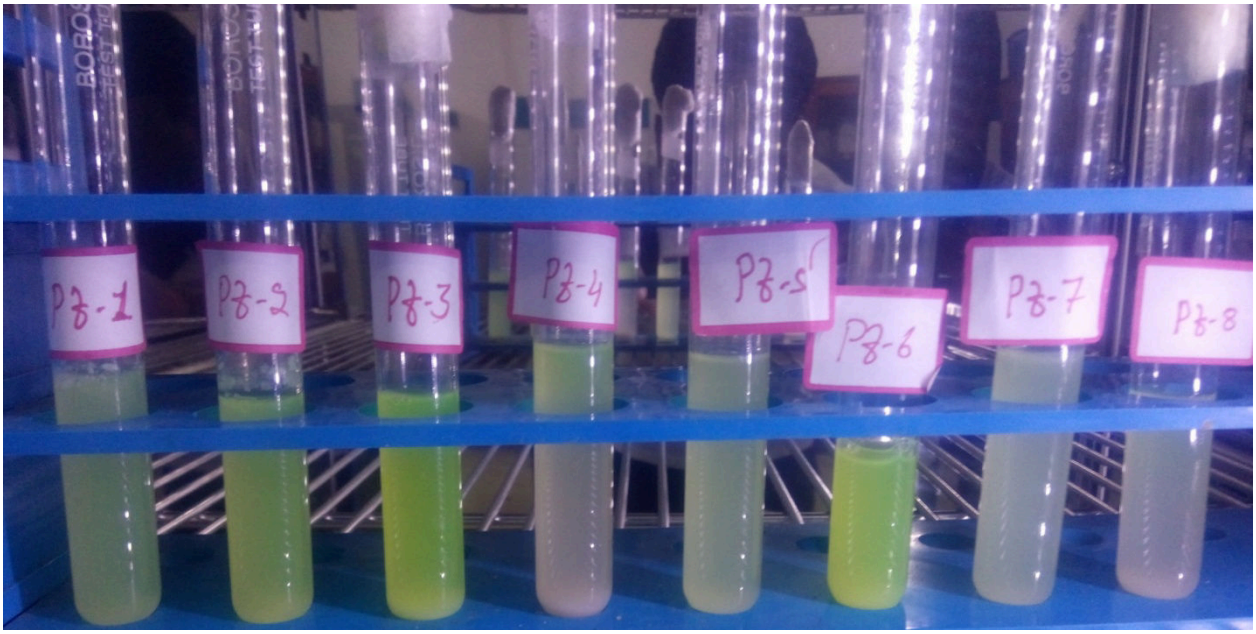


Plate 6 Native *Trichoderma* isolates (T-1 to T-20)



(a) Native *Trichoderma* isolates (T-21 to T-25)



(b) Native *Pseudomonas* isolates (Pf-1 to Pf-8**)**

Plate 7 Native *Trichoderma* and *Pseudomonas* isolates

4.4 Evaluation of antagonists against *P. infestans*

4.4.1 *In vitro* antagonistic effect of *Trichoderma* isolates on radial growth of the pathogen

Altogether 25 isolates of *Trichoderma* were screened for their inhibitory action on the radial growth of *P. infestans* by adopting dual culture technique (Sivakumar *et al.,* 2000) and the data obtained are presented in table 4.2 and fig 4.1. All isolates screened against *P. infestans* were significantly superior over control (Plates 8, 9 and 10). It was found that the growth of the pathogen in dual culture plates progressed until they came in contact with the leading edges of the antagonist. Among the different treatments, least radial mycelial growth of the pathogen was recorded in T-11 (1.97 cm) followed by T-14 (2.50 cm), T-5 (2.63 cm) and T-25 (2.70 cm) respectively. The per cent inhibition over control was calculated and it was observed that T-11 was the most promising isolate against *P. infestans* with 73.73 per cent inhibition after 8 days of incubation. Next best isolate was T-14 (66.67 %) followed by T-5 (64.93 %), T-25 (64.00 %) and the least antagonistic effect was observed in T-17 (51.07 %) at 8 days after incubation at 18±1 °C.

In vitro study was taken up to select the best *Trichoderma* isolates against the test pathogen. Twenty five isolates of *Trichoderma* isolated from various rhizospheres were tested for their antagonistic effect against *P. infestans* by dual culture method. T-11 and T-14 inhibited the pathogen significantly and were found to be the most effective BCAs. Hence, they were selected for further eveluation.

The results of the present findings are in conformity with the findings of earlier workers (Patel and Mukadam, 2011; Zegeye *et al.,* 2011; Kumar *et al*., 2015).

Majaw *et al*. (2016) evaluated native BCAs against *P. infestans*. BCAs were isolated from different agricultural practices of Meghalaya. They found the maximum inhibition of test pathogen growth by *T. viride* (62.22 %) followed by *T. harzianum* (55.55 %). These isolates were obtained from tomato rhizosphere.

Kumar *et al*. (2015) also reported that maximum inhibition of *P. infestans* growth (80.83 %) among biological agents was observed in *T. viride* followed by *T. reesei* (80.33 %), *T. atroviride* (79.17 %), *T. longibrachiatum* (75.00 %), *T. harzianum* (72.92 %), *T. virens* (71.25 %), *T. asperellum* (68.75 %) and *T. koningii* (66.25 %). These isolates were obtained from tomato rhizosphere.

Results obtained by Rai (2017) indicated that *Trichoderma* spp. produce extracellular cell wall degrading enzymes like chitinase, β-1, 3 glucanase, protease, cellulase and lectin, which help them in colonising the host and inhibit soil-borne fungi. *Trichoderma* spp. employs a number of mechanisms to combat the effect of plant pathogens. They are known to secrete secondary metabolites, mycoparasitism, competition for food and space and induction of host defence response (Dennis and Webster, 1971; Kapri and Tewari, 2010; Dixit *et al.,* 2015; Vrieze *et al*., 2018).

In the present investigation, the high inhibitory activity of the antagonists observed on *P. infestans* in dual cultures, the frequency of mycoparasitic activities and antibiosis by the antagonists may be attributed to the production of these toxic metabolites, volatile gases and cell-wall degrading enzymes.

Table 4.2 *In vitro* antagonistic effect of *Trichoderma* isolates on radial growth and per cent inhibition of *P. infestans*

Treatment		Inhibition of *P. infestans* growth		
		Radial growth (cm)	Radial growth (cm) inhibited	Inhibition (%)
T_0	Control *P. infestans* alone	07.50	00.00	00.00 (4.05)*
T_1	*P. infestans* + T-1	02.97	04.53	60.40 (57.0)
T_2	*P. infestans* + T-2	03.43	04.07	54.27 (47.45)
T_3	*P. infestans* + T-3	03.53	03.97	52.93 (46.68)
T_4	*P. infestans* + T-4	03.30	04.20	56.00 (48.45)
T_5	*P. infestans* + T-5	02.63	04.87	64.93 (53.69)
T_6	*P. infestans* + T-6	03.20	04.30	57.33 (49.22)
T_7	*P. infestans* + T-7	03.60	03.90	52.00 (46.15)
T_8	*P. infestans* + T-8	02.90	04.60	61.33 (51.55)
T_9	*P. infestans* + T-9	03.17	04.33	57.73 (49.45)
T_{10}	*P. infestans* + T-10	03.50	04.00	53.33 (46.91)
T_{11}	*P. infestans* + T-11	01.97	05.53	**73.73 (59.17)**
T_{12}	*P. infestans* + T-12	03.47	04.03	53.73 (47.14)
T_{13}	*P. infestans* + T-13	03.00	04.50	60.00 (50.77)
T_{14}	*P. infestans* + T-14	02.50	05.00	**66.67 (54.74)**
T_{15}	*P. infestans* + T-15	03.33	04.17	55.60 (48.21)
T_{16}	*P. infestans* + T-16	03.57	03.93	52.40 (46.38)
T_{17}	*P. infestans* + T-17	03.67	03.83	51.07 (45.61)
T_{18}	*P. infestans* + T-18	03.03	04.47	59.60 (50.54)
T_{19}	*P. infestans* + T-19	02.80	04.70	62.67 (52.34)
T_{20}	*P. infestans* + T-20	02.93	04.57	60.93 (51.32)
T_{21}	*P. infestans* + T-21	03.23	04.27	56.93 (48.99)
T_{22}	*P. infestans* + T-22	03.10	04.40	58.67 (49.99)
T_{23}	*P. infestans* + T-23	03.30	04.20	56.00 (48.45)
T_{24}	*P. infestans* + T-24	03.17	04.33	57.73 (49.45)
T_{25}	*P. infestans* + T-25	02.70	04.80	64.00 (53.13)
SEm±		0.07	0.07	0.91 (0.60)
C.V. (%)		3.70	2.82	2.81 (1.91)
CD (*p*= 0.01)		0.27	0.26	3.45 (2.01)
CD (*p* = 0.05)		0.20	0.19	2.59 (1.51)

***Values in parentheses are angular transformed values.**

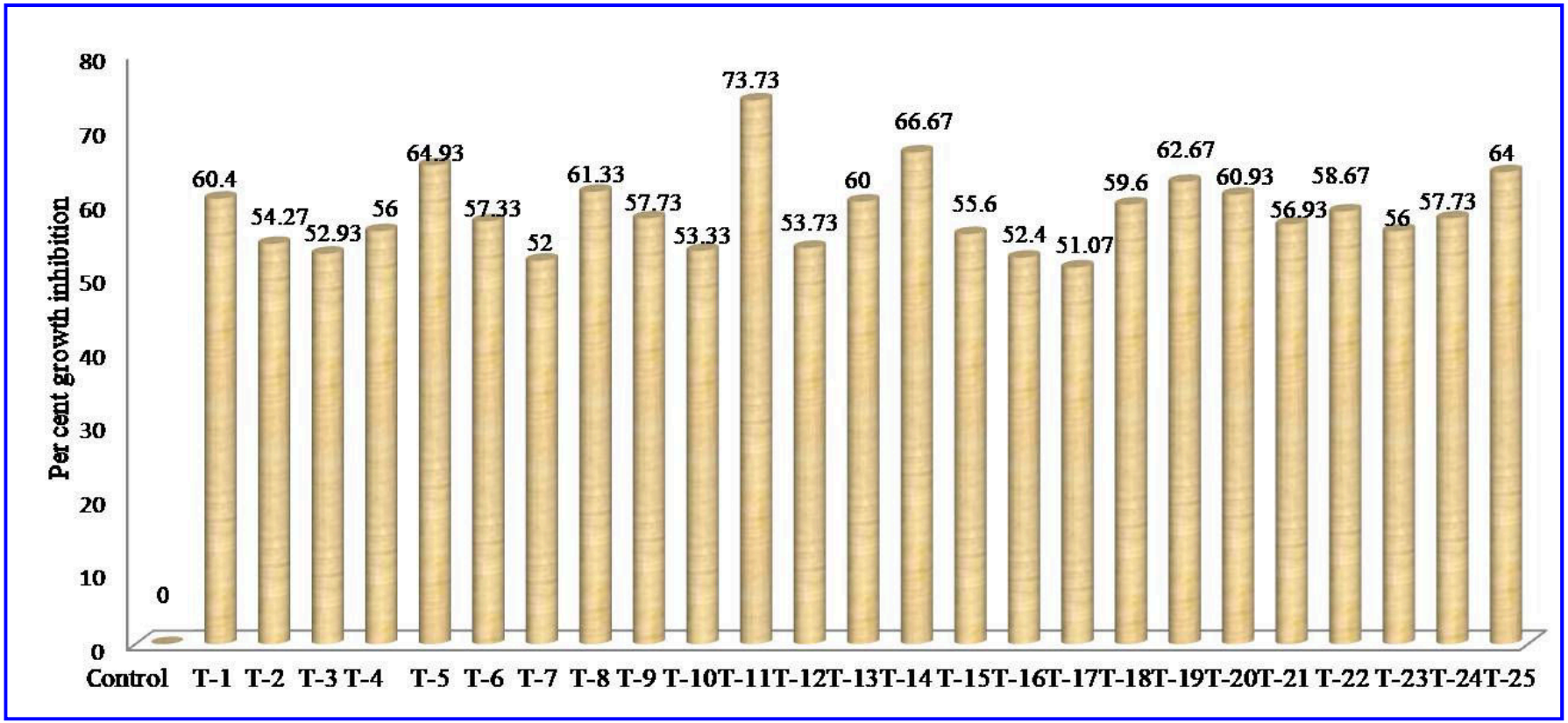


Fig 4.1 *In vitro* antagonistic effect of *Trichoderma* isolates on per cent growth inhibition of *P. infestans*

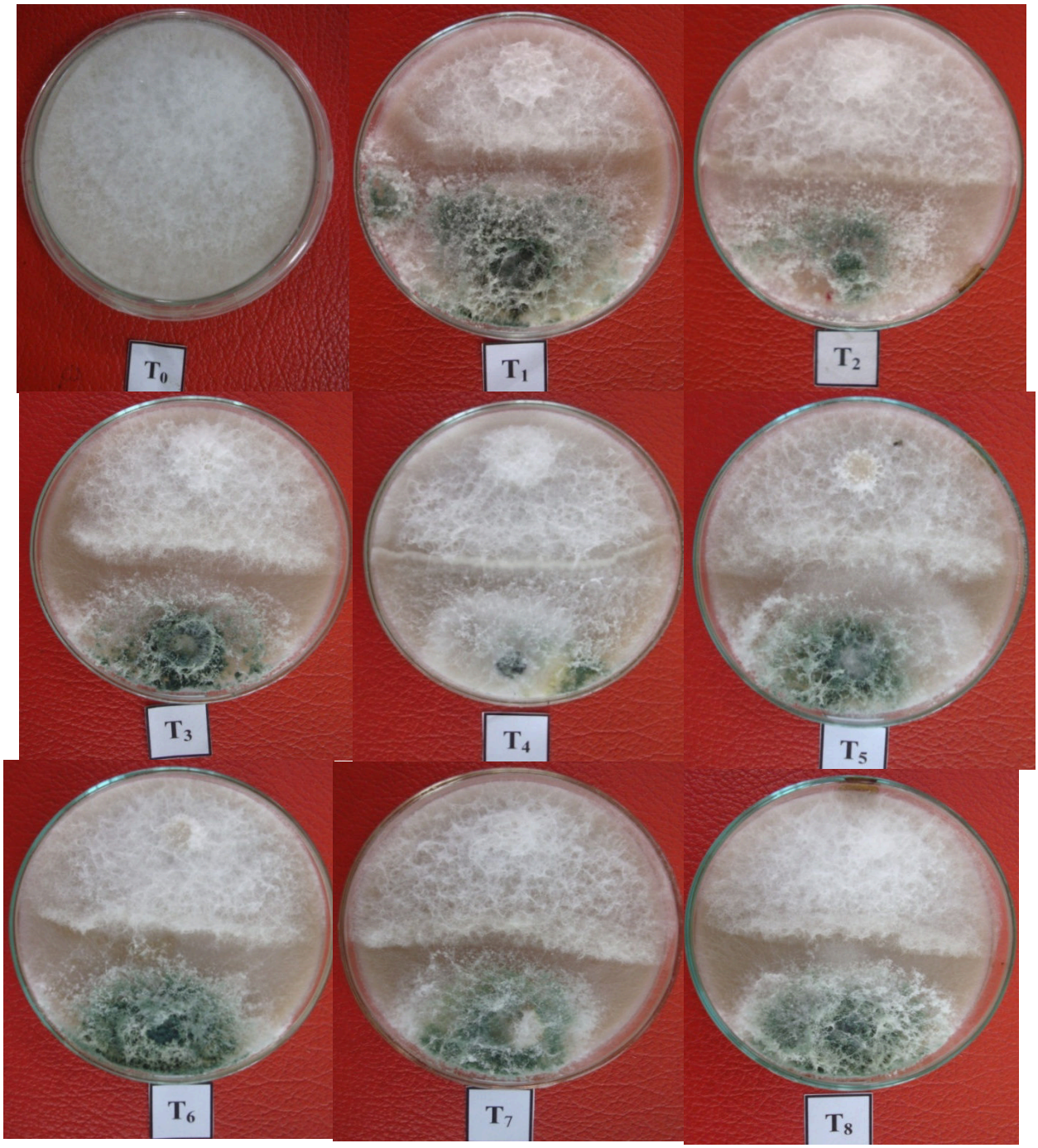


Plate 8 *In vitro* antagonistic effect of *Trichoderma* isolates (T-1 to T-8) on radial growth of *P. infestans*

T_0 (Control- *P. infestans* alone) **T_1** (*P. infestans* + T-1) **T_2** (*P. infestans* + T-2)
T_3 (*P. infestans* + T-3) **T_4** (*P. infestans* + T-4) **T_5** (*P. infestans* + T-5)
T_6 (*P. infestans* + T-6) **T_7** (*P. infestans* + T-7) **T_8** (*P. infestans* + T-8)

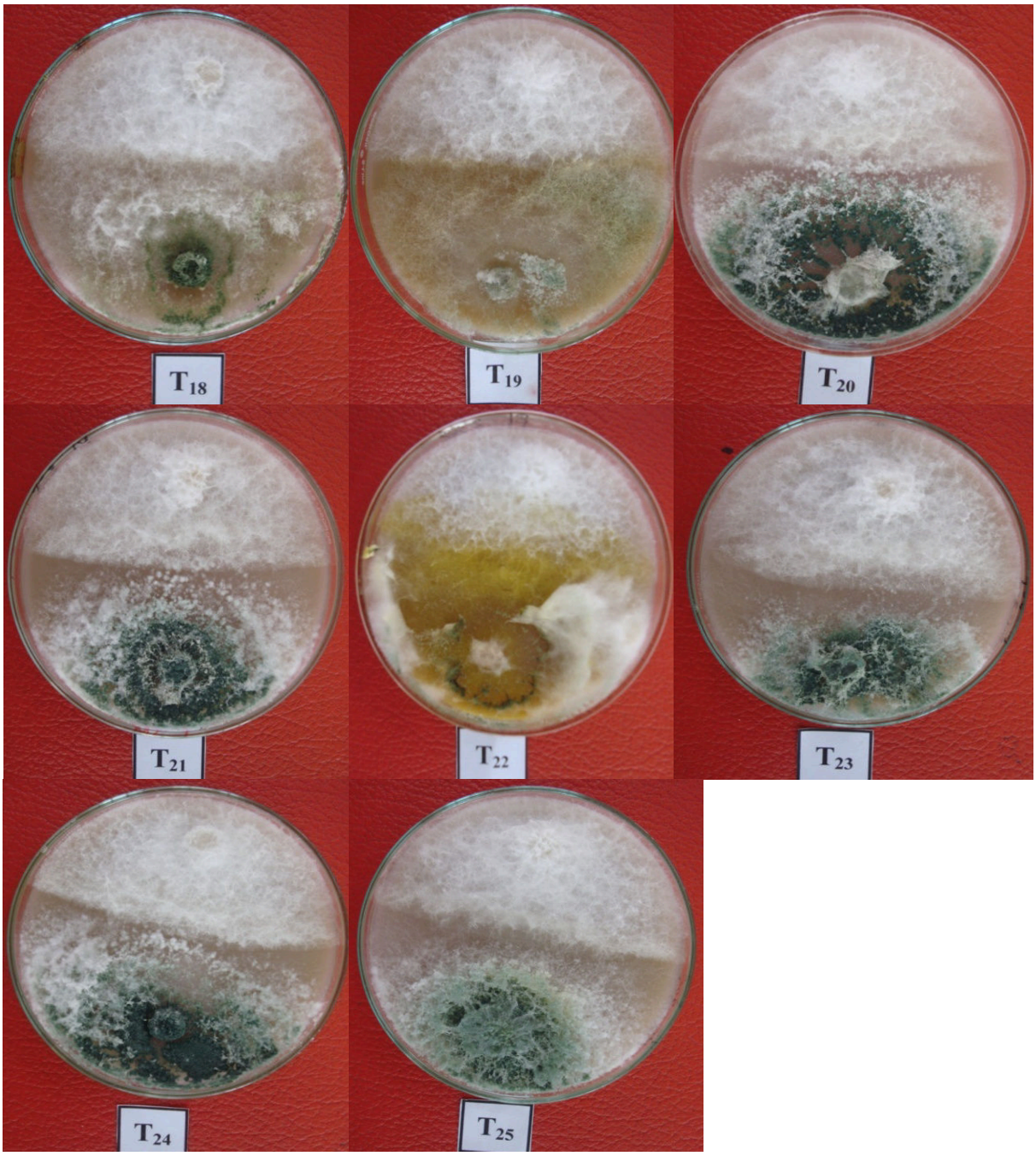


Plate 10 *In vitro* antagonistic effect of *Trichoderma* isolates (T-18 to T-25) on radial growth of *P. infestan*s

T_{18} (*P. infestans* + T-18) **T_{19}** (*P. infestans* + T-19) **T_{20}** (*P. infestans* + T-20)
T_{21} (*P. infestans* + T-21) **T_{22}** (*P. infestans* + T-22) **T_{23}** (*P. infestans* + T-23)
T_{24} (*P. infestans* + T-24) **T_{25}** (*P. infestans* + T-25)

4.4.2 *In vitro* antagonistic effect of *Pseudomonas* isolates on radial growth of the pathogen

The antagonistic effects of *Pseudomonas* isolates were evaluated against *P. infestans* by slight modification of dual culture technique (Georgakopoulos *et al*., 2002) and the data obtained are presented in table 4.3 and fig 4.2. All isolates screened against *P. infestans* significantly inhibited the growth of the fungus as compared to control treatment (Plate 11). Among the *Pseudomonas* isolates, least radial mycelial growth of the pathogen was observed in Pf-2 (1.40 cm) followed by Pf-3 (2.00 cm), Pf-1 (2.30 cm), Pf-7 (2.50 cm) and Pf-4 (2.60 cm) respectively. Out of these isolates maximum per cent inhibition was observed in Pf-2 (81.33 %) which is significantly superior to all other treatments followed by Pf-3 (73.33 %), Pf-1 (69.33 %), Pf-7 (66.67 %) and Pf-4 (65.33 %) at 8 days after incubation at 18±1 °C. The clear zone of inhibition was also observed in the dual culture plate of Pf-1, Pf-2 and Pf-3.

In vitro study was taken up to select the potential *Pseudomonas* isolates against the test pathogen. Eight isolates of *Pseudomonas* isolated from tomato rhizosphere were tested for their antagonistic effect against *P. infestans* by dual culture method. Pf-2 and Pf-3 inhibited the pathogen significantly and were found to be the most effective BCAs. Hence, they were selected for further evaluation.

The findings of present work are in harmony with the findings of earlier workers (Torres-Rubio *et al.,* 2000; Anith *et al.,* 2002; Zegeye *et al*., 2011; Kabir *et al.,* 2013; Lamsal *et al.,* 2013).

Naseby *et al.* (2000) recorded *P. fluorescens* inhibited *P. infestans* mycelial growth (88 %). The clear inhibition zone was also recorded in dual culture plate. This isolate was obtained from tomato rhizosphere.

In the present investigation, the probable reasons of high inhibitory activity of the antagonists observed on *P. infestans* in dual cultures may be due to production of antifungal metabolites such as pyrolnitrin, 2-4 diacetyl phloroglucinol, HCN and siderophores.

Table 4.3 *In vitro* antagonistic effect of *Pseudomonas* isolates on radial growth and per cent inhibition of *P. infestans*

Treatment		Inhibition of *P. infestans* growth		
		Radial growth (cm)	Radial growth (cm) inhibited	Inhibition (%)
T_0	Control *P. infestans* alone	07.50	00.00	00.00 (4.05)*
T_1	*P. infestans* + Pf-1	02.30	05.20	69.33 (56.40)
T_2	*P. infestans* + Pf-2	01.40	06.10	**81.33 (64.44)**
T_3	*P. infestans* + Pf-3	02.00	05.50	**73.33 (58.91)**
T_4	*P. infestans* + Pf-4	02.60	04.90	65.33 (53.93)
T_5	*P. infestans* + Pf-5	02.97	04.53	60.44 (51.03)
T_6	*P. infestans* + Pf-6	03.00	04.50	60.00 (50.77)
T_7	*P. infestans* + Pf-7	02.50	05.00	66.67 (54.78)
T_8	*P. infestans* + Pf-8	02.70	04.80	64.00 (53.14)
SEm±		0.12	0.12	1.65 (0.88)
C.V. (%)		7.15	4.76	4.76 (3.57)
CD (*p*= 0.01)		0.50	0.50	6.72 (4.17)
CD (*p*= 0.05)		0.37	0.37	4.90 (3.04)

***Values in parentheses are angular transformed values.**

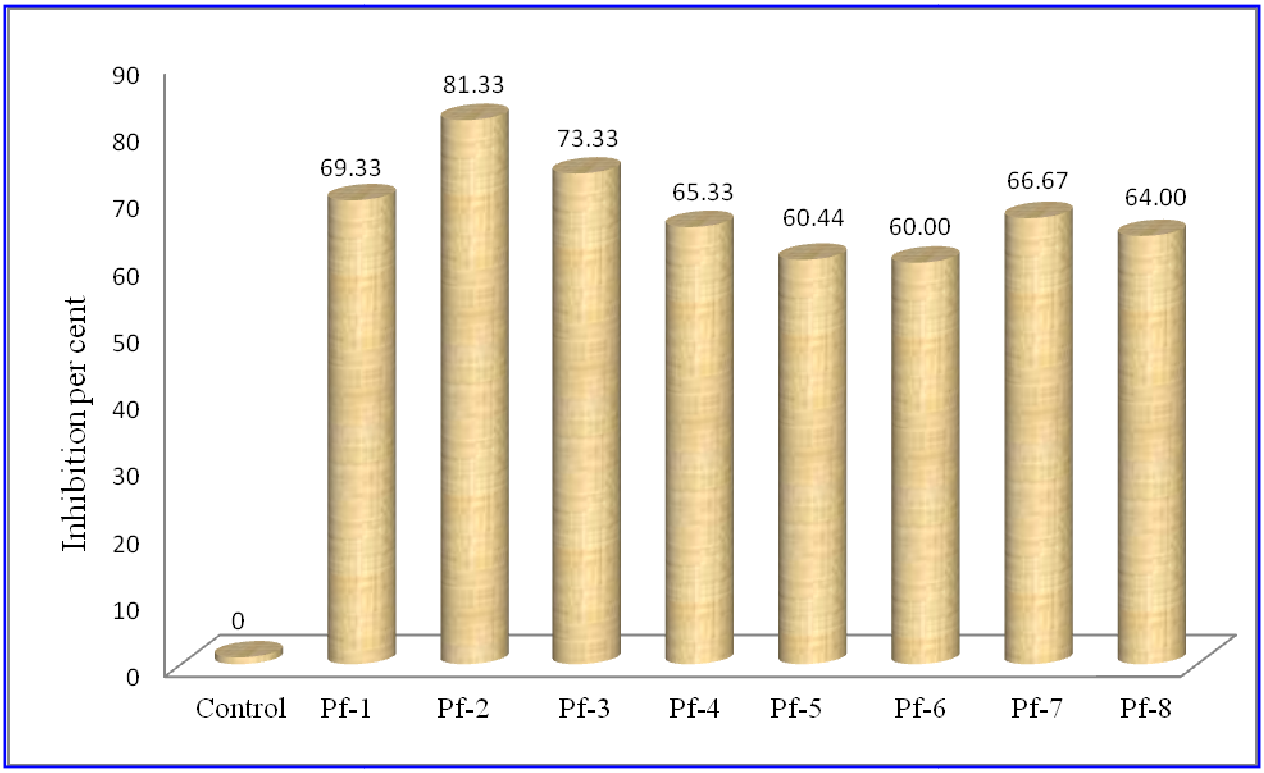


Fig 4.2 *In vitro* antagonistic effect of *Pseudomonas* isolates on per cent growth inhibition of *P. infestans*

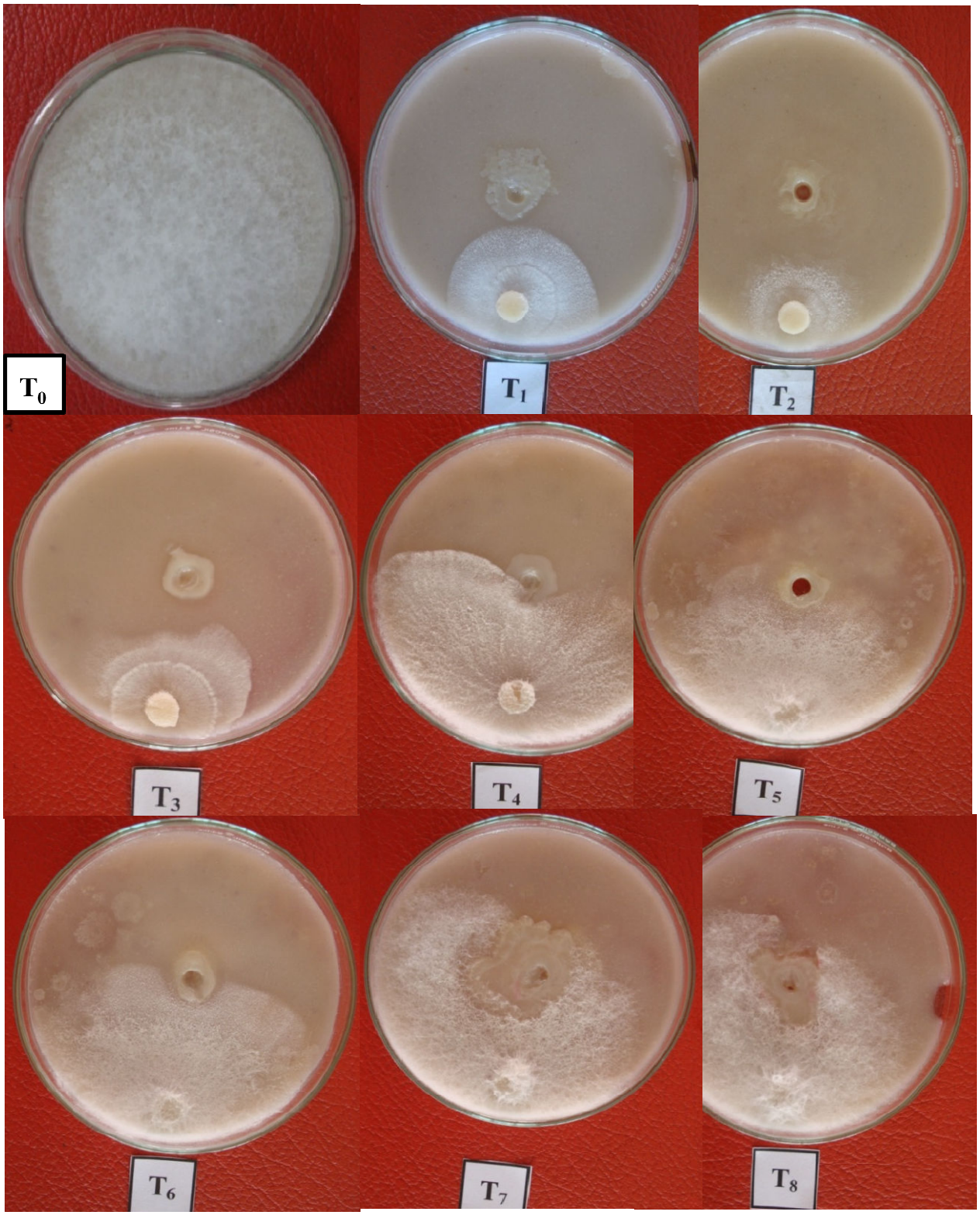


Plate 11 ***In vitro*** **antagonistic effect of** ***Pseudomonas*** **isolates on radial growth of** ***P. infestans***

T0 (Control- *P. infestans* alone) **T1** (*P. infestans* + Pf-1) **T2** (*P. infestans* + Pf-2)
T3 (*P. infestans* + Pf-3) **T4** (*P. infestans* + Pf-4) **T5** (*P. infestans* + Pf-5)
T6 (*P. infestans* + Pf-6) **T7** (*P. infestans* + Pf-7) **T8** (*P. infestans* + Pf-8)

4.5 Investigation on the biocontrol mechanisms of BCAs

4.5.1 Volatile metabolites

The effects of volatile metabolites of *Trichoderma* isolates (25 isolates) were assessed against *P. infestans* by adopting the technique given by Dennis and Webster (1971), and the data thus obtained are presented in table 4.4 and fig 4.3. All isolates assessed against *P. infestans* were significantly superior over control treatment (Plates 12, 13 and 14). Among the different treatments, least mycelial growth of the pathogen was recorded in T-11 (4.90 cm) followed by T-14 (5.80 cm), T-21 (5.83 cm), T-1 (5.87 cm) and T-25 (6.07 cm) respectively at 6 days after incubation at 18±1 °C. The per cent inhibition over control was calculated and it was observed that T-11 was found to be most promising in production of volatile compounds against *P. infestans* with 45.55 per cent inhibition. Next best in order of merit was T-14 (35.55 %) followed by T-21 (35.22 %), T-1 (34.78 %), T-25 (32.55 %) respectively and the least antagonist effect was shown by T-18 (12.22 %).

Data presented in table 4.5 and fig 4.4 represents the effects of volatile metabolites of *Pseudomonas* isolates (8 isolates) assessed against *P. infestans* following the technique given by Dennis and Webster (1971). All isolates assessed against *P. infestans* were also significantly superior over control (Plate 15). Among the *Pseudomonas* isolates, least mycelial growth of test pathogen was observed in Pf-2 (4.17 cm) at 6 days after incubation at 18±1 °C. The next best in order of merit were Pf-3 (4.67 cm), Pf-1 (4.97 cm), Pf-6 (5.77 cm) and Pf-8 (6.70 cm) respectively. Out of these maximum per cent inhibition was observed in Pf-2 (53.67 %) which is significantly superior to all other treatments followed by Pf-3 (48.11 %), Pf-1 (44.78 %), Pf-6 (35.89 %) and Pf-8 (25.56 %) respectively.

The findings of present work corroborate with the works done by earlier workers (Dennis and Webster, 1971; Laha *et al.,* 1996; Kapri and Tewari *et al.,* 2010; Manikandan *et al.,* 2010; Dixit *et al*., 2015). Vrieze *et al*. (2018) tested volatile metabolite of 39 isolates of *Pseudomonas* and 29 isolates of *Trichoderma* against *P. infestans***.** Among tested isolates, *Trichoderma* isolate OTPB3 (92.06 %) and *P. fluorescens* OTPB1 (88.1 %) exhibited maximum inhibition against *P. infestans*. They also reported that different strains of *Trichoderma* spp. produced 15 volatile compounds such as viridian, normal saturated hydrocarbons (C7–C30), cyclopentane, cyclohexane, alcohols, benzene derivatives, trichotoxin, gliotoxin, fatty acids, esters, simple pyrane, sulfurcontaining compounds, trichodermin, paracel icine, trichotoxin, gliotoxin, staldeid and viridian. These isolates were obtained from tomato rhizosphere.

Many volatile metabolites have been recorded to be involved in microbial interactions. Plant associated and soil microorganisms produced one group of volatile metabolites, which are belived to be volatile organic compounds (VOCs). VOCs are high vapour pressure, posses a lipophilic moiety, small, odorous compounds (<C15) with low molecular mass (<300 Da) and low boiling point. Volatile compounds of microbes have the evaporation and diffusion in rhizosphere environment. Microbial VOCs belong to different chemical classes including sulphides, benzenoids, alkenes, alcohols, benzenoids, ketones, pyrazines, terpenes and sulfides (Vespermann *et al*., 2007).

These reports are in agreement with the results of the present investigation, which suggests that the production of volatile metabolites by both *Trichoderma* and *Pseudomonas* isolates have definite influence on the high degree of inhibition of *P. infestans.*

Table 4.4 *In vitro* effect of volatile metabolites of *Trichoderma* isolates on mycelial growth and per cent inhibition of *P. infestans*

Treatment		Inhibition of *P. infestans* growth		
		Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)
T_0	Control *P. infestans* alone	09.00	00.00	00.00 (4.05)*
T_1	*P. infestans* + T-1)	05.87	03.13	34.78 (36.12)
T_2	*P. infestans* + T-2	06.63	02.37	26.33 (30.87)
T_3	*P. infestans* + T-3	07.23	01.77	19.67 (26.29)
T_4	*P. infestans* + T-4	06.10	02.90	32.22 (32.40)
T_5	*P. infestans* + T-5	07.73	01.27	14.11 (22.02)
T_6	*P. infestans* + T-6	07.03	01.97	21.89 (27.88)
T_7	*P. infestans* + T-7	07.70	01.30	14.44 (23.31)
T_8	*P. infestans* + T-8	07.37	01.63	18.11 (25.07)
T_9	*P. infestans* + T-9	06.20	02.80	31.11 (33.89)
T_{10}	*P. infestans* + T-10	06.47	02.53	28.11 (31.97)
T_{11}	*P. infestans* + T-11	04.90	04.10	**45.55 (42.45)**
T_{12}	*P. infestans* + T-12	07.50	01.50	16.67 (24.09)
T_{13}	*P. infestans* + T-13	06.67	02.33	25.89 (30.57)
T_{14}	*P. infestans* + T-14	05.80	03.20	**35.55 (36.60)**
T_{15}	*P. infestans* + T-15	06.30	02.70	30.00 (33.21)
T_{16}	*P. infestans* + T-16	06.57	02.43	27.00 (31.30)
T_{17}	*P. infestans* + T-17	07.63	01.37	15.22 (22.94)
T_{18}	*P. infestans* + T-18	07.90	01.10	12.22 (20.45)
T_{19}	*P. infestans* + T-19	06.70	02.30	25.55 (30.35)
T_{20}	*P. infestans* + T-20	06.87	02.13	23.67 (29.10)
T_{21}	*P. infestans* + T-21	05.83	03.17	35.22 (36.40)
T_{22}	*P. infestans* + T-22	06.67	02.33	25.89 (30.58)
T_{23}	*P. infestans* + T-23	07.83	01.17	13.00 (21.10)
T_{24}	*P. infestans* + T-24	07.50	01.50	16.67 (24.02)
T_{25}	*P. infestans* + T-25	06.07	02.93	32.55 (34.79)
SEm±		0.12	0.12	1.30 (0.81)
C.V. (%)		2.96	9.40	9.42 (6.25)
CD (*p*= 0.01)		0.44	0.44	4.91 (3.89)
CD (*p*= 0.05)		0.33	0.33	3.69 (2.92)

***Values in parentheses are angular transformed values.**

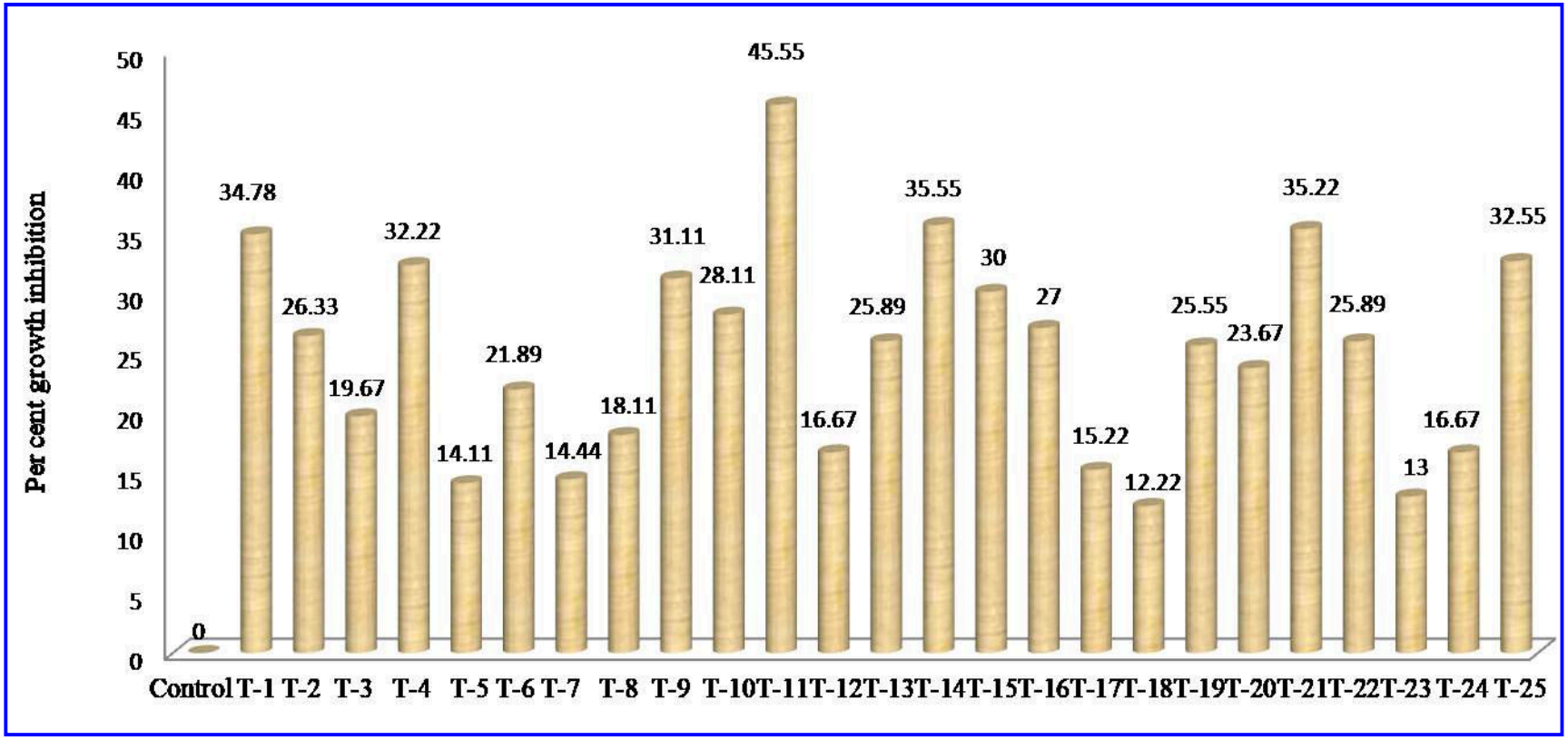


Fig 4.3 *In vitro* effect of volatile metabolites of *Trichoderma* isolates on per cent growth inhibition of *P. infestans*

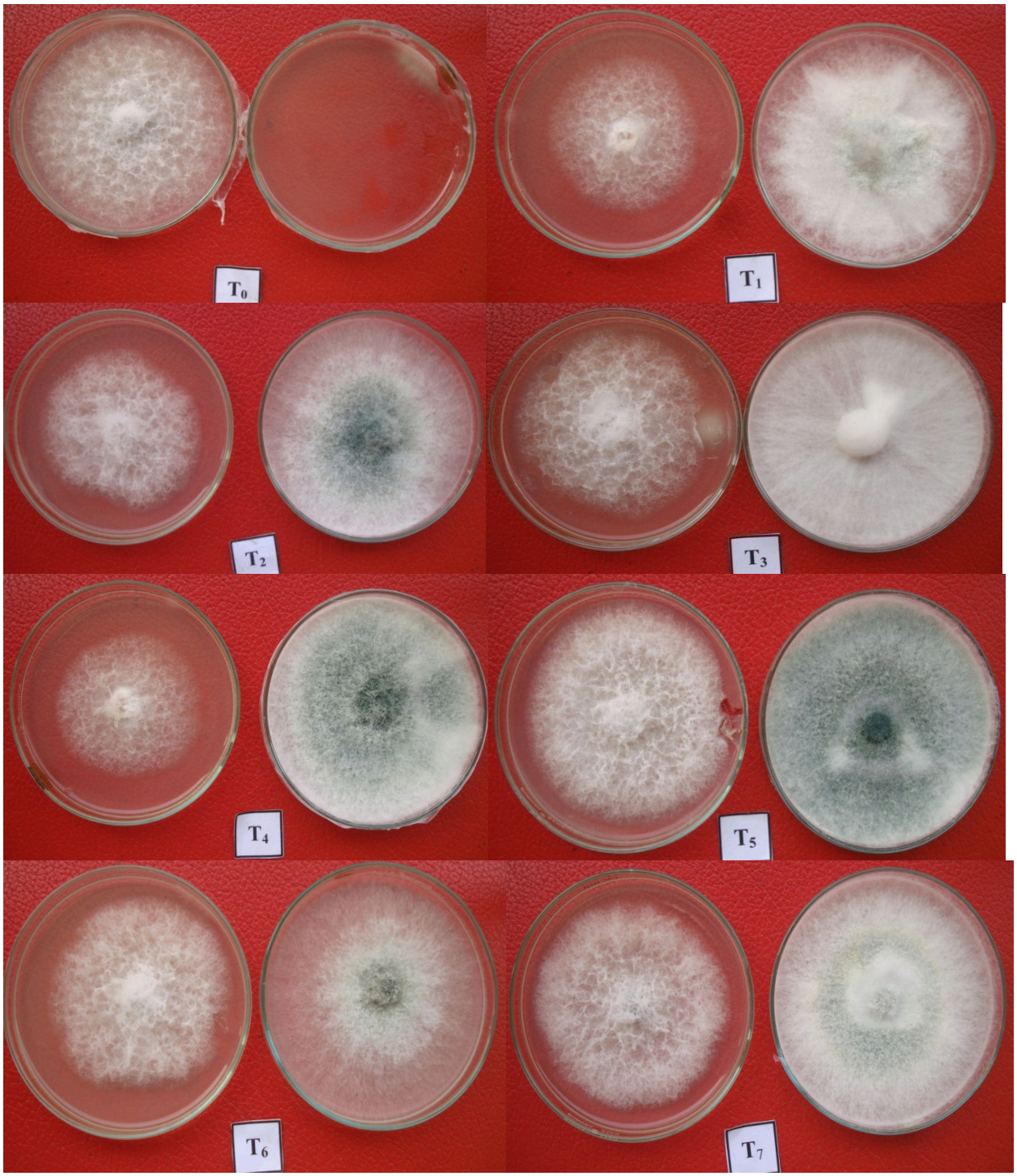


Plate 12 *In vitro* effect of volatile metabolites of *Trichoderma* isolates (T-1 to T-7) on growth of *P. infestans*

T_0 (Control- *P. infestans* alone)

T_1 (*P. infestans* + T-1)

T_2 (*P. infestans* + T-2)

T_3 (*P. infestans* + T-3)

T_4 (*P. infestans* + T-4)

T_5 (*P. infestans* + T-5)

T_6 (*P. infestans* + T-6)

T_7 (*P. infestans* + T-7)

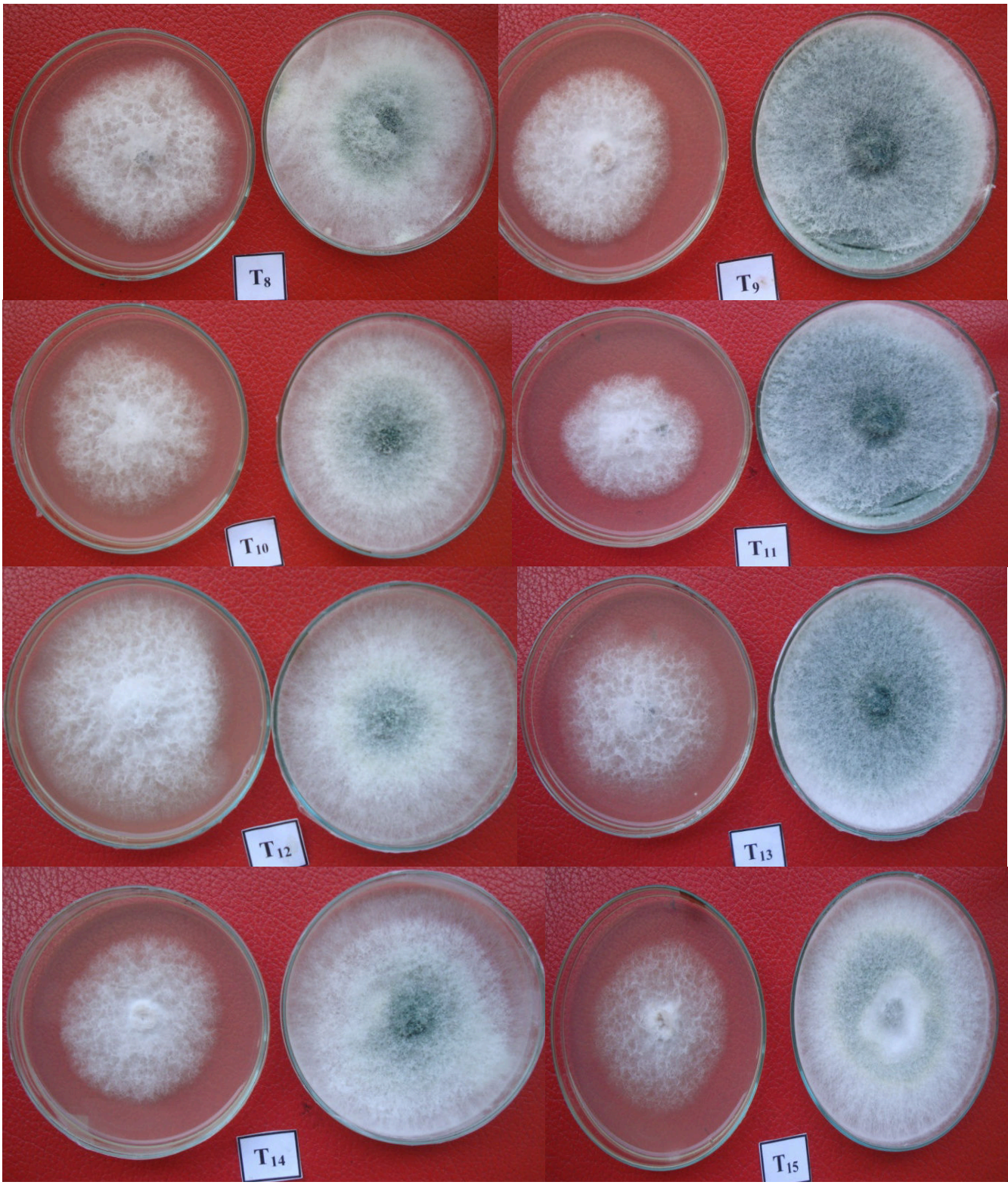


Plate 13 *In vitro* effect of volatile metabolites of *Trichoderma* isolates (T-8 to T-15) on growth of *P. infestan*s

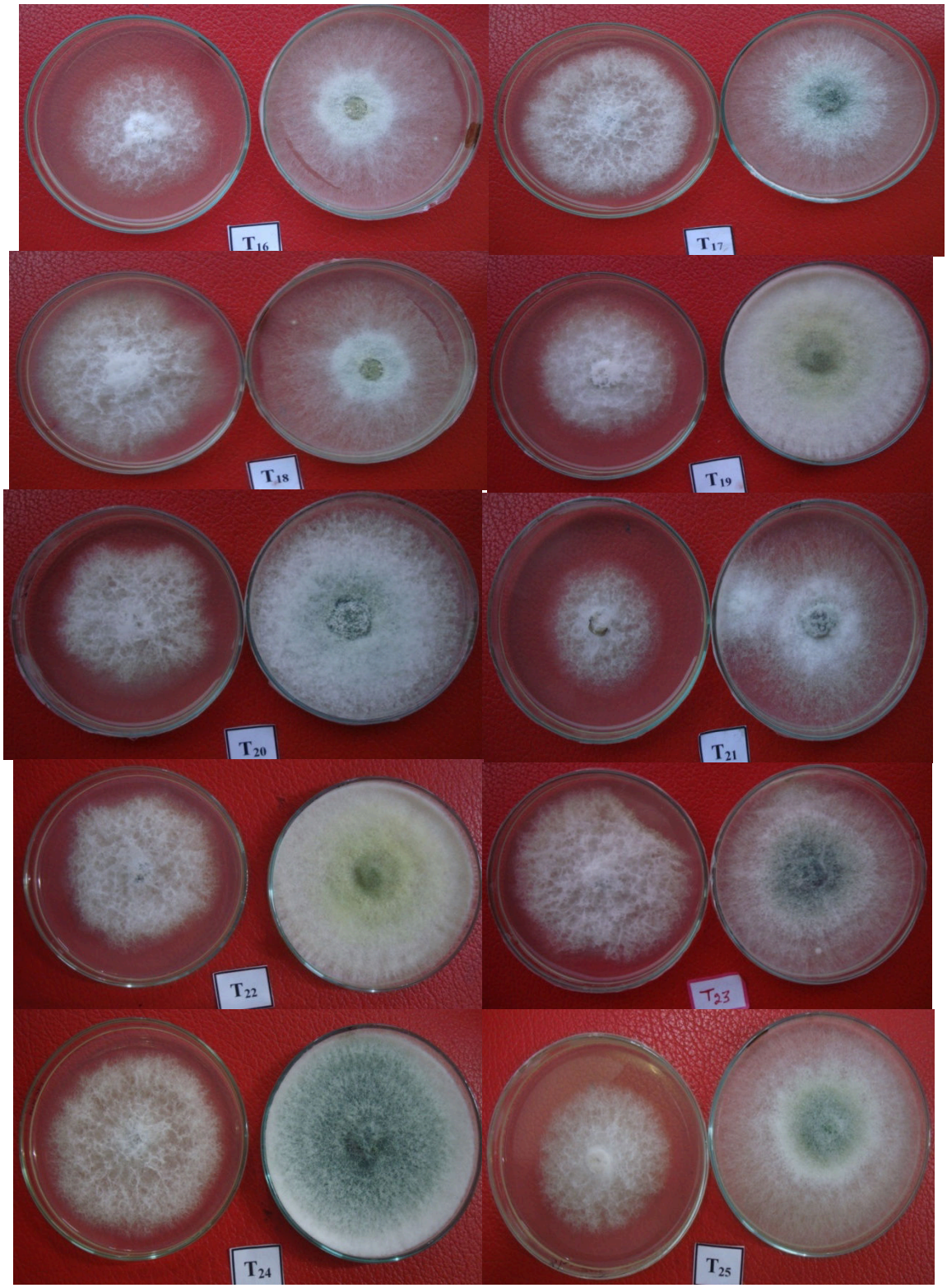


Plate 14 *In vitro* effect of volatile metabolites of *Trichoderma* isolates (T-16 to T-25) on growth of *P. infestans*

Table 4.5 *In vitro* effect of volatile metabolites of *Pseudomonas* isolates on mycelial growth and per cent inhibition of *P. infestans*

Treatment		Inhibition of *P. infestans* growth		
		Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)
T_0	Control *P. infestans* alone	09.00	00.00	00.00 (4.05)*
T_1	*P. infestans* +Pf-1	04.97	04.03	44.78 (42.00)
T_2	*P. infestans* + Pf-2	04.17	04.83	**53.67 (47.10)**
T_3	*P. infestans* + Pf-3	04.67	04.33	**48.11 (43.91)**
T_4	*P. infestans* + Pf-4	06.73	02.27	25.22 (30.13)
T_5	*P. infestans* + Pf-5	06.37	02.63	29.22 (32.72)
T_6	*P. infestans* + Pf-6	05.77	03.23	35.89 (36.79)
T_7	*P. infestans* + Pf-7	06.77	02.23	24.78 (29.83)
T_8	*P. infestans* + Pf-8	06.70	02.30	25.56 (30.34)
SEm±		0.12	0.12	1.31 (0.82)
C.V. (%)		3.32	7.09	7.11 (4.29)
CD (*p*= 0.01)		0.48	0.48	5.33 (3.33)
CD (*p*= 0.05)		0.35	0.35	3.89 (2.43)

***Values in parentheses are angular transformed values.**

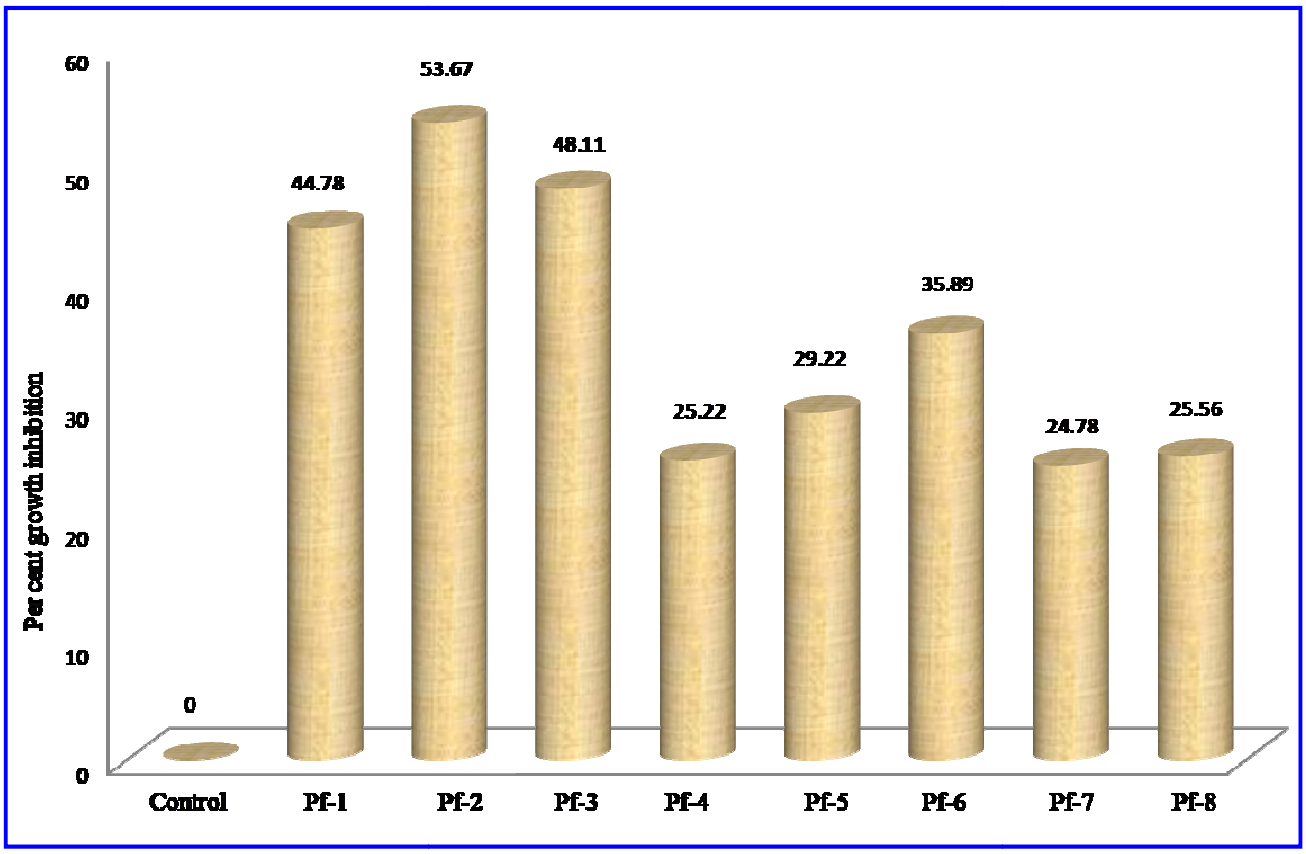


Fig 4.4 *In vitro* effect of volatile metabolites of Pseudomonas isolates on per cent growth inhibition of P. infestans

4.5.2 Ammonia production

Altogether 25 isolates of *Trichoderma* and 8 isolates of *Pseudomonas* were tested for the production of ammonia in peptone water. The results of qualitative assay of ammonia production by different native BCAs are presented in table 4.6.

All, 33 isolates showed positive results for ammonia production. Among the tested isolates, *Pseudomonas* isolates (Pf-3, Pf-4, Pf-7 and Pf-8) and *Trichoderma* isolates (T-1, T-2, T-11, T-14 and T-25) exhibited strong ammonia production by turning initial peptone water broth from yellow to dark brown colour (Plate 16a).

The findings of present work are in harmony with the findings of earlier workers (Bhakthavatchalu *et al.,* 2013; Dixit *et al.,* 2015; Prasad *et al.,* 2017; Rai, 2017). Bhakthavatchalu *et al.* (2013) tested the isolate *P. aeruginosa* FP6 for the production of ammonia, it was found positive. Dixit *et al.* (2015) evaluated 11 isolates of fluorescent *Pseudomonas* for ammonia production. All isolates showed positive result for ammonia production. These isolates were obtained from tomato rhizosphere.

Lalngaihawmi and Bhattacharyya (2019) also evaluated *Trichoderma* spp. for ammonia production and results revealed that all the *Trichoderma* spp. showed positive result. These isolates were obtained from tomato rhizosphere.

These reports are in agreement with the results of the present investigation, which suggests that the production of ammonia by both *Trichoderma* and *Pseudomonas* isolates have positive impact on the plant growth.

Table 4.6 Qualitative assay of ammonia production by different native biocontrol agents (BCAs)

BCAs	Ammonia production	BCAs	Ammonia production
Control	-	T-8	++
(A) *Pseudomonas* isolates		T-9	+++
Pf-1	+++	T-10	+++
Pf-2	+++	T-11	++++
Pf-3	++++	T-12	+
Pf-4	++++	T-13	+
Pf-5	+++	T-14	++++
Pf-6	+++	T-15	+++
Pf-7	++++	T-16	+
Pf-8	++++	T-17	+
(B) *Trichoderma* isolates		T-18	+++
T-1	++++	T-19	+++
T-2	++++	T-20	+++
T-3	+++	T-21	++
T-4	++	T-22	++
T-5	++	T-23	+++
T-6	+++	T-24	+++
T-7	+++	T-25	++++

Whereas

1) - = Negative (No colour)
2) + = Low production (Faint yellow)
3) ++ = Medium production (Deep yellow)
4) +++ = High production (Brownish)
5) ++++ = Extreme production (Brown)

4.5.3 Indole-3-acetic acid (IAA) production

The production of IAA by *Trichoderma* and *Pseudomonas* isolates were determined by qualitative assay (Gordon and Weber, 1951). Development of pink colour indicated IAA production. The results of qualitative assay of IAA production by different native BCAs are presented in table 4.7. The results revealed that *Pseudomonas* isolates (Pf-2, Pf-3 and Pf-8) and *Trichoderma* isolates (T-2, T-6, T-7, T-8, T-9, T-11, T-14, T-15, T-16, T-17, T-19 and T-25) exhibited positive results for IAA production as evidenced by qualitative analysis of culture supernatante (Plate 16b).

The findings of present work are in accord with the works done by earlier workers (El-Tarabilya *et al.,* 1997; Nimnoi and Pongslip, 2009; Gangwar *et al.,* 2012; Bhakthavatchalu *et al.,* 2013; Dixit *et al.,* 2015; Prasad *et al.,* 2017; Rai, 2017).

Lynch (1985) described IAA is one of the most important physiologically active auxins. The rhizospheric PGPR of many crops have an ability to produce many plant growth promoting hormones and nutrients. IAA also helps increased number of root hairs, the production of longer roots and root laterals which are involved in nutrient uptake. IAA also promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting in plants.

Bhakthavatchalu *et al.* (2013) tested the isolate *P. aeruginosa* FP6 for the production of IAA, it was found positive. Dixit *et al.* (2015) evaluated 20 isolates of *Trichoderma* for IAA production. All *Trichoderma* spp. isolates elucidated positive results for IAA production.

Prasad *et al.* (2017) also evaluated 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for IAA production. These isolates were isolated from the rhizosphere of tomato. IAA production was recorded with *Trichoderma* but the highest IAA production was observed with the bacterial isolates *B. subtilis*-5, *P. fluorescens*-2 and 6. These isolates were obtained from tomato rhizosphere.

In the present investigation, 3 isolate of *Pseudomonas* and 12 isolates of *Trichoderma* were observed to produce IAA at varying intensity. This occurrence may be ascribed to the heterogeneous nature of the source and the strains of the antagonists.

Table 4.7 Qualitative assay of IAA production by different native biocontrol agents (BCAs)

BCAs	IAA production	BCAs	IAA production
Control	-	T-8	+
(A) *Pseudomonas* isolates		T-9	+
Pf-1	-	T-10	-
Pf-2	++	T-11	+
Pf-3	+++	T-12	-
Pf-4	-	T-13	-
Pf-5	-	T-14	+
Pf-6	-	T-15	+
Pf-7	-	T-16	+
Pf-8	+	T-17	+
(B) *Trichoderma* isolates		T-18	-
T-1	-	T-19	+
T-2	+	T-20	-
T-3	-	T-21	-
T-4	-	T-22	-
T-5	-	T-23	-
T-6	+	T-24	-
T-7	+	T-25	+

Whereas

1) - = Negative
2) + = Low production
3) ++ = Medium production
4) +++ = Strong production

4.5.4 Phosphate solubility

Phosphate solubility test was conducted qualitatively by inoculating of *Trichoderma* and *Pseudomonas* (10 mm mycelial disc of *Trichoderma* isolates and loop full spot inoculation of *Pseudomonas* isolates) on NBRIP agar medium (Nautiyal, 1999). Indication of positive phosphate solubilization by halo clearing zone around fresh colony after incubating at 28 °C for 7 days. The results of qualitative assay of phosphate solubilization by different native BCAs are presented in table 4.8. The results revealed that *Pseudomonas* isolates (Pf-1, Pf-2, Pf-3, Pf-7 and Pf-8) and *Trichoderma* isolates (T-3, T-5, T-10, T-11, T-14, T-16, T-19 and T-25) elucidated positive results for phosphate solubilization (Plates 17, 18 and 19a).

The findings of present work are supported by the findings of earlier workers (Kapri and Tewari, 2010; Gangwar *et al.,* 2012; Bhakthavatchalu *et al.,* 2013; Prasad *et al.,* 2017; Rai, 2017; Lalngaihawmi and Bhattacharyya, 2019). Kapri and Tewari (2010) tested fungi, actinomycetes and bacteria for phosphate solubilization. These rhizosphere isolates were isolated from the rhizosphere of tomato. Among them bacterial, eleven isolates showed solubilization zone. Bhakthavatchalu *et al.* (2013) tested the isolate *P. aeruginosa* FP6 for the production of phosphate solubilization, it was found positive. Dixit *et al.* (2015) evaluated 20 isolates of *Trichoderma* for phosphate solubilization. All *Trichoderma* spp. isolates elucidated positive results for phosphate solubilization.

Prasad *et al.* (2017) screened 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for phosphate solubilization test. These isolates were isolated from the rhizosphere of tomato. Among 12 isolates of bacterial, eleven isolates showed solubilization zone except *P. fluorescens*-4. The *P. fluorescens*-2 recorded highest solubilization zone followed by *P. fluorescens*-1. These isolates were obtained from tomato rhizosphere.

These reports are in agreement with the results of the present investigation, which suggests that the phosphate solubilization by both *Trichoderma* and *Pseudomonas* isolates have obvious influence on the plant growth.

Table 4.8 Qualitative assay of phosphate solubilization by different native biocontrol agents (BCAs)

BCAs	Phosphate solubility	BCAs	Phosphate solubility
Control	-	T-8	-
(A) *Pseudomonas* isolates		T-9	-
Pf-1	++	T-10	+
Pf-2	+++	T-11	+++
Pf-3	+++	T-12	-
Pf-4	-	T-13	-
Pf-5	-	T-14	+
Pf-6	-	T-15	-
Pf-7	+	T-16	+
Pf-8	++	T-17	-
(B) *Trichoderma* isolates		T-18	-
T-1	-	T-19	+
T-2	-	T-20	-
T-3	+	T-21	-
T-4	-	T-22	-
T-5	+	T-23	-
T-6	-	T-24	-
T-7	-	T-25	+

Whereas

1) - = Negative
2) + = Low solubility
3) ++ = Medium solubility
4) +++ = Strong solubility

4.5.5 Siderophore production

Chrome azurol Sulfonate (CAS) assay was used to detect the production of siderophore of 25 isolates of *Trichoderma* and 8 isolates of *Pseudomonas* following the procedure given by Schwyn and Neilands, 1987. The microbial isolates were inoculated (5 mm mycelium disc of *Trichoderma* and loop full spot inoculation of *Pseudomonas* isolates) and incubated at 28 ºC for 3 days. The colonies with pink/yellow/orange zones were considered as siderophore producing isolates. The results of qualitative assay of siderophore production by different native BCAs are presented in table 4.9.

All, 33 isolates showed positive results for siderophore production. Among the tested isolates, *Pseudomonas* isolates (Pf-3 and Pf-8) and *Trichoderma* isolates (T-3, T-4, T-5, T-7, T-8, T-9, T-10, T-11, T-14, T-15, T-18 and T-21) exhibited strong siderophore production by pink and orange halo colour development (Plates 19b, 20 and 21a).

The findings of present work are in conformity with the works done by earlier workers (Demanage *et al.,* 1987; Yeole *et al.,* 2001; Manikandan *et al.,* 2010; Gangwar *et al.,* 2012; Prasad *et al.,* 2017; Rai, 2017; Lalngaihawmi and Bhattacharyya, 2019). Scher and Bakker (1982) defined siderophores are low molecular weight (<1000 D) iron chelating compounds produced by microorganisms under iron stress conditions. A large number of bacteria and fungi are known to produce siderophores under iron limiting conditions in the soil, based on this mechanism the siderophore producing organisms have been used in biological control of plant pathogens as well as for obtaining higher yield from crop plants.

Bhakthavatchalu *et al.* (2013) recorded positive in siderophore production by *P. aeruginosa* FP6. They also recorded that orange colour in CAS agar medium. This isolate was obtained from tomato rhizosphere. Dixit *et*

al. (2015) evaluated 20 isolates of *Trichoderma* spp. and 11 isolates of fluorescent *Pseudomonas* spp. for siderophore production in CAS solid medium. Among *Trichoderma* spp., isolates T5, T6, T8 and T15 positive production of pink halo colour was deduced in the medium. Among *Pseudomonas* spp., maximum siderophore production was observed in Pf12 followed by Pf27 and Pf28 except Pf25. These isolates were obtained from tomato rhizosphere.

In this present study the strong and positive siderophore production exhibited by *Pseudomonas* isolates (Pf-3 and Pf-8) and *Trichoderma* isolates (T-3, T-4, T-5, T-7, T-8, T-9, T-10, T-11, T-14, T-15, T-18 and T-21) explicate the corresponding inhibited radial growth and high per cent inhibition of *P. infestans*.

Table 4.9 Qualitative assay of siderophore production by different native biocontrol agents (BCAs)

BCAs	Siderophore production	BCAs	Siderophore production
Control	-	T-8	+++
(A) *Pseudomonas* isolates		T-9	+++
Pf-1	++	T-10	+++
Pf-2	++	T-11	+++
Pf-3	+++	T-12	++
Pf-4	++	T-13	++
Pf-5	+	T-14	+++
Pf-6	++	T-15	+++
Pf-7	++	T-16	+
Pf-8	+++	T-17	++
(B) *Trichoderma* isolates		T-18	+++
T-1	+	T-19	++
T-2	+	T-20	++
T-3	+++	T-21	+++
T-4	+++	T-22	+
T-5	+++	T-23	++
T-6	++	T-24	+
T-7	+++	T-25	++

Whereas

1) - = Negative
2) + = Low production
3) ++ = Medium production
4) +++ = Strong production

4.5.6 Qualitative assay of Hydrogen cyanide (HCN) production by *Pseudomonas* isolates

The production of HCN by *Pseudomonas* isolates were determined by modified protocol of Miller and Higgins (1970). *Pseudomonas* isolates were streaked onto tryptic soya agar medium. Filter paper (Whatman No.1) strips were soaked in picric acid solution and placed on the upper lid of each Petri plates. Petri plates were sealed with parafilm and observation was made for 5 days after incubation at 28 °C. Reactions were recorded (Table 4.10) as weak (yellow to light brown), moderate (brown) and strong (reddish brown). The results revealed that *Pseudomonas* isolates (Pf-2, Pf-3 and Pf-8) elucidated positive results for HCN production (Plate 21b).

The findings of present work are in harmony with the findings of earlier workers (Ramette *et al.,* 2003; Manikandan *et al.,* 2010; Bhakthavatchalu *et al.,* 2013; Dixit *et al.,* 2015; Prasad *et al.,* 2017). Dixit *et al.* (2015) evaluated 20 isolates of *Trichoderma* spp. and 11 isolates of fluorescent *Pseudomonas* spp. for HCN production. Among *Trichoderma* spp., isolates T5, T6, T8 and T15 positive production of HCN. Among *Pseudomonas* spp., maximum HCN production was observed in Pf12 followed by Pf27 and Pf28 except Pf25.

Manikandan *et al*. (2010) recorded the HCN production by *P. fluorescens* Pf1. They have tested Pf1 cultures of different ages and it changed the yellow colour of the filter paper to dark brown compared to the control treatment. This isolate was obtained from tomato rhizosphere. Dixit *et al.* (2015) evaluated 11 isolates of fluorescent *Pseudomonas* for HCN production. They found colour change from yellow to dark brown in most of the isolates, except Pf4, Pf6, Pf25. While, Pf20, Pf27 and Pf28 were highly HCN producing isolates as compared to others.

These reports are in agreement with the results of the present investigation, which suggests that the production of HCN by *Pseudomonas* isolates have absolute influence on the high degree of inhibition of *P. infestans.*

Table 4.10 Qualitative assay of HCN production by *Pseudomonas* isolates

***Pseudomonas* isolate**	**HCN production**	***Pseudomonas* isolate**	**HCN production**
Control	**-**	**Pf-5**	**-**
Pf-1	**-**	**Pf-6**	**-**
Pf-2	+	**Pf-7**	**-**
Pf-3	+	**Pf-8**	+
Pf-4	**-**		

Whereas

1) - = Negative.
2) + = Positive.

4.5.7 Mycoparasitism activity of native *Trichoderma* isolates

All 25 isolates of *Trichoderma* under the study were tested for mycoparasitism activity against *P. infestans*. The method consist of inoculating test pathogen and *Trichoderma* antagonist in the same Petri plate containing PDA medium (20 ml) culture having three sterile cover slips in the center of the plate to check interaction between the hyphae (Plate 22A), they were incubated at a temperature of 18±1 °C for ten days in the absence of light. After the incubation period (Plate 22B), cover slips were removed and superimposed on microscope slides with dye cotton blue to verify the interaction between the hyphae. The presence or absence of coiling (Plate 22C) was observed under compound microscope and results are presented in table 4.11. All, 25 isolates showed the presence of coiling as hyphal interactions between *Trichoderma* isolates and *P. infestans.* The mycoparasitic behaviours of *Trichoderma* spp. followed almost the same pattern as has been recorded against *P. infestans* (Ramanujam *et al.,* 2002; Zegeye *et al.*, 2011) *P. cinnamomi* (Pugeg and Ian, 2006) and *P. capsici* (Ezziyyani *et al.,* 2007).

McIntyre *et al*. (2004) described the mycoparasitism is antagonistic interaction between two fungal species, where the direct attack of one fungus on another is known as mycoparasitism by several sequential events, including recognition, attack and subsequent penetration and killing of the host. *Trichoderma* genera may exert necrotrophic mycoparasitism and are considered as effective BCAs due to their antagonistic capacities against a broad range of phytopathogenic fungi. The most evident morphological changes in *Trichoderma* hyphae, such as coiling and development of appressorium like penetration structures, which serve to penetrate the host and contain high concentration of osmotic solutes such as glycerol.

In the present investigation characteristic envelopment and coiling around of the hyphae by all isolates of *Trichoderma* spp. was observed. The hyphae of *Trichoderma* spp. was also observed to grow in close proximity to the hyphae of *P. infestans* before coagulation and disintegration occurred.

Table 4.11 Mycoparasitism activity of native *Trichoderma* isolates

***Trichoderma* isolate**	**Hyphal interaction**	***Trichoderma* isolate**	**Hyphal interaction**
T-1	+	**T-14**	+
T-2	+	**T-15**	+
T-3	+	**T-16**	+
T-4	+	**T-17**	+
T-5	+	**T-18**	+
T-6	+	**T-19**	+
T-7	+	**T-20**	+
T-8	+	**T-21**	+
T-9	+	**T-22**	+
T-10	+	**T-23**	+
T-11	+	**T-24**	+
T-12	+	**T-25**	+
T-13	+		

Whereas, + = the presence of coiling.

4.6 Selection of potential isolates of *Trichoderma* and *Pseudomonas*

Based on *in vitro* antagonistic capabilities of *Trichoderma* and *Pseudomonas* isolates against *P. infestans* and their elucidation for various biocontrol mechanisms, the potent isolates were selected as *Pseudomonas* isolates (Pf-2 and Pf-3) and *Trichoderma* isolates (T-11 and T-14) for further studies. The results of *in vitro* screening of native BCAs against *P. infestans* and their mechanisms are presented in table 4.12. All potent native microbial isolates were capable to release inorganic phosphorus from TCP and showed consistent ability to produce siderophore, HCN, ammonia, IAA, volatile metabolites and mycoparasitism ability.

Harish *et al.* (2008) mentioned that under field conditions alone strain of antagonistic often result in inconsistent disease control. For overcoming such inconsistent performance, mixture of two or more biological agents have diverse mechanisms, promises more efficient disease control. Consortia of biological control agents have ability to produce siderophore, HCN, ammonia, IAA, volatile metabolites, mycoparasitism and lipopeptide antibiotics (Idris *et al.,* 2007). Microbial mixture is much more efficient than single strains of organisms with diverse metabolic capabilities (Yan *et al.,* 2002). Many of these BCAs and PGPRs are known to produce vitamins, amino acids and growth promoting substances like cytokinins, GA and IAA which help in better growth of crop plants (Raupach and Kloepper, 1998).

In this present study, rationale behind the selection of potent isolates of *Pseudomonas* (Pf-2 and Pf-3) and *Trichoderma* (T-11 and T-14) is the deployment of diverse biocontrol mechanisms with highest per cent inhibition of test pathogen.

Table 4.12 *In vitro* screening of native BCAs against *P. infestans* and their mechanisms

BCAs	Per cent inhibition of pathogen growth in dual culture	Biocontrol mechanisms						
		Per cent inhibition of pathogen growth by volatile metabolites	Ammonia prod.	IAA prod.	Phosphate solubility	Siderophore production	HCN prod.	Mycoparasitism
(A) *Pseudomonas* isolates								
Pf-1	69.33	44.78	+++	-	++	++	-	*
Pf-2	**81.33**	**53.67**	+++	++	+++	++	+	*
Pf-3	**73.33**	**48.11**	++++	+++	+++	+++	+	*
Pf-4	65.33	25.22	++++	-	-	++	-	*
Pf-5	60.44	29.22	+++	-	-	+	-	*
Pf-6	60.00	35.89	+++	-	-	++	-	*
Pf-7	66.67	24.78	++++	-	+	++	-	*
Pf-8	64.00	25.56	++++	+	++	+++	+	*
(B) *Trichoderma* isolates								
T-1	60.00	34.78	++++	-	-	+	*	+
T-2	54.27	26.33	++++	+	-	+	*	+
T-3	52.93	19.67	+++	-	+	+++	*	+
T-4	56.00	32.22	++	-	-	+++	*	+
T-5	64.93	14.11	++	-	+	+++	*	+
T-6	57.33	21.89	+++	+	-	++	*	+
T-7	52.00	14.44	+++	+	-	+++	*	+
T-8	61.33	18.11	++	+	-	+++	*	+
T-9	57.73	31.11	+++	+	-	+++	*	+
T-10	53.33	28.11	+++	-	+	+++	*	+
T-11	**73.73**	**45.55**	++++	+	+++	+++	*	+
T-12	53.73	16.67	+	-	-	++	*	+
T-13	60.00	25.89	+	-	-	++	*	+
T-14	**66.67**	**35.55**	+++	+	+	+++	*	+
T-15	55.60	30.00	+++	+	-	+++	*	+
T-16	52.40	27.00	+	+	+	+	*	+
T-17	51.07	15.22	+	+	-	++	*	+
T-18	59.60	12.22	+++	-	-	+++	*	+
T-19	62.67	25.55	+++	+	+	++	*	+
T-20	60.93	23.63	+++	-	-	++	*	+
T-21	56.93	35.22	++	-	-	+++	*	+
T-22	58.67	25.89	++	-	-	+	*	+
T-23	56.00	13.00	+++	-	-	++	*	+
T-24	57.73	16.67	+++	-	-	+	*	+
T-25	64.00	32.55	++++	+	+	++	*	+

Whereas, + = Positive, - = Negative and * = Not tested

4.7 Identification of potential BCAs

4.7.1 Molecular identification and phylogenetic analysis of potential *Trichoderma* isolates

Molecular identification of potential *Trichoderma* isolates (T-11 and T-14) was performed by using Internal Transcribed Spacer (ITS) region of 18S rRNA. Sequence analyses of two isolates (T-11 and T-14) were done to confirm species identity. Comparison of oligonucleotide fragments of 18S rRNA sequences, which included the flanking ITS4 and ITS6 regions, with reference sequences from public databases, showed that they were similar. The ITS forward (ITS6*;* 5'-GAAGGTGAAGTCGTAACAAGG-3') and reverse (ITS4*;* 5'-TCCTCCG CTTATTGATATGC-3') oligonucleotide pairs amplified a single DNA fragment of approximately 600 bp amplicon size in both the isolates (Plate 23). ITS sequences of both the isolates were submitted to NCBI GenBank (MK928414 and MK928417), which showed 100 % similarity with *T. asperellum* during BLAST analysis. This was used for construction of phylogeny tree and subsequently, these isolates were identified as *T. asperellum* (Table 4.13).

The ITS sequence was chosen for this analysis because it has been obsereved to be more accurate with various sections of the genus *Trichoderma* (Kullnig-Gradinger *et al*., 2002). ITS region of 18S rRNA is one of the most consistent targets to identify a *Trichoderma* strain at the species level, but this cannot differentiate all *Trichoderma* spp. (Rifai, 1969).

Kindermann *et al*. (1998) studied the phylogenetic analysis of the whole genus, using sequence analysis of the ITS1 region of the 18S rRNA. Phylogenetic studies of 88 species showed that *Hypocrea* and *Trichoderma* form a single holomorph genus, within which two major clades can be distinguished.

Table 4.13 Internal Transcribed Spacer (ITS) region of rDNA sequence of potential *Trichoderma* isolates

Isolate No.	Accession No.	Primers and their sequence	Sequence of *Trichoderma* isolate	Base pair	Similarity (%)
T-11	MK928414	Forward (ITS6) 5'-GAAGGTGA AGTCGTAACA AGG-3'	GGCCGTTGGAGACGCGGAAGGATCATTACCGAGTTTACAACTCCC AAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCAC GCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGG AACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTA CAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACG GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA CGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAG CGTCATTTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGGGA TCGGGACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGGCGGT CTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCG GGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAA TGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA TCAATAAGGCGGAGGAAA	603	*T. asperellum* (100 %)
		Reverse (ITS4) 5'-TCCTCCGC TTATTGATA TG C-3'	GGACGGGTTCCTACCTGATCCGAGGTCACATTTCAGAAAGTTGGG TGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGC AAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTT CGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATC CCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATG CCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGAT GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGC GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTT TGATTCATTTTGAATTTTTGCTCAGAGCTGTAAGAAATACGTCCGC GAGGGGACTACAGAAAGAGTTTGGTTGGTTCCTCCGGCGGGCGCC TGGTTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCGAGG CAACAGTTTGGTAACGTTCACATTGGGTTTGGGAGTTGTAAACTC GGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGA CTTTCACCTTCAAC	599	

T-14	MK928417	Forward (ITS6) 5’-GAAGGTGAAGTCGTAACAAG G-3’	GGCCTGGGGACAGCGGAGGGACATACCGAGTTTACACTCCCAAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAAGCCGGGAGGAAAAA	604	*T. asperellum* (100%)
		Reverse (ITS4) 5’-TCCTCCGCTTATTGATATGC-3’	GGGGGGGGCCATCAAACCTGATCCGAGGTCACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTTGAATTTTTGCTCAGAGCTGTAAGAAATACGTCCGCGAGGGGACTACAGAAAGAGTTTGGTTGGTTCCTCCGGCGGGCGCCTGGTTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCGAGGCAACAGTTTGGTAACGTTCACATTGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACAACTTTCACCAATCAAG	603	

4.7.2 Identification of potential *Pseudomonas* isolates

The potential *Pseudomonas* isolates (Pf-2 and Pf-3) were examined by their Gram reaction, colony morphology, cell shape and fluorescens. Microscopic observations were also recorded on the basis of their shape, colour, opacity and mucosity. Colony characterization was based on their shape, size, colour and mucosity. Individual isolates were streaked on the King's B medium plates and incubated at 28±2 °C for 4 days to record colony characteristics. The potential *Pseudomonas* isolates (Pf-2 and Pf-3) were identified as *P. fluorescens* based on the following characteristics, gram negative, rod shaped cells, creamy mucoid colony with smooth edges and yellow-green fluorescent pigmentation produced under ultraviolet (UV) light (Plate 24).

Garrity *et al.* (2005) also confirmed the identity of fluorescent pseudomonads by Gram's staining technique, colony morphology, fluorescens and cell shape. Singh *et al*. (2017) also identified *P. fluorescens* on the basis of their cultural, morphological and biochemical characters.

It is inferred from the present study, that taxonomical knowledge on *Pseudomonas* isolates is important for identification and characterization of potential biocontrol species. This is to undeniably avoid potential risk of introducing an unknown bacterial species into the rhizosphere of a given ecosystem.

4.8 *In vitro* testing of compatibility amongst microbial consortia

In vitro compatibility test amongst microbial consortia of potent isolates of *Trichoderma* and *Pseudomonas* was conducted in order to determine whether they can be used in combination or not. Dual culture plate method described by Siddiqui and Shaukat (2003) was employed.

The experiment was carried out in all permutations and combination amongst the potent isolates of *Trichoderma* and *Pseudomonas*. Altogether 11 treatment combinations were tested and compared with growth of Pf-2 (Control-1), Pf-3 (Control-2), T-11 (Control-3) and T-14 (Control-4).

The microorganisms showing positive compatibility among them was recorded, tabulated and selected for further study. The data showed compatibility among all the treatment combinations of the four bioactive microorganisms *in vitro* (Table 4.14). No clear inhibition zone was observed between the tested microbial consortia (Plate 25). Absence of inhibition zone indicated that the potential isolates of *Trichoderma* and *Pseudomonas* were compatible with each other.

The findings of present work are in conformity with the findings of earlier workers (Siddiqui and Shaukat, 2003; Zegeye *et al.,* 2011; Kumar *et al.,* 2014; Nath *et al.,* 2016; Harshita *et al*., 2018; Lalngaihawmi and Bhattacharyya, 2019). Mishra *et al.* (2013) tested *in vitro* compatibility among 40 isolates of fluorescent pseudomonads and 43 isolates of *Trichoderma*. Isolates PBAP-15, PBAP-17, PBAP-27, PBAP-10 of *P. fluorescent* and isolates PBAT-38, PBAT-43, PBAT-6 and PBAT-1 of *Trichoderma* exhibited no or very little antagonism against each other. Nath *et al.* (2016) also recorded *in vitro* compatibility among *T. parareesei*, *P. fluorescens*, *B. subtilis* and *Azotobacter chroococcum* and their consortia. Harshita *et al*. (2018) also tested compatibility among the fungal (*T. harzianum*) and bacterial (*B. subtilis* and *P. fluorescens*) BCAs *in vitro.* Absence of inhibition zone indicated that the BCAs were compatible with each other. These isolates were obtained from tomato rhizosphere.

In this present investigation, all the potent BCAs were compatible with each other. None of the BCAs were observed to inhibit each other and hence surmised that all the BCAs taken are compatible with each other and can be used to develop consortia.

Table 4.14 *In vitro* analysis of compatibility amongst microbial consortia

Treatment	Treatment combination	Compatible/Non-compatible (+/-)
T_1	Control-I Pf-2 alone	*
T_2	Control-II Pf-3 alone	*
T_3	Control-III T-11 alone	*
T_4	Control-IV T-14 alone	*
T_5	Pf-2 + Pf-3	**+ve**
T_6	Pf-3 + T-11	**+ve**
T_7	Pf-3 + T-14	**+ve**
T_8	Pf-2 + T-11	**+ve**
T_9	Pf-2 + T-14	**+ve**
T_{10}	T-11 + T-14	**+ve**
T_{11}	Pf-2 + Pf-3 + T-11	**+ve**
T_{12}	Pf-2 + Pf-3 + T-14	**+ve**
T_{13}	Pf-3 + T-11 + T-14	**+ve**
T_{14}	Pf-2 + T-11 + T-14	**+ve**
T_{15}	Pf-2 + Pf-3 + T-11 + T-14	**+ve**

4.9 *In vitro* antagonistic efficacy of CMC against *P. infestans*

The inhibitory effects of compatible microbial consortia (CMC) were tested *in vitro* against *P. infestans* adopting dual culture bioassay technique in pathogen favourable medium (Rye B agar). A total of 12 treatment combinations were compared. Eleven consortia produced varying inhibitions (%) *in vitro* against *P. infestans* (Table 4.15; Fig 4.5). All consortia tested against *P. infestans* were significantly superior over control (Plate 26). Among the different consortial sets tested *in vitro* the significant highest inhibition of pathogen was recorded in the combination of Pf-2 + Pf-3 + T-11 + T-14 (83.33 %) followed by Pf-2 + Pf-3 + T-11 (78.38 %), Pf-2 + Pf-3 (77.43 %) and Pf-2 + T-14 (76.54 %) respectively at 5 days after incubation at 18 ±1 °C.

An *in vitro* study was taken up to select the two best CMC against the test pathogen. The CMC-1 (Pf-2 + Pf-3 + T-11 + T-14) and CMC-2 (Pf-2 + Pf-3 + T-11) inhibited the pathogen significantly and were found to be the most effective consortia. Hence, they were selected for further eveluation.

These results are in conformity with the findings of earlier workers (Sundaramoorthy and Balabaskar, 2013; Nath *et al.,* 2016; Khan *et al.,* 2018; Vrieze *et al.,* 2018; Lalngaihawmi and Bhattacharyya, 2019). Nath *et al.* (2016) tested the antagonistic potential of CMC of *T. parareesei*, *P. fluorescens*, *B. subtilis* and *Azotobacter chroococcum* against *R. solanacearum.* The highest inhibition of test pathogen was observed from consortia of *B. subtilis* + *T. parareesei* + *P. fluorescens* + *A. chroococcum* (91.10 %) followed by *B. subtilis* + *T. parareesei* + *P. fluorescens* (81.10 %) and *B. subtilis* + *P. fluorescens* + *A. chroococcum* (68.14 %). Vrieze *et al.* (2018) also tested consortia of *Pseudomonas* strains against *P. infestans.* They found triple consortia of R32 + S19 + S35 were best consortia among tested consortia.

Lalngaihawmi and Bhattacharyya (2019) evaluated the antagonistic potential of the three promising *Trichoderma* spp. individually as well as in combinations against *F. o.* f.sp. *cubense.* The result revealed that the efficacy of all the treatments differed significantly with that of control treatment at all the intervals. After 96 hrs of incubation, the per cent inhibition of radial growth of test pathogen *in vitro* was observed highest in the combination of the three *Trichoderma* spp. *viz*., *T. reesei* (RMF-25) + *T. reesei* (RMF-13) + *T. harzianum* (RMF- 28) with 69.18 per cent followed by *T. reesei* (RMF-25) + *T. harzianum* (RMF 28) with 66.86 per cent and *T. reesei* (RMF-13) + *T. harzianum* (RMF 28) with 68.60 per cent.

In this present study, the rationale behind the use of consortia against *P. infestans* is that multiple potent isolates allow the deployment of several different biocontrol mechanisms simultaneously. Hence, based on the results obtained from this study the best two consortia (CMC-1 and CMC-2) were taken up for further studies.

Table 4.15 *In vitro* antagonistic effect of compatible microbial consortia on radial growth and per cent inhibition of *P. infestans*

Treat.	Treatment combination	Inhibition of *P. infestans* growth			
		Radial growth (cm)	Radial growth (cm) inhibited	Individual's Inhibition (%)	Combine Inhibition (%)
T_0	Control *P. infestans* alone	3.90	00.00	00.00	00.00 (4.05)*
T_1	Pf-2 + Pf-3	0.83 + 0.93	3.07 + 2.97	78.72 + 76.15	**77.43 (61.66)**
T_2	Pf-3 + T-11	1.30 + 0.93	2.60 + 2.97	66.67 + 76.15	71.41 (57.67)
T_3	Pf-3 + T-14	0.90 +1.00	3.00 + 2.90	76.92 + 74.36	75.64 (60.43)
T_4	Pf-2 + T-11	0.93 + 1.07	2.97 + 2.83	76.15 + 72.56	74.36 (59.58)
T_5	Pf-2 + T-14	0.87 + 0.96	3.03 + 2.94	77.69 + 75.38	76.54 (61.05)
T_6	T-11 + T-14	0.90 + 1.20	3.00 + 2.70	76.92 + 69.23	73.08 (58.76)
T_7	Pf-2 + Pf-3 + T-11	0.70 + 0.90 + 0.93	3.20 + 3.00 + 2.97	82.05 +76.92 +76.15	**78.38 (62.29)**
T_8	Pf-2 + Pf-3 + T-14	1.03 +1.23 +1.30	2.87 + 2.67 + 2.60	73.59 + 68.46+66.67	69.57 (56.33)
T_9	Pf-3 + T-11 + T-14	1.03 + 1.07 +1.27	2.87 + 2.83 + 2.63	73.59 +72.56+67.44	71.20 (57.56)
T_{10}	Pf-2 + T-11 + T-14	0.97 + 0.87 + 1.10	2.93 + 3.03 + 2.80	75.13+77.69+71.79	74.87 (59.91)
T_{11}	Pf-2 + Pf-3 + T-11 + T-14	0.63 + 0.67 + 0.60 + 0.70	3.27 + 3.23 + 3.30 + 3.20	83.85+82.82+84.61+82.05	**83.33 (65.90)**
				SEm±	0.91 (0.60)
				C.V. (%)	2.29 (1.86)
				CD (*p*= 0.01)	3.60 (2.36)
				CD (*p*= 0.05)	2.66 (1.74)

***Values in parentheses are angular transformed values.**

4.10 *In vitro* effect of CMC on tomato seedlings

In vitro study conducted to check out the efficacy of selected two best consortia (CMC-1 and CMC-2) on seed germination, seedling vigour index, fresh and dry weight of shoot and fresh and dry weight of root, seedlings shoot and root length was carried out by Standard filter paper method (three layered moistened filter papers in Petri plates; ISTA, 1993). The healthy seeds of tomato cv. Pusa Ruby were selected for experimental purpose. The surface sterilized seeds (1.0 % sodium hypochlorite for 2 min) were soaked with liquid formulations of consortia (1.0 %) and shade dried in laminar air flow for 5 hrs. For chemical control treatment; the surface sterilized seeds were treated with captan 50 % WP (seed dressing @ 3 mg/1 g seed or 0.3 %). For control treatment, the surface sterilized seeds were soaked in sterile distilled water (@1 ml/1 g seed) and shade dried in laminar air flow for 5 hrs (Srinivasan and Mathivanan, 2009).

4.10.1 Per cent germination at 10 DAS

Observation on per cent germination of different treatments was observed on 10^{th} DAS. Tomato seed germination per cent was significantly higher in the seed treatment with CMC-1 (89.17 %) followed by CMC-2 (80.00 %) and chemical control (74.16 %). The lowest seed germination per cent was observed in control treatment (72.50 %) (Table 4.16; Plate 27). This experimental result revealed that the CMC-1 significantly increased seed germination per cent (22.99 %) over control treatment (Table 4.17; Fig 4.6). Next in order of merit was CMC-2 (10.34 %) and chemical control (2.29 %) but these were statistically non-significant.

The results of the present findings are in harmony with the findings of earlier workers (Zaidi and Singh, 2004; Sharma *et al.,* 2009; Manikandan *et al*., 2010; Someshwar and Sitansu, 2010; Ananthi *et al*., 2013).

The application of consortia of *Trichoderma* spp., significantly increased the tomato seed germination per cent at 10 DAS. Maximum seed germination per cent was obtained from seed treated with *T. harzianum* + *T. asperellum* + *T. viride* (92.00 %) followed by *T. harzianum* + *T. asperellum* (90.0 %) as compared to control (73.8 %) (Murthy *et al.,* 2013).

4.10.2 Seedlings shoot length and root length (cm) at 10 DAS

The root length and shoot length of individual tomato seedlings (60 seedlings/treatment) were measured. The data on shoot length is depicted in table 4.16. Shoot length was longer in all the treatments as compared to the control (Plate 27). Among these treatments, significantly longer shoot was recorded in seed treated with CMC-1 (5.54 cm) than the other treatments tested. Next best in order of merit was CMC-2 (5.0 cm) and chemical control (3.32 cm). Minimum shoot length was observed in control (3.02 cm). This experimental result revealed that the CMC-1 significantly increased shoot length (83.44 %) over control treatment (Table 4.17; Fig 4.6). Next in order of merit was CMC-2 (65.56 %) and chemical control (9.93 %) but these were statistically non-significant.

Root length was also longer in all the treatments as compared to the control (Table 4.16; Plate 27). Among these treatments, significantly higher root was recorded in seed treated with CMC-1 (5.84 cm) than the other treatments tested. Next best in order of merit was CMC-2 (5.21 cm) and chemical control (3.59 cm). Minimum root length was observed in control (3.22 cm). This experimental result revealed that the CMC-1 also significantly increased root length (81.37 %) over control treatment (Table 4.17; Fig 4.6). Next in order of merit was CMC-2 (61.80 %) and chemical control (11.49 %) but these were statistically non-significant.

The results of the present findings are in agreement with the findings of earlier workers (Manoranjitham and Prakasam, 1999. Raj *et al*., 2004; Eutesari *et al.,* 2013; Kabir *et al.,* 2013; Kumar *et al.,* 2015; Singh *et al.,* 2016).

In corroboration to the present work, the application of consortia of *Trichoderma* spp., significantly increased the shoot length and root length of tomato seedling at 10 DAS. It was reported that, maximum shoot length was obtained from seed treated with *T. harzianum* + *T. asperellum* + *T. viride* (5.76 cm) followed by *T. harzianum* + *T. asperellum* (5.63 cm) as compared to control (4.36 cm). Maximum root length was obtained from seed treated with *T. harzianum* + *T. asperellum* + *T. viride* (8.55 cm) followed by *T. harzianum* + *T. asperellum* (8.36 cm) as compared to control (6.75 cm) (Murthy *et al.,* 2013).

4.10.3 Seedling vigour index (SVI) at 10 DAS

Seedling vigour index was higher in all the treatments as compared to the control (Table 4.16). Among these, significantly higher seedling vigour index was recorded in seed treated with CMC-1 (1014.75) than the other treatments tested. Next best in order of merit was CMC-2 (816.80) and chemical control (512.44) respectively. Minimum seedling vigour index was observed in control (452.40). This experimental result revealed that the CMC-1 also significantly increased vigour index of tomato seedlings (124.30 %) over control treatment (Table 4.17; Fig 4.6). Next in order of merit was CMC-2 (80.55 %) and chemical control (13.27 %) but these were statistically non-significant.

The findings of present work are in harmony with the findings of earlier workers (Raj *et al*., 2004; Ananthi *et al*., 2013; Bhakthavatchalu *et al.,* 2013; Sudharani *et al*. 2014; Biam and Majumder, 2019).

Manikandan *et al*. (2010) recorded plant growth promotion by liquid formulation of *P. fluorescens* Pf1. The 2 day old culture of Pf1 resulted in increased vigour index (1868) of tomato seedlings. They found that it significantly increased tomato plant growth compared to control treatment. This isolate was obtained from tomato rhizosphere. This isolate was obtained from tomato rhizosphere.

Murthy *et al.* (2013) reported that application of consortia of *Trichoderma* spp., significantly increased the tomato seedling vigour index at 10 DAS. Maximum vigour index was obtained from seed treated with *T. harzianum* + *T. asperellum* + *T. viride* (1321.34) followed by *T. harzianum* + *T. asperellum* (1259.18) as compared to control (938.56). These isolates were obtained from tomato rhizosphere.

Sudharani *et al*. (2014) also evaluated the plant growth promoting abilities of BCAs and PGPRs. The combination of *Azotobacter chroococcum* + *B. megaterium* + *P. fluorescens* + *B. subtilis* + *T. harzianum* showed enhanced cabbage seedling vigour. These isolates were obtained from cabbage rhizosphere.

4.10.4 Fresh weight (mg) of seedling shoot and root at 10 DAS

The fresh weight of root and shoot of individual tomato seedlings (60 seedlings/treatment) were measured. Fresh weight of seedling shoot was higher in all the treatments as compared to the control (Table 4.16). Among these treatments, significantly higher fresh weight of shoot was recorded in seed treated with CMC-1 (14.44 mg) than the other treatments tested. Next best in order of merit was CMC-2 (9.17 mg) and chemical control (5.35 mg)

respectively. Minimum shoot fresh weight was observed in control (4.85 mg). This experimental result also revealed that the CMC-1 significantly increased shoot fresh weight (197.73 %) over control treatment (Table 4.17; Fig 4.7). Next in order of merit was CMC-2 (89.07 %) and chemical control (10.31 %) but these were statistically non-significant.

Root fresh weight was also higher in all the treatments as compared to the control (Table 4.16). Among these treatments, significantly higher fresh weight of root was recorded in seed treated with CMC-1 (0.38 mg) than the other treatments tested. Next best in order of merit was CMC-2 (0.25 mg), chemical control (0.14 mg). Minimum root fresh weight was observed in control (0.10 mg). This experimental result revealed that the CMC-1 significantly increased root fresh weight (280.0 %) over control treatment (Table 4.17; Fig 4.7). Next in order of merit was CMC-2 (150.0 %) and chemical control (40.0 %).

The findings of present work are in conformity with the findings of earlier workers (Kabir *et al.,* 2013; Lamsal *et al.,* 2013; Sandheep *et al.,* 2013; Kumar *et al.,* 2015; Khan *et al.,* 2018). Sudharani *et al*. (2014) also evaluated the plant growth promoting abilities of BCAs and PGPRs. The combination of *Azotobacter chroococcum* + *B. megaterium* + *P. fluorescens* + *B. subtilis* + *T. harzianum* showed enhanced fresh weight of cabbage seedlings. These isolates were obtained from cabbage rhizosphere.

Murthy *et al.* (2013) evaluated that application of consortia of *Trichoderma* spp., significantly increased the fresh weight of shoot at 10 DAS. Maximum fresh weight of shoot was obtained from seed treated with *T. harzianum* + *T. asperellum* + *T. viride* (1.25 mg) followed by *T. harzianum* + *T. asperellum* (1.26 mg) as compared to control (0.75 mg).

4.10.5 Dry weight (mg) of seedling shoot and root at 10 DAS

The dry weight of root and shoot of individual seedlings (60 seedlings /treatment) were measured after oven drying at 60° C (when constant weight

obtained) for 24 hrs. Dry weight of seedling shoot was also higher in all the treatments as compared to the control (Table 4.16). Among these treatments, significantly higher dry weight of shoot was recorded in seed treated with CMC-1 (0.76 mg) than the other treatments tested. Next best in order of merit was CMC-2 (0.65 mg) and chemical control (0.45 mg). Minimum shoot dry weight was observed in control (0.43 mg). This experimental result revealed that the CMC-1 significantly increased shoot dry weight (76.74 %) over control treatment (Table 4.17; Fig 4.7). Next in order of merit was CMC-2 (51.16 %) and chemical control (4.65 %).

Root dry weight was also higher in all the treatments as compared to the control (Table 4.16). Among these treatments, significantly higher dry weight of root was recorded in seed treated with CMC-1 (0.05 mg) than the other treatment tested. Next best in order of merit was CMC-2 (0.03 mg) and chemical control (0.02 mg). Minimum root dry weight was observed in control (0.017 mg). This experimental result also revealed that the CMC-1 significantly increased root dry weight (194.12 %) over control treatment (Table 4.17; Fig 4.7). Next in order of merit was CMC-2 (76.47 %) and chemical control (17.64 %).

The findings of present work are in harmony with the findings of earlier workers (Manoranjitham and Prakasam, 1999; Kabir *et al.,* 2013; Lamsal *et al.,* 2013; Sandheep *et al.,* 2013; Khan *et al.,* 2018).

The report of Murthy *et al.* (2013), that the application of consortia of *Trichoderma* spp., significantly increased the dry weight of shoot at 10 DAS attests the findings of the present investigation. They reported that maximum dry weight of shoot was obtained from seed treated with *T. harzianum* + *T. asperellum* + *T. viride* (0.36 mg) followed by *T. harzianum* + *T. asperellum* (0.27 mg) as compared to control (0.08 mg).

Table 4.16 *In vitro* effects of CMC on tomato seed germination (%), seedling shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and vigour index at 10 DAS

Treatment	Seed Germination (%) At 10 DAS	Seedling shoot at 10 DAS			Seedling root at 10 DAS			Seedling Vigour index at 10 DAS
		Shoot Length (cm)	Shoot Fresh wt. (mg)	Shoot Dry wt. (mg)	Root Length (cm)	Root Fresh wt. (mg)	Root Dry wt. (mg)	
T_1 (CMC-1)	89.17 (71.88)*	5.54	14.44	0.76	5.84	0.38	0.050	1014.75
T_2 (CMC-2)	80.00 (63.74)	5.00	09.17	0.65	5.21	0.25	0.030	816.80
T_3 (Chemical control)	74.16 (59.94)	3.32	05.35	0.45	3.59	0.14	0.020	512.44
T_4 (Control)	72.50 (58.68)	3.02	04.85	0.43	3.22	0.10	0.017	452.40
SEm±	3.74 (1.88)	0.13	0.56	0.04	0.17	0.05	0.00	30.76
C.V. (%)	11.60 (10.85)	7.38	16.35	17.60	9.24	13.59	14.19	10.78
CD (*p*=0.01)	12.90 (10.52)	0.51	2.27	0.17	0.68	0.18	0.03	123.76
CD (*p*=0.05)	11.03 (8.31)	0.37	1.66	0.12	0.50	0.14	0.02	90.74

***Values in parentheses are angular transformed values.**

Table 4.17 *In vitro* effects of CMC on per cent increase of tomato seed germination (%), shoot and root length, shoot fresh and dry weight, root fresh and dry weight and seedling vigour index at 10 DAS

Treat.	Per cent increase of plant growth promotion over control at 10 DAS							
	Seed germination (%)	Seedling shoot			Seedling root			Seedling Vigour index
		Shoot Length	Shoot Fresh wt.	Shoot Dry wt.	Root Length	Root Fresh wt.	Root Dry wt.	
T_1 (CMC-1)	22.99	83.44	197.73	76.74	81.37	280.00	194.12	124.30
T_2 (CMC-2)	10.34	65.56	89.07	51.16	61.80	150.00	76.47	80.55
T_3 (Chemical control)	02.29	09.93	10.31	04.65	11.49	40.00	17.64	13.27
T_4 (Control)	-	-	-	-	-	-	-	-

4.11 *In vivo* effect of CMC on tomato seedlings

In vivo study was conducted to check out the efficacy of selected two best consortia (CMC-1 and CMC-2) on seed germination, seedling vigour index, fresh and dry weight of shoot and fresh and dry weight of root, seedlings shoot and root length during 2017-18 and 2018-19. The healthy seeds of tomato cv. Pusa Ruby were selected for the experimental purpose. Tomato seeds were sown in pre sterilized nursery beds (Plate 28) with 2 per cent formalin. The surface sterilized seeds (1.0 % sodium hypochlorite for 2 min) were soaked with liquid formulations of consortia (1.0 %) and shade dried in laminar air flow for 5 hrs. For chemical control treatment; the surface sterilized seeds were treated with captan 50 % WP (seed dressing @ 3 mg/1 g seed or 0.3 %). For control treatment, the surface sterilized seeds were soaked in sterile distilled water (@1 ml/1 g seed) and shade dried in laminar air flow for 5 hrs (Srinivasan and Mathivanan, 2009).

4.11.1 Per cent germination at 10 DAS

Tomato seed germination per cent was significantly higher in the seed treatment with CMC-1 (86.76 %) followed by CMC-2 (78.19 %) and chemical control (73.10 %). The lowest seed germination per cent was observed in control (71.85 %) treatment (Table 4.18). This experimental result also revealed that the CMC-1 significantly increased seed germination per cent (20.75 %) at 10 DAS over control treatment (Table 4.22; Fig 4.8). Next in order of merit was CMC-2 (8.82 %) and chemical control (1.74 %) but these were statistically non-significant.

The results of the present findings confirms the findings of earlier workers (Raj *et al*., 2004; Sharma *et al.,* 2009; Manikandan *et al*., 2010; Someshwar and Sitansu, 2010; Nazir *et al.,* 2011; Bhakthavatchalu *et al.,* 2013; Eutesari *et al.,* 2013). Manikandan *et al*. (2010) recorded plant growth promotion by liquid formulation of *P. fluorescens* Pf1. The 2 day old culture of Pf1 resulted in increased seed germination per cent (92 %). They found that it significantly increased tomato plant growth compared to control treatment.

Sudharani *et al*. (2014) also evaluated the plant growth promoting abilities of BCAs and PGPRs. The combination of *Azotobacter chroococcum* + *B. megaterium* + *P. fluorescens* + *B. subtilis* + *T. harzianum* showed enhanced cabbage seedling germination per cent. These isolates were obtained from cabbage rhizosphere.

Biam and Majumder (2019) reported the biopriming of tomato seeds with *Trichoderma* isolates (*T. harzianum* strain DIS 326F (TR 122), *T. harzianum* strain CEN693 (TR 55), *T. harzianum* strain US10 (TR 66) and *T. harzianum* (TR 136) showed considerable enhancing in seed germination percentage over control treatment, with maximum germination percentage recorded in TR 55 (75.13 %). The least was observed in control (62 %).

4.11.2 Seedlings shoot length and root length (cm) at 20 DAS and 30 DAS

The root length and shoot length of individual tomato seedlings (60 seedlings/treatment) were measured at 20 DAS and 30 DAS (Plate 28). The data on shoot length is depicted in tables 4.18 and 4.20. Shoot length was longer in all the treatments as compared to the control. Among these treatments, significantly longer shoot was recorded in seed treated with CMC-1 (4.88 cm at 20 DAS and 15.22 cm at 30 DAS) than the other treatment tested. Next best in order of merit was CMC-2 (3.87 cm at 20 DAS and 14.45 cm at 30 DAS) and chemical control (2.95 cm at 20 DAS and 11.72 cm at 30 DAS). Minimum shoot length was observed in control (2.92 cm at 20 DAS and 11.38 cm at 30 DAS). This experimental result revealed that the CMC-1 significantly increased shoot length (67.12 % at 20 DAS and 33.74 % at 30 DAS) over control treatment (Table 4.22; Figs 4.8 and 4.10). Next in order of merit was CMC-2 (32.53 % at 20 DAS and 26.98 % at 30 DAS) and chemical control (1.03 % at 20 DAS and 2.99 % at 30 DAS).

Root length was also longer in all the treatments as compared to the control (Tables 4.18 and 4.20). Among these treatments, significantly higher root was recorded in seed treated with CMC-1 (3.21 cm at 20 DAS and 4.68 cm at 30 DAS) than the other treatment tested. Next best in order of merit was CMC-2 (2.30 cm at 20 DAS and 3.30 cm at 30 DAS) and chemical control (1.60 cm at 20 DAS and 1.92 cm at 30 DAS). Minimum root length was observed in control (1.58 cm at 20 DAS and 1.89 cm at 30 DAS). This experimental result revealed that the CMC-1 also significantly increased root length (103.16 % at 20 DAS and 147.62 % at 30 DAS) over control treatment (Table 4.22; Figs 4.8 and 4.10). Next in order of merit was CMC-2 (45.57 % at 20 DAS and 74.60 % at 30 DAS) and chemical control (1.26 % at 20 DAS and 1.59 % at 30 DAS).

The results of the present findings are in agreement with the findings of earlier workers (Manoranjitham and Prakasam, 1999; Raj *et al*., 2004; Eutesari *et al.,* 2013; Kabir *et al.,* 2013; Sandheep *et al.,* 2013; Kumar *et al.,* 2015; Singh *et al.,* 2016; Khan *et al.,* 2018; Biam and Majumder, 2019).

Manikandan *et al*. (2010) recorded tomato growth promotion by liquid formulation of *P. fluorescens* Pf1. The 2 day old culture of Pf1 resulted in increased shoot length (6.2 cm) and root length (14.11 cm) of tomato seedlings. They found that it significantly increased tomato plant growth compared to control treatment.

Kumar *et al.* (2015) developed a seed coating formulation of *B. subtilis* OTPB1 and *T. harzianum* OTPB3 and their consortia. The tomato seeds treated with a mixture of *T. harzianum* OTPB3 and *B. subtilis* OTPB1 or singly, exhibited increase in seedling growth attributes significantly compared to Dithane M-45 (0.2 %) and control. The consortium increased root and shoot lengths by 56.3 and 40.9 % respectively as compared to the control seedlings.

4.11.3 Seedling vigour index (SVI) at 20 DAS and 30 DAS

Seedling vigour index was higher in all the treatments as compared to the control at 20 DAS and 30 DAS (Tables 4.18 and 4.20). Among these treatments, significantly higher seedling vigour index was recorded in seed treated with CMC-1 (701.90 at 20 DAS and 1727.05 at 30 DAS) than the other treatments tested. Next best in order of merit was CMC-2 (483.20 at 20 DAS and 1387.86 at 30 DAS) and chemical control (332.94 at 20 DAS and 997.22 at 30 DAS). Minimum seedling vigour index was observed in control (323.65 at 20 DAS and 953.43 at 30 DAS). This experimental result revealed that the

CMC-1 also significantly increased vigour index of tomato seedlings (116.87 % at 20 DAS and 81.14 % at 30 DAS) over control treatment (Table 4.22; Figs 4.8 and 4.10). Next in order of merit was CMC-2 (49.30 % at 20 DAS and 45.56 % at 30 DAS) and chemical control (2.87 % at 20 DAS and 4.59 % at 30 DAS).

The findings of present work are in agrrement with the findings of earlier workers (Raj *et al*., 2004; Ananthi *et al*., 2013; Bhakthavatchalu *et al.,* 2013; Sudharani *et al*. 2014; Biam and Majumder, 2019). Kumar *et al.* (2015) developed a seed coating formulation of *B. subtilis* OTPB1 and *T. harzianum* OTPB3 and their consortia. The tomato seeds treated with a mixture of *T. harzianum* OTPB3 and *B. subtilis* OTPB1 or singly, exhibited increase in seedling growth attributes significantly compared to Dithane M-45 (0.2 %) and control. The consortium increased root and shoot lengths by 56.3 and 40.9 % respectively as compared to the control seedlings. These isolates were obtained from tomato rhizosphere.

Manikandan *et al*. (2010) recorded plant growth promotion by liquid formulation of *P. fluorescens* Pf1. The 2 day old culture of Pf1 resulted in increased vigour index (1868) of tomato seedlings. They found that it significantly increased tomato plant growth compared to control treatment.

Sudharani *et al*. (2014) also evaluated the plant growth promoting abilities of BCAs and PGPRs. The combination of *Azotobacter chroococcum* + *B. megaterium* + *P. fluorescens* + *B. subtilis* + *T. harzianum* enhanced cabbage seedling vigour. These isolates were obtained from cabbage rhizosphere.

Biam and Majumder (2019) reported the biopriming of tomato seeds with 4 *Trichoderma* isolates (*T. harzianum* strain DIS 326F (TR 122), *T. harzianum* strain CEN693 (TR 55), *T. harzianum* strain US10 (TR 66) and *T. harzianum* (TR 136) showed considerable increase in vigour index over control, with vigour index recorded in TR 55 (47.99 %).

4.11.4 Fresh weight (mg) of seedling shoot and root at 20 DAS and 30 DAS

The fresh weight of root and shoot of individual tomato seedlings (60 seedlings/treatment) were measured. Fresh weight of seedling shoot was higher in all the treatments as compared to the control at 20 DAS and 30 DAS (Tables 4.19 and 4.21). Among these treatments, significantly higher fresh weight of shoot was recorded in seed treated with CMC-1 (110.92 mg at 20 DAS and 1141.03 mg at 30 DAS) than the other treatments tested. Next best in order of merit was CMC-2 (92.12 mg at 20 DAS and 794.46 mg at 30 DAS) and chemical control (63.84 mg at 20 DAS and 639.76 mg at 30 DAS). Minimum shoot fresh weight was observed in control (62.21 mg at 20 DAS and 570.12 mg at 30 DAS). This experimental result also revealed that the CMC-1 significantly increased shoot fresh weight (78.30 % at 20 DAS and 100.14 % at 30 DAS) over control treatment (Table 4.22; Figs 4.9 and 4.11). Next in order of merit was CMC-2 (48.08 % at 20 DAS and 39.44 % at 30 DAS) and chemical control (2.62 % at 20 DAS and 12.21 % at 30 DAS).

Root fresh weight was also higher in all the treatments as compared to the control (Tables 4.19 and 4.21). Among these treatments, significantly higher fresh weight of root was recorded in seed treated with CMC-1 (10.76 mg at 20 DAS and 93.67 mg at 30 DAS) than the other treatments tested. Next best in order of merit was CMC-2 (7.82 mg at 20 DAS and 51.72 mg at 30 DAS) and chemical control (4.52 mg at 20 DAS and 42.47 mg at 30 DAS). Minimum root fresh weight was observed in control (4.47 mg at 20 DAS and 39.62 mg at 30 DAS). This experimental result also revealed that the CMC-1 significantly increased root fresh weight (140.71 % at 20 DAS and 136.42 % at 30 DAS) over control treatment (Table 4.22; Figs 4.9 and 4.11). Next in order of merit was CMC-2 (74.94 % at 20 DAS and 30.54 % at 30 DAS) and chemical control (1.12 % at 20 DAS and 7.19 % at 30 DAS).

The findings of present work are in agreement with the findings of earlier workers (Sandheep *et al.,* 2013; Kumar *et al.,* 2015; Khan *et al.,* 2018).

Sandheep *et al.* (2013) also evaluated combination of rhizobacteria for plant growth promoting on vanilla plants. Combined inoculation of *P. fluorescens* + *T. harzianum* registered the highest dry weight of shoot (4.56 g plant^{-1}) and dry weight of roots (2.08 g plant^{-1}). The heighest percentage of growth increase was recorded in the consortia of *P. fluorescens* + *T. harzianum* treatment followed by *P. fluorescens* + *P. putida* + *T. harzianum* + *T. virens*, respectively in decreasing order.

Kumar *et al.* (2015) developed a seed coating formulation of *B. subtilis* OTPB1 and *T. harzianum* OTPB3 and their consortia. The tomato seeds treated with a mixture of *T. harzianum* OTPB3 + *B. subtilis* OTPB1 or singly, exhibited increase in seedling growth attributes significantly compared to Dithane M-45 (0.2 %) and control. The consortium increased fresh weight of roots and shoots by 56.9 and 50.2 % respectively as compared to the control seedlings. These isolates were obtained from tomato rhizosphere.

4.11.5 Dry weight (mg) of seedling shoot and root at 10 DAS and 30 DAS

The dry weight of root and shoot of individual seedlings (60 seedlings /treatment) were measured after oven drying at 60° C (when constant weight obtained) for 24 hrs. Dry weight of seedling shoot was also higher in all the treatments as compared to the control at 20 DAS and 30 DAS (Tables 4.19 and 4.21). Among these treatments, significantly higher dry weight of shoot was recorded in seed treated with CMC-1 (6.26 mg at 20 DAS and 62.27 mg at 30 DAS) than the other treatments tested. Next best in order of merit was CMC-2

(5.31 mg at 20 DAS and 42.83 mg at 30 DAS) and chemical control (4.53 mg at 20 DAS and 32.83 mg at 30 DAS). Minimum shoot dry weight was observed in control (4.29 mg at 20 DAS and 30.48 mg at 30 DAS). This experimental result revealed that the CMC-1 significantly increased shoot dry weight (45.92 % at 20 DAS and 104.30 % at 30 DAS) over control treatment (Table 4.22; Figs 4.9 and 4.11). Next in order of merit was CMC-2 (23.78 % at 20 DAS and 40.52 % at 30 DAS) and chemical control (5.59 % at 20 DAS and 7.71 % at 30 DAS).

Root dry weight was also higher in all the treatments as compared to the control at 20 DAS and 30 DAS (Tables 4.19 and 4.21). Among these treatments, significantly higher dry weight of root was recorded in seed treated with CMC-1 (0.86 mg at 20 DAS and 4.84 mg at 30 DAS) than the other treatments tested. Next best in order of merit was CMC-2 (0.74 mg at 20 DAS and 2.75 mg at 30 DAS) and chemical control (0.47 mg at 20 DAS and 2.44 mg at 30 DAS). Minimum root dry weight was observed in control (0.44 mg at 20 DAS and 2.23 mg at 30 DAS). This experimental result also revealed that the CMC-1 significantly increased root dry weight (95.45 % at 20 DAS and 117.04 % at 30 DAS) over control treatment (Table 4.22; Figs 4.9 and 4.11). Next in order of merit was CMC-2 (68.18 % at 20 DAS and 23.32 % at 30 DAS) and chemical control (6.82 % at 20 DAS and 9.42 % at 30 DAS).

The findings of present work are in confirmation with the findings of earlier workers (Manoranjitham and Prakasam, 1999; Kabir *et al.,* 2013; Lamsal *et al.,* 2013; Sandheep *et al.,* 2013; Kumar *et al.,* 2015).

Sandheep *et al.* (2013) also evaluated combination of rhizobacteria for plant growth promoting on vanilla plants. Combined inoculation of *P. fluorescens* + *T. harzianum* registered the highest dry weight of shoot (4.56 g plant^{-1}) and dry weight of roots (2.08 g plant^{-1}). The heighest percentage of growth increase was recorded in the consortia of *P. fluorescens* + *T. harzianum* treatment followed by *P. fluorescens* + *P. putida* + *T. harzianum* + *T. virens*, respectively in decreasing order.

Khan *et al.* (2018) also tested three compatible microbial bioagents, *viz. T. viride, B. thuringiensis* and *P. fluorescens* for plant growth parameters in lettuce plant. There was significant increase in shoot dry weight and root dry weight of lettuce plants in treatments where bioformulations of *T. viride* + *B. thuringiensis* + *P. fluorescens* were applied as combination of root treatment (2.0 %) and foliar treatment (1.0 %). The highest shoot dry weight (16.41 g) and root dry weight (5.62 g) was recorded when lettuce plants were treated with application of consortia of *T. viride* + *B. thuringiensis* + *P. fluorescens*.

In this present investigation, an attempt has been made to study the effect of CMC on tomato seedlings in the plant growth promotion aspect in which effective results were obtained with CMC-1 in both *in vitro* as well as *in vivo* experiments. The improvement in tomato seed germination might be due to reserve mobilization of food materials. The enhanced in average tomato seedling dry weight upon CMC-1 treatment may be due to higher metabolic activity that leads to the better mobilization efficiency of stored food that might contribute for the better growth of seedlings which in turn result in enhanced seed germination, root length and shoot length and hence increase in the mean seedling dry weight. The increase in seedling vigour index upon CMC-1 treatment may be due to enhanced germination percentage, root length, shoot length and dry weight of tomato seedlings.

Table 4.18 *In vivo* effects of CMC on tomato seed germination (%) at 10 DAS, seedling shoot length, root length and vigour index at 20 DAS

Treatment	Seed Germination (%) at 10 DAS			Seedling shoot length (cm) at 20 DAS			Seedling root length (cm) at 20 DAS			Seedling Vigour index at 20DAS		
	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-17	Pooled	2017-18	2018-19	Pooled
T_1 (CMC-1)	86.92 (69.06)*	86.61 (68.67)	86.76 (68.82)	4.70	5.06	4.88	3.19	3.23	3.21	685.80	718.00	701.90
T_2 (CMC-2)	78.46 (62.48)	77.93 (62.05)	78.19 (62.26)	3.83	3.92	3.87	2.20	2.41	2.30	473.11	493.30	483.20
T_3 (Chemical control)	73.85 (59.45)	72.36 (58.42)	73.10 (58.93)	2.92	2.99	2.95	1.57	1.63	1.60	331.59	334.30	332.94
T_4 (Control)	72.31 (58.46)	71.39 (57.81)	71.85 (58.12)	2.89	2.95	2.92	1.55	1.62	1.58	321.06	326.25	323.65
SEm±	2.75 (1.88)	2.27 (1.53)	2.44 (1.59)	0.11	0.15	0.09	0.09	0.11	0.09	18.78	15.10	14.72
C.V. (%)	8.65 (7.41)	7.21 (6.07)	7.73 (6.54)	7.71	10.30	6.32	10.67	12.39	10.57	10.16	7.91	7.83
CD (*p*=0.01)	11.06 (7.59)	9.13 (6.16)	9.83 (6.66)	0.45	0.63	0.38	0.37	0.45	0.38	75.56	60.77	59.22
CD (*p*=0.05)	8.11 (5.56)	6.70 (4.51)	7.21 (4.88)	0.33	0.46	0.28	0.27	0.33	0.28	55.40	44.56	43.42

***Values in parentheses are angular transformed values.**

Table 4.19 *In vivo* effects of CMC on tomato seedling shoot fresh and dry weight, root fresh and dry weight at 20 DAS

Treatment	Seedling shoot at 20 DAS						Seedling root at 20 DAS					
	Fresh wt. (mg)			Dry wt. (mg)			Fresh wt. (mg)			Dry wt. (mg)		
	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1 (CMC-1)	107.62	114.23	110.92	06.25	06.28	06.26	10.57	10.98	10.76	0.88	0.84	0.86
T_2 (CMC-2)	89.25	95.00	92.12	05.32	05.30	05.31	07.49	08.15	07.82	0.76	0.72	0.74
T_3 (Chemical control)	64.54	63.15	63.84	04.66	04.40	04.53	04.47	04.57	04.52	0.41	0.53	0.47
T_4 (Control)	61.46	62.96	62.21	04.25	04.33	04.29	04.33	04.61	04.47	0.37	0.51	0.44
SEm±	5.30	3.75	3.60	0.28	0.21	0.21	0.52	0.63	0.45	0.07	0.03	0.04
C.V. (%)	16.16	10.95	10.72	13.64	10.18	10.07	19.15	15.84	15.92	11.27	18.18	16.25
CD (*p*=0.01)	21.32	15.07	14.49	1.15	0.85	0.84	2.11	2.54	1.80	0.30	0.14	0.17
CD (*p*=0.05)	15.63	11.05	10.62	0.84	0.62	0.62	1.55	1.86	1.32	0.22	0.10	0.12

Table 4.20 *In vivo* effects of CMC on tomato seedling shoot length, root length and vigour index at 30 DAS

Treatment	Seedling shoot length (cm) at 30 DAS			Seedling root length (cm) at 30 DAS			Seedling vigour index at 30 DAS		
	2017-18	2018-19	Pooled	2017-18	2018-17	Pooled	2017-18	2018-19	Pooled
T_1 (CMC-1)	15.08	15.37	15.22	4.79	4.57	4.68	1727.10	1727	1727.05
T_2 (CMC-2)	14.15	14.75	14.45	3.24	3.36	3.30	1364.42	1411.31	1387.86
T_3 (Chemical control)	11.80	11.64	11.72	1.93	1.91	1.92	1013.96	980.48	997.22
T_4 (Control)	11.31	11.45	11.38	1.92	1.86	1.89	956.66	950.20	953.43
SEm±	0.50	0.37	0.32	0.15	0.16	0.11	54.55	44.71	42.37
C.V. (%)	9.35	6.90	5.97	12.85	13.26	8.99	10.56	8.64	8.20
CD (*p*=0.01)	2.01	1.51	1.29	0.63	0.64	0.43	219.49	179.11	170.49
CD (*p*=0.05)	1.47	1.10	0.95	0.46	0.47	0.32	160.49	131.91	125.01

Table 4.21 *In vivo* effects of CMC on tomato seedling shoot fresh and dry weight, root fresh and dry weight at 30 DAS

Treatment	Seedling shoot						Seedling root					
	Fresh wt. (mg) at 30 DAS			Dry wt. (mg) at 30 DAS			Fresh wt. (mg) at 30 DAS			Dry wt. (mg) at 30 DAS		
	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1 (CMC-1)	1103.70	1178.37	1141.03	58.57	65.97	62.27	88.58	98.76	93.67	4.47	5.21	4.84
T_2 (CMC-2)	790.00	798.93	794.46	42.60	43.07	42.83	50.66	52.79	51.72	2.73	2.77	2.75
T_3 (Chemical control)	648.50	631.03	639.76	34.68	30.99	32.83	43.38	41.57	42.47	2.53	2.36	2.44
T_4 (Control)	528.90	611.35	570.12	30.65	30.23	30.48	39.40	39.84	39.62	2.24	2.22	2.23
SEm±	42.94	35.04	33.18	2.50	3.14	2.02	4.06	4.06	3.43	0.25	0.28	0.21
C.V. (%)	13.70	10.66	10.34	14.71	10.09	11.80	17.90	12.39	14.79	19.25	16.54	16.67
CD (*p*=0.01)	172.75	141.0	133.50	10.06	12.66	8.15	16.32	16.35	13.82	0.99	1.11	0.84
CD (*p*=0.05)	126.67	103.38	97.89	7.37	9.28	5.78	11.92	11.99	10.13	0.73	0.81	0.61

Table 4.22 *In vivo* effects of CMC on per cent increase of tomato seed germination (%), shoot and root length, shoot fresh and dry weight, root fresh and dry weight and seedling vigour index

Treat.	Per cent increase of plant growth promotion over control														
	Seed ger. (%) At 10 DAS	Seedling shoot						Seedling root						Seedling Vigour index	
		Length		Fresh wt.		Dry wt.		Length		Fresh wt.		Dry wt.			
		At 20 DAS	At 30 DAS	At 20 DAS	At 30 DAS	At 20 DAS	At 30 DAS	At 20 DAS	At 30 DAS	At 20 DAS	At 30 DAS	At 20 DAS	At 30 DAS	At 20 DAS	At 30 DAS
T_1 (CMC-1)	20.75	67.12	33.74	78.30	100.14	45.92	104.30	103.16	147.62	140.71	136.42	95.45	117.04	116.87	81.14
T_2 (CMC-2)	08.82	32.53	26.98	48.08	39.44	23.78	40.52	45.57	74.60	74.94	30.54	68.18	23.32	49.30	45.56
T_3 (Chemical control)	01.74	01.03	02.99	02.62	12.21	05.59	07.71	01.26	01.59	01.12	07.19	06.82	09.42	02.87	04.59
T_4 (Control)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4.12 Field evaluation of CMC against late blight of tomato

A field study conducted to check out the efficacy of selected two best consortia (CMC-1 and CMC-2) on no of leaves per plant, no of branches per plant, no of fruits per plant, plant height, fresh weight of fruit, marketable fruit yield, root length, late blight disease severity and tomato plant mortality per cent was carried out during 2017-18 and 2018-19 at experimental farm of Department of Plant Pathology, SASRD, Nagaland University, Medziphema.

4.12.1 Plant height (cm)

Plant height of the selected plants (30 plants per treatment) was recorded (Plate 29). The mean plant height was expressed in cm. The plant height was recorded at harvest (122 DAS) and it is depicted on table 4.23. The data of plant height revealed that all the treatments were significantly superior over the control. The highest plant height was recorded in treatment CMC-1 (45.33 cm) followed by CMC-2 (35.65 cm), chemical control (26.08 cm) and least plant height was recorded with control treatment at harvest (22.53 cm). The CMC-2 was found to be significantly superior to other treatments.

This experimental result also revealed that the CMC-1 significantly increased plant height (101.20 %) over control treatment (Fig 4.12). Next in order of merit was CMC-2 (58.23 %) and chemical control (15.76 %) respectively.

The findings of present work are in validation with the findings of earlier workers (Maiyappan *et al*., 2010; Manikandan *et al*., 2010; Muthukumar *et al.,* 2010; Rajasekar and Elango, 2011; Sharma *et al.,* 2015).

The application of BCAs or PGPR is considered as an important approach in crop protection against plant pathogens. Several microbes have been studied extensively as BCAs against various plant pathogens and these also showed plant growth promotion activity (Singh *et al.,* 2003; Lucy *et al.,* 2004; Mathivanan *et al.,* 2005; Srinivasan, 2007). PGPR strains were also tested individually and in combinations (two/more strains) against multiple plant pathogens (Raupach and Kloepper, 1998; Yan *et al.,* 2002; Idris *et al.,* 2007; Harish *et al.,* 2008).

Analogous to the observations in the present study, tomato plants treated with *T. hamanatum* (ANR-1) isolate showed a significant stimulatory effect on height (73.62 cm) of tomato plants in comparison to other isolates and untreated control. The increased in plant height may be due to the increase in the levels of growth hormones *viz*., IAA, gibberellic acid and defense enzymes *viz*., peroxidase, polyphenol oxidase and superoxide dismutase (Biam and Majumder, 2019).

4.12.2 Number of leaves per plant

The data concerning to effect of various treatment in relation to number of leaves per plant is depicted in table. 4.23. The results indicated that CMC-1 treated plants have more number of leaves (Plate 29) than in control. At harvest (122 DAS), it was recorded that CMC-1 recorded the highest number of leaves per plant (28.90) and was found to be significant over other treatments. Least number of leaves per plant was recorded in control (13.35).

This experimental result also revealed that the CMC-1 significantly increased number of leaves per plant (116.48 %) over control treatment (Fig 4.12). Next in order of merit was CMC-2 (64.04 %) and chemical control (12.36 %).

The findings of present work are in agreement with the findings of earlier workers (Maiyappan *et al*., 2010; Muthukumar *et al.,* 2010; Sharma *et al.,* 2015; Biam and Majumder, 2019).

The influence of *Trichoderma* isolates (*T. harzianum* strain DIS 326F (TR 122), *T. harzianum* strain CEN693 (TR 55), *T. harzianum* strain US10 (TR 66) and *T. harzianum* (TR 136) on the growth parameters of tomato such as numbers of leaves under greenhouse conditions has also been demonstrated by Biam and Majumder (2019). The number of leaves recorded after 45 DAS ranged from 32 to 57.20 numbers, when compared to control with 30 numbers. Maximum number of leaves/plant was recorded in TR 55 (45), followed by TR 122 (43.20), TR 66 (36) and TR 136 (34.46).

4.12.3 Number of branches per plant

The number of branches per plant (30 selected plants per treatment) were counted and recorded. The data concerning to effect of various treatments in relation to number of brances per plant is depicted in table. 4.23. The results indicated that CMC-1 treated plants have more number of branches (Plate 29) than in control. At harvest (122 DAS), it was recorded that CMC-1 recorded the highest number of branches per plant (8.26) and was found to be significant over other treatments. Least number of branches per plant was recorded in control (3.25).

This experimental result also revealed that the CMC-1 significantly increased number of branches per plant (146.57 %) over control treatment (Fig 4.12). Next in order of merit was CMC-2 (77.01 %) and chemical control (24.78 %).

Microbial consortia are known to enhance plant growth, which can result in development of various plant parts and higher growth leads to more branching, more number of leaves, flowers and fruits. The increased in plant growth may be due to the increase in the levels of growth hormones *viz*., indole-3-acetic acid (IAA) and gibberellic acid and defense enzymes *viz*., peroxidase, polyphenol oxidase and superoxide dismutase. Presence of consortia in the rhizoshphere increases the availability of nutrients through solubilization of insoluble sparingly soluble minerals have better nutrient uptake thereby enhancing plant growth (Raupach and Kloepper, 1998; Idris *et al.,* 2007; Harish *et al.,* 2008; Biam and Majumder, 2019).

4.12.4 Number of fruits per plant

The data concerning to effect of various treatment in relation to number of fruits per plant is depicted in table. 4.24. The results indicated that CMC-1 treated plants have more number of fruits per plant (Plate 29) than in control. At harvest (122 DAS), it was recorded that CMC-1 recorded the highest number of fruits per plant (35.69) and was found to be significant over other treatments. Least number of fruits per plant was recorded in control (12.50).

This experimental result also revealed that the CMC-1 significantly increased number of fruits per plant (185.52 %) over control treatment (Fig 4.12). Next in order of merit was CMC-2 (99.84 %) and chemical control (52.08 %).

Indole-3-acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L- tryptophan metabolism produced by several microorganisms including PGPR (Lynch, 1985). The microorganisms isolated from rhizosphere region of various crop have an ability to produce IAA as secondary metabolites due to rich supply of substrates. IAA helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake. It promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting (Raupach and Kloepper, 1998; Yan *et al.,* 2002; Idris *et al.,* 2007; Harish *et al.,* 2008).

4.12.5 Fresh weight of fruit (g fruit^{-1})

The data concerning to effect of various treatment in relation to fresh weight of fruit is depicted in table. 4.24. The results indicated that CMC-1 treated plants have highest fresh weight of tomato fruit than in control. At harvest (126 DAS), it was recorded that CMC-1 recorded the highest fresh weight of fruit (33.01 g) and was found to be significant over other treatments. Least fresh weight of fruit was recorded in control (23.15 g).

This experimental result also revealed that the CMC-1 significantly increased fresh weight of fruit (42.59 %) over control treatment (Fig 4.13). Next in order of merit was CMC-2 (32.31 %) and chemical control (9.37 %).

Manikandan *et al*. (2010) tested *P. fluorescens* Pf1 against *Fusarium* wilt of tomato under glasshouse and field. They recorded significantly enhanced the fresh weight of tomato fruit (38.96 g under glasshouse and 43.67 g under field conditions) over control by seed treatment + seedling dip + soil drenching. This isolate was obtained from tomato rhizosphere.

4.12.6 Marketable fruit yield (g plant^{-1}) and calculated marketable fruit yield (t ha^{-1})

The yield of ripened marketable fruits harvested at different dates from sampling plants was computed and their average yield per plant was noted in gram. The data concerning to effect of various treatment in relation to yield of tomato fruit is depicted in table. 4.25. The data revealed that CMC-1 treated plants recorded highest yield (1166.78 g plant^{-1}) followed by CMC-2 (735.45 g plant^{-1}) and chemical control (475.31 g plant^{-1}). The lowest yield was recorded in control with 282.50 g plant^{-1}.

The actual mean yield per plant in replication was converted in t ha^{-1}. The data concerning to effect of various treatment in relation to calculated marketable fruit yield of tomato is depicted in table. 4.25. The data revealed that CMC-1 treated plants recorded highest calculated marketable fruit yield (43.21 t ha^{-1}) followed by CMC-2 (27.23 t ha^{-1}) and chemical control (17.60 t ha^{-1}). The lowest yield was recorded in control with 10.46 t ha^{-1}.

This experimental result also revealed that the CMC-1 significantly increased marketable tomato fruit yield (313.02 %) over control treatment (Fig 4.13). Next in order of merit was CMC-2 (160.34 %) and chemical control (68.25 %).

This is in line with the findings of Manikandan *et al*. (2010), Hema and Selvaraj (2011) and Khan *et al.* (2018).

Srivastava *et al*. (2010) evaluated a consortium of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *T. harzianum* formulation against *F. oxysporum* f. sp. *lycopersici* for the management of tomato wilt and found that combination of all three bioagents with cow dung compost significantly reduced disease in pots and field, respectively and enhanced the yield.

Nath *et al.* (2016) developed a biointensive strategy for the management of bacterial wilt of tomato caused by *R. solanacearum* using four compatible PGPM. Significantly maximum yield (1.692 kg plant^{-1}) of tomato was recorded in treatment comprising of *A. chroococcum* + *B. subtilis* + *T. parareesei* + *P. fluorescens*.

Biam and Majumder (2019) reported the influence of *Trichoderma* isolates (*T. harzianum* strain DIS 326F (TR 122), *T. harzianum* strain CEN693 (TR 55), *T. harzianum* strain US10 (TR 66) and *T. harzianum* (TR 136) on the yields under greenhouse conditions. The yield enhanced in all the treatments compared to control (1.4 kg). The highest yield was recorded in TR 55 (2.25 kg), followed by TR 122 (1.76 kg), TR 136 (1.74 kg) and TR 66 (1.69 kg). Among the treatments, maximum yield was recorded in T_3 (2.07 kg), followed by T_2 (1.65 kg) and T_1 (1.58 kg).

4.12.7 Root length (cm)

The root length was measured with the help of a scale from uprooted tagged plants. The data concerning to effect of various treatment in relation to root length of tomato plant is depicted in table. 4.24. The results indicated that CMC-1 treated plants have highest root length (Plate 30) than in control. After harvest (128 DAS), it was recorded that CMC-1 recorded the highest root length (44.63 cm) and was found to be significant over other treatments. Least root length was recorded in control (26.68 cm).

This experimental result also revealed that the CMC-1 significantly increased root length of tomato plant (67.28 %) over control treatment (Fig 4.13). Next in order of merit was CMC-2 (36.54 %) and chemical control (10.38 %).

Indole-3-acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L- tryptophan metabolism produced by several microorganisms including PGPR. The microorganisms isolated from rhizosphere region of various crop have an ability to produce IAA as secondary metabolites due to rich supply of substrates. IAA helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (Lynch, 1985).

Rajasekar and Elango (2011) studied the effect of microbial consortium consisting of *Azotobacter, Azospirillum, Bacillus* and *Pseudomonas* were evaluated separately and consortia on *Withania somnifera* for two repeated years. Consortia of above PGPR strains significantly increased alkaloid content, root length and plant height. The combinations of above mentioned PGPR strains significantly increased plant height, root length and alkaloid content.

Sharma *et al.* (2015) tested consortia of *Bacillus* strains against *P. capsici* in tomato under net house conditions. Consortial application of four strains of *Bacillus* (S_{21} + S_{25} + B_6+ A_{10}) had significant effect in achieving biocontrol efficacy of 66.7 %. Combination of 4 strains of bacteria resulted in significant enhanced in plant parameters including fresh and dry weight, shoot and root length, fresh weight and dry weight over control treatment.

Biam and Majumder (2019) reported the influence of *Trichoderma* isolates (*T. harzianum* (TR 136), *T. harzianum* strain US10 (TR 66), *T. harzianum* strain CEN693 (TR 55) and *T. hamatum* strain DIS 326F (TR 122) on the root length of tomato under greenhouse conditions. Heighest root lenght was observed in TR 122 (18.35 cm), followed by TR 66 (16 cm) and TR 136 (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).

4.12.8 Disease severity and mortality per cent

The late blight disease severity was evaluated visually on fruits, stems, and leaves of all plants of each replication following scale 0-5 (Irzhansky and Cohen, 2006) when total late blight infestation had occurred in the control plot under natural epiphytotic condition (Plate 30). The severity grades were converted into percentage disease index (PDI) for analysis (Wheeler, 1969). The disease severity was recorded at 105 DAS and it is depicted in table 4.25. The data of disease severity revealed that least disease severity (PDI) was recorded in chemical treatment (6.25 PDI) and CMC-1 (12.08 PDI) which is statistically significant whereas highest disease severity was recorded in control with 77.36 PDI.

This experimental result revealed that the chemical control significantly reduced or decreased late blight severity (91.92 %) over control treatment (Fig 4.14). Next in order of merit was CMC-1 (84.38 %) and CMC-2 (77.20 %).

Observations for mortality per cent all plants in each replication were observed when total late blight infestation had occurred in the control plot under natural epiphytotic condition. The mortality per cent was recorded at 105 DAS and it is depicted on table 4.25. The data of mortality per cent revealed that least mortality per cent was recorded in all the treatment (0.00 %) except control (24.30 %). The mortality per cent significantly reduced or decreased in all the treatments (100 %) over control treatment (Fig 4.14).

Application of PGPR or BCAs is an important strategy considered in crop protection against wide range of plant pathogens. PGPRs andl BCAs have been reported as plant growth promotion activity against wide range of various plant pathogens (Singh *et al.,* 2003; Mathivanan *et al.,* 2005; Srinivasan, 2007). PGPR strains were also tested individually and in combinations against multiple plant pathogens (Raupach and Kloepper, 1998; Yan *et al.,* 2002; Idris *et al.,* 2007; Harish *et al.,* 2008).

Srivastava *et al*. (2010) also tested a combination of AM fungus, *P. flourescens* and *T. harzianum* for the management of tomato wilt (*F. oxysporum* f. sp. *lycopersici*). They found that triple consortia of bioagents with cow dung compost significantly reduced wilt disease of tomato in field and pots. For the management of tomato wilt and found that combination of all three bioagents with cow dung compost significantly reduced disease in pots and field, respectively.

Kumar *et al.* (2015) also evaluated the efficacy of seed treatment with *T. harzianum* OTPB3 + *B. subtilis* OTPB1 + *P. putida* OPf1 strain and Dithane M-45 as chemical control with foliar sprays of *P. putida* OPf1 and fenamidone-mancozeb against late blight of tomato under pot culture conditions. Late blight disease severity was 73.1 % in seed treatment with OTPB1 + OTPB3 followed by foliar spray of OPf1. Seed treatment with OTPB3 + OTPB1 followed by foliar spray of OPf1 significantly reduced late blight severity by 73.1 % compared to untreated controls.

Sharma *et al.* (2015) also tested consortia of *Bacillus* strains against *P. capsici* in tomato under net house conditions. Consortial application of four strains of Bacillus (S_{21} + S_{25} + B_6 + A_{10}) had significant effect in achieving biocontrol efficacy of 66.7 %.

In this present investigation, the results clearly indicate that the CMC-1 could reduce the late blight severity as well as enhance the growth of tomato plants. The significant decrease of late blight severity and increase in tomato yield in two best treatments, *i.e.*, CMC-1 as well as CMC-2 applied as combinations of seed, soil and foliar treatments might have occurred due to direct effects of the bioagents on suppression of the pathogen population in the plant rhizosphere. The increased yield and biomass production may be attributed to the good vegetative growth due to the efficiency of consortia, in supplying nutrients and production of IAA, GA and cytokinin and their synergistic action of antagonists.

Table 4.23 *In vivo* effects of CMC on tomato plant height, number of leaves and branches per plant at 122 DAS

Treatment	Plant height (cm) at 122 DAS				No. of leaves per plant at 122 DAS				No. of branches per plant at 122 DAS			
	2017-18	2018-19	Pooled	% increase over control	2017-18	2018-19	Pooled	% increase over control	2017-18	2018-19	Pooled	% increase over control
T_1 (CMC-1)	43.40	47.27	45.33	101.20	27.57	30.23	28.90	116.48	08.20	08.33	08.26	146.57
T_2 (CMC-2)	34.10	37.20	35.65	58.23	20.87	22.93	21.90	64.04	06.17	05.50	05.83	77.01
T_3 (Chemical control)	29.33	22.83	26.08	15.76	14.00	16.00	15.00	12.36	04.20	04.17	04.18	24.78
T_4 (Control)	24.43	20.63	22.53	-	13.03	13.67	13.35	-	03.23	03.47	03.25	-
SEm±	1.52	1.74	1.17	-	1.32	1.61	1.22	-	0.43	0.41	0.38	-
C.V. (%)	11.38	13.31	8.89	-	17.20	19.02	15.19	-	19.18	18.76	17.15	-
CD (*p*=0.01)	6.35	7.24	4.90	-	5.52	6.70	5.11	-	1.78	1.71	1.58	-
CD (*p*=0.05)	4.59	5.24	3.54	-	3.99	4.85	3.70	-	1.29	1.24	1.14	-

Table 4.24 *In vivo* effects of CMC on number of tomato fruit per plant, fresh weight of fruit and root length

Treatment	No. of fruit per plant at 122 DAS				Fresh weight of fruit (g) at 126 DAS				Root length (cm) at 128 DAS			
	2017-18	2018-19	Pooled	% increase over control	2017-18	2018-19	Pooled	% increase over control	2017-18	2018-19	Pooled	% increase over control
T_1 (CMC-1)	35.65	35.73	35.69	185.52	33.30	32.73	33.01	42.59	44.93	44.33	44.63	67.28
T_2 (CMC-2)	24.60	25.37	24.98	99.84	29.73	31.54	30.63	32.31	37.07	35.80	36.43	36.54
T_3 (Chemical control)	19.53	18.50	19.01	52.08	24.33	26.31	25.32	09.37	30.20	28.70	29.45	10.38
T_4 (Control)	11.07	13.93	12.50	-	23.07	23.24	23.15	-	27.30	26.07	26.68	-
SEm±	2.11	2.06	1.98	-	1.41	1.55	1.22	-	1.23	1.59	1.04	-
C.V. (%)	17.90	19.21	18.02	-	14.24	15.51	13.80	-	8.61	11.54	7.47	-
CD (*p*=0.01)	8.80	8.57	8.24	-	5.88	6.48	6.24	-	3.69	6.62	4.36	-
CD (*p*=0.05)	6.36	6.20	5.96	-	4.25	4.68	4.69	-	5.11	4.79	3.15	-

Table 4.25 *In vivo* effects of CMC on per cent decrease of late blight severity and mortality per cent and per cent increase of marketable tomato fruit yield over control

Treat.	Disease severity at 105 DAS (PDI)				Mortality per cent at 105 DAS				Marketable fruit yield (g plant^{-1})				Calculated marketable fruit yield (t ha^{-1})			
	2017-18	2018-19	Pooled	% decrease over control	2017-18	2018-19	Pooled	% decrease over control	2017-18	2018-19	Pooled	% increase over control	2017-18	2018-19	Pooled	% increase over control
T_1 (CMC-1)	12.50 (20.58)	11.67 (19.89)	12.08 (20.28)	84.38	00.00 (4.05)*	00.00 (4.05)	00.00 (4.05)	100	1185.70	1147.87	1166.78	313.02	43.91	42.51	43.21	313.10
T_2 (CMC-2)	19.44 (26.09)	15.83 (23.32)	17.64 (24.80)	77.20	00.00 (4.05)	00.00 (4.05)	00.00 (4.05)	100	718.00	752.90	735.45	160.34	26.59	27.88	27.23	160.32
T_3 (Chemical control)	06.94 (15.16)	05.55 (13.50)	06.25 (14.39)	91.92	00.00 (4.05)	00.00 (4.05)	00.00 (4.05)	100	467.83	482.80	475.31	68.25	17.32	17.88	17.60	68.26
T_4 (Control)	77.78 (61.97)	76.94 (61.35)	77.36 (61.62)	-	23.61 (28.51)	25.00 (29.79)	24.30 (29.36)	-	243.67	321.33	282.50	-	09.02	11.90	10.46	-
SEm±	1.18 (0.91)	1.34 (1.08)	0.75 (0.36)	-	2.26 (1.58)	1.52 (1.02)	1.46 (0.98)	-	92.79	61.16	72.92	-	3.47	2.26	2.70	-
C.V. (%)	9.95 (7.17)	11.98 (8.97)	6.54 (4.94)	-	19.82 (7.85)	11.95 (4.69)	15.69 (6.36)	-	16.85	17.10	16.86	-	17.56	14.46	16.86	-
CD (*p*=0.01)	4.94 (3.78)	5.61 (4.51)	3.15 (2.54)	-	9.42 (6.60)	6.34 (4.25)	6.11 (3.97)	-	386.70	254.91	303.91	-	14.47	9.44	11.25	-
CD (*p*=0.05)	3.57 (2.73)	4.05 (3.26)	2.28 (1.84)	-	6.81 (4.77)	4.58 (3.07)	4.42 (2.87)	-	279.63	184.33	219.76	-	10.46	6.83	8.14	-

***Values in parentheses are angular transformed values.**

4.13 Field evaluation of native tomato genotypes for their resistance to late blight under natural epiphytotic condition

A field study conducted to evaluate the native tomato genotypes for their resistance to late blight under natural epiphytotic condition was carried out during 2017-18 and 2018-19 at experimental farm of Department of Plant Pathology, SASRD, Nagaland University, Medziphema.

4.13.1 Collection of tomato genotypes

Ripened fruits of 20 native tomato genotypes were collected from different tomato growing areas of Nagaland, Manipur and Arunachal Pradesh (Plate 31). Known susceptible hybrid (Arka Rakshak, F_1) and susceptible variety (Pusa Ruby) seeds were also collected from IIHR, Bangalore and local market, Dimapur, respectively. Characterization of tomato genotypes (Plates 32 and 33) and their collection locations are mentioned in table 4.26. Result revealed that most of the collected genotypes were indeterminate in nature except Pusa Ruby (***semi-determinate) and*** T_2 (determinate). Fruit shape of collected genotepes were oblong o***val*** (Arka Rakshak F_1), flattish round (Pusa Ruby), small round cherry type (T_2, T_3, T_4, T_9, T_{16}, T_{17}, T_{18}, T_{19} and T_{20}), round medium size (T_5), plum type (T_6, T_{10}, T_{11} and T_{14}), pointed small grape type (T_7), small round (T_8, T_{12}, T_{15}), round (T_{13}) and Heirloom (T_{21}).

Various research workers collected and characterized different cultivars/ hybrids of tomato, cultivars with spherical, smooth fruit especially John Bear, Main Crop, Sunshine, Victory and Matchless (Bondartzeva, 1926), which were reported to possess resistance to late blight of tomato. Yimchunger *et al.* (2018) also recorded different shapes of cherry tomato from Nagaland. They reported that shape among the traits like round, oblong, heart shape was noticed in different genotypes. They also mentioned that variations in different shape of fruit are influenced by the genetic makeup of the genotype.

Table 4.26 Characterization of tomato genotypes and their collection location

Genotypes Code	Type	Fruit type	Growth habits	Collected from	District	State
T_0	Arka Rakshak, F_1 hybrid	Oblong o***val***	Determinate	IIHR	Bangalore	Karnataka
T_1	Tomato cv. Pusa Ruby	Flattish round	***Semi-determinate***	Local market	Dimapur	Nagaland
T_2	Cherry tomato	Small round	Determinate	Horticulture farm, SASRD, Medziphema	Dimapur	Nagaland
T_3	Cherry tomato	Small round	Indeterminate	Near C.V. Raman Hostel, SASRD, Medziphema	Dimapur	Nagaland
T_4	Cherry tomato	Small round	Indeterminate	Daily vegetable market, Kohima town	Kohima	Nagaland
T_5	Tomato	Round medium size	Indeterminate	Daily vegetable market, Risethsi	Kiphre	Nagaland
T_6	Tomato	Plum type	Indeterminate	Daily vegetable market, Merema	Kohima	Nagaland
T_7	Tomato	Pointed small Grape type	Indeterminate	Daily vegetable market, Phekerkriema	Kohima	Nagaland
T_8	Tomato	Small round	Indeterminate	Daily vegetable market, Merema	Kohima	Nagaland
T_9	Cherry tomato	Small round	Indeterminate	Daily vegetable market, Tsiesema	Kohima	Nagaland

T_{10}	Tomato	Plum type	Indeterminate	Daily vegetable market, Wokha town	Wokha	Nagaland
T_{11}	Tomato	Plum type	Indeterminate	Daily vegetable market, Pfutsero	Phek	Nagaland
T_{12}	Tomato	Small round	Indeterminate	Daily vegetable market, Wokha town	Wokha	Nagaland
T_{13}	Tomato	Round	Indeterminate	Daily vegetable market, Pfutsero	Phek	Nagaland
T_{14}	Tomato	Plum type	Indeterminate	Daily vegetable market, Kohima town	Kohima	Nagaland
T_{15}	Tomato	Small round	Indeterminate	Daily vegetable market, Kohima town	Kohima	Nagaland
T_{16}	Cherry tomato	Small round	Indeterminate	Daily vegetable market, Kohima town	Kohima	Nagaland
T_{17}	Cherry tomato	Small round	Indeterminate	Daily vegetable market, D' sector, Itanagar	Papum Pare	Arunachal Pradesh
T_{18}	Cherry tomato	Small round	Indeterminate	Daily vegetable market, Keishamthong	Imphal West	Manipur
T_{19}	Cherry tomato	Small round	Indeterminate	Daily vegetable market, Moa gate	Senapati	Manipur
T_{20}	Cherry tomato	Small round	Indeterminate	Daily vegetable market, Pisum Oinam Leikai	Imphal West	Manipur
T_{21}	Tomato	Heirloom	Indeterminate	Daily vegetable market, Risethsi	Kiphre	Nagaland

4.13.2 Fruit yield (g plant^{-1}) and calculated fruit yield (t ha^{-1})

The yield of ripened fruits harvested at different dates from all plants was computed and their average per plant was noted in gram. The data concerning to yield of tomato genotypes is depicted in table 4.27. The data revealed that Arka Rakshak, F_1 hybrid recorded highest yield (3433.55 g plant^{-1}) followed by T_5 (645.35 g plant^{-1}) and T_{21} (628.61 g plant^{-1}). The lowest yield was recorded in T_{19} with 268.15 g plant^{-1}.

The actual mean yield per plant in replication was converted in t ha^{-1}. The data revealed that Arka Rakshak, F_1 hybrid recorded highest yield (105.65 t ha^{-1}) followed by T_5 (19.85 t ha^{-1}) and T_{21} (19.34 t ha^{-1}). The lowest yield was recorded in T_{19} with 8.25 t ha^{-1}.

These results are in similarity with the findings of Swaroop and Suryanarayana (2005), Ahmed *et al.* (2007), Doreswamy *et al.* (2011), Dar and Sharma (2011) and Narolia *et al.* (2012).

Bhati, (2017) also evaluated tomato genotypes for growth, yield and quality traits under foothills condition of Nagaland. It is evident from this study that there was significant difference in yield attributes among various genotypes. It was revealed from this study that yield per hectare profoundly affected by the genotypes. Maximum yield was recorded in genotype of TODVAR-8 (46.62 t ha^{-1}) followed by TODVAR-1 (33.14 t ha^{-1}). The minimum yield was recorded by genotype H-86 (12.41 t ha^{-1}).

4.13.3 Disease severity

The late blight disease severity was evaluated visually on fruits, stems and leaves of all plants of each replication following scale 0-5 (Irzhansky and Cohen, 2006) when total late blight infestation had occurred in the control plot under natural epiphytotic condition (Plate 34). The severity grades were converted into percentage disease index (PDI) for analysis (Wheeler, 1969). In all, 22 genotypes were screened against the late blight disease under natural epiphtotic condions in field and disease reaction was recorded at 124 DAS and presented in table 4.27. The data of disease severity revealed that least disease severity (PDI) was recorded in T_3 (16.81 PDI) and T_{17} (35.99 PDI) whereas highest disease severity was recorded in Arka Rakshak, F_1 control with 89.72 PDI (Fig 4.15).

This experimental results revealed that most of the genotypes (20 genotypes) reacted as highly susceptible, while T_3 (16.81 PDI) and T_{17} (35.99 PDI) were found resistant and tolerant respectively under natural epiphytotic condition. These results clearly indicate that a good source of resistance to late blight is available in the genotype T_3 (cherry tomato). The resistant genotype thus obtained through this experiment shows a potential disease resistance trait contributor for tomato breeding against late blight disease.

This is in line with the findings of Islam *et al*. (2001) who evaluated 15 advanced lines of tomato against late blight under natural epiphytotic condition. They observed 2 enteries were moderately resistant (V-385 and V-187), two resistant (V-259 and V-426), two tolerant (V-422 and V-282), four moderately susceptible (V-138, V-378, V-258 and BARI-10), three susceptible (V-201, V-330 and Manik) and two highly susceptible (V-215 and V-52), but none were highly resistant. Two highly susceptible (V-52 and V-215), but none were found highly resistant.

Nazrul *et al*. (2001) also evaluated 15 advanced lines of tomato but none of them were resistant. While only two advanced lines (V-187 and V-385) were moderately resistant.

Khalid *et al*. (2012) evaluated 82 tomato genotypes against late blight using whole-plant assays and detached-leaf. None of the test genotypes were immune or highly resistant. Of the 82 genotypes only TMS-2 was resistant with severity index of 2.4 in the detached-leaf assay on 0-5 scale and 23.3 PDI under the whole-plant assay. Variable levels of the PDI were found in all the genotypes under the whole-plant assay. The overall screening results indicate that TMS-2 is a good source of resistance and it can be useful for the development of tomato hybrid cultivars resistant to late blight.

Table 4.27 Late blight disease severity, disease reaction and fruit yield of tomato genotypes under natural epiphytotic condition

Genotypes	Disease severity at 124 DAS (PDI)				Fruit yield (g plant^{-1})			Calculated Fruit yield (t ha^{-1})		
	2017-18	2018-19	Pooled	Disease reaction	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_0 Arka Rakshak F_1 (Control)	92.22	87.22	89.72	HS	3450.44	3416.67	3433.55	106.17	105.13	105.65
T_1 Pusa Ruby (Control)	84.44	90.00	87.22	HS	275.56	271.33	273.44	08.48	08.35	08.41
T_2	88.88	78.89	83.88	HS	327.50	276.67	302.08	10.08	08.51	09.29
T_3	22.65	10.98	16.81	R	329.50	256.00	292.75	10.14	07.88	09.01
T_4	47.01	79.18	63.09	HS	286.67	311.67	299.17	08.82	09.59	09.20
T_5	53.87	83.94	68.90	HS	634.03	656.67	645.35	19.51	20.20	19.85
T_6	55.44	87.96	71.70	HS	418.73	393.33	406.03	12.88	12.10	12.49
T_7	62.75	86.48	74.61	HS	504.63	423.33	463.98	15.53	13.02	14.27
T_8	58.63	68.00	63.31	HS	541.41	446.67	494.04	16.66	13.74	15.20
T_9	50.73	78.57	64.65	HS	371.69	343.33	357.51	11.44	10.56	11.00
T_{10}	67.97	79.76	73.86	HS	514.78	463.33	489.05	15.84	14.26	15.05

T_{11}	73.96	81.11	77.53	**HS**	340.75	355.33	348.04	10.48	10.93	10.70
T_{12}	76.39	86.46	81.42	**HS**	462.86	408.67	435.76	14.24	12.57	13.40
T_{13}	67.39	80.22	73.80	**HS**	339.41	285.67	312.54	10.44	08.79	09.61
T_{14}	60.00	87.53	73.76	**HS**	300.67	291.33	296.00	09.25	08.96	09.10
T_{15}	66.07	76.94	71.50	**HS**	331.81	306.67	319.24	10.21	09.43	09.82
T_{16}	53.52	79.35	66.43	**HS**	334.61	266.67	300.64	10.29	08.20	09.24
T_{17}	38.33	33.65	35.99	**T**	238.85	325.33	282.09	07.35	10.01	08.68
T_{18}	59.61	78.34	68.97	**HS**	254.82	293.33	274.07	07.84	09.02	08.43
T_{19}	53.70	78.70	66.20	**HS**	282.97	253.33	268.15	08.71	07.79	08.25
T_{20}	51.59	81.11	66.35	**HS**	284.02	283.33	283.67	08.74	08.72	08.73
T_{21}	71.67	81.20	76.43	**HS**	693.89	563.33	628.61	21.35	17.33	19.34
SEm±	4.06	3.30	2.52	-	57.25	38.44	36.03	1.76	1.19	1.11
C.V. (%)	11.41	7.50	6.33	-	18.49	13.05	12.25	18.94	13.35	12.26
CD (*p*=0.01)	15.50	12.59	9.62	-	218.46	146.66	137.48	6.72	4.56	4.23
CD (*p*=0.05)	11.59	9.42	7.19	-	163.40	109.70	102.83	5.03	3.41	3.17

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present investigations on "**Development of microbial consortia for sustainable management of late blight (*Phytophthora infestans* (Mont.) de Bary) in tomato (*Solanum lycopersicum* L.)**" was carried out under *in vitro* as well as field condition in the experimental farm of the Department of Plant Pathology, SASRD, Nagaland University, Medziphema Campus, located on foot hills of Nagaland at an altitude of 310 m from the mean sea level with the geographical location of 25° 45′ 45′′ North latitude and 93° 51′ 45" East longitude. The investigations were carried out to study the efficacy of native compatible microbial consortia (CMC) against the pathogen causing late blight of tomato and field evaluation of native tomato genotypes for their resistance to late blight in order to formulate an effective, sustainable, eco-friendly and economical disease management strategy.

The findings obtained from the experiment are summarized as follows:

- Typical initial foliar symptoms of late blight of tomato were recorded on lower leaves as small, irregular, light to dark green and water soaked spots. Under favourable weather conditions, these spots develop into large brown necrotic lesions with indefinite borders. On the ventral surface of the leaves, a fluffy, white downy growth of the fungus appeared around the necrotic tissues. The affected leaves soon turned brown in colour, shriveled and later dried off.
- Symptoms also appeared on petioles and stems as brown streaks. In moist weather, disease progressed very rapidly and infects the whole foliage.
- On tomato fruits, the symptoms appeared only on green fruits as a dark olivaceous, greasy spots which gradually covers the entire fruit. Initially, the tissue of the affected fruit remain firm with varying depths of discoloured

tissue below the skin but when soft rot appeares after the blight attack, fruit disintegration rapidly.

- The pathogen causing late blight of tomato was isolated from infected leaves, stems and fruits showing typical symptoms and pathogenicity test was carried out to establish the ability of fungal isolate to produce signs and symptoms of the pathogen under natural condition. The pathogen was re-isolated for confirmation.

- The mycelium of isolated fungus, *P. infestans* was grown on rye-B agar medium and incubated at 18±1 °C. It was hyaline, coenocytic, moderately thick hyphae, fluffy cottony with slightly striated pattern and profusely branched. Sporangiophores were compound, sympodial, with a small swelling at the base of each branch. Sporangia were terminal or lateral, ellipsoid, ovoid or limoniform, semipapillate, deciduous and pedicelless. Chlamydospores of the pathogen were also observed from diseased specimens.
- The pathogen was identified as *P. infestans* based on the nature of disease observed, cultural and morphological characters seen under the microscope.
- A field survey was undertaken for the collection of tomato rhizosphere soil samples from tomato growing areas *viz.,* Merema, Tsiesema, SASRD, campus and CIH polyhouse, Medziphema.
- Sixteen isolates (8 isolates of *Pseudomonas* and 8 isolates of *Trichoderma*) were isolated from tomato rhizosphere by soil dilution plate technique.
- Seventeen isolates of *Trichoderma* were also obtained from department of Plant Pathology, SASRD, Nagaland University, Medziphema.
- For obtaining better insight in the antagonistic potential of native BCAs, 33 isolates were evaluated against *P. infestans* by dual culture technique.
- Upon *in vitro* screening of the varied isolates, highest growth inhibition of pathogen was recorded among *Pseudomonas* isolates [Pf-2 (81.33 %), Pf-3

(73.33 %) and Pf-1 (69.33 %)] followed by *Trichoderma* isolates [T-11 (73.73 %), T-14 (66.67 %) and T-5 (64.93 %)].

- The effects of volatile metabolites of 25 isolates of *Trichoderma* and 8 isolates of *Pseudomonas* were assessed against *P. infestans* by Dennis and Webster (1971) technique. Among the *Trichoderma* isolates, the maximum per cent inhibition was observed in T-11 (45.55 %) followed by T-14 (35.55 %) and T-21 (35.22 %) respectively. Among the *Pseudomonas* isolates, the maximum per cent inhibition was observed in Pf-2 (53.67 %) which is significantly superior to all other treatments followed by Pf-3 (48.11 %) and Pf-1 (44.78 %) at 6 days after incubation at 18±1 °C.
- Altogether 25 isolates of *Trichoderma* and 8 isolates of *Pseudomonas* were tested for the production of ammonia in peptone water. All, 33 isolates showed positive results for ammonia production. Among the tested isolates, *Pseudomonas* isolates (Pf-3, Pf-4, Pf-7 and Pf-8) and *Trichoderma* isolates (T-1, T-2, T-11, T-14 and T-25) exhibited strong ammonia production by turning initial peptone water broth from yellow to dark brown colour.
- The production of IAA by *Trichoderma* and *Pseudomonas* isolates were determined by qualitative assay (Gordon and Weber, 1951). The results revealed that *Pseudomonas* isolates (Pf-2, Pf-3 and Pf-8) and *Trichoderma* isolates (T-25, T-19, T-17, T-16, T-15, T-14, T-11, T-9, T-8, T-7, T-6 and T-2) elucidated positive results for IAA production.
- Phosphate solubility test was conducted qualitative by inoculating of *Trichoderma* and *Pseudomonas* on NBRIP agar medium. For the indication of presence of halo clearing zone around fresh colony after incubating at 28 °C for 7 days was used as an indicator for positive P solubilization. The results revealed that *Pseudomonas* isolates (Pf-1, Pf-2, Pf-3, Pf-7 and Pf-8) and *Trichoderma* isolates (T-3, T-5, T-10, T-11, T-14, T-16, T-19 and T-25) elucidated positive results for phosphate solubilization.

- Chrome azurol sulfonate (CAS) assay was used to detect the production of siderophore of 25 isolates of *Trichoderma* and 8 isolates of *Pseudomonas* following the procedure given by Schwyn and Neilands, 1987. All, 33 isolates showed positive results for siderophore production. Among the tested isolates, *Pseudomonas* isolates (Pf-3 and Pf-8) and *Trichoderma* isolates (T-21, T-18, T-15, T-14, T-11, T-10, T-9, T-8, T-7, T-5, T-4 and T-3) exhibited strong siderophore production by pink and orange halo colour.
- The production of HCN by *Pseudomonas* isolates were determined by modified protocol of Miller and Higgins (1970). The results revealed that *Pseudomonas* isolates (Pf-2, Pf-3 and Pf-8) elucidated positive results for HCN production.
- All 25 isolates of *Trichoderma* under the study were tested for mycoparasitism activity against *P. infestans*. All, 25 isolates showed the presence of coiling as hyphal interactions between *Trichoderma* isolates and *P. infestans.*
- Based on *in vitro* antagonistic capabilities of *Trichoderma* and *Pseudomonas* isolates against *P. infestans* and their elucidation for various biocontrol mechanisms, the potent isolates were selected as *Pseudomonas* isolates (Pf-2 and Pf-3) and *Trichoderma* isolates (T-11 and T-14) for further studies.
- All potent native microbial isolates were capable to release inorganic p from TCP and showed consistent ability to produce siderophore, HCN, ammonia, IAA, volatile metabolites and mycoparasitism ability.
- Molecular identification of potential *Trichoderma* isolates (T-11 and T-14) was performed by using Internal Transcribed Spacer (ITS) region of 18S rRNA.

- Sequence analyses of two isolates (T-11 and T-14) were done to confirm species identity. ITS sequences of both the isolates were submitted to NCBI GenBank (MK928414 and MK928417), which showed 100 % similarity with *T. asperellum* during BLAST analysis. This was used for construction of phylogeny tree and subsequently, these isolates were identified as *T. asperellum*.
- The potential *Pseudomonas* isolates (Pf-2 and Pf-3) were identified as *P. fluorescens* based on the following characteristics, gram negative, rod shaped cells, creamy mucoid colony with smooth edges and yellow-green fluorescent pigmentation produced under ultraviolet (UV) light.
- *In vitro* compatibility test amongst microbial consortia of potent isolates of *Trichoderma* and *Pseudomonas* was conducted in order to determine whether they can be used in combination or not. No clear inhibition zone was observed between the tested microbial consortia. Absence of inhibition zone indicated that the potential isolates of *Trichoderma* and *Pseudomonas* were compatible with each other.
- The inhibitory effects of compatible microbial consortia (CMC) were tested *in vitro* against *P. infestans* adopting dual culture bioassay technique. Among the different consortial sets tested *in vitro* the significant highest inhibition of pathogen was recorded in the combination of Pf-2 + Pf-3 + T-11 + T-14 (83.33 %) followed by Pf-2 + Pf-3 + T-11 (78.38 %), Pf-2 + Pf-3 (77.43 %) and Pf-2 + T-14 (76.54 %) respectively at 5 days after incubation at 18±1 °C.
- Based on *in vitro* antagonistic potential of compatible microbial consortia (CMC), the best two consortia were selected as CMC-1 (*P. fluorescens* Pf-2 + *P. fluorescens* Pf-3 + *T. asperellum* T-11 + *T. asperellum* T-14) and CMC-2 (*P. fluorescens* Pf-2 + *P. fluorescens* Pf-3 + *T. asperellum* T-11).
- *In vitro* study conducted to check out the efficacy of selected two best consortia (CMC-1 and CMC-2) on plant growth promoting activities like

seed germination, seedling vigour index, shoot length and root length was carried out by Standard filter paper method (three layered moistened filter papers in Petri plates; ISTA, 1993).

- *In vitro* result shows that, CMC-1 significantly increased vigour index of tomato seedlings (124.30 %), including germination per cent (22.99 %), shoot length (83.44 %) and root length (81.37 %) over control at 10 DAS.
- *In vivo* study also conducted to check out the efficacy of selected two best consortia (CMC-1 and CMC-2) on plant growth promoting activities like seed germination, seedling vigour index, shoot length and root length was carried out during 2017-18 and 2018-19.
- *In vivo* experimental results also revealed that, CMC-1 significantly increased seedling vigour index (116.87 % at 20 DAS and 81.14 % at 30 DAS), germination per cent (20.75 % at 10 DAS), shoot length (67.12 % at 20 DAS and 33.74 % at 30 DAS) and root length (103.16 % at 20 DAS and 147.62 % at 30 DAS) over control.
- A field study conducted to check out the efficacy of selected two best consortia (CMC-1 and CMC-2) on number of branches per plant, number of leaves per plant, plant height, fresh weight of fruit, number of fruits per plant, marketable fruit yield, root length, late blight disease severity and tomato plant mortality per cent was carried out during 2017-18 and 2018-19.
- Liquid formulations were prepared using the best two consortia which were utilized for the management of late blight through seed treatment (1.0 %), soil application (1.0 % in FYM) at 10 DBT and foliar sprays (1.0 %) at 15, 30 and 45 DAT under field condition.
- Significantly highest reduction of late blight severity was recorded in chemical control treatment (91.92 %) followed by CMC-1 (84.38 %) and CMC-2 (77.20 %).

- The CMC-1 also significantly promoted the tomato plant height (101.20 %), number of leaves per plant (116.48 %), number of branches per plant (146.57 %), fresh weight of fruit (42.59 %), number of fruits per plant (185.52 %), root length (67.28 %) and marketable fruit yield (313.02 %) over control treatment.
- Based on field study, the best consortium was identified as CMC-1. This promising indigenous consortium enhanced the quality of the tomato seedlings from the point of vigour and health of the plants. The seedling vigour of tomato plants determined on their establishment in field. CMC-1 improved fruit yield and also suppressed the losses due to late blight disease in an eco-friendly and sustainable. Hence, exhibiting tremendous potential for its commercial exploitation.
- Ripened fruits of 20 native tomato genotypes were collected from different tomato growing areas of Nagaland, Manipur and Arunachal Pradesh.
- Known susceptible hybrid (Arka Rakshak, F_1) and susceptible variety (Pusa Ruby) seeds were also collected from IIHR, Bangalore and local market, Dimapur respectively.
- Characteristics of tomato genotypes were recorded and result revealed that most of the collected genotypes were indeterminate in nature except Pusa Ruby (***semi-determinate) and*** T_2 (determinate).
- Fruit shape of collected genotepes were oblong o***val*** (Arka Rakshak F_1), flattish round (Pusa Ruby), small round cherry type (T_2, T_3, T_4, T_9, T_{16}, T_{17}, T_{18}, T_{19} and T_{20}), round medium size (T_5), plum type (T_6, T_{10}, T_{11} and T_{14}), pointed small grape type (T_7), small round (T_8, T_{12}, T_{15}), round (T_{13}) and Heirloom (T_{21}).
- A field study also conducted to evaluate the native tomato genotypes for their resistance to late blight under natural epiphytotic condition was carried out during 2017-18 and 2018-19.

- The late blight disease severity was recorded visually on fruits, leaves and stems of all plants of each replication following scale 0-5 (Irzhansky and Cohen, 2006) when total late blight infestation had occurred in the control plot under natural epiphytotic condition.
- This experimental result revealed that most of the genotypes (20 genotypes) reacted as highly susceptible, while T_3 (16.81 PDI) and T_{17} (35.99 PDI) was found resistant and tolerant respectively under natural epiphytotic condition.
- These results clearly indicate that a good source of resistance to late blight is available in the genotype T_3 (cherry tomato). The resistant genotype thus obtained through this experiment shows a potential disease resistance trait contributor for tomato breeding against late blight disease.

Conclusions

Late blight of tomato caused by *Phytophthora infestans* (Mont.) de Bary is destructive and wide spread in nature. It is an economically important disease of tomato worldwide including India. An indigenous liquid formulation of compatible microbial consortium-1 was developed in order to combat late blight disease in tomato. *P. infestans* has intensified its genetic variation in recent years, comprising isolates that are highly aggressive and highly virulent. Some isolates are resistant to phenylamides fungicide even. In order to subdue this trait, it is imperative to explore and find durable resistance. Several commercial tomato varieties/hybrids commonly grown in Nagaland are highly susceptible to late blight disease and show considerable yield losses under disease favourable conditions. The present investigation was carried out in Nagaland for the first time to determine the level of resistance of *Solanum lycopersicum* against late blight and its possible utilization in breeding programs to develop late blight resistant cultivars. On the other hand, native bioagents (*P. fluorescens* Pf-2 + *P. fluorescens* Pf-3 + *T. asperellum* T-11 + *T.*

asperellum T-14) based liquid compatible microbial consortium-1 is sustainable, eco-friendly, enhances tomato plant growth and protect the plants from late blight disease because of these obvious high level of antagonistic ability against *P. infestans*. However, integration of CMC-1 with resistant genotype (T_3, cherry tomato) proved to be the best combination in managing the late blight of tomato.

In future, the consortium to develop may be studied further with the aim of developing a commercially viable and effective formulation by taking into consideration all the necessary field tests, for the tomato growers in this region. A collaborative study involving Department of Plant Pathology, Nagaland University and commercial tomato growers may be initiated to popularize the use of microbial consortia for efficient disease management. This study will not only help in benefiting the tomato growers but it will also reduce the dependence on fungicides and problem of environmental pollution.

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APPENDICES

APPENDIX

ANOVA TABLE FOR *IN VITRO* EXP.01
(RADIAL GROWTH (Cm) OF PATHOGEN)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	25	66.662	2.666	179.298	0.000
Error	52	0.773	0.015	-	-
Total	77	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.01
(RADIAL GROWTH INHIBITED)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	25	66.606	2.664	188.920	0.000
Error	52	0.733	0.014	-	-
Total	77	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.01
(INHIBITION %)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	25	11839.833	473.593	189.942	0.000
Error	52	129.655	2.493	-	-
Total	77	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.02
(RADIAL GROWTH (Cm) OF PATHOGEN)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	8	74.403	9.300	202.508	0.000
Error	18	0.827	0.046	-	-
Total	26	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.02
(RADIAL GROWTH INHIBITED)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	8	74.403	9.300	202.508	0.000
Error	18	0.827	0.046	-	-
Total	26	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.02
(INHIBITITION %)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	8	13227.191	1653.399	202.381	0.000
Error	18	147.055	8.170	-	-
Total	26	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.03
(DIAMETER GROWTH-FUNGAL)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	25	57.815	2.313	56.194	0.000
Error	52	2.140	0.041	-	-
Total	77	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.03
(DIAMETER GROWTH INHIBITED-FUNGAL)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	25	57.815	2.313	56.194	0.000
Error	52	2.140	0.041	-	-
Total	77	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.03
(INHIBITION %-FUNGAL)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	25	7126.811	285.072	56.257	0.000
Error	52	263.502	5.067	-	-
Total	77	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.03
(DIAMETER GROWTH-BACTERIAL)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	8	50.605	6.326	152.493	0.000
Error	18	0.747	0.041	-	-
Total	26	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.03
(DIAMETER GROWTH INHIBITED-BACTERIAL)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	8	50.605	6.326	152.493	0.000
Error	18	0.747	0.041	-	-
Total	26	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.03
(INHIBITION %-BACTERIAL)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	8	6236.335	779.542	151.536	0.000
Error	18	92.597	5.144	-	-
Total	26	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.04
(COMBINED INHIBITION %)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	11	15950.370	1450.034	583.668	0.000
Error	24	59.624	2.484	-	-
Total	35	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(SEED GERMINATION %)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	1019.792	339.931	4.049	0.021
Error	20	1679.167	83.958	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(SHOOT LENGTH)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	27.632	9.211	94.913	0.000
Error	20	1.941	0.097	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(ROOT LENGTH)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	28.521	9.507	55.729	0.000
Error	20	3.412	0.171	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(SEEDLING VIGOUR INDEX)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	1255106.295	418368.765	73.694	0.000
Error	20	113541.807	5677.090	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(FRESH WEIGHT OF SHOOT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	354.075	118.025	61.823	0.000
Error	20	38.182	1.909	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(DRY WEIGHT OF SHOOT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	0.449	0.150	14.631	0.000
Error	20	0.205	0.010	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(FRESH WEIGHT OF ROOT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	0.327	0.109	8.598	0.001
Error	20	0.253	0.013	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VITRO* EXP.05
(DRY WEIGHT OF ROOT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	0.008	0.003	8.864	0.001
Error	20	0.006	0.000	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(SEED GERMINATION %)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	825.560	275.187	7.676	0.001
Error	20	717.006	35.850	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(SHOOT LENGTH AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	15.448	5.149	96.473	0.000
Error	20	1.067	0.053	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(SHOOT LENGTH AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	66.870	22.290	35.876	0.000
Error	20	12.426	0.621	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(ROOT LENGTH AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	10.537	3.512	66.409	0.000
Error	20	1.058	0.053	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(ROOT LENGTH AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	31.730	10.577	150.479	0.000
Error	20	1.406	0.070	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(SEEDLING VIGOUR INDEX AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	562540.418	187513.473	144.239	0.000
Error	20	26000.349	1300.017	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(SEEDLING VIGOUR INDEX AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	2384194.747	794731.582	73.764	0.000
Error	20	215479.588	10773.979	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(FRESH WEIGHT OF SHOOT AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	9960.692	3320.231	42.669	0.000
Error	20	1556.291	77.815	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(FRESH WEIGHT OF SHOOT AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	1164673.436	388224.479	58.766	0.000
Error	20	132124.990	6606.249	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(DRY WEIGHT OF SHOOT AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	14.245	4.748	18.027	0.000
Error	20	5.268	0.263	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(DRY WEIGHT OF SHOOT AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	3768.054	1256.018	51.006	0.000
Error	20	492.501	24.625	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(FRESH WEIGHT OF ROOT AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	163.874	54.625	45.360	0.000
Error	20	24.085	1.204	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(FRESH WEIGHT OF ROOT AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	11313.410	3771.137	53.302	0.000
Error	20	1415.012	70.751	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(DRY WEIGHT OF ROOT AT 20 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	0.763	0.254	24.417	0.000
Error	20	0.208	0.010	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR *IN VIVO* EXP.01
(DRY WEIGHT OF ROOT AT 30 DAS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	25.941	8.647	33.139	0.000
Error	20	5.219	0.261	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(PLANT HEIGHT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	59.965	11.993	1.446	0.265
Treatments	3	1890.510	630.170	75.970	0.000
Error	15	124.425	8.295	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(NUMBER OF LEAVES PER PLANT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	37.989	7.598	0.841	0.541
Treatments	3	911.171	303.724	33.621	0.000
Error	15	135.506	9.034	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(NUMBER OF BRANCHES PER PLANT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	2.998	0.600	0.697	0.634
Treatments	3	84.528	28.176	32.733	0.000
Error	15	12.912	0.861	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(NUMBER OF FRUITS PER PLANT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	95.376	19.075	0.813	0.559
Treatments	3	1746.719	582.240	24.808	0.000
Error	15	352.053	23.470	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(FRESH WEIGHT OF FRUITS)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	214.566	42.913	1.453	0.263
Treatments	3	1742.731	580.910	19.667	0.000
Error	15	443.054	29.537	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(MORTALITY PERCENT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	64.418	12.884	1.000	0.451
Treatments	3	2657.934	885.978	68.768	0.000
Error	15	193.254	12.884	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(DISEASE SEVERITY)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	37.853	7.571	2.208	0.108
Treatments	3	19619.380	6539.793	1907.221	0.000
Error	15	51.434	3.429	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(MARKETABLE FRUIT YIELD PER PLANT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	141982.134	28396.427	0.890	0.512
Treatments	3	2634214.395	878071.465	27.521	0.000
Error	15	478583.178	31905.545	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(MARKETABLE FRUIT YIELD PER HECTARE)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	194.586	38.917	0.889	0.512
Treatments	3	3613.325	1204.442	27.526	0.000
Error	15	656.342	43.756	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.01
(ROOT LENGTH)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	6.580	1.316	0.201	0.957
Treatments	3	1157.190	385.730	58.782	0.000
Error	15	98.430	6.562	-	-
Total	23	-	-	-	-

ANOVA TABLE FOR FIELD EXP.02
(DISEASE SEVERITY)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	2	66.825	33.413	1.754	0.186
Treatments	21	15899.856	757.136	39.737	0.000
Error	42	800.247	19.053	-	-
Total	65	-	-	-	-

ANOVA TABLE FOR FIELD EXP.02
(YIELD PER PLANT)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	2	9894.053	4947.027	1.270	0.291
Treatments	21	27663025.068	1317286.908	338.238	0.000
Error	42	163571.521	3894.560	-	-
Total	65	-	-	-	-

ANOVA TABLE FOR FIELD EXP.02
(YIELD PER HECTARE)

ANOVA TABLE					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	2	9.355	4.677	1.267	0.292
Treatments	21	26190.015	1247.144	337.781	0.000
Error	42	155.071	3.692	-	-
Total	65	-	-	-	-