BIOLOGICAL REMEDIATION IN THE MANAGEMENT OF COLLAR ROT OF FRENCH BEAN (*Phaseolus vulgaris* L.)

Thesis

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NAGALAND UNIVERSITY

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of

DOCTOR OF PHILOSOPHY

in

PLANT PATHOLOGY

by

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2022

Affectionately
Dedicated
To My
Beloved Family
&
Friends

DECLARATION

I, Valenta Kangjam, 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.

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This is to certify that the thesis entitled **"Biological remediation in the management of collar rot of French bean (*Phaseolus vulgaris* 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 Miss. Valenta Kangjam, Registration No. Ph.D./PPL/00118, 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.

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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

ABSTRACT

French bean (*Phaseolus vulgaris* L.) is an important vegetable which has high nutritional values. It has possibility to be grown round the year specially in the North eastern region of India. French bean is affected by number of diseases. Among the diseases collar rot (*Sclerotium rolfsii*) being most devastating one and cause a substantial yield loss up to 55-70 per cent. An indigenous compatible microbial consortium was developed in order to combat collar rot in French bean. For obtaining better insight in the antagonistic potential of native BCAs, 32 isolates were evaluated against *S. rolfsii* by dual culture technique. Upon *in vitro* screening of the varied isolates, the highest growth inhibition of pathogen was recorded among *Pseudomonas* isolates [Pf-2 (87.55 %), Pf-12 (87.11 %) and Pf-10 (84.00 %)]. In *Trichoderma* isolates highest inhibition per cent was found in isolates [T-8 (85.7 %) and T-20 (84.8%)]. Efficient mycoparasitism ability was assessed in all *Trichoderma* isolates. All potent native microbial isolates were able to release inorganic phosphorus from tri-calcium phosphate and showed consistent ability to produce siderophore, ammonia, IAA and volatile and non volatile metabolites and microbial enzymes. The sequencing of potential isolates of *Trichoderma* (T-8 and T-20) were analyzed and identified by ITS region of 18S rRNA as *T. asperellum*. *In vitro* compatibility analysis of microbial consortia showed positive interaction. Different consortial sets were tested *in vitro* and highest inhibition of pathogen was recorded in the combination of Pf-2 + Pf-12 + T-8 + T-20 (78.20 %) followed by Pf-2 + Pf-12 + T-8 (76.32%) and Pf-2 + T-8 (75.63 %) respectively. The selected best consortia was further compared with single BCAs for plant growth promoting activity *in vitro* and result shows that, CMC significantly increased vigour index of French bean seedlings (111.1 %), including germination per cent (34.08 %), shoot length (60.64 %) and root length (54.69 %) over control at 10 DAS at 10 DAS. *In vivo* experimental results also revealed that, T_1 (seed

treatment + soil drenching at 0, 15 and 30 DAS) significantly increased vigour index (187.0 % at 20 DAS and 183.5% at 60 DAS), germination per cent (42.46 % at 10 DAS), shoot length (104.5 % at 20 DAS and 110.5 % at 60 DAS) and root length (93.1 % at 20 DAS and 92.60 % at 60 DAS) over control. Liquid formulations were prepared using the best consortia which was utilized for the management of collar rot through seed treatment (1.0 %), soil drenching (1.0 %) under pot condition. Significantly highest reduction of collar rot disease incidence was recorded in chemical control treatment (91.09 %) followed by T_1 (81.38 %), T_2 and T_4 (70.85 %) over control treatment. The treatment T_1 significantly increased the French bean number of leaves per plant (152.80 %), number of branches per plant (82.44 %), length of pod (53.05%), number of pod per plant (146.89 %), fresh weight of pod (51.23 %) and marketable yield (114.44 %) over control treatment. Among the CMC treated treatment, T_1 (seed treatment + soil drenching at 0, 15 and 30 DAS) indicating better plant growth promoting potential and disease reduction potential and thus exhibiting tremendous potential for its commercial exploitation.

Key words: Compatible microbial consortia, ITS, 18S rRNA, mycoparasitism, volatile, non volatile, siderophore, IAA, microbial enzymes, collar rot, disease management.

CHAPTER I

INTRODUCTION

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most important leguminous vegetable crops. It is native of South America and belongs to the Fabaceae family and started domestication in Colombia, Mexico and Peru about 8000 years ago. It has evolved from wild growing vine distributed in the high lands of Andes and Middle-America. These two domestications led to two groups of cultivars with contrasting agronomic characteristics. During this evolution, some marked changes has affected this plant from climbing to dwarf type, which has taken place both in the middle American and Andean domestication centres as reported by Schoonhoven and Vosyest, 1991. It is widespread and cultivated as a major food crop in many tropical, sub-tropical and temperate areas of the America, Europe, Africa and Asia. Spanish and Portuguese explorers introduced the legume to the Europe and Africa. In 19th century the slim pods became common in France as *Haricot verts*, which mean slender pod and hence being referred to as "French" beans. It is widely cultivated in tropics, sub-tropics and temperate regions. In India and most of the tropical Asia, it is a major vegetable crop (Athikho *et al*., 2019).

The common bean is recognized as strategic crop, especially in South America, Africa, and Asia (Torres *et al*., 2009). It is considered one of the most important protective foods because of its exceptionally high nutritive value and versatile food use (Noureen *et al*., 2010). It plays not only a vital role in nourishment of human population, but also improves soil fertility to a greater extent by virtue of being highly nitrogen fixing crop. 100 g green pods contain 1.7 g protein, 0.1 g fat, 4.5 g carbohydrate, 1.8 g fibre and are also rich in minerals and vitamins. It offers some medicinal properties in control of diabetes, cardiac problems and natural cure for bladder burn. It has carminative and reparative properties against constipation and diarrhea as reported by Duke (1981).

French bean is a tender warm season vegetable which cannot tolerate frost, high temperature and rainfall. Its seeds do not germinate below 15°C and a most favorable soil temperature for its seed germination ranged from 18-24°C. A mean air temperature of 25-30°C is optimum for its growth and high pod yield. French bean is grown during winter in plains, while it can be grown round the year except winter in hilly regions. Although it can be grown on all types of soil, but loams and clay loams are best for obtaining high yield. The soil and climatic conditions of this region is highly suitable for cultivation of French bean. It is also grown both under field as well as green house conditions throughout the year supplying in the fresh market as well as for processing purposes (Bhati and Kanaujia, 2014) and is a popular vegetable grown under irrigated conditions almost throughout the year.

Worldwide production of bean is 28.3 million tonnes (Anon., 2019) and China is the largest producer sharing about 76 % of world green bean production followed by Indonesia and India. China alone produced the green beans mounted to 17.96 million tones and start gaining a lot of importance due to its short duration and high production potential to fetch premium price in market as compared to other vegetables.

In India, vegetables occupy about an area of 9,068 million ha and with the production of about 196.27 million tonnes (Anon., 2020). Among vegetable, common beans is one of the highest yielding vegetable crop in the world followed by pea and major beans crops cultivated globally (Annon, 2010). This vegetable is largely grown in HP, UP, Bihar, Gujarat, MP, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu (Tiwari *et al.*, 2017) and is grown for tender vegetable purpose while in other country like USA it is grown for processing in large quantities. Among Indian state, Gujarat produced highest with total bean production of 751.99 lakh tonnes with total share of 29.84% and Nagaland stands 18th position with total production of 17.60 lakh tones and share of 0.70 % (Anon., 2021).

Nagaland like other north east states is bequeathed with agro-climatic condition which is suitable for cultivation of all types of vegetable crops grown in the region. In north eastern region of India, French bean is grown for tender vegetable, shelled green beans and dry beans (rajmah). In this region, it is cultivated in rice fallow during spring summer and on hill slopes during autumn winter season. It has possibility to be grown round the year in this region where irrigation facilities are available during dry period. The soil and climatic conditions of this region is highly suitable for cultivation of French bean. Although it can be grown on all types of soil, loams and clay loams are better for obtaining high yield.

Though French bean 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 disease incidence. Number of diseases *viz*., Leaf spot (*Alternaria alternata*), Collar rot (*Sclerotium rolfsii*), Anthracnose (*Colletotrichum lindemuthianum*), Root rot (*R. solani*), Fusarium root rot (*Fusarium solani* f. sp. *phaseoli*), Root knot nematode (*Meloidogyne sp.*), Bacterial brown spot (*Pseudomonas syringae* pv. *syringae*), Common blight (*Xanthomonas campestris* pv. *phaseoli*), Halo blight (*Pseudomonas syringae* pv. *phaseolicola)*, Bean yellow mosaic disease etc. affected on French bean (Kumar *et al*, 2018). Fungal pathogens which considered a potent cause of soil-borne plant diseases and more than 1200 fungal species were implicated in plant diseases or crop failure in various major crops (Consolo *et al*., 2012). Collar rot and root rot diseases in different regions of the world may be caused by several fungal pathogens and fungal-like organisms including *Fusarium solani*, *Rhizoctonia solani*, *Pythium* spp., and *Sclerotium rolfsii* (Ronquillo-López *et al*., 2010).

French bean is infected with various fungal, bacterial and viral diseases during cultivation. Among the pathogen, *S. rolfsii* Sacc. causing collar rot is the most devastating soil-borne pathogen and infects more than 500 plant species (Aycock, 1966; Punja, 1985). The most common hosts are legumes, crucifers, cucurbits, etc. It can infect stems, roots, leaves, fruits, seeds, seedlings and mature plants in the field but fresh vegetables and rhizomes while in storage and transit. It occurs worldwide and can destroy agricultural crops and cause significant yield losses in most of tropical and sub-tropical regions of the world. The fungus, *Sclerotium rolfsii,* was first observed in USA by Peter Henry Rolf (1892) as a causal agent of tomato blight in Florida. Later, the fungus was named *S. rolfsii* by Saccardo (1911). This disease can cause substantial yield losses ranging from 25 %, but sometimes it reaches 80-90 % (Grichar and Bosweel, 1987).

S. rolfsii causing collar rot is widespread in India and also prevails in north eastern states like Nagaland, Assam, Manipur, Tripura, Arunachal Pradesh etc. French beans production sustained losses of 10-20 million dollars annually due to this pathogen (Kator *et al.*, 2015). But this disease is more severe in Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Orissa and Tamil Nadu. Mostly *S. rolfsii* diseases have been reported on dicotyledonous hosts but several monocotyledonous species have also been infected by this fungus species. Sign of infection include development of dark- coloured water soaked lesions at the base of stems, stem thinning and eventually wilting of whole plant and yellowing of leaves. A coarse white strand of mycelium growing in a fan-shape pattern on collar region and light brown to tan coloured sclerotial bodies are formed as disease progresses on the soil. Humid weather is conducive to sclerotial germination and mycelial growth. It also causes blight, root rot, stem rot, foot rot, collar rot, rhizome rot etc. of many crop plants. The pathogen infects the root and base of the crop plants that cause rotting.

Disease of various names has been applied because of large and diverse number of plants and plant parts are attacked by *S. rolfsii*. Common beans are continually exposed to diverse root rot pathogens with typical disease symptoms of reddish-brown lesions on the hypocotyl and tap roots, vascular discoloration, foliar chlorosis and wilt, and seedling death (Ronquillo- López *et al*. 2010). Perhaps the three most commonly used name in the United States for field phases of the disease are Southern blight; Southern wilt and Southern stem rot. Other common usages are wilt; sclerotial disease; stem rot; Collar rot; sclerotial blight and southern root rot (Rolfs, 1897 and Garren, 1959). The *S. rolfsii* causes severe damage during any stage of crop growth (Ganesan *et al*., 2007) and attacks all parts of the plant but the stem or collar infection is more common.

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. The use of large volumes of chemical fungicides facilitates the development of resistance in fungi, which reduces their efficacy (Apaliya *et al.*, 2017) as well as hazardous to environment and human health. Under such conditions, the most effective method is the biological control which considered both safe and environmental friendly (Dukare *et al*., 2019). Additionally, bio-agents can combat plant pathogens by multiple mode of action and development of resistance by pathogens (Wallace *et al*., 2018).

In recent years large number of antagonistic BCAs have been identified that effectively inhibit the soil and seed-borne pathogens under both *in vitro* and *in vivo* conditions, including *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp., and *Streptomyces* spp. (Caulier *et al.* 2018). The use of plant beneficial microorganisms as biological control agents (BCAs) of pests and diseases emerges as a viable alternative to the abusive use of agrochemicals (Ab- Rahman *et al*., 2018; Rändler-Kleine *et al*., 2020). They

are considered key players in modern crop management programs aiming to increase sustainability in agriculture (Compant *et al*., 2019). Bio-priming of seeds of many crops with bio control agents such *Trichoderma* spp., *Bacillus subtillus*, *Psedomonas fluorescens* was also found effective in controlling seed and root rot pathogens (Begum *et al*., 2011).

The mechanisms of disease management by biocontrol antagonists includes competition for space and nutrients (Haran, 1995), mycoparasitism (Harman *et al.*, 2004), production of antifungal agents and hydrolytic enzymes, inactivation of the pathogen's enzymes, induce resistance and enhancement of plant growth (Howell, 2006).

Current strategies for management of plant disease involve integrated biological control by applying single antagonistic microorganism or by developing consortia. Single antagonistic strains often result in inconsistent disease control under field conditions and for overcoming such inconsistent performance, mixture of two or more biological agents or microbial consortia promises more efficient in disease control (Harish *et al.*, 2008).

Microbial consortia have the potential to colonize more effectively in the rhizosphere, express more consistent beneficial traits under various soil conditions and control a wide range of plant pathogens than singly used agents due to their ability to produce various lipopeptide antibiotics (Idris *et al*., 2007). Many of these biological control agents and PGPRs are known to produce amino acids, enzymes, siderophores, phosphate and other growth promoting substances like GA, IAA and cytokinins which help in better growth of crop plants (Raupach and Kloepper, 1998).

Exploring for eco-friendly, safe, long-lasting and effective ways to protect crops from pests and phytopathogens becomes imperative (Boukaew *et al*., 2013), and biological control should be the main focus. A strong increase in registered microbial biocontrol agents worldwide in recent years serves as good evidence (van Lenteren *et al*., 2017).

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 beencarried out on collar rot of French bean under Nagaland condition. Hence, the present investigation entitled “Biological remidiation in the management of collar rot of French bean (*Phaseolus vulgaris* L.)” was undertaken with the following objectives:

1. To isolate the native rhizospheric microbes and test their antagonistic activity against the pathogen under *in-vitro*
2. To investigate the antagonistic mechanism of native rhizospheric microbes
3. To study the compatibility of potential antagonists and test the efficacy of their combination against the pathogen
4. To evaluate the efficacy of compatible microbial consortia against collar rot of French bean under pot condition.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The pertinent literature available on the following aspects of collar rot of French bean is reviewed here as under:

2.1 History

Collar rot caused by *S. rolfsii* is the most destructive soil-borne fungus, facultative parasite and omnipathogenic organism which occurs worldwide and infects more than 500 plant species (Punja, 1985) including tomato, cucumber, brinjal, soybean, maize, groundnut, French bean, watermelon, sunflower, papermint etc. This fungus was first reported by Rolfs (1892) as a cause of tomato blight in Florida. Later, Saccardo (1911) named the fungus as *S. rolfsii.* Higgins (1927) worked in detail physiology and parasitism of *S. rolfsii*. Later Curzi (1931) discovered that the teleomorph was a corticioid fungus and accordingly placed the species in the form gen *Corticium*. With a move to a more natural classification of fungi, *Corticium rolfsii* was transferred to *Athelia* in 1978 (Kator *et al*., 2015).

But in India, Shaw and Ajrekar (1915); Mundkar (1934) successfully isolated the fungus from rotten potatoes and identified as *Rhizoctonia detruens*. Later, Ramakrishnan (1930) confirmed that the fungus involved was *S. rolfsii*. Higgins (1927) worked in detail on physiology and parasitism of *S. rolfsii*. The fungus produces white cottony mycelial growth on potato dextrose agar medium. Initially, it produces white colored sclerotia later their color changes from white to off-white, light brown and dark brown as they attained maturity (Punja, 1985). Hammad and Omar (2021) reported that root rot pathogens caused considerable losses in both the quality and productivity of common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.).

2.2 Geographical distribution

S. rolfsii is a cosmopolitan fungi commonly occuring in the tropics, subtropics, and other warm temperate regions, especially the southern United States, Central and South America, the West Indies, European countries bordering the Mediterranean, Africa, India, Japan, Philippines, and Hawaii. It occurs rarely where winter temperatures fall below 0 ºC (Kator *et al*., 2015).

2.3 Pathogen life-cycle

Punja *et al*. (1981) was of the view that during favorable weather conditions, sclerotia resume activity by either eruptive or hyphal germination. An external food source is not required for this type of germination. Sclerotia can germinate eruptively only after being induced by dry conditions or volatile compounds.

Punja (1985) found out that the hyphal growth resumes from infected tissues and germinating sclerotia in the presence of volatile compounds from decaying organic matter under warm and moist conditions. When hyphae come into contact with host tissues, direct penetration occurs by producing enzymes and facilitates infection.

Mehan *et al*. (1994) reported that sclerotia survive for 3-4 years at or near the soil surface but survive poorly when buried deep because of it high oxygen demand.

Muller (2001) reported that sclerotia of *S. rolfsii* easily spread in soil attached to shoes, hand tools, vehicle tires, or machinery, or in splashing water. Long-distance movement occurs by means of sclerotic in plant material or soil during shipment.

2.4 Biology

S. rolfsii grows, survives, and attacks plants at or near the soil line. Before the pathogen penetrates host tissue it produces a considerable mass of

mycelium on the plant surface, a process which can take 2 to 10 days. Sclerotia either undergo hyphal or eruptive germination. The quantity of mycelia growth and the energy needed for infection is dictated by the type of *sclerotia* germination that takes place. A food base of non living organic matter must be present for hyphal germination of sclerotia to infect host tissue because mycelia growth is sparse. However, mycelium from eruptive germinating *sclerotia* can infect host tissue without an exogenous food base (Mehan *et al*., 1994).

Mycelia growth and sclerotia germination occurs rapidly in continuous light and will occur in darkness if other conditions are favourable (Edmund *et al.*, 2003).

Kator *et al.* (2015) stated that *S. rolfsii* has a sexual fruiting stage which develops on the margins of lesions and in locations that are shaded from the sun.

2.5. Host range

Aycock (1959) stated that host range of *S. rolfsii* is very wide and includes not only many important horticultural and agronomic crops but many of non economic importance. Nevertheless the soil borne plant pathogenic fungus *S. rolfsii* infected more than 500 spp. of plants belonging to over 100 families.

Madhavi *et al*. (2011) reported that chilli is recently affected by dry root rot disease caused by *S. rolfsii* (Sacc.) under rain fed conditions at Andhra Pradesh, India.

Anushya (2016) stated that *S. rolfsii* has a very extensive host range including rice, maize, wheat, barley, groundnut, sunflower, pigeon pea, chickpea tomato, potato, chilli, capsicum, brinjal, cucurbits, onion, carrot chrysanthemum, crossandra, marigold etc, fruits and ornamental crops. Disease caused by this pathogen lead to heavy losses in vegetable crop especially

during the wet season when weather conditions are favourable for both crop production and for the growth and dissemination of the sclerotia of the pathogen.

2.6 Survival of the pathogen

Aycock (1966) reported that sclerotia of *S. rolfsii* survived from 2 months to 7 years in field soil depending on experimental conditions.

Smith (1972) demonstrated that stimulation of eruptive germination during drying following wetting was associated with rind cracking and leakage of nutrients, permitting activity of lytic microorganisms and also found out that survival of *S. rolfsii* decreased when depth of burial was greater than 2.5 cm.

Elad *et al*. (1982) reported that factors such as drying, wetting, and heating that increase activity of soil microorganisms near sclerotia and predispose sclerotia to antagonism may accelerate their mortality rate For *Trichoderma*, this process is facilitated by production of the enzymes β–1, 3 glucanase and chitinase impact of size of sclerotia on survival.

Punja and Jenkins (1984) reported that cycles of drying and wetting, as well as cycles of freezing may decrease survival of sclerotia of *S. rolfsii* and also attributed to increasing gravitational pressure at greater depths. Soil texture and pH may affect the survival of sclerotia.

Matti (1988) found that only 11% of sclerotia survived on the soil surface, whereas 94% survived at 10-cm soil depth, after alternating 7-day cycles of wetting and drying over 8 weeks. The relative absence of soil drying at 10-cm depth might account for greater survival than at the soil surface.

Deacon (2006) studied that sclerotia are the primary structures that enable *S. rolfsii* to survive during unfavorable conditions.

2.7 Environmental factors

Beute and Rodriguez-Kabana (1981) reported that temperature and moisture may interact in influencing survival of *S. rolfsii* under field conditions.

Matti (2001) found out little difference in the proportion of viable sclerotia of *S. rolfsii* recovered under a range of controlled temperature (0 to 40 °C) or under moderate to low soil water holding capacity.

Edmund *et al.* (2003) studied that *S. rolfsii* is able to survive within a wide range of environmental conditions. Growth is possible within a broad pH range, though best on acidic soils. The optimum pH range for mycelia growth is 3.0 to 5.0, and sclerotia germination occurs between 2.0 and 5.0. Germination is inhibited when pH is above 7.0. Maximum mycelia growth occurs between 25 and 35 ^{o}C with little or none at 10 or 40 ^{o}C. High moisture is required for optimal growth of the fungus. *Sclerotia* fail to germinate when the relative humidity is much below saturation. Mycelial growth and sclerotia germination occur rapidly in continuous light, though occurs in darkness if other conditions are favourable.

2.8 Symptomatology

Mehan and McDonald (1990) reported that the primary symptoms of stem rot are browning and wilting of leaves and branches which are still attached with the plant. The fungus preferentially infects stem by forming a whitish mycelial mat around the stem, but it can also infect any part of the plant including root, leaf and pod.

Taylor and Rodriguez (1999) reported that *S. rolfsii* primarily attacks host stems, although it may infect any part of a plant under favourable environmental conditions including roots, fruits, petioles, leaves, and flowers.

Tsahouridou and Thanassoulopoulos (2002) observed that lower stems decay, a white mat of mycelium develops at the lesion site. This white mat often spread out onto the nearby soil surface. These mustard-seed-sized structures, called sclerotia, soon become smooth and light tan, brown or black in colour. Sclerotia serve as overwintering bodies and may be seen in the mycelium, on diseased tissues above or below ground, on soil surfaces, or in soil crevices.

Kumar *et al.* (2013) stated that in heavy soils, fungus damages plants near the soil surface but in light soils it can reach up to pod level, causing severe damage. Leaves of infected plants turn brown, dry and often remain attached to the dead stem. Drying or shrivelling of the affected branches ultimately lead to death of the complete plants after wilting.

Kator *et al.* (2015) reported that seedlings are very susceptible and die quickly once they become infected. Invaded tissues are pale brown and soft, but not watery. The first symptom usually noticed by the homeowner or grower is wilt. Wilted plants often decline and die rapidly as a result of an extensive lower stem rot.

Mahadevakumar *et al*. (2018) reported that the disease symptoms of collar rot include yellowing of plant leaves, formation of dark brown lesions at collar region near the soil line which further lead to wilting of the whole plant. It produces enormous sclerotia, which persist in soil for many years in the form of infected plant debris.

2.9 Isolation and maintenance of the pathogen

Ramarao and Usharaja (1980) reported that *S. rolfsii* can also be maintained on potato sucrose agar medium.

S. rolfsii can be isolated from different plant parts, *viz.*, collar (Goud, 2011) and stem (Kumar and Sen, 2000) region of the affected portion of the plant tissue.

Potato dextrose agar (PDA) was found to be the best supporting medium for isolation of *S. rolfsii* (Shukla, 2008; Ozgonen, 2010; Rakh, 2011).

2.10 Pathogenicity test of the pathogen

Artificial inoculation of pathogen through soil infestation method was employed by Dange (2006) and Datur and Bindu (1974) proved the pathogenicity of *S. rolfsii*.

Siddaramaiah and Chandrappa (1988) proved the pathogenicity of *S. rolfsii* on cardamom in pot culture studies by inoculating the sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week after inoculation.

Further Senthilraja *et al.* (2010) proved the pathogenicity test of *S. rolfsii* through artificial inoculation with inoculum prepared on sterilized maize bran medium and observed maximum mortality in 15 days old plants and the least mortality in 105 days old in groundnut plants.

Vaishali *et al*. (2022) proved pathogenicity of *S. rolfsii* on tomato in pot and after 24 hours of post-pathogen inoculation, visible disease symptoms were observed after 48 hours.

2.11 Characterization and identification of the pathogen

Punja and Damiani (1996) studied the morphological characteristic features of *S. rolfsii* which includes the fluffy nature of mycelia on PDA and formation of abundant sclerotia which was more than 250 to 350 per plate.

Kokub *et al*. (2007) studied the morphological characteristics of 8 *S. rolfsii* strains grown on PDA plates revealed that mycelial growth rate of different strains varied considerably upto three days. Mycelia of most strains

showed fluffy appearance. The whole plate was covered with mycelium within 3 days. Further they observed the formation of sclerotia initiated after 72 hours of incubation and continued till 168 hours. In initial stage, white colored sclerotia were formed then changed from white to off-white, light brown and dark brown as they attained maturity. The change in color of sclerotia might also be due to utilization/exhaustion of nutrients.

Mahadevakumar *et al*. (2015) characterized the pathogen *S. rolfsii* which was isolated on potato dextrose agar (PDA) medium and observed the development of whitish fast-growing mycelia with numerous reddish brown sclerotia. The sclerotia were initially white and turned dark brown upon maturation.

2.12 Collection and isolation of rhizospheric antagonists

Beneficial microorganism present in the rhizhosphere restrict the growth of soil borne pathogens, they produce antifungal substances, act as mycoparasite against the pathogenic fungi and secrete the lytic enzyme (Weller, 1988).

Arya and Mathiew (1993) studied on the rhizhosphere microflora of pigeon pea and revealed and that out of 14 fungal species isolated from rhizosphere soils, three genera belonged to Zygomycetes, three of Ascomycetes and five of Deuteromycetes indicating the number and quality of fungi present in the rhizhosphere soil.

Thakare *et al*. (2002) isolated highest antagonistic activity of *Trichoderma* and *Aspergillus* sp. from groundnut rhizhosphere against *S. rolfsii* and *Rhizoctonia batiticola.*

Siddiqui and Shaukat (2003) found out some species of *Trichoderma* and *Pseudomonas* isolated from rhizosphere soil used as biocontrol agents that have shown efficacy in controlling a number of fungal diseases.

Singh *et al*. (2022) isolated 25 *Trichoderma* spp and 8 *Pseudomonas* spp from rhizosphere soil soil and used as biocontrol agents for controlling late blight of tomato.

2.13 *In vitro* screening of rhizospheric antagonists against *S. rolfsii*

2.13.1 Fungal antagonists

The majority of work done on biocontrol of plant disease was related to soilborne diseases using either bacteria or fungal antagonists. The efficacy of several microorganisms, including bacteria, actinomycetes, a mycorrhizal fungus, and *Trichoderma* spp. against *S. rolfsii* under *in vitro* conditions was reported by Punja (1985).

The potential use of fungal antagonists as biocontrol agents against plant diseases was suggested by Weindling (1932).

Bandyopodhyay *et al*. (2003) reported that *Trichoderma* strains inhibited the growth of *S. rolfsii* and *R. solani* by 76.6 and 73.3 % respectively.

Yaqub and Shahzad (2005) reported that *T. harzianum* and *T. longibrachiatum* restricted the growth of *S. rolfsii* under *in vitro* condition by coiling around mycelium of *S. rolfsii* which leads in lysis of hyphae.

Further Anand and Harikesh (2005) found different isolates of *Trichoderma* and two isolates of *Gliocladium virens* highly antagonist against *S. rolfsii* under *in vitro* conditions.

Bhuiyan *et al*. (2012) reported that *T. harzianum* isolate Th-18 showed the highest (83.09%) reduction of the radial growth against *S. rolfsii*. This might be due to the production of secondary metabolites and antibiotics production, which diffused into the PDA which showed detrimental effect towards growth of *S. rolfsii* as well as due to higher antagonistic ability of potential *Trichoderma* mutants.

Asad *et al*. (2014) evaluated the biocontrol capability of 3 *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *Trichoderma* spp.) against *R. solani*

Kator *et al*. (2015) evaluated the antagonistic potential of *Trichoderma* species *in vitro. T. harzianum* gave the highest mycelia inhibition of 74.50 % while *T. viride* gave an inhibition o f 68.75 % against *S. rolfsii*.

Prajapati *et al*. (2015) observed that among different *Trichoderma* species tested against *S. rolfsii* through dual culture technique, *T. asperellum* showed strong antagonistic effect in terms of mycelia growth inhibition *i.e.* 61.48, 75.00 and 73.33 % at 4, 6 and 8 days of incubation, respectively.

Singh *et al*. (2016) reported that the inhibition ability against root rot pathogens varied depended on both the specific *Trichoderma* species and pathogen.

Vrieze *et al*. (2018) concluded the reason behind antagonistic property employed by *Trichoderma* spp. and other bioagents as competition as an indirect mechanism, where in pathogens is excluded by depletion of food or by physical occupation of sites.

Sharma *et al*. (2020) evaluated four potential *Trichoderma* mutants against *S. rolfsii*. Among the four potential *Trichoderma* mutants tested the maximum inhibition was observed in BARC mutant (81.50 %) over control which was followed by mutants M-136 (81 %), M-23 (80.5 %) and M-18 (79 %) respectively.

2.13.2 Bacterial antagonists

Dwivedi (1987) recorded the *in vitro* efficacy of *B. subtilis* and *Pseudomonas aeruginosa* against *S. rolfsii* with 58 and 36 % inhibition over control respectively in dual culture.

Likewise, Ongena and Jacques (2008) reported the highest inhibition of radial growth of various soil borne pathogens including *S. rolfsii* using antagonistic ability of bacterial species.

Pastor *et al*. (2010) reported that *Pseudomonas* spp. isolated from rhizosphere soil of groundnut plants showed highest antagonistic activity against *S. rolfsii*.

Rakh (2011) isolated 11 *Pseudomonas spp*. from rhizospheric soil and evaluated for their antagonistic activity against *S. rolfsii*.

Prasada and Paramageetham (2013) reported that *P. fluorescens* isolate PATPT 6 was found to be potential antagonist against *S. rolfsii*.

Sahni *et al*. (2019) proved that *Pseudomonas* spp. was highly effective in controlling *S. rolfsii*. The antifungal potential of *P. fluorescnec* was due to its ability to produce hydrogen cyanide, hydrolytic enzyme I, phosphate solubilization, and siderophore to suppress the pathogen's mycelial growth.

Muthukumar *et al*. (2019) who reported eight native *Pseudomonas* species (I1 to I8) and one introduced *P. fluorescens* (I9) were evaluated to test the antagonism against *S. rolfsii* under *in vitro* conditions. Out of the eight antagonists tested, isolate-I7 (PFP) recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii* accounting for 74.25 per cent reduction in the mycelial growth over control (89.33 mm) and it was on par with I9 (PFC). This was followed by isolate-I4 (PFK).

Sharf *et al*. (2021) tested three bacterial strains, *viz. B. megaterium* OSR3, *B. megaterium* ZMR6, and *P. fluorescence* PF-097, were selected for their *in vitro* antagonistic assessment against *S. rolfsii* by dual culture technique on potato dextrose agar medium. OSR3 showed the highest antagonistic potential (68 %), followed by PF-097 (54 %) and ZMR6 (33 %).

Lal *et al*. (2022) tested 22 isolates, among them 2 isolates Pf10 and Pf14 showed different degree of inhibition of *R. solani*, *S. sclerotiorum*, *S. rolfsii* and *Fusarium* spp. Isolate Pf14 displayed a strong growth inhibitory activity ranged from 62.2 to 59.3 % against different fungal pathogens

2.14 Biocontrol mechanisms of native rhizospheric BCAs

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

2.14.1 Volatile metabolites production

Dennis and Webster (1971) reported the some *Trichoderma* isolates were found to produce volatile inhibitory components to the growth of pathogen. These isolates possessed a characteristic odour, especially members of the *T. viride*. The susceptibility of the test fungi varied widely. Acetaldehyde was identified tentatively as one inhibitory metabolite of *T. viride.*

Fravel (1988) reported the production of different volatile compounds likes alkyl pyrones, ethanol, isobutanol, isoamyl alcohol and isobutyric acid by *Trichoderma* spp having antagonistic activity against *S. cepivorum and S. rolfsii*. Additionally, the volatile metabolites produced by this *Trichoderma* spp. were both fungicidal and fungistatic.

The inhibitory effect of volatile metabolites produced by *P. fluorescens* and *B. subtilis* against *S. rolfsii* was well documented by Laha *et al.* (1996). Further, the production of specific antifungal volatile compounds (AFV) against *S. rolfsii* by the bacterial antagonists was observed by Knox *et al.* (2000).

Similarly Srinivasulu *et al.* (2005) reported the production volatile substances *T. viride, T. hamatum* and *T. harzianum* against *S. rolfsii* under *in vitro* conditions.

Vespermann *et al*. (2007) reported that the microbial volatile organic compounds belong to different chemical classes including alkenes, alcohols, ketones, benzenoids, pyrazines, sulfides and terpenes.

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. oxysporum* and *Helminthosporium* sp. about 75 % and 25 %, respectively.

Similarly Kotasthane *et al.* (2014) recorded the highest antagonism by *Trichoderma viride* isolate against two soil borne plant pathogens *Scelrotium rolfsii* and *Rhizoctonia solani*. They further conclude that, the antagonistic ability of the isolate was due to the 6-Pentyl pyrone which is one of the best secondary metabolites having both plant growth-promoting traits and antifungal activities.

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.

Likewise, Li *et al.* (2016) evaluated VOCs produced by *Bacillus* strain against *Fusarium solani* in sealed petri dishes and found 56 to 82 per cent growth inhibition of pathogen. They later characterised in detail the chemical nature of these VOCs and found to be ketones, alcohols, aldehydes, pyrazines, acids, esters, pyridines and benzene compounds.

Vrieze *et al*. (2018) tested volatile metabolite production of 39 isolates of *Pseudomonas* and 29 isolates of *Trichoderma* spp. They found out that 15 volatile compounds such as normal saturated hydrocarbons (C7–C30), cyclohexane, cyclopentane, fatty acids, alcohols, esters, sulfur containing compounds, simple pyrane, benzene derivatives, trichodermin, paracel icine, trichotoxin, gliotoxin, staldeid and viridian.

Lal *et al*. (2022) evaluated 22 *Pseudomonas* isolates for effect of volatile production test in sealed plate assay. Isolate Pf14 significantly inhibited the radial mycelia growth of *R. solani*, *S. sclerotiorum*, *S. rolfsii* and *Fusarium* spp. ranged from 80 to 50 %. as compared to control.

2.14.2 Non volatile metabolites production

Jeyarajan and Nakkeerun (1988) reported that the inhibitory effect of culture filtrate of *Trichoderma harzianum* against *S. rolfsii*. They further opined that, the inhibiting effect of culture filtrate was due to presence of antibiotics such as trichodermin, dermadin, trichoviridin and sesquiterpene heptalic acid.

O' Dowling and O 'Gara (1994) reported that the antifungal metabolites produced by *P. fluorescens* might be attributed due to production of an array of low-molecular weight metabolites which helps in the growth reduction of the pathogen.

Saxena *et al*. (1995) revealed that the cell free extracts of fluorescent pseudomonads effectively inhibited the growth of *R. solani*.

Revathy and Muthusamy (2003) showed the culture filtrate of *P. fluorescens* was the most effective in inhibiting the mycelial growth of *S. rolfsii.*

Nagarajkumar *et al.* (2005) revealed the presence of several antifungal proteins in the culture filtrate of *Pseudomonas* sp. and *Bacillus* sp. which limited the mycelial growth and sclerotial production by *Rhizoctonia solani*.

They are capable of inhibiting the numerous pathogens under *in vitro* conditions. Among the 5 potential isolates of *Bacillus* sp. significantly highest inhibition of germination of sclerotia of virulent isolate of *S. rolfsii* (SrGj-3) over control was recorded with culture filtrate of B1 (94.00 %) followed by B2 isolate (87.00 %) and significantly least inhibition was observed with B5 isolate (59.00 %).

Similarly, Paramasivan (2006) reported the production of non-volatile compounds by *T. viride* and *T. harzianum* and were found antagonistic to *S. rolfsii.*

Muthukumar *et al*. (2010) reported that the culture filtrate of *P. fluorescens* isolate EBS 20 and EBR 4 totally inhibited mycelial growth of *Pythium aphanidermatum* at a concentration of 15% *in vitro.*

Chanutsa *et al*. (2014) reported that the culture filtrate of *P. florescence* inhibited 100 per cent in the mycelial growth of *S. rolfsii.*

Muthukumar *et al*. (2019) showed the effect of culture filtrate of *Pseudomonas* isolates on the mycelial growth of *S. rolfsii* under *in vitro* conditions revealed that the culture filtrate of the isolate-I7 totally (100 %) inhibited the mycelial growth of *S. rolfsii* at 15 % concentration under *in vitro* conditions followed by the isolate-I4 (90.67 %).

Lal *et al*. (2022) studied the effect of non volatile metabolite production by the selected 22 *Pseudomona*s isolates inhibited the mycelial growth ranged from 68.9 to 42.6 % of the tested pathogens *R. solani, S. sclerotiorum, S. rolfsii* and *Fusarium* spp.with 48 hours old culture filtrate.

2.14.3 Ammonia production

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

Malleswari *et al* (2013) studied 219 bacterial strains isolated from the rhizosphere soils of different medicinal and aromatic plants and tested for *in vitro* specific plant growth promotion activities under *in vitro* condition. Results revealed that four isolates showed maximum plant growth promoting activities such as ammonia production, IAA production, phosphate solubilization, HCN production and antifungal activity.

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.

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.

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.

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 medium amount of ammonia.

2.14.3 Indole acetic acid (IAA) production

Lynch (1985) described Indole acetic acid (IAA) as 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. It promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting in plants.

Nimnoi and Pongslip (2009) demonstrated that the isolates of IAA synthetic bacteria enhanced root and shoot development of *Raphanus sativus* and *Brassica oleracea* more than fivefold when compared with control.

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

Bhakthavatchalu *et al.* (2013) tested the isolate of *P. aeruginosa* FP6 for the production of IAA which was found to be positive. A significant increase in IAA production was also observed in the presence of the precursor, L-tryptophan *i.e.,* 80 µg/ml when compared to its absence (16 µg/ml). IAA production was increasing up to 96 hrs when bacteria reached stationary phase of growth and then decreased slowly which may be due to release of IAA degrading enzymes. Increased amount of IAA production in the presence of the precursor, L-tryptophan shows that the isolate is dependent on the L-tryptophan precursor and probably synthesized IAA through Trp pathways.

Ahemad and Kibret (2014) reported that BCAs have the ability to produce plant growth promoting substances like Indole Acetic Acid (IAA) and antifungal substances, which favours better growth of crop plants. They

facilitate the plant growth directly or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of BCAs.

Dixit *et al.* (2015) investigated 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 .

Tiwari *et al*. (2016) reported that bacterial isolates PN10, PN11, PN13, PN14, PN15, PN17 and PN18 induced the production of IAA in the presence of tryptophan.

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 observed with *Trichoderma* but the highest IAA production was noticed with the bacterial isolates *B. subtilis*-5, *P. fluorescens*-2 and 6.

Rai (2017) studied 20 isolates of *Trichoderma* for IAA production. All isolates were found positive for IAA production.

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.

2.14.4 Phosphate solubility

Quecine *et al*. (2008) reported that *P. fluorescens* is capable of solubilizing phosphate and producing IAA that may enhance its potential use as an effective biological control agent to contribute to the control of *S. rolfsii*.

Kapri and Tewari (2010) evaluated 14 strains of *Trichoderma* spp. for its phosphate (P) solubilizing potential. These strains were isolated from the forest tree rhizospheres of pinus, deodar, bamboo, guava and oak. These isolates were tested for their *in vitro* P-solubilizing potential using National Botanical Research Institute Phosphate (NBRIP) broth containing tri-calcium phosphate (TCP) as the sole P source, and compared with a standard culture of *T. harzianum*. All the cultures were found to solubilize TCP but invariably showed very good mycelial growth in NBRIP broth, with simultaneous disappearance of TCP within 72 hrs in most of the cases.

Gangwar *et al*. (2012) also evaluated 45 isolates of endophytic actinomycetes from surface sterilized root, stem and leaf tissues of rice. Qualitative determination of phosphate solubilizing capacity of the tested actinomycetes strains showed that 20 of the tested 45 strains were able to solubilize phosphate. The quantitative estimation of phosphate solubilization showed diverse levels of phosphate solubilizing activity.

Bhakthavatchalu *et al.* (2013) observed the *P. aeruginosa* FP6 showed clear visible halos around the colonies on Pikovskaya agar medium after 3 days of incubation. Solubilization index was also observed to be 2.26. The maximum amount of soluble phosphates released was 270 μg/ml.

Tiwari *et al*. (2016) found only PN13 isolate to give clear zone on Pikovskaya agar containing insoluble mineral phosphate such as tri-calcium phosphate. But this isolate was also found to be medium producer of IAA.

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 twelve

bacterial isolates, 11 bacterial isolates showed solubilization zone except *P. fluorescens*-4. The *P. fluorescens*-2 recorded highest 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.

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 estimation of phosphate solubilization for all the *Trichoderma* spp. did not show any clear zone on Pikovskaya's Agar after incubation at room temperature for 0-7 days.

Thi *et al*. (2020) reported 14 out of 15 rhizobacterial isolates were able to solubilize phosphate.

Lal *et al*. (2022) reported that phosphate solubilisation potential of bacterial isolates Pf14 (5.65) showed maximum phosphate solubilization, followed by Pf10 (4.50) and Pf11 (4.50). Similarly, highest SE was observed by Pf14 (82.29%), followed by Pf10 (77.78%) and Pf11 (77.78%).

2.14.5 Siderophore production

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.

Scher and Bakker (1982) defined siderophores as low molecular weight iron chelating compounds produced by microorganisms under iron stress conditions.

Demanage *et al.* (1987) reported that fluorescent pseudomonads are characterized by the production of yellow-green pigments termed pyoverdines which fluoresce under UV light and function as siderophores.

Kamensky *et al*. (2003) reported that siderophore productions are correlated with antifungal activity.

Mahesh (2007) suggested that fungal growth is mainly inhibited by siderophore production and HCN production.

Manikandan *et al*. (2010) also reported siderophore production by *P. fluorescens* Pf1. They 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 isolates where 15 strains produced siderophores.

Bhakthavatchalu *et al.* (2013) recorded change in the color of the CAS agar from blue to orange red by the isolate confirmed the ability of *P. aeruginosa* FP6 to produce siderophore. The maximum production of siderophore (85.7 μM) was recorded after 36 hrs of incubation.

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.

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.

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. *T. viride*-9 and *T. harzianum*-2 showed lower production of siderophores.

Rai (2017) screened 20 isolates of *Trichoderma* for siderophore production. Around 25 % of isolates showed remarkably higher siderophore production and rest of them were moderate producer.

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).

2.14.6 Hydrogen cyanide (HCN) production

Corbett (1974) described that the cyanide ion is exhaled as HCN and metabolized to a lesser degree into other compounds. HCN first inhibits the

electron transport and the energy supply leading to the disruption of the cell, leading to the death of the organisms.

Ramette *et al*. (2003) reported production of a broad spectrum antimicrobial compound, hydrogen cyanide by many plant associated fluorescent pseudomonads, involved in biological control of root rot disease.

Mahesh (2007) suggested that fungal growth is mainly inhibited by HCN production and siderophore production.

Manikandan *et al*. (2010) recorded the HCN production by *P. fluorescens* Pf1. They tested Pf1 cultures of different ages where the yellow colour of the filter paper change to dark brown compared to the control.

Bhakthavatchalu *et al.* (2013) also reported the microbial production of HCN as an important antifungal trait to control root infecting fungi. Strong HCN production was recorded by isolate *P. aerugonisa* FP6, as evidenced by change in colour of filter paper from yellow to reddish-brown after 2-3 days of incubation.

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 and Pf25. While, Pf20, Pf27 and Pf28 were highly HCN producing isolates as compared to others.

Tiwari *et al*. (2016) found out the production of HCN was detected in only single isolate PN11 out of nine bacterial isolates.

Prasad *et al.* (2017) screened 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates for HCN production. All the bioagents exhibited positive results for HCN production. Highest HCN production was observed with the *T. viride* isolates-2, 9, 10, *T. harzianum*-2, 5, 6, 11 and bacterial isolates of *B. subtilis*-2, *P. fluorescens*-2 and 3.

2.14.7 Microbial Enzymes Production

2.14.7.1 Pectolytic enzymes production

Benhamou and Chet (1993) demonstrated that enzymes of *Trichoderma* cause localized lysis of cell walls of phytopathogenic fungi at points of contact between the antagonist and the host.

Marcia *et al.* (1999) reported that 102 isolates were positive for pectinase production. Among them, 30 % produced considerable pectinolytic activity

Maria *et al.* (2001) found out that *T. harzianum*, *T. viride* and *T. koningii* produced extracellular pectinases.

Similarly El-Katatny *et al.* (2001) recorded the production of hydrolytic enzymes in culture filtrate of *T. harzianum* (T24) which was found toxic to phytopathogenic basidiomycete *S. rolfsii.*

Kashyap *et al*. (2001) reported that most of the fungal pectinases are optimal at the acidic conditions and bacterial pectinases have optimum activity in alkaline conditions.

Nagrajkumar (2004) have demonstrated that the lytic enzymes of bacteria are involved in the control mechanisms against plant root pathogen including *Fusarium oxysporum* and *Rhizooctonia solani.*

Qualhato *et al.* (2013) reported the production of cell wall degrading enzymes by *T. harzianum* and *T. asperellum* when grown in liquid cultures with cell walls of *F. solani*, *R. solani* and *S. sclerotiorum.* Further these enzymes were found highly toxic against the above pathogens.

Yannam *et al*. (2014) found out the important pectinases producing fungi and bacterial species.

Tsegaye *et al* (2019) reported two rhizobacterial isolates were positive for pectinase production.

Thi *et al*. (2020) reported 66.67 % of selected bioinoculants could produce pectolytic enzyme.

2.14.7.2 Cellulose enzymes production

Benhamou and Chet (1997) reported that large amounts of cellulytic enzymes are produced and play a key role in breaching the host cell walls at sites of attempted penetration of *Trichoderma* into the host cell walls.

Kumar and Gupta (1999) reported the enzymatic degradation of cell wall of fungal pathogens by biocontrol agents.

Jayaraj *et al*. (2005) who reported the production of extracellular lytic enzymes by bacterial antagonists against fungal plant pathogens was well documented.

Khalid *et al.* (2006) reported that the fungi grown on the selective media supported the growth of the fungi by using cellulose as the carbon source

Jain and Dhawan (2008) found out the role of cellulase in pathogenesis.

Mishra (2010) observed that *Trichoderma* spp exhibited highest cellulase activity of 3.6µ/ml and it was found to suppress the mycelial growth of *P. aphanidermatum.*

Kamala and Indira (2014) reported that cellulase and the -1, 3-glucanase are the two enzymes that play important role in the enzymatic degradation of cell walls of phytopathogenic fungi like *P. ultimum* during mycoparasitic interaction.

El-Komy *et al.* (2015) reported the production of extracellular enzymes by *Trichoderma* isolates.

Parmar *et al*. (2015) showed the specific activities of cell wall degrading enzyme cellulase by *Trichoderma* spp.

Tsegaye *et al* (2019) reported 37 rhizobacterial isolates evaluated showed positive results for cellulase production.

Thi *et al*. (2020) reported 80 % of selected bioinoculants produced cellulose enzyme.

2.14.7.3 Amylase production

Maria *et al.,* (2001) that *T. harzianum, T. viride* and *T. koningii* produced extracellular cellulose, amylase and pectinases. Amylases are also employed in the starch processing industries for the hydrolysis of polysaccharides.

Malleswari *et al* (2013) reported that amylase activity was observed in rhizobacterial isolate (Cf 60).

Verma and Shahi (2015) isolated 23 bacteria from rhizosphere soils and found out19 bacterial isolates were positive for amylase production.

Goswami *et al* (2016) reported the production of cell wall degrading enzymes used as a mechanism by biocontrol agents to control soil-borne pathogens.

Tsegaye *et al* (2019) reported 95 isolates of rhizosphere antagonists were positive for amylase production.

Thi *et al.* (2020) reported 73.33% of selected bioinoculants produced amylase.

Blake *et al.* (2021) reportd that enzymes such as chitinase, amylase and protease secreted by biocontrol strains provoke a direct inhibitory effect on the mycelium growth of fungal pathogens by degrading their cell wall.

2.14.7.4 Catalase production

Malleswari *et al* (2013) reported the production of catalase was exhibited by all the 219 isolates of rhizobacteria. Catalase activity may be potentially very advantageous for plant growth promotion activities.

Verma and Shahi (2015) evaluated 23 bacteria from rhizosphere soils and found out that 16 bacterial isolates showed positive for catalase activity. Bacterial strains showing catalase activity might be highly resistant to environmental, mechanical and chemical stress.

2.14.8 Mycoparasitism

Coley-Smith and Cooke (1971) first reported the clamydospore production by *T. hamatum* invading sclerotia of *S. delphinii*, whereas Henis *et al.* (1982) first reported on clamydospore production by *T. harzianum* in sclerotia of *S. rolfsii*. Degraded sclerotia became dark in colour, soft, empty and disintegrated even under slight pressure.

The direct mycoparasitic activity of *Trichoderma* species has been proposed as one of the major mechanisms for their antagonistic activity against phytopathogenic fungi (Baker, 1987; Chet, 1990).

Trichoderma spp. attach to the host hyphae by coiling, hooks or apressorium like structures and penetrate the host cell walls by secreting hydrolytic enzymes such as a basic proteinase (Geremia *et al*., 1993).

Elad *et al.* (1980) observed the interaction between *T. harzianum* and *S. rolfsii* by using the scanning electron microscope and fluorescent microscope where they observed that the lysed sites and penetration holes appeared on the hyphae of the pathogenic fungi following removal of parasite hyphae.

Similarly, Elad and Chet, (1983) observed the formation of coiling hooks and appresoria by *T. harzianum* while attacking the hyphae of *S. rolfsii.*

Further, Henis *et al.* (1982) recorded the penetration of *Trichoderma* into the rind and cortex of sclerotia leading to its lysis.

Likewise, Singh *et al*. (1999) noticed destructive parasitism *i.e.*, coiling around hypae with direct penetration leading to lysis of fungal hyphae by *T. harzianum* and *Gliocladium virens*.

Srivastava and Singh (2000) observed the extent of lysis of purified cell wall of *Rhizoctonia solani* by enzyme preparation of *T. harzianum, T. viride, T. hamatum* and *A. flavus*. The observations showed maximum lysis of 86 % by *T. harzianum*, followed by *T. viride* (45 %), *T. hamatum* (15 %) and *A. flavus* (6 %).

Kubicek *et al*. (2001) reported that mycoparasitism as the major antagonistic mechanisms in *Trichoderma* spp. After host recognition, *Trichoderma* confers to the host hyphae *via.* coiling; penetrate the cell wall by wide range of lytic enzymes and a versatile metabolites production. On the other hand, lysis of protoplasm was uniformly observed in the hyphae during the interaction.

Saravanakumar (2002) observed the zone of interaction between *Trichoderma* spp and *S. rolfsii* which revealed the hyperparasitism of antagonist on the test pathogen. This may result in disorganization or digestion of protoplasm contents or directly penetrated the hypae of *S. rolfsii*.

McIntyre *et al*. (2004) described that mycoparasitism as the antagonistic interaction between two fungal species, where the direct attack of one fungus on another occurs by several sequential events, including recognition, attack and subsequent penetration and killing of the host.

2.15 Molecular identification and phylogenetic analysis of *Trichoderma*

Kindermann *et al*. (1998) attempted 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 *Trichoderma* and *Hypocrea*

form a single holomorph genus, within which two major clades can be distinguished.

Kullnig-Gradinger *et al*. (2002) reported that the ITS sequence was chosen for this analysis because it has been showed to be more informative with various genus *Trichoderma*. They further added that used 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.

Druzhinina *et al*. (2006) reported that *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.

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 (partial sequence); ITS1, 5.8S rRNA gene and ITS2 (complete sequence) and 28S rRNA gene (partial sequence) 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*).

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.

2.16 Identification of *Pseudomonas* isolates

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

Malleswari and Bagyanarayana (2013) reported that four isolates which showed maximum plant growth promoting activities were identified on the basis of colony morphology, gram staining and biochemical tests. These PGPR isolates were further characterized through 16S rRNA gene sequencing which led to their identification as *Pantoea* sp. (Cf 7), *Bacillus* sp. (Cf 60) and *Pseudomonas* sp. (Te1, Av 30) respectively

Ahmadloo *et al*. (2014) reported that rhizobacteria are largely represented by the species of *Pseudomonas* and *Bacillus* genus and some bacteria belonging to the family of Enterobacteriaceae such as Serratia, Pantoea and Enterobacter strains found in the area of the rhizoplane of leguminous plants.

Verma *et al* (2015) identified bacterial strains as *Pseudomonas plecoglossicida, Stenotrophomonas maltophilia, Achromobacter insolitus, Pseudomonas aeruginosa, Chryseobacterium hispalense, Bacillus pumilus, Enterobacter hormaechei, Comamonas testosteroni, Enterobacter roggenkampii, Delftia acidovoran, Pseudomonas putida, Agrobacterium fabrum, Flavobacterium anhuiense, Acinetobacter soli*. They further revealed that isolates which shows round shaped and raised colonies having smooth shiny surface with smooth margin, only few produces filamentous, umbonate flat with rough and filamentous irregular margin observed in the colonies of agar plate. The isolates were gram negative except few were gram positive and they differed in colour but all were odourless.

Singh *et al*. (2022) also identified *P. fluorescens* on the basis of their cultural, morphological characters and biochemical test. They also identified through molecular characterization.

2.17 Compatibility of potential rhizospheric antagonists

Use of different biocontrol agents with several mechanisms of control fits in well with the concept of integrated disease management, in which several means of disease suppression are applied concurrently. When one or more means of mechanisms are not effective, the others may compensate for the former absence.

Siddiqui and Shaukat (2003) reported *in vitro* compatibility between *P. aeruginosa* and *Pochonia chlamydosporia* and between *T. harzianum* and *Streptomyces rochei*.

Rini and Sulochana (2007) evaluated 26 *Trichoderma* isolates and eight *P. fluorescens* for compatibility test and found out that *T. viride/T. harzianum* and *P. fluorescens* were compatible and also improved plant growth.

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 40 isolates of fluorescent pseudomonads and 43 isolates of *Trichoderma*. These isolates were isolated from rhizosphere and non-rhizosphere soils of tomato, chickpea, lentil, peas, rice and soybean. Isolates PBAP-10, PBAP-15, PBAP-17, PBAP-27 of fluorescent pseudomonads and isolates PBAT-1, PBAT-6, PBAT-38 and PBAT-43 of *Trichoderma* exhibited no or very little antagonism against each other. Fungal isolate PBAT-43 (*T. harzianum*) and bacterial isolate PBAP-27 (*P. fluorescens*) emerged as most compatible and efficient and therefore were used for development of mixed formulation.

Kumar *et al.* (2014) evaluated compatibility between *T. harzianum* OTPB3 and *B. subtilis* OTPB1 *in vitro*. When one loop of culture broth was

streaked on PDA broth *B. subtilis* and *T. harzianum* exhibited growth on PDA without any antagonistic activity after 72 hrs of incubation. They also did not exhibit inhibitory effects on each other when spot inoculated on PDA.

Nath *et al.* (2016) 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.

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. was found to be compatible with each other in all combinations without inhibiting each other.

2.18 *In vitro* antagonistic efficacy of compatible microbial consortia

Dandurand and Knudsen (1993) studied compatibility of *T. harzianum* and *P. fluorescens* 2-79 RN10 and they found that mycelial growth of *T. harzianum* was stimulated in presence of *P. fluorescens* 2-79 RN10.

Microbial mixture is much more efficient than single strains of organisms with diverse metabolic capabilities (Yan *et al.*, 2002). Many of these biological control agents and PGPRs are known to produce amino acids, vitamins and growth promoting substances like IAA, GA and cytokinins which help in better growth of crop plants.

Manjula *et al*. (2004) observed that fluorescent pseudomonads did not reduce the biocontrol ability of *T. harzianum* under *in-vitro* conditions.

Sundaramoorthy and Balabaskar (2013) reported that the native microbial consortia of *P. fluorescens* and *B. subtilis* against *F. oxysporum* f. sp.

lycopersici. They had tested singly or in consortia and the highest inhibition of test pathogen was recorded 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.

Nath *et al.* (2016) evaluated the antagonistic potential of *T. parareesei*, *P. fluorescens*, *B. subtilis* and *Azotobacter chroococcum* against *R. solanacearum.* They had tested *in vitro* singly or in consortia and the highest inhibition of test pathogen was recorded against consortia of *T. parareesei* + *P. fluorescens* + *B. subtilis* + *A. chroococcum* (91.10 %) followed by *T. parareesei* + *P. fluorescens* + *B. subtilis* (81.10 %) and *P. fluorescens* + *B. subtilis* + *A. chroococcum* (68.14 %).

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.* Bioagents applied alone or as consortia, significantly reduced bacterial wilt pathogen *in vitro* producing varying sizes of inhibition zones in TTC medium. The inhibition produced by the combination of three antagonists *T. viride* + *B. thuringiensis* + *P. fluorescens* was significantly highest (70.27 %) followed by combination of two bioagents *T. viride* + *B. thuringiensis* (63.83 %). This was followed by *T. viride* + *P. fluorescens* (59.84 %).

Vrieze *et al.* (2018) also tested consortia of *Pseudomonas* strains and found triple consortia of R32 + S19 + S35 was best among tested consortia.

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).

Mahendra *et al*. (2022) reported the efficacy of combine application of *Trichoderma harzianum* (GRT4) and *Pseudomonas fluorescens* (PF4) isolated from the groundnut rhizosphere against *Macrophomina phaseolina* and *Sclerotium rolfsii*.

2.19 *In vitro* evaluation of plant growth promoting ability of microbial isolates

Zaidi and Singh (2004) carried out experiment on bio-priming seed with *T. harzianum* on soybean, chickpea and tomato and found out the population of bioagent on surface of bioprimed seeds increased by almost 10000 folds at 48 hours after incubation. Higher germination of seeds than non-bioprimed seeds was also observed in all tested crops.

Raja *et al*. (2006) studied the effect of individual and microbial consortium of *Azospirillum lipoferum, B. megaterium* var. *phosphaticum* and *P. fluorescens* Pf-1 on rice exudates and plant growth under hydroponic culture which enhanced the plant growth positively.

Kumar *et al.* (2010) found that combined application of *T. harzianum* and P. *fluorescens* as seed biopriming resulted in significant growth of seedling (18.38 cm at 30 DAS) in sweet pepper.

Nazir *et al.* (2011) reported that *T. viride* and *T. harzianum* showed significant increased in seed germination, shoot length and fresh weight of tomato and chilli.

Murthy *et al.* (2013) reported the application of consortia of *Trichoderma* spp., significantly increased the French bean 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 %). They further found out the application of consortia significantly increased the shoot length and root length of French bean 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). They further reported 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).

Bhakthavatchalu *et al.* (2013) also recorded a significant difference in cowpea seeds treated with *P. aeruginosa* FP6 and non-treated plants, showed increased effects on seed germination per cent in bacterized seeds (92.0 %), which germinated earlier in comparison to control (72.0 %).

Eutesari *et al.* (2013) reported that three fungal biocontrol agents *T. harzianum, T. virens, T. viride* and a bacterium; *P. fluorescens* showed increase

soybean seed germination per cent and seedling growth promoting traits including root length, seedling length, fresh and dry weight of root.

Kabir *et al.* (2013) also tested five isolates of PGPR; all of the isolates (BI 06, BI 09, BI 11, BI 15 and BI 18) were capable of enhancing different growth parameters (shoot/root length, fresh biomass and dry matter) in comparison with non-inoculated control plants.

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 index.

Verma *et al* (2015) evaluated bio-primed seed with CMC and showed highest seed germination per cent, seed vigor index, number of root and seedling fresh weights.of green-gram under *in vitro* condition.

Singh *et al*. (2019) reported the *in vitro* efficacy of indigenous liquid compatible microbial consortia (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) on plant growth promoting activities like seed germination %, seedling vigour index, shoot length, root length, dry and fresh weight of shoot, dry and fresh weight of root and the results showed that CMC-1 increased vigour index of tomato seedlings (124.30 %), germination per cent (22.99 %), shoot length (83.44 %) and root length (81.37 %) over control at 10 DAS.

2.20 *In vivo* evaluation of compatible microbial consortia (CMC)

As seeds germinate and roots grow through the soil, the loss of organic material provides the driving force for the development of active microbial populations around the root, known as the rhizosphere effect (Whipps, 1992).

A variety of application methods has been evaluated in the field that would improve the integration of PGPR-mediated ISR into conventional agriculture and in some cases with improved efficacy.

Delivery systems for formulated product include seed treatment reported by Mew and Rosales (1986) and foliar application Chatterjee *et al.* (1996) in various crops.

Likewise, Chamswarng and Sangkaha (1988) recorded the enhanced efficacy of *Bacillus* spp. and *Pseudomonas* spp. in controlling tomato stem rot caused by *S. rolfsii* under field conditions.

Manoranjitham and Prakasam (1999) reported the seed treatment with *T. viride* and *P. fluorescens* showed a reduction in pre and post emergence damping off when compared to control. The treatment also increased the shoot length, root length and dry matter production of chilli seedlings.

Nandakumar *et al*. (2001) applied PGPR strains to rice plants through seed, root, soil or foliar application or in combinations found that combinations of three or four application methods were more effective than single methods in controlling sheath blight of rice in field trials.

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

Haas and Défago (2005) studied Plant growth-promoting rhizobacteria have been shown to control plant pathogens through antibiosis, reduction of pathogen virulence, competition for iron, plant growth promotion, and ISR. Most reported PGPR antagonists were from the genera *Bacillus* and *Pseudomonas*.

The more effectiveness of combination of different methods of application compared to single method of application was also noted by Saravanakumar (2006) in different crops.

Srinivasan (2007) reported that application of BCAs against various soil-borne plant pathogens and enhanced plant growth promotion activity.

Yigit and Dikilitas (2007) reported the control of *Fusarium* wilt by combination of *P. fluorescens,* non-pathogenic *Fusarium* and *T. harzianum* in greenhouse conditions.

Harish *et al.,* (2008) reported that PGPR strains were tested individually and in combinations (two/more strains) against multiple plant pathogens.

Sharma *et al.* (2009) reported that biopriming of cumin seeds with *T. harzianum* increased the germination of seeds while *T. viride* showed good shoot-root ratio in pot condition against cumin wilt.

Maiyappan *et al*. (2010) studied the efficacy of four species microbial consortium *viz., Bacillus* sp., *Streptomyces* sp., *Azotobacter* sp. and *Frauteria* sp. for its plant growth promoting efficacy and its antagonistic activities against select pathogens in blackgram. Pot culture studies against *S. rolfsii, F. oxysporum* and *R. solani* recorded high percentage of seed germination, maximum radical length and effective antagonistic activity.

Muthukumar *et al.* (2010) also reported the biocontrol efficacy of *T. viride* and *P. fluorescens* against *Pythium aphanidermatum*, the causal agent of chilli damping-off and increased the plant growth and yield, shoot length and root length of chilli when compared to control.

Srivastava *et al*. (2010) conducted an experiment using 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.

Akrami *et al*. (2011) studied the biocontrol effects of *Trichoderma* isolates alone and in combination against *F. oxysporum*. *T. harzianum* and *T.*

asperellum isolates and their combination were more effective than other treatments in controlling the disease.

Hema and Selvaraj (2011) conducted a green house nursery study to assess the interaction between arbuscular mycorrhizal (AM) fungus, *Glomus aggregatum* and a consortium of PGPRs, *B. coagulans and T. harzianum*, in soil and their consequent effect on growth, nutrition and content of secondary metabolities of *Solanum viarum* seedlings. Triple inoculation of *G. aggregatum* + *B. coagulans* + *T. harzainum* with *Solanum viarum* resulted in maximum plant biomass and yield.

Rajasekar and Elango (2011) concluded that the effect of microbial consortium consisting of PGPR like *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* tested separately and in combination on *Withania somnifera*. The combinations of above mentioned PGPR strains significantly increased plant height, root length and alkaloid content.

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.

Jain *et al*. (2012) studied the potentiality of three rhizospheric microorganisms *P. fluorescens* PJHU15, *T. harzianum* TNHU27 and *B. subtilis* BHHU100 in suppression of *Sclerotinia* rot in pea in consortia mode and their impact on host defence responses. It was reported that microbial consortia triggered defence responses in an enhanced level in pea than the microbes alone and provided better protection against *Sclerotinia* rot.

Ananthi *et al*. (2013) evaluated chilli (*Capsicum annuum* L.) seed bio-priming with *T. viride* and *P. fluorescens* which showed enhanced seed germination and seedling vigour index.

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. Seed germination index was higher in bacterized seeds (92.0 %), which germinated earlier in comparison to control (72.0 %) and seed vigour indices were good as overall as compared to control.

Eutesari *et al.* (2013) evaluated that three fungal biocontrol agents *T. harzianum, T. virens, T. viride* and a bacterium; *P. fluorescens* for their impact on soybean seed germination and seedling growth and chlorophyll amount. Studies reveal that seed factors such as germination rate and seedling growth indices including root length, seedling length and dry weight of root showed improvement. Also increased seedlings and leaf area per plant and total chrolophyll amount was also reported

Kabir *et al.* (2013) also tested five isolates of PGPR and concluded that all the isolates (BI 06, BI 09, BI 11, BI 15 and BI 18) were capable of enhancing different growth parameters (shoot/root length, fresh biomass and dry matter) in comparison with non-inoculated 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 highest 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. *In vivo* assay, all of the bacterial isolates were capable of enhancing different growth parameters (shoot/root length, fresh biomass and dry matter) in comparison with non-inoculated control plants. AB 17-treated plants in particular showed the highest enhancement in fresh biomass with 27 and 32 % increments in the root and shoot biomass, respectively. However, isolate AB 10 showed the highest shoot and root growth with 18 and 26 % increments, respectively.

Sandheep *et al.* (2013) also evaluated the plant growth promoting efficiency of combined inoculation of rhizobacteria on vanilla plants. The

maximum percentage of growth enhancement were observed in the combination of *T. harzianum* with *P. fluorescens* treatment followed by *P. fluorescens, T. harzianum, P. putida* and *T. virens,* respectively in decreasing order. Combined inoculation of *T. harzianum* and *P. fluorescens* registered the maximum length of vine (82.88 cm), highest number of leaves (26.67/plant), recorded the highest fresh weight of shoots (61.54 g plant^{-1}), 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.*

Contreras-Cornejo *et al*., 2014) reported an increase in total biomass, chlorophyll content and acceleration of flowering by isobutyl alcohol, isopentyl alcohol and 3-methylbutanal from application of *T. viride.*

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.

Kotasthane *et al.* (2014) evaluated 5 potential isolates of rhizobacterial species against *S. rolfsii.* The B1 isolates was found most effective with significantly highest reduction in number of sclerotia produced (84.32%) over control treatment.

Sharma *et al.* (2015) reported the consortial application of four strains of bacterial species (S_{21} + S_{25} + B_6 + A_{10}) had significant effect in achieving biocontrol efficacy of 66.7 %. Efficacies of seven strains of *Pseudomonas fluorescens* plant growth promoting rhizobacteria (PGPR) were tested for their ability to protect chickpea against *S. rolfsii* infection in potcondition. Consortium of the four strains resulted in significant increase in most plant parameters including root length, shoot length, plant fresh weight and plant dry weight over un-inoculated control.

Singh *et al.* (2016) tested bioprimed seeds of tomato with different spore doses of *T. asperellum* BHUT8 ranging from 10^2 to 10^8 spores ml^{-1}. The effective spore dose for enhancement in seed germination and radicle length was found to be 10^3 spores ml^{-1}. 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.

Khan *et al.* (2018) also studied 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.

Kumar *et al*. (2018) showed that seed treatment with *Trichoderma viride* @ 5.0 g/kg seed showed maximum seed germination (85.82%) and minimum pre- emergence (13.93%) and post- emergence (17.00%) root rot incidence of field pea and the maximum yield (63.44 q/ha) was also recorded by seed treatment with *T. veride* @ 5.0 g/10-6 spores/ml followed by *Aspergillus niger* @ 5.0 g/10-6 spores/ml (62.33 q/ha).

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

Hammad and Omar (2021) studied for managing common bean and pea root rot disease using different *Trichoderma* species (*T. harzianum, T. hamatum, T. viride, T. koningii, T. asperellum, T. atroviridae, T. lignorum, T. virens, T. longibrachiatum, T. cerinum*, and *T. album*) were reported both *in vitro* and *in vivo* with promotion of plant growth and induction of systemic defense. The wide scale application of selected metabolites produced by *Trichoderma spp*. to induce host resistance and/or to promote crop yield, may represent a powerful tool for the implementation of integrated pest management strategies.

Singh *et al*. (2022) reported the used of microbial consortia for managing late blight disease using different *Trichoderma* species and *Pseudomonas fluorescens* species. *In vivo* 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. The 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 %), number of fruits per plant (185.52 %), fresh weight of fruit (42.59 %), root length (67.28 %) and marketable fruit yield (313.02 %) over control treatment.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

All the experiments related to research work entitled "**Biological remediation in the management of collar rot of French bean (*Phaseolus vulgaris* L.)**" were carried out in the laboratory and pot experiment site of the Department of Plant Pathology, School of Agricultural Science and Rural Development (SASRD), Nagaland University, Medziphema Campus, Nagaland during 2018 and 2019. The details of materials used and the research methodology followed during the 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 mean sea level.

3.1.2 Climate

The site of pot 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.

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.

Table 3.1 Meteorological data recorded during the pot experiments (September-November, 2018 and 2019)

Month	Temperature (˚C)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Max.	Min.	
2018					
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
2019					
September, 2019	32.7	23.9	94	72	173.4
October, 2019	30.3	21.7	95	73	244.8
November, 2019	28.8	16.3	97	64	52.9

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

3.2.2 Glasswares, materials and chemicals

Throughout the experimental work glasswares made of Borosil *viz*., Petri plates, flasks, test tubes, pipette, beakers, funnels, glass rod, glass slides, cover slips and measuring cylinders, etc. were used. Petri plates and micro tips made of Tarson's disposable were used for serial dilution. Polyethylene bags of different sizes, rubber bands, non absorbent cotton, tags, parafilm were used in various experiments.

3.2.3 Cleaning of glasswares

The glasswares were cleaned with 6 per cent chromic acid (Potassium dichromate ($K_2Cr_2O_7$) 60 g dissolved in 1000 ml of distilled water and 60 ml conc. sulphuric acid (H_2SO_4) added with constant stirring), followed by cleaning with cleaning powder and several washing in running tap water and finally cleaned with distilled water and air dried in hot air oven before use.

3.2.4 Sterilization

Glassware used in the present investigation were wrapped in thick paper and kept for sterilization in hot air oven at 170 °C for 60 min. Surface of laminar air flow chamber was sterilized by wiping with tissue paper sprinkled with alcohol prior to research work. Culture media and water were sterilized in an autoclave at 121 °C (15 lb psi) for 15 min (Dhingra and Sinclair, 1995). The cork borer, inoculation needle and loop, forceps etc. were initially dipped in the rectified spirit then 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 and distributed equally into 250 ml. conical flasks @ 200 ml/flask and autoclaved at 121 °C (15 lb psi) pressure for 15 min.

Potato dextrose agar (PDA) medium (HIMEDIA)

Ingredients	**Grams/Litre**
Potatoes	200 g
Dextrose	20 g
Agar-agar	15 g
Distilled water	1000 ml

Potato dextrose broth (HIMEDIA)

Potatoes	200 g
Dextrose	20 g

***Trichoderma* selective medium** (HIMEDIA)

$MgSO_4$	0.2 g	Glucose	3.0 g
K_2HPO_4	0.9 g	Rose Bengal	0.15 g
NH_4NO_3	1.0 g	Agar-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-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	

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

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

3.3.1 Collection of diseased specimens

Diseased specimens of collar rot of French bean were collected from French bean field, Horticulture farm, SASRD, Nagaland University, Medziphema campus showing typical symptoms of collar rot (Plate 1). 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

Isolation of *S. rolfsii* was followed by tissue segment method using Potato dextrose agar medium (Rangaswami, 1993). The infected plants parts showing characteristic symptoms of disease presence of white mycelial mat with small round brown sclerotia near the collar region were pulled out and gently tapped to remove the soil particle. The infected portions of diseased

Plate 1 Collection of typical collar rot diseased specimens

were cut into small pieces of 1 cm size using sterilized scalpel. These pieces were then surface sterilized with 0.1 per cent sodium hypochloride for one minute and washed thrice in sterile distilled water. Then placed at equal distance in a Petri plate containing solidified Potato dextrose agar medium and were incubated in a BOD at 25±2°C for five days and observed for hyphal growth of the fungus. After initiation of growth, a portion from the periphery having single hyphal tip was transferred to PDA slants under aseptic conditions, and pure culture of *S. rolfsii* were prepared. Stock culture of *S. rolfsii* were maintained on PDA slants and stored at 4 °C. These isolates were used for pathogenicity test.

3.3.3 Pathogenicity test

Soil was sterilized in an autoclave (121.6 ^{0}C) for 30 minutes (Williams and Ewel, 1984). The plastic pots of 7 cm diameter were filled with sterilized soil, sand and vermicompost. Susceptible French bean cv. Anupama was grown in polybag under green shade net house conditions. The seeds were surface sterilized with 0.1 % sodium hypochloride and sown @ 5 seeds per polybag. In order to prove the Koch´s postulate of *Sclerotium rolfsii*, inoculums was artificially inoculated prior one week of sowing of seed (5 seed per bag) at the rate of 5g/Kg of sterilized soil and polybag without pathogen inoculation were served as a control. Re-isolation was done from parts showing typical symptoms of collar rot. The culture obtained from re-isolation was kept on PDA slants for further investigation.

3.3.4 Characterization and identification of the pathogen

Morphological characters of the pathogen were studied on host as well as in pure culture maintained on PDA medium. Infected plants were brought to the laboratory and sections from diseased portions were examined under microscope. The fungus growing on PDA medium was observed

microscopically and principle taxonomic characters such as fungus colony colour, colony texture, growth pattern, septation, branching of hyphae and formation of sclerotia and shape etc. was studied.

3.4 Collection and isolation of native biocontrol agents

3.4.1 Collection of French bean rhizosphere soil samples

A field survey was undertaken for the collection of French bean rhizosphere soil samples from French bean growing areas *viz.*, Kohima, Mao, Merema, Tsiesema, SASRD campus and CIH polyhouse. Soil samples were taken from the rhizosphere of healthy French bean plants. The soil samples were collected from the healthy French bean growing area and dug at an around a depth of about 10-15 cm. The soils were collected close to the root of the French bean plant and kept in paper 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 French bean 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 KB medium (King *et al.*, 1954). The plates were then incubated in BOD at 25±2 °C for three days.

3.5 Maintenance of the cultures

The pure culture of the *S. rolfsii*, *Trichoderma* and *Pseudomonas* isolates were maintained throughout the period of investigation on the Petri plates and test tubes slants of PDA and KB medium, respectively in BOD

incubator. Regular interval of sub-culturing was done to maintain the purity of the cultures.

3.6 Evaluation of antagonists against *S. rolfsii*

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 *S. rolfsii* by dual culture technique given by Sivakumar *et al.* (2000). A 10 mm diameter mycelial disc of *S. rolfsii* (5 days old) was placed on one side of a Petri plate (90 mm diameter) containing PDA medium (20 ml). Simultaneously, 10 mm diameter disc of *Trichoderma* isolates (5 days old) were placed on another side 60 mm away from the pathogen leaving 10 mm from both periphery on the dual plates, whereas sterile PDA disc was placed in the control plates and incubated at 25±2°C. The radial growth of the pathogen was measured after fully grown of control plate.

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 mycelia growth of the test pathogen.

Per cent inhibition of the growth of pathogen by BCAs over control was calculated as per the formula given by Vincent, 1927.

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

Where C = Radial growth of *S. rolfsii* (cm) in control plate

T = Radial growth of *S. rolfsii* (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- *S. rolfsii* alone	T_{13}	- *S. rolfsii* + T-13
T_1	- *S. rolfsii* + T-1	T_{14}	- *S. rolfsii* + T-14
T_2	- *S. rolfsii* + T-2	T_{15}	- *S. rolfsii* + T-15
T_3	- *S. rolfsii* + T-3	T_{16}	- *S. rolfsii* + T-16
T_4	- *S. rolfsii* + T-4	T_{17}	- *S. rolfsii* + T-17
T_5	-*S. rolfsii* - + T-5	T_{18}	- *S. rolfsii* + T-18
T_6	- *S. rolfsii* + T-6	T_{19}	- *S. rolfsii* + T-19
T_7	- *S. rolfsii* + T-7	T_{20}	- *S. rolfsii* + T-20
T_8	- *S. rolfsii* + T-8		
T_9	- *S. rolfsii* + T-9		
T_{10}	- *S. rolfsii* + T-10		
T_{11}	- *S. rolfsii* + T-11		
T_{12}	- *S. rolfsii* + T-12		

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 *S.rolfsii* by dual culture technique given by Morton and Straube. (1955). A 20 µl of an overnight culture (12 hrs old) of *Pseudomonas* isolates were streaked at the centre of the Petri plate containing equal amount of KB agar medium and PDA medium (10 ml each). Simultaneously, 10 mm disc of *S. rolfsii* (5 days old) was placed at either side of the bacterial culture. The inoculated plates were incubated at 25 ±2 °C. Linear mycelial growth of pathogen was recorded after the control plate was completely covered by the test pathogen and zone of inhibition of pathogen by BCAs over control was calculated as per formula given in 3.61(Vincent, 1927).

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- *S. rolfsii* alone	T_7	- *S. rolfsii* + Pf-7
T_1	- *S. rolfsii* + Pf-1	T_8	- *S. rolfsii* + Pf-8
T_2	- *S. rolfsii* + Pf-2	T_9	- *S. rolfsii* + Pf-9
T_3	- *S. rolfsii* + Pf-3	T_{10}	- *S. rolfsii* + Pf-10
T_4	- *S. rolfsii* + Pf-4	T_{11}	- *S. rolfsii* + Pf-11
T_5	- *S. rolfsii* + Pf-5	T_{12}	- *S. rolfsii* + Pf-12
T_6	- *S. rolfsii* + Pf-6		

3.7 Investigation on the biocontrol mechanisms of BCAs

3.7.1 Qualitative assay of volatile metabolites production

The effects of volatile metabolites of BCAs were assessed following Dennis and Webster (1971) technique. The pathogen *S. rolfsii* was inoculated (10 mm diameter disc) at the centre of a Petri plate containing PDA medium (20 ml). After 3 hrs of incubation at 25±2 °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 25±2 °C. Diameter mycelial growth was measured 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 as per the formula given in 3.61(Vincent, 1927).

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 *Pseudomonas* isolates

T_0	- Control- *S. rolfsii* alone	T_7	- *S. rolfsii* + Pf-7
T_1	- *S. rolfsii* + Pf-1	T_8	- *S. rolfsii* + Pf-8
T_2	- *S. rolfsii* + Pf-2	T_9	- *S. rolfsii* + Pf-9
T_3	- *S. rolfsii* + Pf-3	T_{10}	- *S. rolfsii* + Pf-10
T_5	- *S. rolfsii* + Pf-5	T_{11}	- *S. rolfsii* + Pf-11
T_6	- *S. rolfsii* + Pf-6	T_{12}	- *S. rolfsii* + Pf-12

For *Trichoderma* isolates

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

3.7.2 Qualitative assay of non-volatile production

For testing non volatile test of *Trichoderma* and *Pseudomonas* isolates was followed with the protocol given by You *et al.*, 2016. The isolates of bacterial and fungal antagonists were inoculated in 100 ml sterile nutrient broth and potato dextrose broth in 250 ml conical flask. Inoculated flasks were then incubated at 25±2 ^{0}C for 15 days. Supernatant of the liquid culture was prepared by filtering through a 0.22-µm filter, then mixed to unsolidified PDA (40 ^{0}C) at a ratio of 10 % (v/v). Control plates was maintained without amending the culture filtrate of bio-control agents Then, a mycelial block (10 mm) of *S. rolfsii* was inoculated on poured media plates, and the plates were kept for six days at 25±2 ^{0}C. Colony diameter of mycelial growth was measured when control plate was completely covered and calculated as per the formula given in 3.61 (Vincent, 1927).

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:

For *Trichoderma* isolates

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

For *Pseudomonas* isolates

T_0	- Control- *S. rolfsii* alone	T_7	- *S. rolfsii* + Pf-7
T_1	- *S. rolfsii* + Pf-1	T_8	- *S. rolfsii* + Pf-8
T_2	- *S. rolfsii* + Pf-2	T_9	- *S. rolfsii* + Pf-9
T_3	- *S. rolfsii* + Pf-3	T_{10}	- *S. rolfsii* + Pf-10
T_5	- *S. rolfsii* + Pf-5	T_{11}	- *S. rolfsii* + Pf-11
T_6	- *S. rolfsii* + Pf-6	T_{12}	- *S. rolfsii* + Pf-12

3.7.3 Qualitative assay of 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).

3.7.4 Qualitative assay of 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, Yeast extract 3 g, Malt extract 3 g, Peptone 5 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.

3.7.5 Qualitative assay of 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, $MgCl_2.6H_2O$ 5 g, $MgSO_4.7H_2O$ 0.25g, KCl 0.2 g, $(NH_4)_2SO_4$ 0.1 g, agar 15 g and distilled water 1 L) according to Nautiyal (1999). The presence of halo clearing zone around growing colony after incubating at 28 °C for 7 days was used as an indicator for positive P solubilization.

3.7.6 Qualitative assay of 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.

3.7.7 Qualitative assay of HCN production

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 as weak (yellow to light brown), moderate (brown) and strong (reddish brown).

3.7.8Qualitative assay of microbial enzymes production

3.7.8.1 Qualitative screening of pectinolytic enzyme producing isolates

Pectinolytic activity of *Trichoderma* and *Pseudomonas* isolates were carried out on solid medium. The medium was aseptically poured to Petri dishes and inoculated with a 10 mm disc from 5-day old *Trichoderma* isolates separately. For *Pseudomonas* isolates which were pin point on the media and incubated at 25 ± 2°C in darkness. After 24-48 hours growth, plates were flooded with Gram's iodine solution (2 g KI and 1g I2 crystals dissolved in 100 ml of water). A clear zone around the colony indicated the pectinolytic activity (Kaur *et al*., 1988).

3.7.8.2 Qualitative screening of cellulose producing isolates

For qualitative cellulose production assay (Sadasivam and Manickam, 1992), the *Trichoderma* and *Pseudomonas* isolates were grown on the Czapek-Mineral Salt Agar Medium (KH_2PO_4 1.00 g, $MgSO_4.7H_2O$ 0.50 g, $NaNO_3$ 2.00 g, KCl 0.50 g, Peptone 2.00 g, and Agar 20.00g, Distilled water 1000ml) supplemented with Carboxy Methyl Cellulose (CMC) 5.00g. The medium was aseptically transferred to Petri dishes and inoculated with a 10 mm agar disc cut from 5-day old fungal culture of each strain separately.

For bacterial isolates, 48 hours old inoculums was streaked on medium containing Petri plates and incubated at 25 ± 2°C in darkness for 3 to 5 days. The plates were flooded with aqueous Congo red (2 % w/v) solution for 15 min. Production of cellulase was observed by the formation of yellow-opaque area around the colonies.

3.7.8.3 Qualitative screening for amylase producing isolates

The qualitative screening of amylase production by *Pseudomonas* isolates was ascertained using the starch agar medium (Toye, 2009). The colonies which formed clear zones around them were recorded and the strains

showing high amylolytic potential (depending upon the zone diameter) were selected and screened further for efficient amylase production.

Amylase production test (Hankin and Anagnostakis, 1975) was assessed by growing the *Trichoderma* isolates on Starch Agar Medium (Starch 20.00g, Beef extract 3.00g, Peptone 5.00g, Agar 16.00g, Distilled water 1000 ml). The medium was aseptically transferred to petri dishes and inoculated with a 6mm agar disc cut from 5-day old fungal culture of each strain separately and incubated at 25 ± 2°C in darkness for 3 to 5 days. The plates were then flooded with 1 % iodine in 2 % potassium iodide. The clear zone formed surrounding the colony was considered positive for amylase production.

3.7.8.4 Qualitative screening for catalase producing isolates

The qualitative screening of catalase production by *Pseudomonas* isolates was ascertained (Aneja, 2003). One loopful culture of each isolates was put on the slide and adds few drop of H_2O_2 separately. The evolution of oxygen in the form of bubble indicates positive for catalase production.

3.7.9 Mycoparasitism activity of native *Trichoderma* isolates

This method consisted of inoculating the 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. Mycelial 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 25±2 °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 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-8 and T-20) 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 mol 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-8 and T-20 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

3.8.2.1. Cultural and morphological identification of bacterial isolates

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

3.8.2.2 Molecular characterization of selected bacterial isolates

Based on 16s rRNA Gene Sequence Analysis Bacterial isolates identification were initially performed using gram staining reactions and examined under electron microscope. Then, molecular identification of the isolates was determined on the basis of 16S rDNA sequence analysis. Bacterial isolates were cultured for 48 hrs and the DNA of the isolates was extracted according to the procedure described by Sambrook *et al.,* 2001. The extracted bacterial genomic DNA was visualized in 1% agarose gel electrophoresis stained with ethidium bromide at 100 V for 45 minutes and the DNA was viewed under UV light and stored at −20˚C for further use. DNA template for PCR amplification was prepared by picking individual colony of each strain and amplification of 16S rRNA gene. Amplification of the gene was carried out by PCR using universal primer ITS1 (5'-AAGTCGTAACAAGGTAG-3') and ITS2 (5'-GACCATATATAACCCCAAG-3') was used for amplifying and sequencing of Pseudomonas isolates according to Turner *et al*., 1999. Reaction mixture (25 µL), prepared for full-length 16S rRNA gene amplification was initially denatured at 94˚C for 2 min, followed by 30 cycles consisting of denaturation at 94˚C for 1 min; primer annealing at 52˚C for 1:30 min. and primer extension at 72˚C for 2 min and finally extension at 72˚C for 10 min in a thermal cycler. Amplified PCR products of 16S ribosomal gene were separated on 1% agarose gel in 0.5X TE (Tris-EDTA) buffer containing 2 µL ethidium bromide (20 mg/mL). λ Hind-III ladder was used as a size marker. The gel was viewed under UV light and photographed using gel documentation system. Amplified PCR products of full length 16S rRNA genes were sent to the sequencing company with the service of PCR purification. For species identification, ITS1 and ITS2 gene sequences were submitted to BLAST (Basic local alignment search tool) interface by using the BLASTN program from NCBI web site (http://www.ncbi.nlm.nih.gov).

3.9 *In vitro* compatibility of potential rhizospheric antagonists

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-12 were streaked on one side of the Petri plates (90 mm diameter) containing equal volume of KB agar and PDA medium (10 ml each). The other side of the Petri plates was inoculated with 10 mm diameter disc of T-8 and T-20 (5 days old). The plates were incubated at 25±2 °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	- Pf-2 + Pf-12	T_7	- Pf-2 + Pf-12 + T-8
T_2	- Pf-12 + T-8	T_8	- Pf-2 + Pf-12 + T-20
T_3	- Pf-12 + T-20	T_9	- Pf-12 + T-8+ T-20
T_4	- Pf-2+ T-8	T_{10}	- Pf-2 + T-8 + T-20
T_5	- Pf-2+ T-20	T_{11}	- Pf-2 + Pf-12 + T-8 + T-20
T_6	- T-8 + T-20		

3.10 *In vitro* antagonistic efficacy of compatible microbial consortia (CMC) against *S. rolfsii*

The slight modification of bioassay technique (Morton and Straube., 1955) was used for the testing of compatible microbial consortia against *S. rolfsii.* A 10 mm diameter mycelial disc of pathogen (5 days old) was placed at centre of Petri plate (90 mm diameter) containing equal amount of PDA and KB agar medium (10 ml each). Simultaneously, 10 mm diameter disc of T-8 and T-20 isolates (9 days old) and 20 µl of an overnight culture (12 hrs old, 2 ml) of Pf-2 and Pf-12 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 25±2 °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 as per the formula given in 3.61 (Vincent, 1927).

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, *S.rolfsii* alone	T_6	- T-8 + T-20
T_1	- Pf-2 + Pf-12	T_7	- Pf-2 + Pf-12 + T-8
T_2	- Pf-12 + T-8	T_8	- Pf-2 + Pf-12 + T-20
T_3	- Pf-12 + T-20	T_9	- Pf-12 + T-8 + T-20
T_4	- Pf-2+ T-8	T_{10}	- Pf-2 + T-8 + T-20
T_5	- Pf-2+ T-20	T_{11}	- Pf-2 + Pf-12 + T-8 + T-20

3.11 Preparation of liquid compatible microbial consortia (CMC)

Liquid suspension of each selected *T. asperellum* isolates (T-8 and T-20) was prepared from 7 days old cultured PDA medium plates. The plates were rinsed with sterile distilled (10 ml) 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.

In case of bacteria, 250 ml of each selected *P. fluorescens* isolates (Pf-2 and Pf-12) 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 1×10^9 cfu/ml (O.D. 600= 1) (Mulya *et al.*, 1996). Liquid consortium was prepared by mixing equal

volume (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 French bean seed

The healthy seed of French bean cv. Anupama was selected for experimental purpose. The seeds were obtained from local market. French bean seeds were surface sterilized with 1.0 % sodium hypochlorite for 2 min for all treatments, followed by three rinses with sterile distilled water.

3.12.1 Wet seed treatment

The surface sterilized seeds were soaked with liquid formulations of consortia @ 1.0 % or 15 ml formulation of CMC in 1 kg seed (French bean 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: 7
- No. of treatment : 7

- No. of replication: 4
- No. of seeds/ Petri plate: 5 seeds
- Method: Standard filter paper method (three layered moistened filter papers in Petri plates) (ISTA, 1993).

Treatments:

T_1- T-8 isolate

T_2 –T-20 isolate

T_3 – Pf-2 isolate

T_4 – Pf 12 isolate

T_5 – CMC (T-8+T-20+Pf-2+Pf-12)

T_6-Chemical control

T_7- Control

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

The per cent increase over control was calculated using the formula:

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

3.13 *In vivo* effect of compatible microbial consortia (CMC) on French bean in pot experiment

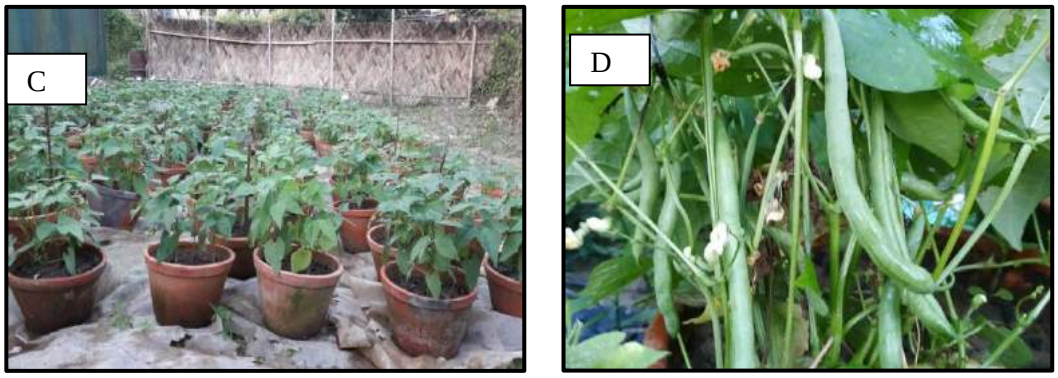
Pot experiments was conducted to test the efficacy of potential isolates of *Trichoderma* sp. and *Pseudomonas* sp. compatible microbial consortia formulations through seed treatment (ST) and soil drenching in controlling collar rot of French bean.

3.13.1 Details of experiment under pot condition

The experiment was conducted in a completely randomized block design with seven treatments having four replicates (Plate 2). Based on the previous *in vitro* studies one best compatible microbial consortium (T-8+T-20+ Pf-2+ Pf-12) was selected for further analysis.

- Crop : French bean
- Variety : Anupama (popular and susceptible)
- Experimental design : Complete randomized design (CRD)
- Number of treatment : 7 (Seven)
- Number of replication : 4 (Four)
- Time of sowing : Sep. - Nov (Rabi season)
- Date of sowing: 19/09/2018 and 19/09/2019
- No. of pot per replication : 5 (Five)
- No. of experiment : Twice
- Number of seed per pot: 7 (Seven)

A) General view of pot experiment before emergence
B) Close view of pot after emergence



C) General view of pot experiment at 30 DAS
D) Pod bearing French bean plant

Plate 2 General view of pot experiment

The treatment combination was laid out as follows:

1. $T_{1.....}$ Seed treatment + soil drenching (at the time of sowing +15 DAS +30 DAS)
2. T_2 : Seed treatment + soil drenching (at the time of sowing + 15 DAS)
3. T_3 : Seed treatment + soil drenching (at the time of sowing + 30 DAS)
4. T_4 : Seed treatment + soil drenching (15 DAS + 30 DAS)
5. T_5 : Chemical control
6. T_6 : Positive control (Inoculated)
7. T_7 Negative control (Uninoculated)

3.13.2 Preparation of inoculum of *S. rolfsii*

The sand-maize (3:1) medium was added to 250 ml conical flask with 20 ml distilled water autoclaved for 15 min at 15 psi (Dubey *et al.*, 2012). To mass multiply the pathogen, each sterilized flask was inoculated with 5mm disc of 7 days old culture of *Sclerotium rolfsii* and incubated at 25±2 for 15 days (Plate 3).

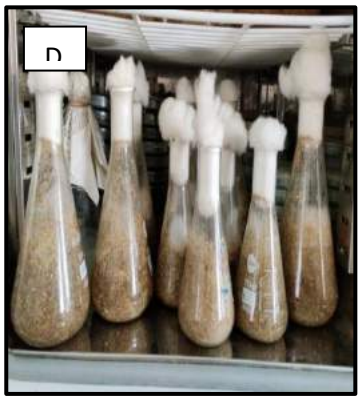
3.13.3. Preparation of sick soil

Soil, sand and FYM were disinfected with 1 % formalin solution and left covered with polythene sheet for 24 hours, then allowed to dry it until odor of formalin disappeared from the soil (Plate 4). All earthern pots were disinfected by dipping in formalin solution before filling up of soil (Miller, 1950). Disinfected soil, sand and FYM were filled in the earthen pots of 30 cm diameter with 6 kg of soil, 2 kg of sand and FYM in the ratio 3:1:1. The inoculum of *S. rolfsii* grown on sterilized sand maize bran medium was mixed with the disinfected soil (5 g of inoculums per kg of soil) one week before sowing (Datur and Bindu. 1974). Suitable control without inoculation was

Plate 3 Preparation of mass multiplication of pathogen inoculum

A) Disinfectant of soil, FYM and sand

B) Disinfectant soil covered with polythene, (C) Pot filling



D)30 days old mass pathogen inoculum, E) Inoculation of *S.rolfsii* on the soil and F) Growth of white fluffy mycelial of pathogen after inoculation

Plate 4 Preparation of sick

maintained. The pots were placed under natural environmental conditions in open sunlight.

3.13.4Applications of treatment

3.13.4.1 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 and shade dried in laminar air flow for 5 hrs (Srinivasan and Mathivanan, 2009).

3.13.4.2 For chemical control treatment

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

3.13.4.3 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.

3.13.4.4 Soil drenching

For T_1, T_2, T_3 and T_4 (three soil drenching @ 1.0 % of CMC or 50 ml per pot at 0, 15, 30 DAS) (Wang *et al*., 2019).

For chemical control (three foliar sprays of Captan 50% WP @ 0.2 % or 0.375 g / 50 ml of water per pot at 0, 15, and 30 DAS).

For control (three foliar sprays of sterile distilled water @ 50 ml /pot).

3.14 Observation and recording procedures

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

3.14.1 Per cent germination at 10 DAS

Per cent germination was calculated using the following formula –

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

3.14.2 Seedlings shoot length and root length (cm) at 20 and 60 DAS

The root length and shoot length of individual seedlings (5 seedlings/ replication) were measured. The shoot length was measured from collar region to the tip of the seedling with the help of a scale and the mean shoot length was expressed in cm. The root length measured from collar region to the tip of primary root with the help of a scale and the mean root length was expressed in cm.

3.14.3 Plant vigour index at 20 and 60 DAS

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

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

3.14.4 Fresh weight (g) and dry weight of shoot at 20 and 60 DAS

The fresh weight (g) and dry weight of shoot of individual seedlings (5 seedlings/ replication) were measured and expressed in gram.

3.14.5 Fresh and dry weight (g) of root at 20 and 60 DAS

The fresh and dry weight of root of individual plant (5 plants/ replication) were measured after oven drying at 60° C (when constant weight obtained) for 24 hrs. The weights of root were recorded and mean dry weight was calculated and was expressed in gram.

3.14.6 Per cent increase of plant growth promotion over positive control at 20 DAS and 60 DAS

Per cent increase of plant growth promotion over positive control was calculated using the following formula:

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

3.14.7 Number of leaves per plant

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

3.14.8 Number of branches per plant

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

3.14.9 Number of pod per plant

The numbers of pod harvested from five randomly tagged plants were counted from which total number of pod per plant was calculated.

3.14.10 Fresh weight of pod (g pod^{-1})

Fresh weight of randomly selected 25 pods from each replication was recorded and divided by number of pod to get the average weight of the pod.

3.14.11 Marketable pod yield (g plant^{-1})

The yield of immature marketable pod harvested from sampling plants was computed and their average per plant was noted in g.

3.14.12 Calculated marketable pod yield (g treatment^{-1})

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

3.14.13 Disease incidence

The disease incidence was assessed at 30 DAS visually on development of white fluffy mycelium on collar or stems region just above soil of all plants of each replication in regular interval. The percentage disease index (PDI) for analysis (Wheeler, 1969) was adopted as given below:

PDI = Total sum of diseased plants / Total number of plants examined x 100.

3.14.14 Mortality per cent

Pre emergence and post emergence mortality per cent observations of plants in each replication were recorded. Mortality per cent was calculated as per the formula given below:

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

3.14.15 Per cent reduction over positive control

Disease reduction over positive control is calculated by using the following formula:

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

3.14.16 Per cent increase over positive control

Per cent increase over positive control is calculated by using the following formula:

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

3.15 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) 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.

RESULTS AND DISCUSSION

Observations and results of the present investigation, "**Biological remediation in the management of collar rot of French bean (*Phaseolus vulgaris* L.)**" were analyzed statistically to assess the effectiveness 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

French bean plant of all the growth stages were attacked by the pathogen and occurs during warm, humid weather and symptoms commonly observed on stem or collar region are described as under:

The symptoms develop on lower stem, roots and collar region of French bean. It also caused rotting of pods which is in contact with the soil. Typical early visible symptoms of collar rot of French bean were recorded as progressive yellowing and wilting of leaves. Following this, the pathogen produces abundant white, fluffy mycelium on infected tissues and the soil. Shortly after the mycelia mat develops, small, white, round, fuzzy mycelial bodies called sclerotia begin to appear. They are of relative uniform size, roundish and white when immature then later becoming dark brown to black. Infected collar parts formed woody tissue which are gradually girdled by lesions and eventually die (Plate 5).

Symptoms appeared on pod only at or near soil surface. Pod becomes soft, sunken lesions and shortly covered with white mycelium, eventually developing sclerotia spreads over the infected pod surface.

Typical collar rot symptoms appeared on different stages of plant

Sclerotial development on collar region and pod of French bean

Plate 5 Typical symptoms of collar rot of French bean

Plate 5 Typical symptoms of collar rot of French bean

The symptoms of the collar rot of French bean as observed in present studies are in conformity with the symptoms described by previous workers Taylor and Rodriguez (1999); Mehan and McDonald (1990); Tsahouridou and Thanassoulopoulos (2002); Kumar *et al.* (2013); Kator *et al.* (2015) and Mahadevakumar *et al*. (2018).

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

4.2.1 Collection of diseased specimens

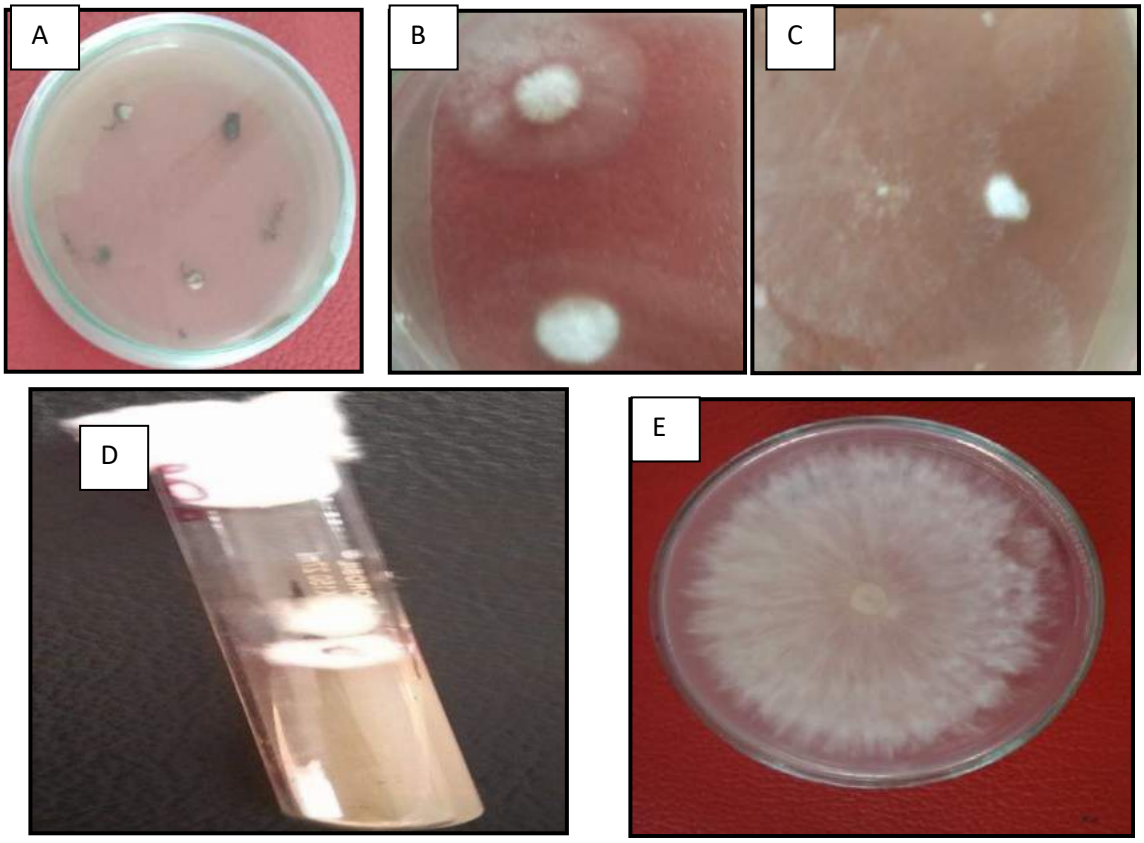
Diseased specimens of collar rot of French bean were collected from French bean field, Horticulture farm, SASRD, Nagaland University, Medziphema campus showing typical symptoms of collar rot of French bean.

4.2.2 Isolation and purification of the pathogen

The causal pathogen from naturally infected tissues of French bean were isolated (Plate 6a) by tissue isolation technique. The typical pathogen colonies developed within 48 hrs (Plate 6b). *S. rolfsii* produced white cottony, compact fluffy mycelial growth on PDA medium. The pure culture of the fungus was obtained by hyphal tip isolation technique (Plate 6c, d and e) and each isolate thus obtained was coded and stored in test tube stants. The results are in conformity with the similar kind of observation by previous workers Ramarao and Usharaja (1980); Kumar and Sen (2000); Shukla (2008); Ozgonen (2010); Goud (2011) and Rakh (2011).

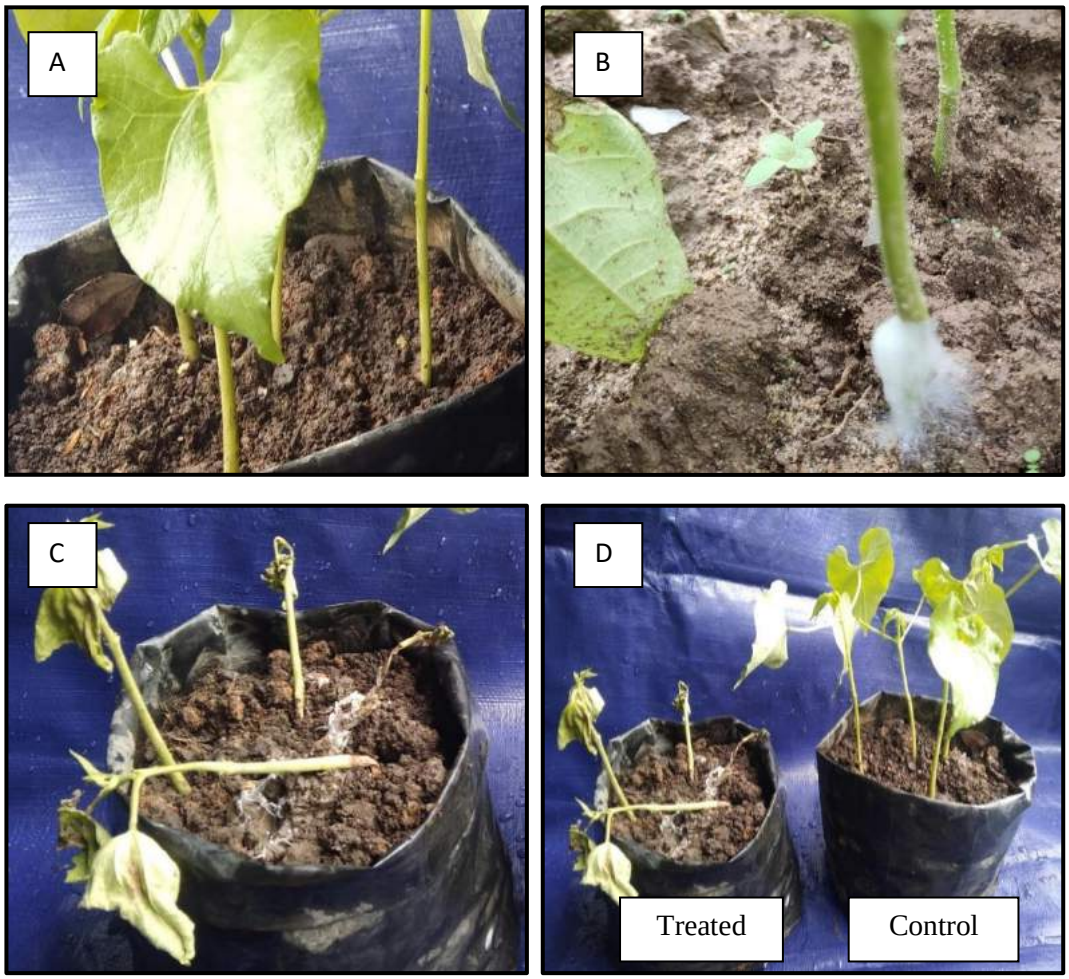
4.2.3 Pathogenicity test

The pathogen obtained was subjected to pathogenicity test (Plate 7). A pot culture experiment was conducted to test the pathogenicity. For this purpose, the pathogen was inoculated on susceptible French bean cv Anupama seedlings. After 48 hours, the observation on disease development was



A. Isolation of pathogen from infected plant parts
B. Growth of pathogen on PDA after 3 days
C. Hyphal tip growth of pure pathogen
D. Pure growth of pathogen on PDA slants
E. Growth of pathogen at 7 Days

Plate 6 Different procedure for isolation and purification of the pathogen



A. Close view of control pot
B. Close view of development of white fluffy mycelium on pathogen inoculated pot
C. Typical symptoms and signs observed on inoculated pot
D. Comparison of treated seedlings with control.

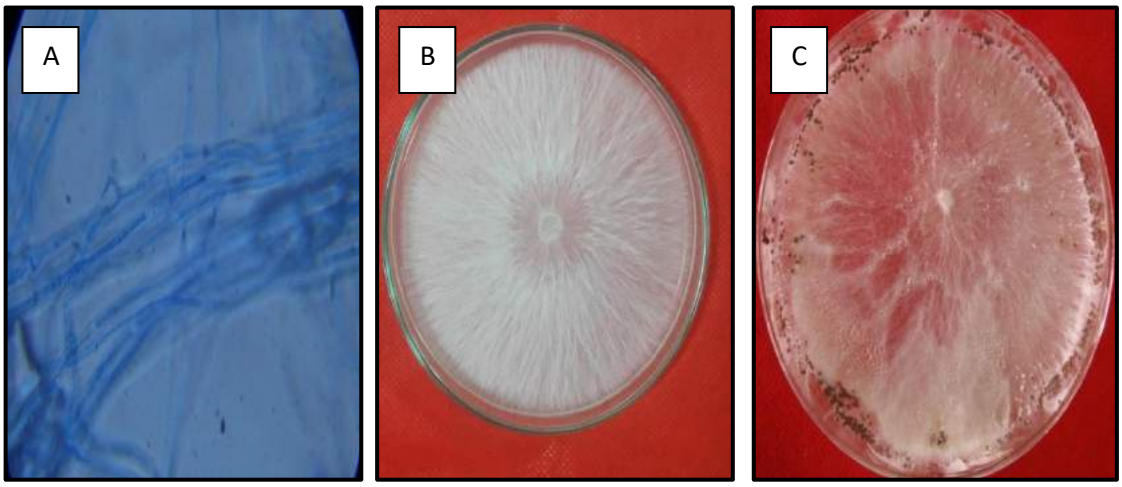
Plate 7 Pathogenicity test

recorded where lesions appeared near the collar region subsequently followed with the withering and yellowing of leaf. Then after 96 hours, full wilting of the plant was observed. The pathogen was re-isolated from the same infected tissue on a PDA plate and when compared they were observed to be akin with the original pathogen. No symptoms were observed on un-inoculated seedlings. The pathogen was then undergoing for further characterization studies.

The results of the present findings are in agreement with the findings of earlier workers Siddaramaiah and Chandrappa (1988); Dange (2006); Senthilraja *et al.* (2010) and Vaishali *et al*. (2022).

4.2.4 Characterization and identification of the pathogen

The pure culture isolates obtained from the diseased specimens were identified as *Sclerotium rolfsii* based on cultural and morphological characters of the isolates. *S. rolfsii* produced white cottony mycelial growth on potato dextrose agar medium and the fluffy colony. Initially, the pathogen produced white colored sclerotia and then their colour gradually changed from white to off-white, light brown and tan when they attained maturity. The sclerotia were mostly globose and sometimes spherical in shape and the surface of sclerotia was smooth (Plate 8). Kator *et al*. (2015) isolated the pathogen and identified according to morphological and cultural characters as *S. rolfsii*. Further they noted the fungus grew very rapidly on PDA and the colony colour was white. The white mycelium formed many narrow mycelia strands in the aerial mycelium and they measured 4.2-8.4um in width. The sclerotia were formed between 18-21 days and were small and globoid. They were white at first but became dark brown after maturity and ranged from 1-3mm.



A. Microscopic view of hyaline, septate, branch mycelium

B. Abundant white, fluffy aerial mycelium

C. Brown, globose , smooth sclerotia developed on Petri plate.

Plate 8 Characterization of the pathogen

The present findings of the cultural and morphological characters exhibited by the pathogen was compared and identified as *Sclerotium rolfsii* compared on description given by Punja and Damiani (1996). Additionally, the description of pathogen was also in conformity with the report of earlier workers Kokub *et al.* (2007) and Mahadevakumar *et al*. (2015).

4.3 Collection and isolation of native rhizosphere antagonists

A field survey was undertaken for the collection of French bean rhizosphere soil samples from different French bean growing areas *viz.*, Kohima, Mao, SASRD and CIH campus Medziphema (Table 4.1). Soil samples were taken from the rhizosphere of healthy French bean plants (Plate 9a).

For the isolation of *Trichoderma* the soil samples were serially diluted (10^{-4}) and plated on *Trichoderma* selective medium (Plate 9b). The fluorescent pseudomonads were isolated by plating the serially diluted (10^{-6}) on King's B medium (Plate 9c).

Totally 32 isolates (20 isolates of fungi and 12 bacterial isolates) were subjected to preliminary screening against *S. rolfsii*, to test their biocontrol ability. The isolates were designated serially as T-1 to T-20 (Plate 10 and 11a) for fungal isolates and Pf-1 to Pf-12 for bacterial isolates (Plate 11b). Among 32 isolates, the most promising 4 isolates (2 isolates of antagonistic fungi and 2 isolates of antagonistic bacteria) were selected for further microbial consortia studies. The present work done are in similar with the earlier workers Arya and Mathiew (1993), Thakare *et al*. (2002), Siddiqui and Shaukat (2003) and Singh *et al*. (2022) isolated antagonists from rhizosphere soils.

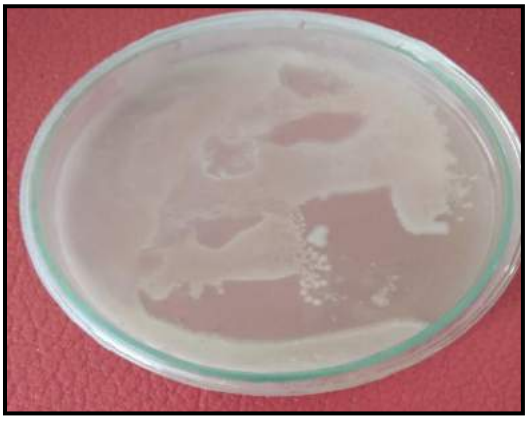
Table 4.1 Native rhizospheric microbes and their collection locations

S.No.	Isolate code	Isolation from	Location	District	State
(A) Native *Pseudomonas* isolates					
01	Pf-1	French beans Rhizosphere	French bean Agronomy field,SASRD	Chumoukedima	Nagaland
02	Pf-2	French beans Rhizosphere	AICRP field, SASRD	Chumoukedima	Nagaland
03	Pf-3	French beans Rhizosphere	French bean Entomology field, SASRD	Chumoukedima	Nagaland
04	Pf-4	French beans Rhizosphere	Horticulture farm (Sample-1), SASRD	Chumoukedima	Nagaland
05	Pf-5	French beans Rhizosphere	Horticulture farm (Sample II), SASRD	Chumoukedima	Nagaland
06	Pf-6	French beans Rhizosphere	Farmers' field (Sample-I), Kohima	Kohima	Nagaland
07	Pf-7	French beans Rhizosphere	Farmers' field (Sample-II), Kohima	Kohima	Nagaland
08	Pf-8	French beans Rhizosphere	Farmers' field (Sample-III), Kohima	Kohima	Nagaland
09	Pf-9	French beans Rhizosphere	Farmer's field, Medziphema	Chumoukedima	Nagaland
10	Pf-10	French beans Rhizosphere	Farmers' field, Mao	Senapati	Manipur
11	Pf-11	French beans Rhizosphere	Kitchen garden,SASRD	Chumoukedima	Nagaland
12	Pf-12	French beans Rhizosphere	Soil science,SASRD	Chumoukedima	Nagaland
(B)Native *Trichoderma* isolates					
13	T-1	French beans rhizosphere	French bean Agronomy field, SASRD	Chumoukedima	Nagaland
14	T-2	French beans rhizosphere	AICRP field, SASRD	Chumoukedima	Nagaland
15	T-3	French beans rhizosphere	French bean Entomology field, SASRD	Chumoukedima	Nagaland
16	T-4	French beans rhizosphere	Horticulture farm (Sample-1), SASRD	Chumoukedima	Nagaland

17	T-5	French beans rhizosphere	Horticulture farm (Sample II), SASRD	Chumoukedima	Nagaland
18	T-6	French beans rhizosphere	Farmers' field (Sample-I), Kohima	Kohima	Nagaland
19	T-7	French beans rhizosphere	Farmers' field (Sample-II), Kohima	Kohima	Nagaland
20	T-8	French beans rhizosphere	Farmers' field (Sample-III), Kohima	Kohima	Nagaland
21	T-9	French beans rhizosphere	Farmer's field, Medziphema	Chumoukedima	Nagaland
22	T-10	French beans rhizosphere	Farmers' field, Mao	Senapati	Manipur
23	T-11	French beans rhizosphere	Kitchen garden,SASRD	Chumoukedima	Nagaland
24	T-12(*T. harzianum*)	Virgin forest	DBT project site, Dziilakie	Kohima	Nagaland
25	T-13(*T. virens)*	Virgin forest	DBT project site, Dziilakie	Kohima	Nagaland
26	T-14(*T. asperellum)*	Virgin forest	DBT project site, Dziilakie	Kohima	Nagaland
27	T-15	Tomato rhizosphere	Polyhouse (Sample-I), CIH, Medziphema	Chumoukedima	Nagaland
28	T-16	Tomato rhizosphere	Tomato field (Sample-I), Horticulture farm, SASRD	Chumoukedima	Nagaland
29	T-17	Tomato rhizosphere	Farmers' field (Sample-I), Merima	Kohima	Nagaland
30	T-18	Tomato rhizosphere	Farmers' field (Sample-I), Tsiesma	Kohima	Nagaland
31	T-19	Tomato rhizosphere	Polyhouse (Sample-II), CIH, Medziphema	Chumoukedima	Nagaland
32	T-20	Tomato rhizosphere	Tomato field (Sample-II), Horticulture farm, SASRD	Chumoukedima	Nagaland

a) Different steps for soil sampling from different places

b) *Trichoderma* colonies on TSM medium



c) *Pseudomonas* colonies on King's B medium

Plate 9 Collection of French bean rhizosphere soil samples and isolation of French bean rhizospheric microbes

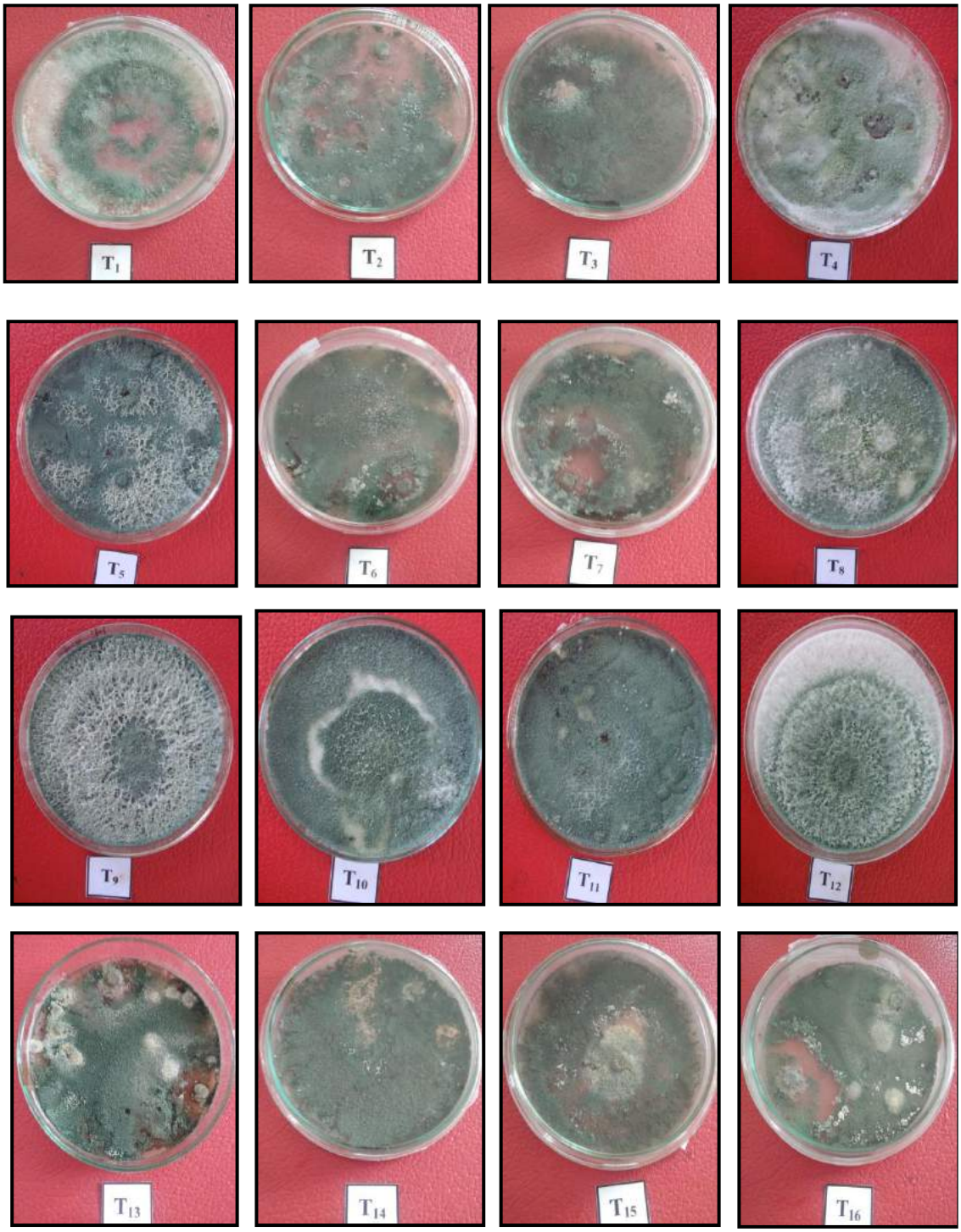
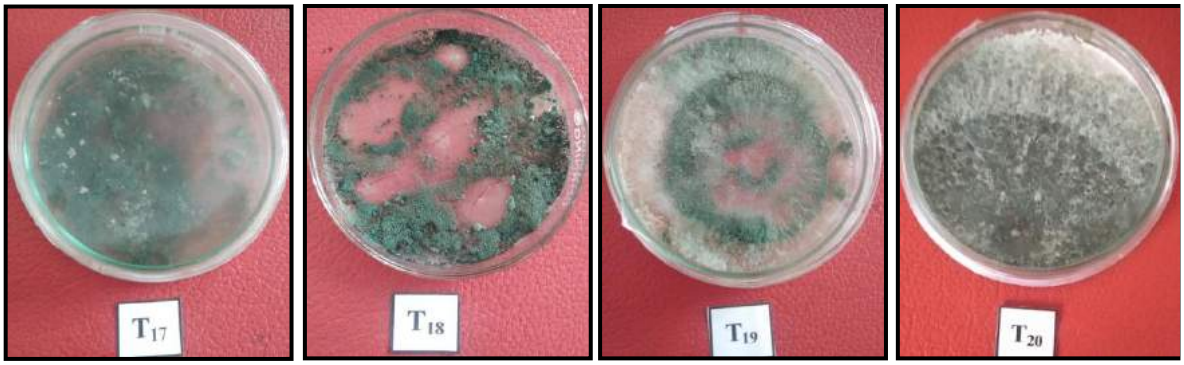
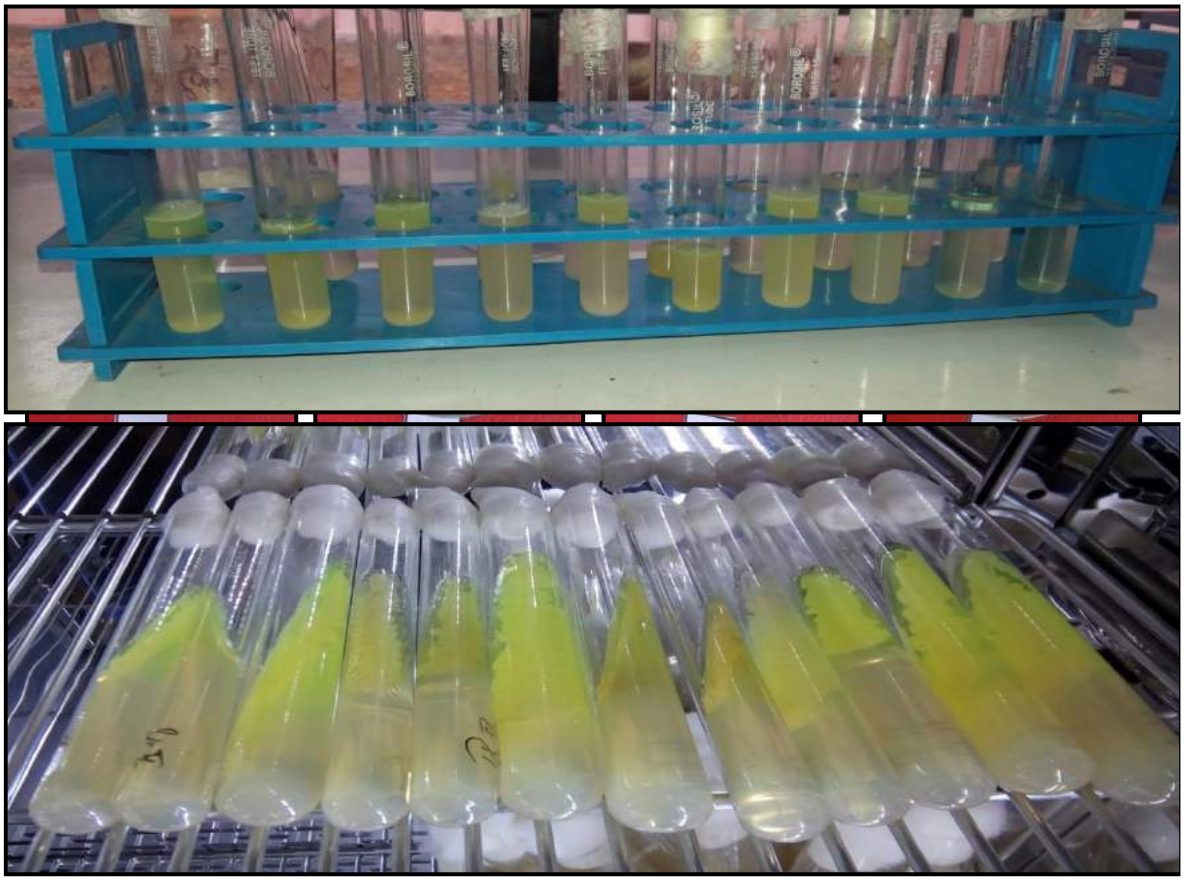


Plate 10 Native rhizospheric *Trichoderma* isolates (T_1-T_{16})



a) Native rhizospheric*Trichoderma* isolates (T_{17}-T_{20})



b) Bacterial isolates grown on KB broth and KB agar slants

Plate 11 Native rhizospheric *Trichoderma* and *Pseudomonas* isolates

4.4 *In-vitro* screening of rhizospheric antagonists against *S. rolfsii*

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

Altogether 20 promising isolates of *Trichoderma* were screened for their inhibitory action on the radial growth of *S. rolfsii* by adopting dual culture technique (Sivakumar *et al.*, 2000) and the data obtained are presented in Table 4.2 and illustrated in Fig 4.1. The isolates of fungal antagonists screened and exhibited varied level of biocontrol traits against the virulent *S. rolfsii* and showed significantly superior over control (Plate 12 and 13). 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_8 (1.06 cm) which is statistically at par with T_{20} (1.10 cm) followed by T_5 (1.33 cm) and T_{13} (2.03 cm) respectively.

The per cent inhibition over control was calculated and it was observed that highest inhibition per cent was recorded in T_8 (85.77 %) which is statistically at par with T_{20} (84.88%) followed by T_5 (82.22 %), T_{13} (72.88 %) and the least antagonistic effect was observed in T_{19} (49.77 %) at 5 days after incubation at 25±2 °C.

In the present investigation, the probable reasons of high inhibitory activity of the *Trichoderma* spp against *S. rolfsii* in dual cultures may be due to the fact that *Trichoderma* spp. produce extracellular cell wall degrading enzymes like chitinase, β-1, 3 glucanase, protease, cellulase and lectin, competition, mycoparasitic activity like coiling of mycelium which help them in colonising the host and inhibit soil-borne fungi.

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

Treatment	Inhibition of *S. rolfsii* growth		
	Radial growth (cm)	Radial growth (cm) inhibited	Inhibition (%)
T_0(Control)	7.50	0.00	0.00 (4.05)
T_1(*S. rolfsii* + T-1)	3.33	4.16	55.56 (48.19)
T_2(*S. rolfsii* + T-2)	3.00	4.50	60.00 (50.76)
T_3(*S. rolfsii* + T-3)	3.03	4.36	58.22 (49.73)
T_4(*S. rolfsii* + T-4)	2.26	5.23	69.77 (56.65)
T_5(*S. rolfsii* + T-5)	1.33	6.16	82.22 (65.06)
T_6(*S. rolfsii* + T-6)	2.93	4.56	60.89(51.29)
T_7(*S. rolfsii* + T-7)	2.93	4.56	60.88 (51.28)
T_8(*S. rolfsii* + T-8)	**1.06**	**6.43**	**85.77 (67.84)**
T_9(*S. rolfsii* + T-9)	3.66	3.83	51.11(45.63)
T_{10}(*S. rolfsii* + T-10)	2.66	4.83	64.44 (53.39)
T_{11}(*S. rolfsii* + T-11)	3.36	4.13	55.11 (47.93)
T_{12}(*S. rolfsii* + T-12)	2.96	4.53	60.44 (51.02)
T_{13}(*S. rolfsii* + T-13)	2.03	5.46	72.88(58.62)
T_{14}(*S. rolfsii* + T-14)	3.46	4.03	53.77 (47.16)
T_{15}(*S. rolfsii* + T-15)	3.36	4.13	55.11 (47.93)
T_{16}(*S. rolfsii* + T-16)	2.53	4.96	66.22 (54.46)
T_{17}(*S. rolfsii* + T-17)	2.56	4.93	65.77 (54.19)
T_{18}(*S. rolfsii* + T-18)	2.46	5.03	67.11 (55.00)
T_{19}(*S. rolfsii* + T-19)	3.76	3.73	49.77 (44.87)
T_{20}(*S. rolfsii* + T-20)	**1.10**	**6.40**	**84.88 (67.12)**
SEm±	3.98	3.68	0.05
C.V. (%)	2.58	1.78	1.80
CD (*p*= 0.01)	0.16	0.17	2.42

*Values in parentheses are angular transformed values

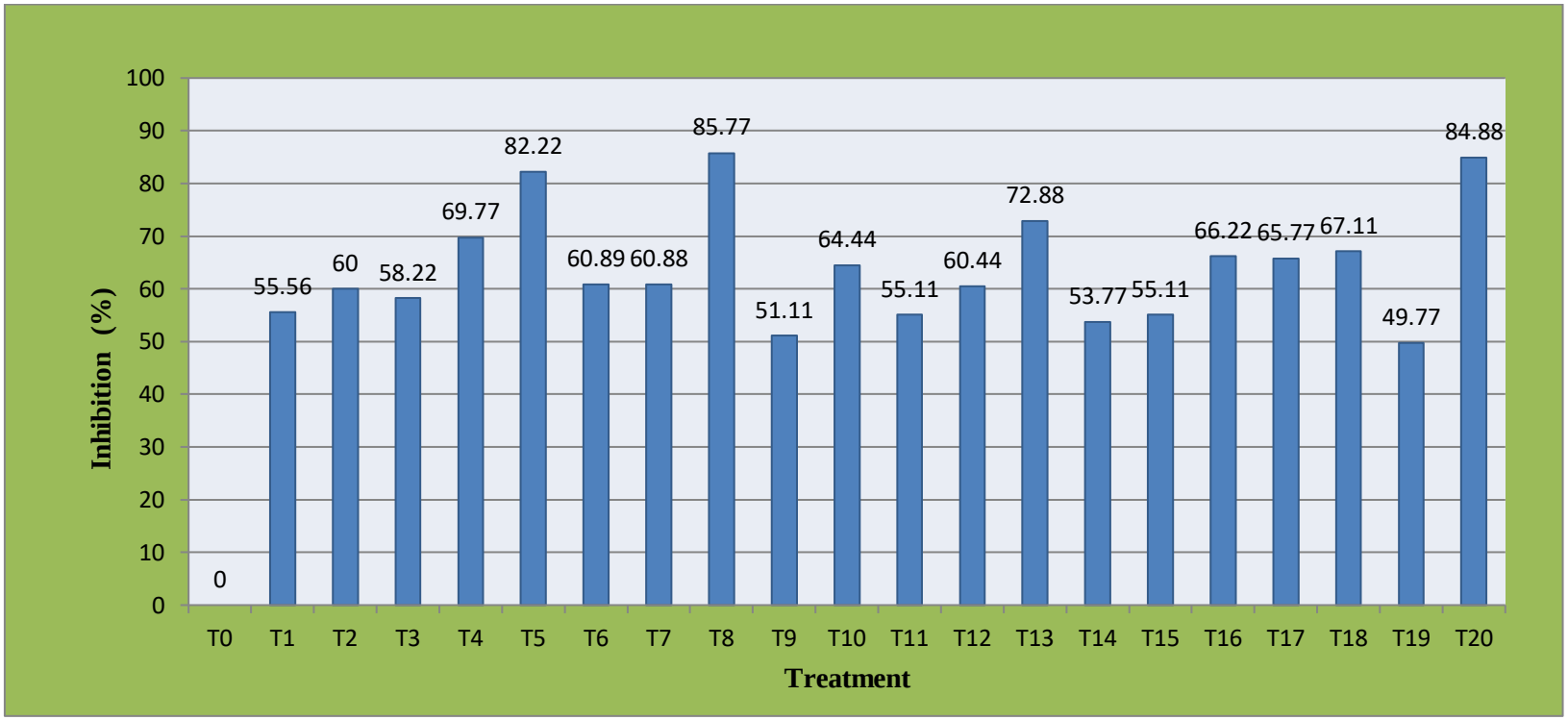
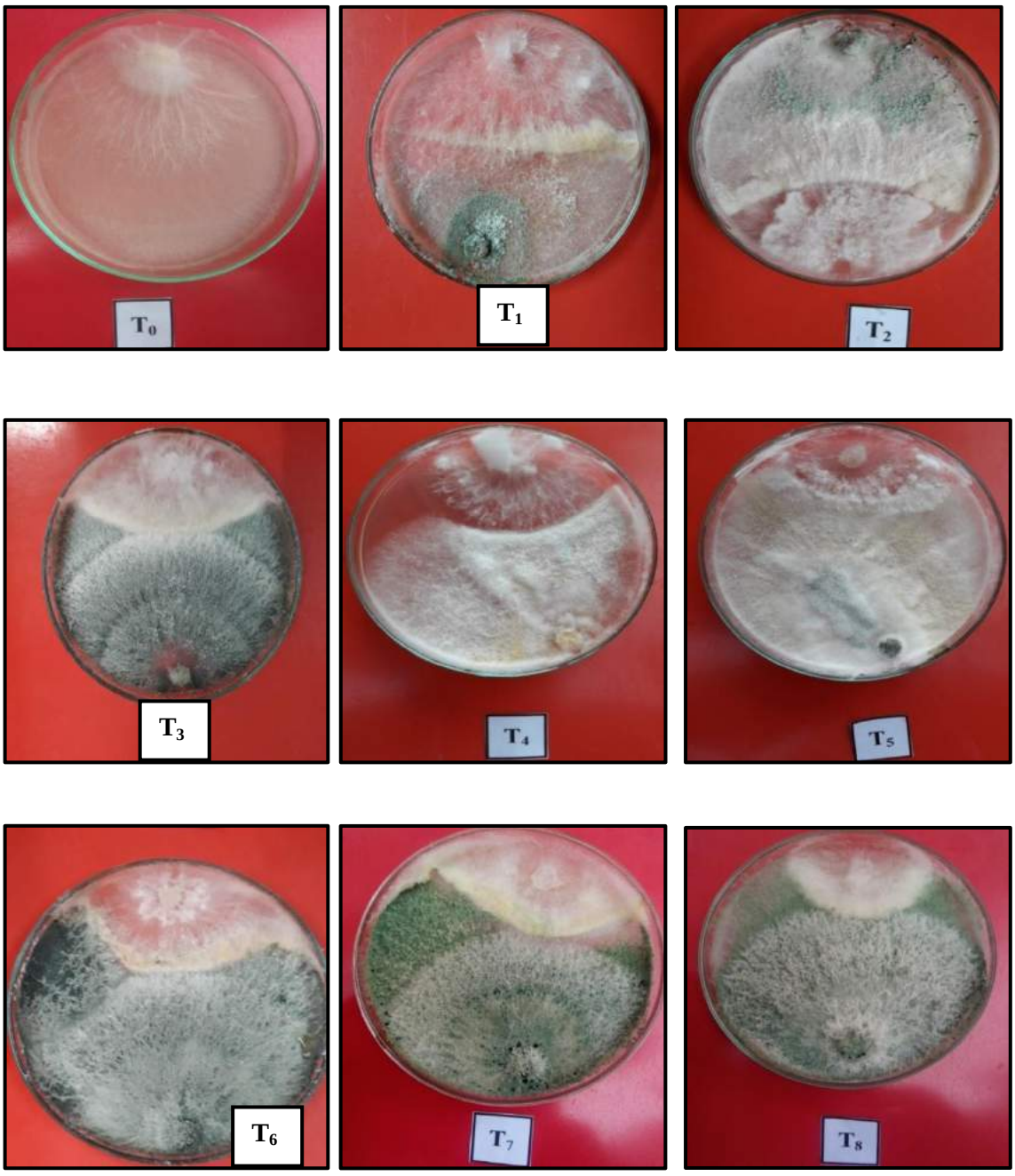


Fig 4.1 *In vitro* antagonistic effect of *Trichoderma* isolates on per cent growth inhibition of *S. rolfsii*



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

Plate 12 *In vitro* antagonistic effect of *Trichoderma* isolates (T-1 to T-8) on radial growth of *S. rolfsii*

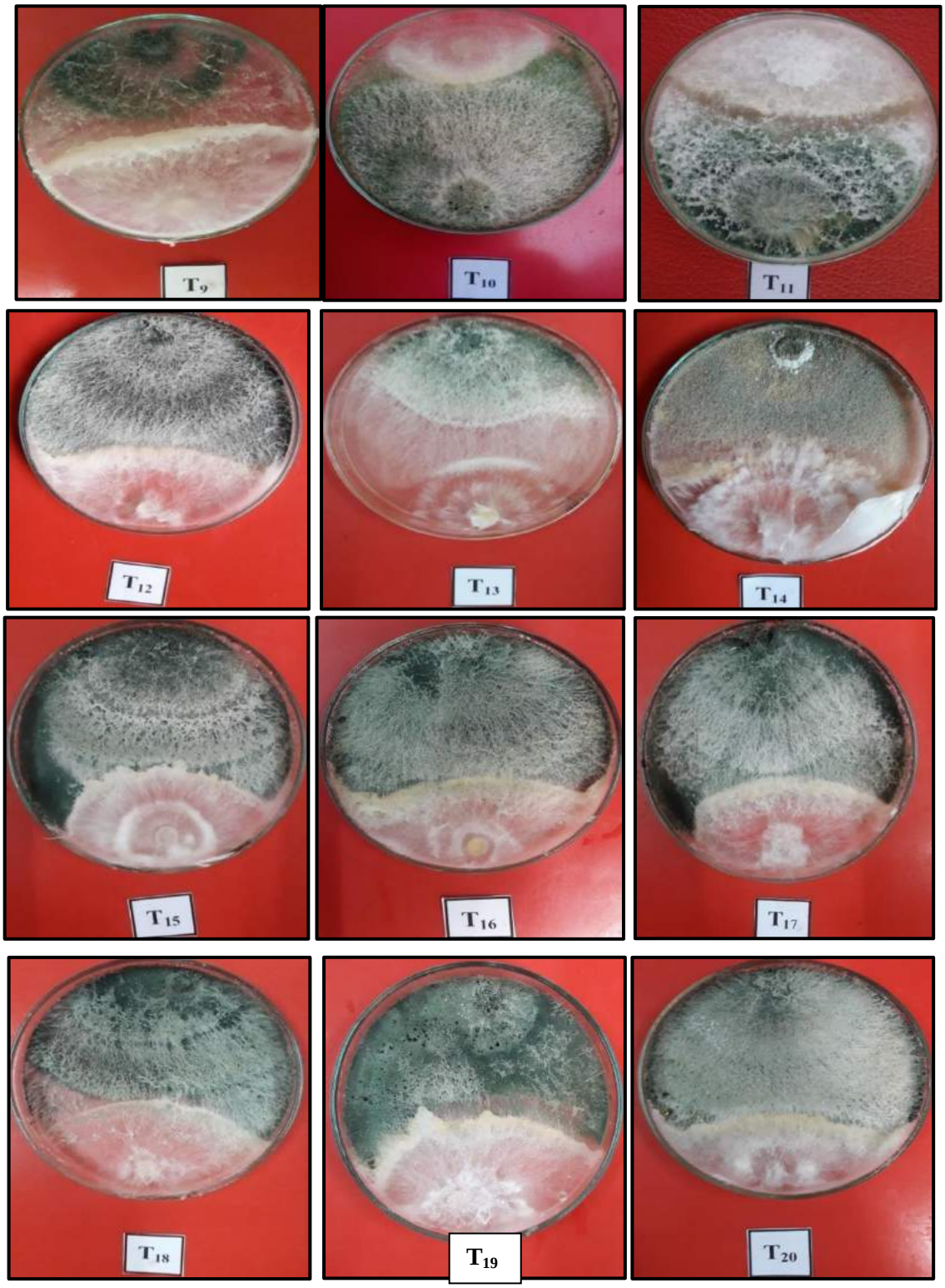


Plate 13 *In vitro* antagonistic effect of *Trichoderma* isolates (T-10 to T-20) on radial growth of *S. rolfsii*

Prajapati *et al*. (2015) observed that among different *Trichoderma* species tested against *S. rolfsii* through dual culture technique, *T. asperellum* showed strong antagonistic effect in terms of mycelia growth inhibition *i.e.* 61.48, 75.00 and 73.33 per cent at 4, 6 and 8 days of incubation, respectively. Sharma *et al*. (2020) evaluated four potential *Trichoderma* mutants against *S. rolfsii*. Among the four potential *Trichoderma* mutants tested the maximum inhibition was observed in BARC mutant (81.50 %) over control which was followed by mutants M-136 (81 %), M-23 (80.5 %) and M-18 (79 %) respectively. Similar trend of present studies was observed by earlier workers Bandyopodhyay *et al*. (2003); Yaqub and Shahzad (2005); Anand and Harikesh (2005) and Bhuiyan *et al*. (2012).

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 *S. rolfsii* by slight modification of dual culture technique given by Georgakopoulos *et al*. (2002) and the data obtained are presented in Table 4.3 and illustrated in Fig 4.2. All isolates screened against *S. rolfsii* significantly inhibited the growth of the fungus as compared to control treatment (Plate 14). Among the *Pseudomonas* isolates, least radial mycelial growth of the pathogen was observed in Pf_{12} (0.93 cm) which is statistically at par with Pf_2 (0.96 cm). Further followed by Pf_{10} (1.20 cm) and Pf_1 (1.80 cm) respectively.

Out of these isolates maximum per cent inhibition was observed in Pf_{12} and Pf_2 (87.55 and 87.11 %) which was statistically at par with each other. Further followed by Pf_{10} (84.00 %) and Pf_1 (76.00 %) respectively at 5 days after incubation at 25±2°C.

In the present investigation, the probable reasons of high inhibitory activity of the bacterial antagonists may be due to production of antifungal metabolites, siderophores, HCN and microbial enzymes.

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

Treatment	Inhibition of *S. rolfsii* growth		
	Radial growth (cm)	Radial growth (cm) inhibited	Inhibition (%)
T_0 (Control)	7.50	0.00	0.00 (4.05)
T_1 (*S. rolfsii* + Pf-1)	1.80	5.70	76.00 (60.66)
T_2 (*S. rolfsii* + Pf-2)	**0.96**	**6.53**	**87.11 (68.96)**
T_3 (*S. rolfsii* + Pf-3)	2.76	4.73	63.11 (52.60)
T_4 (*S. rolfsii* + Pf-4)	2.86	4.63	61.78 (51.81)
T_5 (*S. rolfsii* + Pf-5)	2.43	5.06	67.56 (55.27)
T_6 (*S. rolfsii* + Pf-6)	4.46	3.03	40.44 (39.49)
T_7 (*S. rolfsii* + Pf-7)	2.43	5.06	67.56 (55.27)
T_8 (*S. rolfsii* + Pf-8)	2.36	5.13	68.44 (55.82)
T_9 (*S. rolfsii* + Pf-9)	2.90	4.60	61.33 (51.54)
T_{10} (*S. rolfsii* + Pf-10)	1.20	6.30	84.00 (66.43)
T_{11} (*S. rolfsii* + Pf-11)	4.53	2.96	39.56 (38.97)
T_{12} (*S. rolfsii* + Pf-12)	**0.93**	**6.56**	**87.55 (69.35)**
SEm±	0.00	0.00	0.05
C.V. (%)	1.94	1.19	1.19
CD (*p*= 0.01)	0.12	0.12	1.67

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

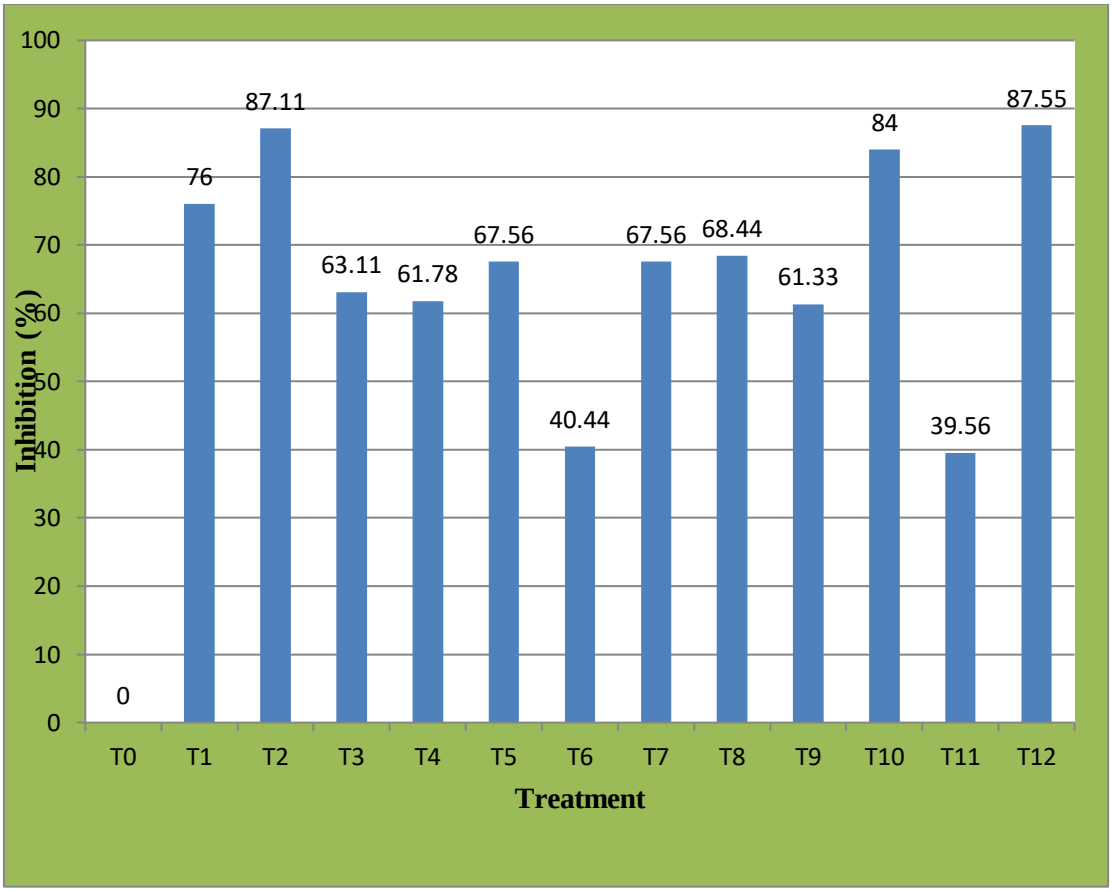
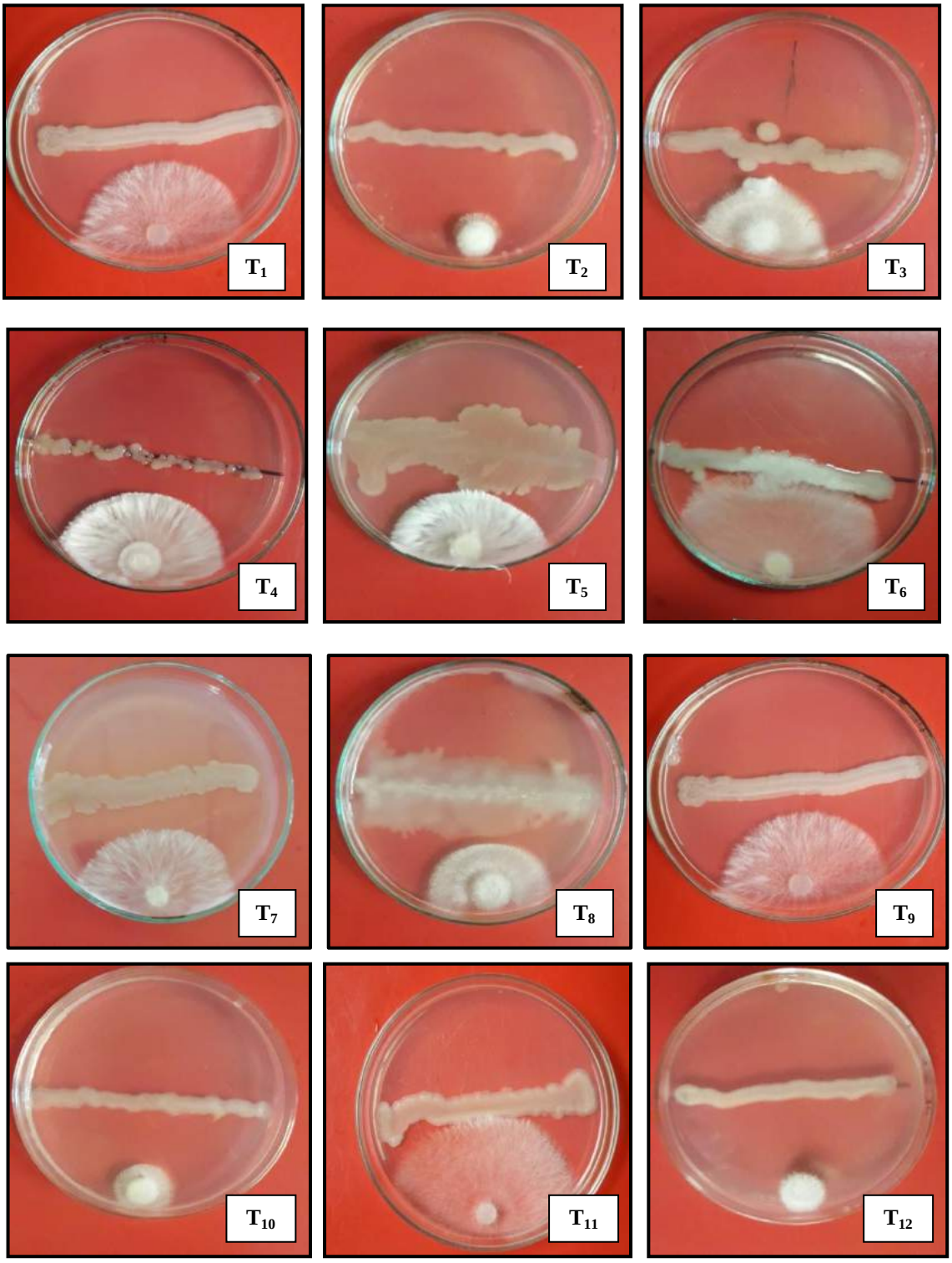


Fig 4.2 *In vitro* antagonistic effect of *Pseudomonas* isolates on per cent growth inhibition of *S. rolfsii*



T_1 (*S. rolfsii*+ Pf-1); T_2 (*S. rolfsii*+ Pf-2); T_3 (*S. rolfsii*+ Pf-3); T_4 (*S. rolfsii*+ Pf-4) T_5 (*S. rolfsii* + Pf-5); T_6 (*S. rolfsii*+ Pf-6); T_7 (*S. rolfsii*+ Pf-7); T_8 (*S. rolfsii*+ Pf-8); T_9 (*S. rolfsii*+ Pf-9); T_{10} (*S. rolfsii*+ Pf-10); T_{11} (*S. rolfsii*+ Pf-11); T_{12} (*S. rolfsii*+

Plate 14 *In vitro* antagonistic effect of *Pseudomonas* isolates on radial growth of *S. rolfsii*

Similar findings was observed by Muthukumar *et al*. (2019) who reported that out of eight native *Pseudomonas* species (I1 to I8) and one introduced *P. fluorescens* (I9) tested against *S. rolfsii*, isolate-I7 (PFP) recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii* accounting for 74.25 per cent reduction in the mycelial growth over control (89.33 mm) and it was on par with I9 (PFC). This was followed by isolate-I4 (PFK). The least mycelial growth inhibition was observed with the isolate-I3 (BSE). Further, the present findings are also supported by the observation of Lal *et al*. (2022) who reported that isolate Pf14 showed the highest mycelial inhibitory potential (ranged from 62.2 to 59.3 %) against *R. solani*, *S. sclerotiorum*, *S. rolfsii* and *Fusarium* spp. The findings of present work are also in conformity with the findings of earlier workers who showed that bacterial antagonists are known to suppress the growth of soil borne plant pathogens (Dwivedi, 1987; Ongena and Jacques, 2008; Sahani *et al*., 2019 and Sharf *et al*., 2021).

4.5 Investigation on the biocontrol mechanisms of native rhizospheric BCAs

4.5.1 *In vitro* screening of volatile metabolites production

The effects of volatile metabolites of selected twenty *Trichoderma* isolates were assessed against *S. rolfsii* by following the technique given by Dennis and Webster (1971), and the data thus obtained are presented in Table 4.4 and illustrated in Fig 4.3. All isolates assessed against *S. rolfsii* were significantly superior over control treatment (Plates 15 and 16). Among the different treatments, least mycelial growth of the pathogen was recorded in T_8 (**0.90** cm) which was found to be statistically at par with T_{20} (**0.93** cm) and T_{15} (1.03 cm) .This was followed by T_9, T_{17} and $T_{18}$with 1.10 cm respectively.

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

Treatment		Inhibition of *S. rolfsii* growth		
		Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)
T_0	T_0 (Control)	9.00	0.00	0.00 (4.05)
T_1	T_1(*S. rolfsii* + T-1)	1.30	7.66	85.18 (67.36)
T_2	T_2(*S. rolfsii* + T-2)	2.10	6.90	76.67 (61.11)
T_3	T_3(*S. rolfsii* + T-3)	6.63	2.36	26.30 (30.85)
T_4	T_4(*S. rolfsii* + T-4)	2.63	6.36	70.74 (57.25)
T_5	T_5(*S. rolfsii* + T-5)	6.30	2.70	30.00 (33.21)
T_6	T_6(*S. rolfsii* + T-6)	3.66	5.33	59.26 (50.33)
T_7	T_7(*S. rolfsii* + T-7)	5.90	3.10	34.44 (35.93)
T_8	T_8(*S. rolfsii* + T-8)	**0.90**	**8.10**	**90.00 (71.58)**
T_9	T_9(*S. rolfsii* + T-9)	1.10	7.90	87.78 (69.57)
T_{10}	T_{10}(*S. rolfsii* + T-10)	8.03	0.96	10.74 (19.12)
T_{11}	T_{11}(*S. rolfsii* + T-11)	2.73	6.26	69.63 (56.55)
T_{12}	T_{12}(*S. rolfsii* + T-12)	1.23	7.70	85.56 (67.66)
T_{13}	T_{13}(*S. rolfsii* + T-13)	2.76	6.23	69.26 (56.32)
T_{14}	T_{14}(*S. rolfsii* + T-14)	4.33	4.66	51.85 (46.06)
T_{15}	T_{15}(*S. rolfsii* + T-15)	1.03	7.96	88.52 (70.21)
T_{16}	T_{16}(*S. rolfsii* + T-16)	1.13	7.86	87.78 (69.22)
T_{17}	T_{17}(*S. rolfsii* + T-17)	1.10	7.90	87.78 (69.55)
T_{18}	T_{18}(*S. rolfsii* + T-18)	1.10	7.90	87.78 (69.53)
T_{19}	T_{19}(*S. rolfsii* + T-19)	8.30	0.83	9.26(17.67)
T_{20}	T_{20}(*S. rolfsii* + T-20)	**0.93**	**8.03**	**89.63 (71.21)**
SEm±		4.51	3.68	0.43
C.V. (%)		2.42	1.46	1.46
CD (*p*= 0.01)		0.18	0.17	1.99

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

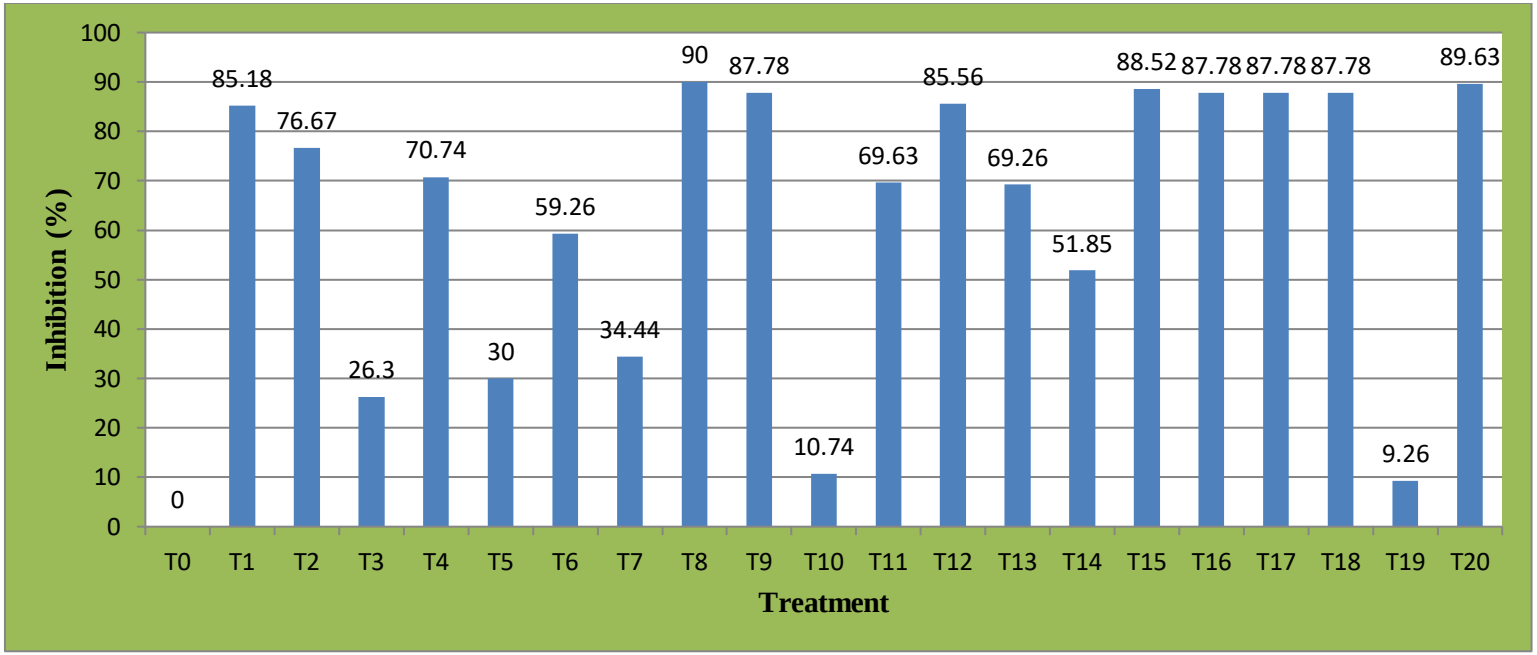


Fig 4.3 *In vitro* effect of volatile metabolites of *Trichoderma* isolates on per cent growth inhibition of *S. rolfsii*

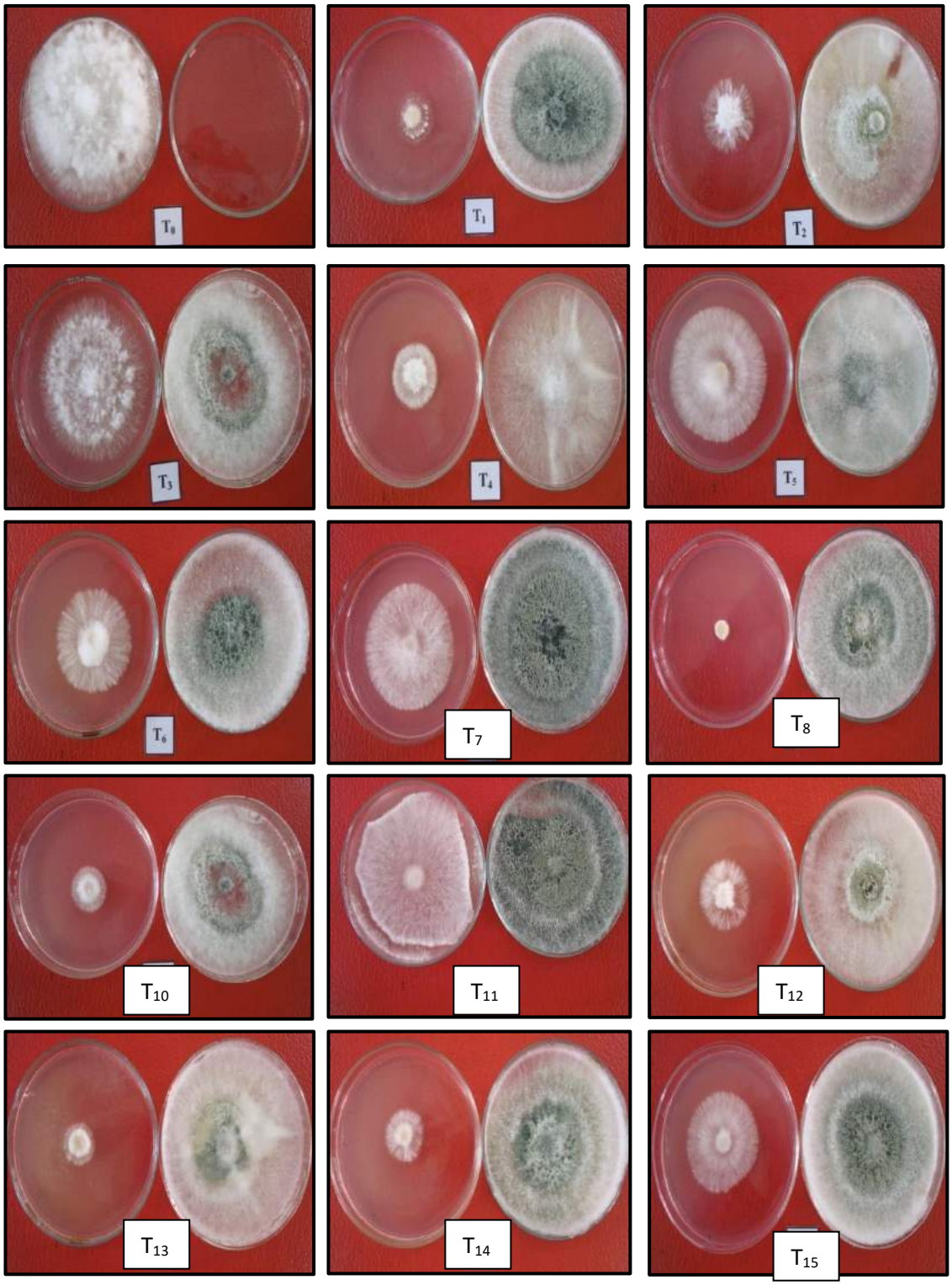


Plate 15 *In vitro* effect of volatile metabolites of *Trichoderma* isolates (T-1 to T-14) on growth of *S. rolfsii*

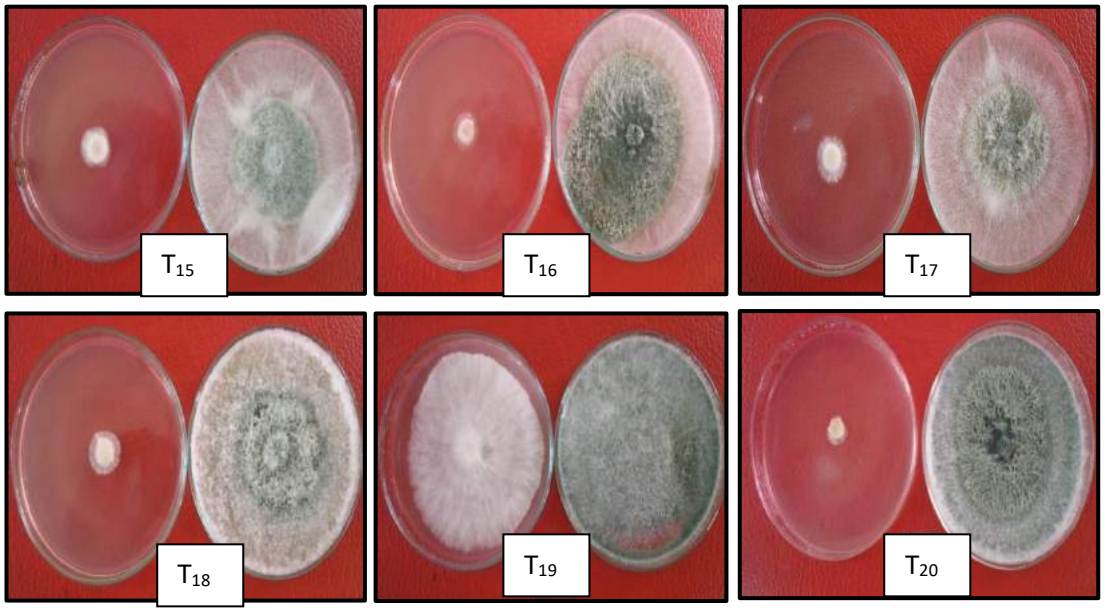


Plate 16 *In vitro* effect of volatile metabolites of *Trichoderma* isolates (T-15 to T-20) on growth of *S. rolfsii*

The per cent inhibition over control was calculated and it was observed that $T_8$with 90.00 % were found to be most promising in production of volatile compounds by *Trichoderma* isolates against *S. rolfsii* which was statistically at par withT_{20} (89 %). The least antagonist effect was shown in T_{19} with 9.26 %.

Data presented in table 4.5 and illustrated inFig 4.4 represents the effect of volatile metabolites of *Pseudomonas* isolates (12 isolates) assessed against *S. rolfsii* (Plate 17). Among the *Pseudomonas* isolates, least mycelial growth of test pathogen was observed in T_2 (1.33 cm) and T_{12} (1.46 cm) which is statistically at par with each other. The next best in order of merit were T_{11} (2.20 cm), T_6 (3.30 cm) and T_8 (4.86 cm) respectively. Out of these, maximum per cent inhibition was observed in T_2 (85.18%) which is statistically at par with T_{12} (83.70 %) and were significantly superior to all other treatments. 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 *S. rolfsii.*

Similar trend was observed by Kotasthane *et al.* (2014) who reported the efficacy of 5 potential isolates of *Bacillus* sp. against *S. rolfsii* in metabolic assay. The B1 isolate was found most effective with significantly highest reduction of radial growth (74.22 %). Similarly 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. The results are in conformity with the report of Fravel (1988); Srinivasulu *et al.* (2005); Kapri and Tewari (2010); Li *et al.* (2016); Vrieze *et al*. (2018) and Lal *et al*. (2022) who observed the effect of volatile metabolites of *Trichoderma* spp and *Pseudomonas* spp against *S. rolfsii.*

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

Treatment		Inhibition of *S. rolfsii* growth		
		Diameter growth (cm)	**Diameter growth (cm) inhibited**	**Inhibition (%)**
T_0	**(Control)**	9.00	0.00	0.00 (4.05)
T_1	**(*S. rolfsii* + Pf-1)**	7.33	1.66	18.52 (25.48)
T_2	**(*S. rolfsii* + Pf-2)**	**1.33**	**7.66**	**85.18 (67.36)**
T_3	**(*S. rolfsii* + Pf-3)**	5.03	3.96	44.07 (41.59)
T_4	**(*S. rolfsii* + Pf-4)**	8.00	1.00	11.11 (19.45)
T_5	**(*S. rolfsii* + Pf-5)**	6.86	2.13	23.70 (29.13)
T_6	**(*S. rolfsii* + Pf-6)**	3.30	5.70	63.33 (52.73)
T_7	**(*S. rolfsii* + Pf-7)**	7.73	1.26	14.07 (22.02)
T_8	**(*S. rolfsii* + Pf-8)**	4.86	4.13	45.93 (42.66)
T_9	**(*S. rolfsii* + Pf-9)**	5.06	3.93	43.70 (41.38)
T_{10}	**(*S. rolfsii* + Pf-10)**	6.46	2.53	28.15 (32.04)
T_{11}	**(*S. rolfsii* + Pf-11)**	2.20	6.80	75.56 (60.37)
T_{12}	**(*S. rolfsii* + Pf-12)**	**1.46**	**7.53**	**83.70 (66.19)**
SEm±		2.43	2.44	0.06
C.V. (%)		1.48	2.10	2.11
CD (*p*= 0.01)		0.17	0.18	1.97

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

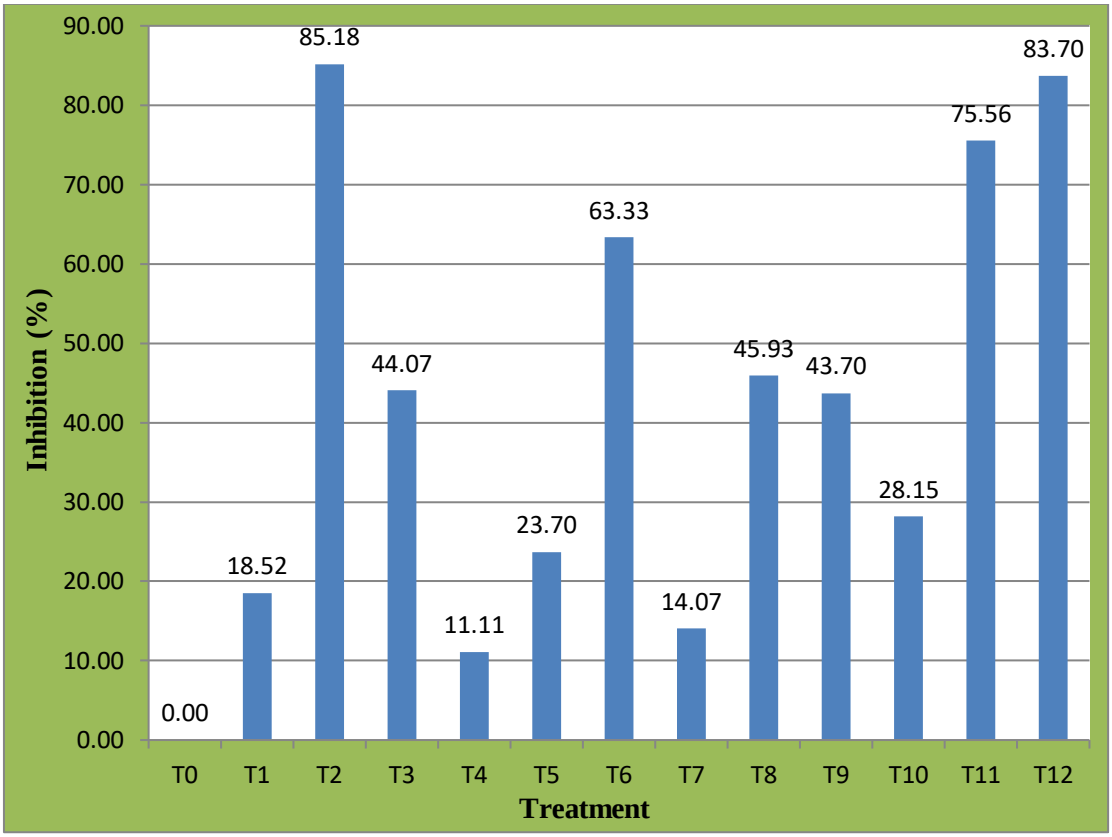


Fig 4.4 *In vitro* effect of volatile metabolites of *Pseudomonas* isolates on per cent growth inhibition of *S. rolfsii*

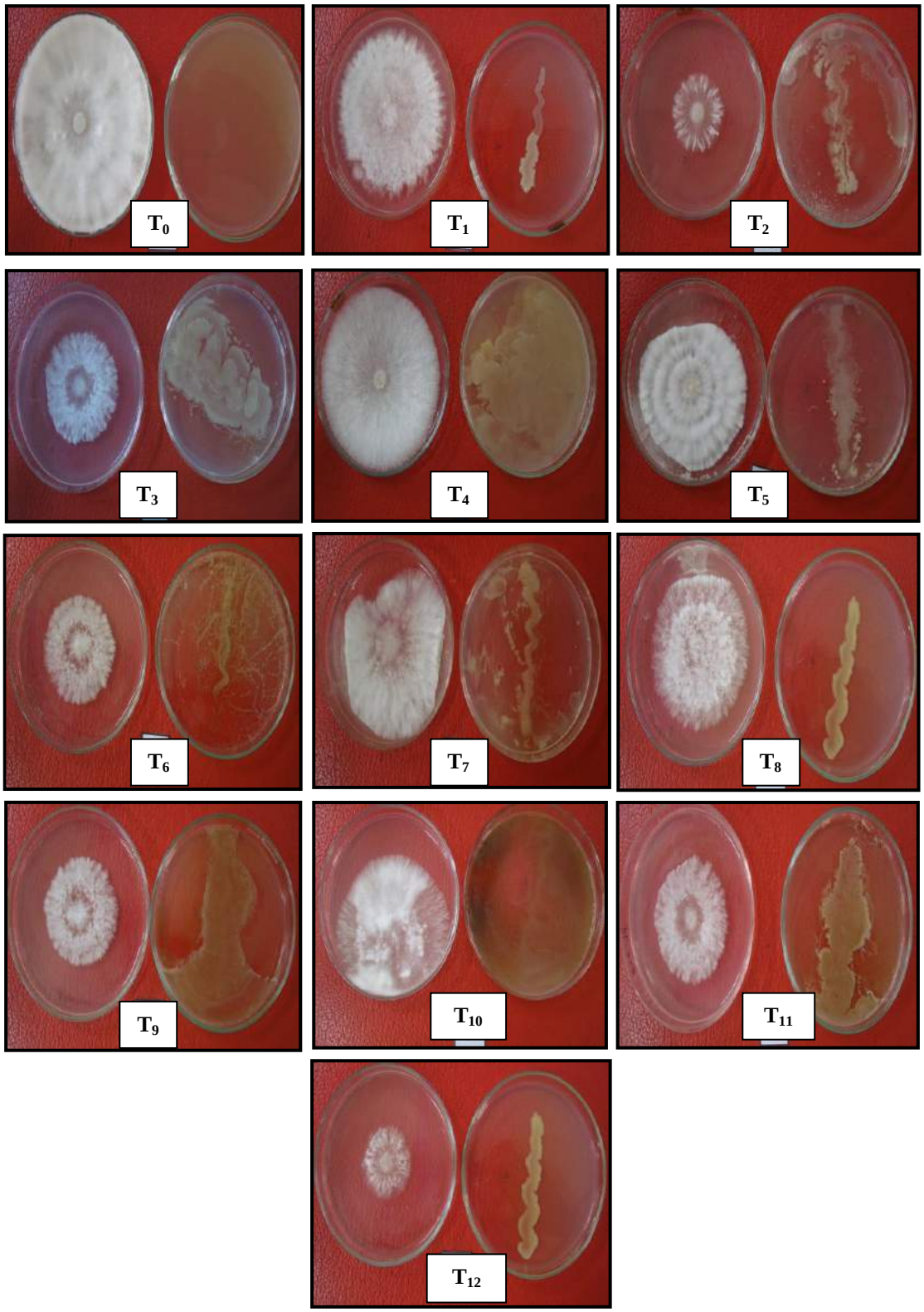


Plate 17 *In vitro* effect of volatile metabolites of *Pseudomonas* isolates (Pf-1 to Pf-12) on growth of *S. rolfsii*

4.5.2 *In vitro* screening of non volatile metabolites production

Non volatile (culture filtrates) production of 20 potential isolates of *Trichoderma* sp. tested for their inhibitory effect on mycelial growth of *S. rolfsii* arepresented in Table 4.6 and illustrated in Fig 4.5. All isolates assessed against *S. rolfsii* were significantly superior over control treatment (Plates 18 and 19). Among the different treatments, no mycelial growth of the pathogen was recorded in T_1, T_6, T_8, T_{13}, T_{14}, T_{15}, T_{16}, T_{18} and T_{20} (**0.00** cm).

The per cent inhibition over control was calculated and it was observed that T_1, T_6, T_8, T_{13}, T_{14}, T_{15}, T_{16}, T_{18} and T_{20} were found total inhibition 100.00 % of the growth of *S. rolfsii*.

Data presented in Table 4.7 and Fig 4.6 represents the effects of non volatile metabolites of *Pseudomonas* isolates (12 isolates) assessed against *S. rolfsii* following the technique given by (You *et al.*, 2016). All isolates assessed against *S. rolfsii* were also significantly superior over control (Plate 20 and 21). Among the *Pseudomonas* isolates, least mycelial growth of test pathogen was observed in Pf_{12} (1.07 cm) which is statistically at par with Pf_2 (1.06 cm). Out of these maximum per cent inhibition was observed in Pf_{12} (88.15%) which is statistically at par with Pf_2 (88.1.4 %) and were significantly superior to all other treatments followed by Pf_3(74.80%), Pf_{10}(72.90%) and Pf_9 (71.15 %) respectively.

The results of the present investigation on the production of non-volatile metabolites by both *Trichoderma* and *Pseudomonas* isolates have definite influence on the high degree of inhibition of *S. rolfsii.* This might be due to the presence of several antifungal proteins in the culture filtrate of *Pseudomonas*

Table 4.6 *In vitro* effect of non volatile metabolites of *Trichoderma* isolates on mycelial growth and per cent inhibition of *S. rolfsii*

Treatment		Inhibition of *S. rolfsii* growth		
		Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)
T_0	T_0 (Control)	9.00	0.00	0.00 (4.05)
T_1	T_1(*S. rolfsii* + T-1)	0.00	9.00	100.00 (85.94)
T_2	T_2(*S. rolfsii* + T-2)	2.36	6.63	73.10 (59.16)
T_3	T_3(*S. rolfsii* + T-3)	2.80	6.20	68.89 (56.09)
T_4	T_4(*S. rolfsii* + T-4)	3.06	5.93	65.93 (54.28)
T_5	T_5(*S. rolfsii* + T-5)	5.63	3.36	37.41 (37.70)
T_6	T_6(*S. rolfsii* + T-6)	0.00	9.00	100 .0(85.94)
T_7	T_7(*S. rolfsii* + T-7)	1.93	7.06	78.52 (62.40)
T_8	T_8(*S. rolfsii* + T-8)	0.00	9.00	**100. 00(85.94)**
T_9	T_9(*S. rolfsii* + T-9)	0.73	8.26	91.85 (73.42)
T_{10}	T_{10}(*S. rolfsii* + T-10)	0.00	9.00	100.00 (85.94)
T_{11}	T_{11}(*S. rolfsii* + T-11)	0.80	8.20	91.11 (72.65)
T_{12}	T_{12}(*S. rolfsii* + T-12)	0.96	8.03	89.26 (70.87)
T_{13}	T_{13}(*S. rolfsii* + T-13)	0.90	9.00	100.00 (85.94)
T_{14}	T_{14}(*S. rolfsii* + T-14)	0.00	9.00	100.00 (85.94)
T_{15}	T_{15}(*S. rolfsii* + T-15)	0.00	9.00	100.00 (85.94)
T_{16}	T_{16}(*S. rolfsii* + T-16)	0.00	9.00	100.00 (85.94)
T_{17}	T_{17}(*S. rolfsii* + T-17)	2.63	6.36	70.74 (57.75)
T_{18}	T_{18}(*S. rolfsii* + T-18)	0.00	9.00	100.00 (85.94)
T_{19}	T_{19}(*S. rolfsii* + T-19)	1.33	7.63	85.18 (67.36)
T_{20}	T_{20}(*S. rolfsii* + T-20)	0.00	9.00	100.00 (85.94)
SEm±		0.00	0.00	0.04 (0.02)
C.V. (%)		4.86	0.94	0.94 (0.77)
CD (*p*= 0.01)		0.16	0.15	1.74 (120)

***Values in parentheses are angular transformed value**

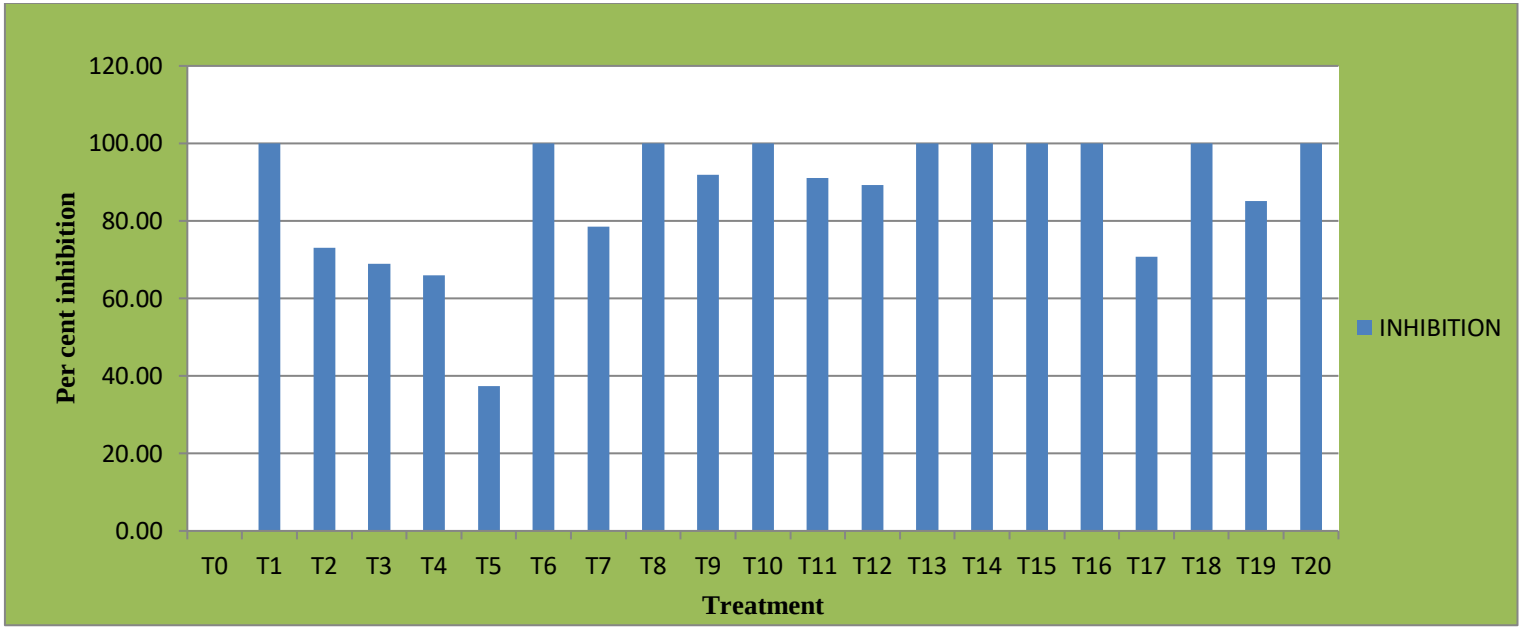


Fig 4.5 Per cent inhibition of *S. rolfsii* of non volatile metabolites production by *Trichoderma* isolates

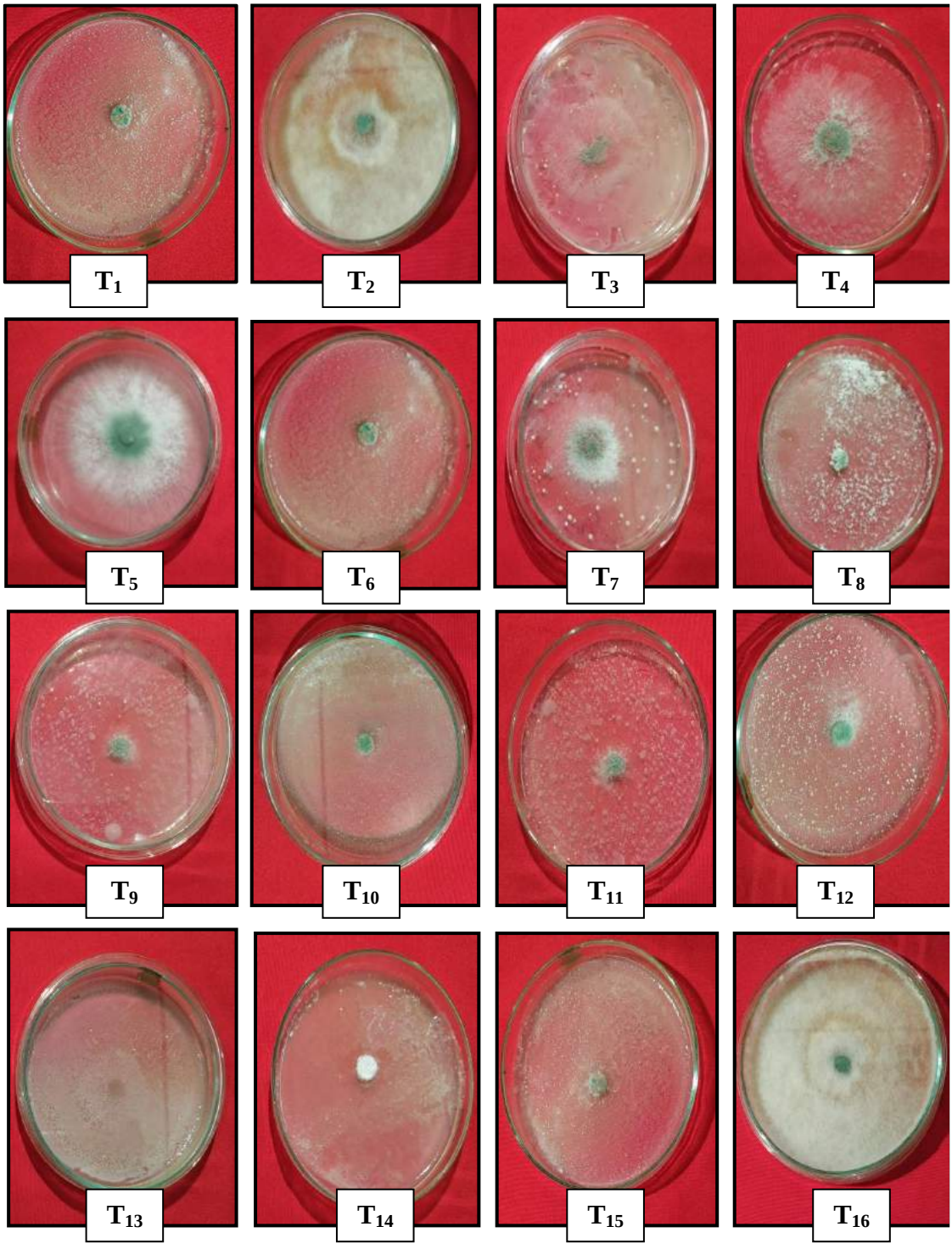


Plate 18 *In vitro* effect of non- volatile metabolites of *Trichoderma* isolates (T-1 to T-16) on growth of *S. rolfsii*

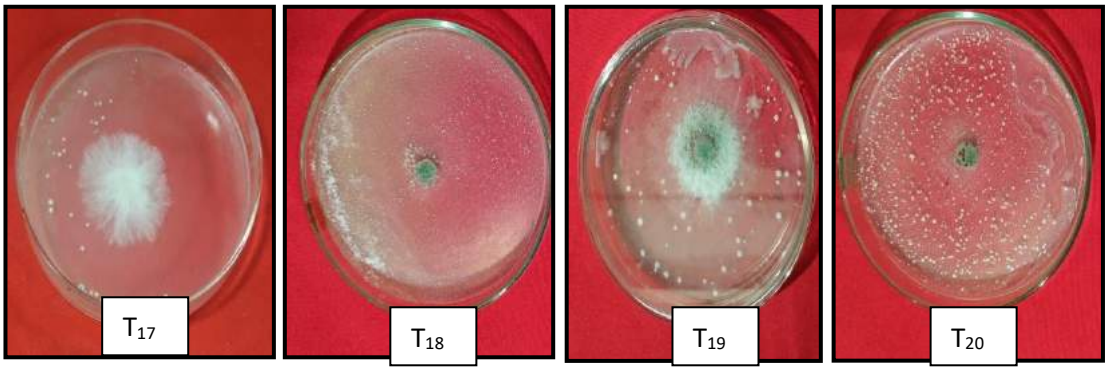


Plate 19 *In vitro* effect of non-volatile metabolites of *Trichoderma* isolates (T-17 to T-20) on growth of *S. rolfsii*

Table 4.7 *In vitro* effect of non volatile metabolites of *Pseudomonas* isolates on mycelial growth and per cent inhibition of *S. rolfsii*

Treatment		Inhibition of *S. rolfsii* growth		
		Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)
T_0	(Control)	9.00	0.00	0.00 (4.05)
T_1	(*S. rolfsii* + Pf-1)	2.70	2.70	70.0 (56.8)
T_2	(*S. rolfsii* + Pf-2)	1.06	7.93	88.2 (69.9)
T_3	(*S. rolfsii* + Pf-3)	2.26	6.73	74.8 (59.9)
T_4	(*S. rolfsii* + Pf-4)	3.60	5.40	60.0 (50.8)
T_5	(*S. rolfsii* + Pf-5)	3.20	5.80	64.4 (53.4)
T_6	(*S. rolfsii* + Pf-6)	2.73	6.26	69.6 (56.6)
T_7	(*S. rolfsii* + Pf-7)	2.63	6.36	70.7 (57.3)
T_8	(*S. rolfsii* + Pf-8)	1.86	7.13	79.3 (62.9)
T_9	(*S. rolfsii* + Pf-9)	2.60	6.40	71.1 (57.5)
T_{10}	(*S. rolfsii* + Pf-10)	2.43	6.56	72.9 (58.7)
T_{11}	(*S. rolfsii* + Pf-11)	3.00	6.00	66.7 (54.7)
T_{12}	(*S. rolfsii* + Pf-12)	1.06	7.93	88.15 (69.9)
SEm±		0.02	0.02	0.07
C.V. (%)		2.72	1.39	1.32
CD (*p*= 0.01)		0.19	0.19	2.02

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

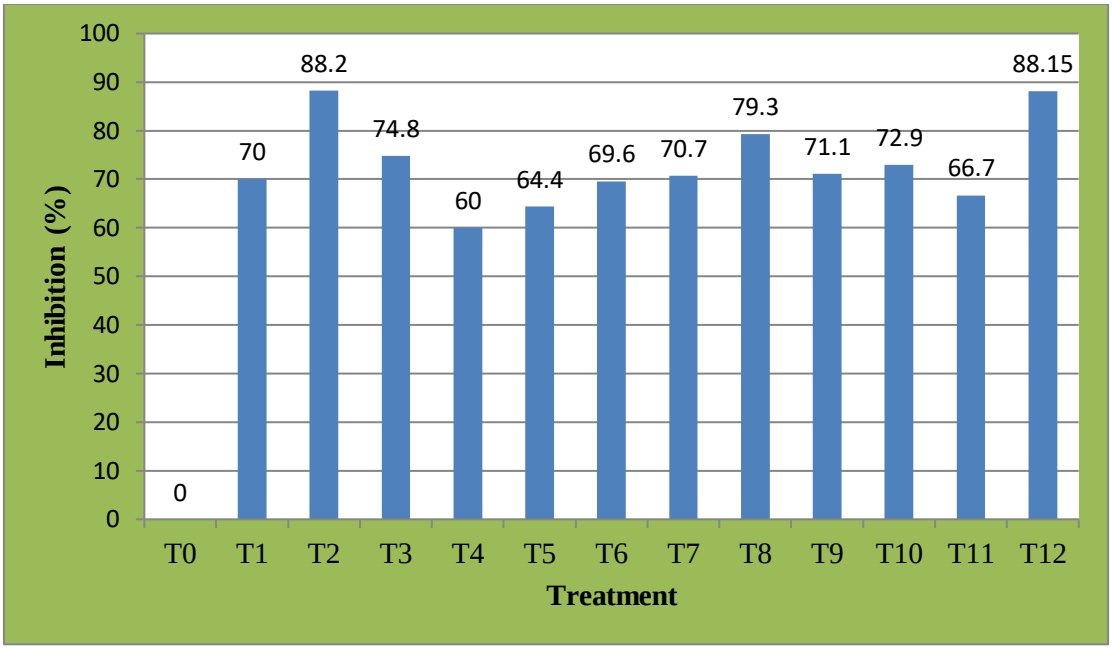


Fig 4.6 *In vitro* effect of non volatile metabolites of *Pseudomonas* isolates on per cent growth inhibition of *S. rolfsii*

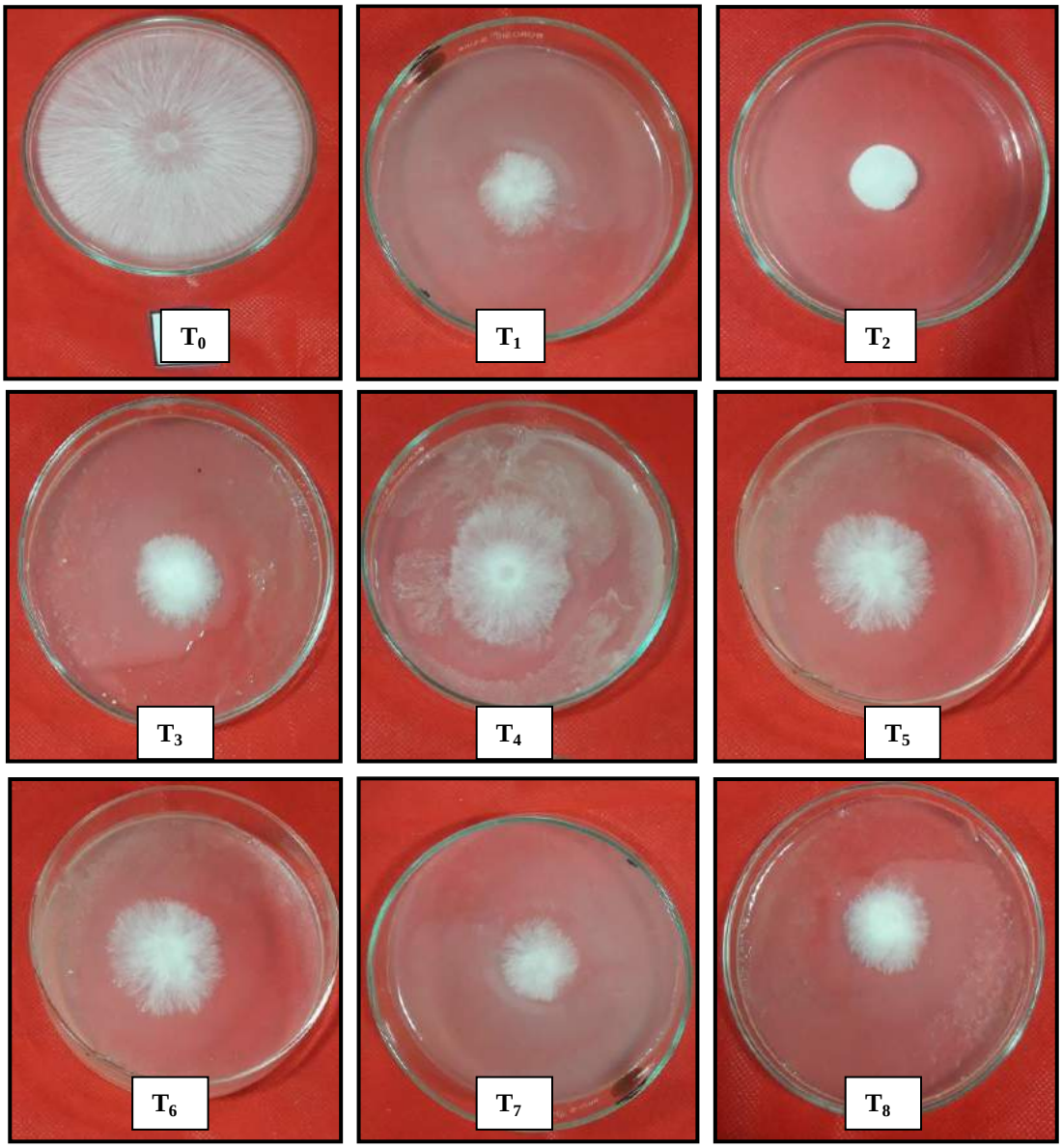


Plate 20 *In vitro* effect of non volatile metabolites of *Pseudomonas* isolates (Pf-1 to Pf-8) on growth of *S. rolfsii*

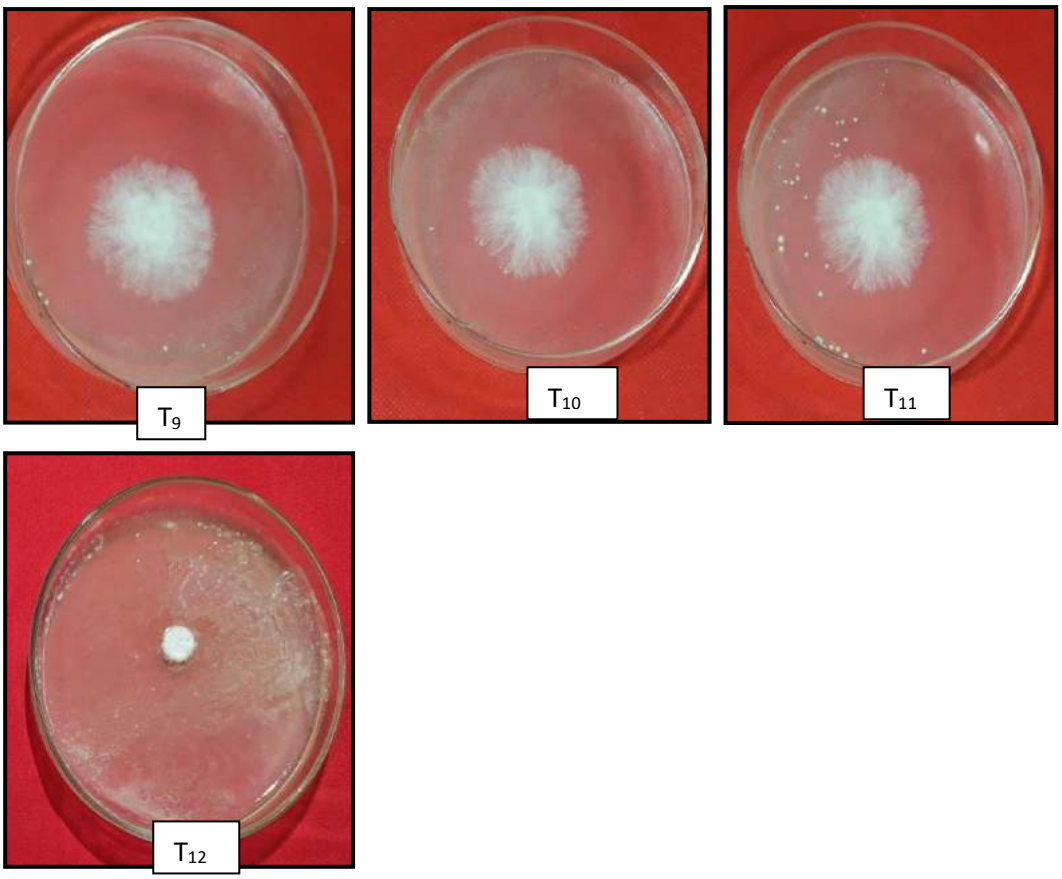


Plate 21 *In vitro* effect of non volatile metabolites of *Pseudomonas* isolates (Pf-9 to Pf-12) on growth of *S. rolfsii*

spp. and *Trichopderma* spp. which limited the mycelial growth under *in vitro* condition.

The results are in conformity with the reports of earlier workers Saxena *et al*. (1995); Revathy and Muthusamy (2003); Nagarajkumar *et al.* (2005); Paramasivan (2006) and Muthukumar *et al*. (2010). Chanutsa *et al*. (2014) reported that the culture filtrate of *P. florescence* inhibited 100 per cent in the mycelia growth of *S. rolfsii* Muthukumar *et al*. (2019) showed the effect of culture filtrate of *Pseudomonas* isolates on the mycelial growth of *S. rolfsii* under *in vitro* conditions revealed that the culture filtrate of the isolate-I7 totally (100 %) inhibited the mycelial growth of *S. rolfsii* at 15 % concentration under *in vitro* conditions followed by the isolate-I4 (90.67 %).

4.5.3 Qualitative assay of Ammonia production

Altogether 20 isolates of *Trichoderma* and 12 isolates of *Pseudomonas* were tested for the production of ammonia in peptone water. Development of brown to yellow color was a positive test for ammonia production (Cappuccino and Sherman, 1992). The results of qualitative assay of ammonia production by different native BCAs are presented in Table 4.8.

All 32 isolates showed positive results for ammonia production. Among the tested isolates, *Pseudomonas* isolates (Pf-1, Pf-2, Pf-3, Pf-4, Pf-5, Pf-6, Pf-7, Pf-8, Pf-9 and Pf-12) and *Trichoderma* isolates (T-1, T-2, T-3, T-6, T-7,T-8, T-11, T-12, T-13 and T-20) exhibited strong ammonia production by turning initial peptone water broth from yellow to dark brown colour (Plate 22 a and b).

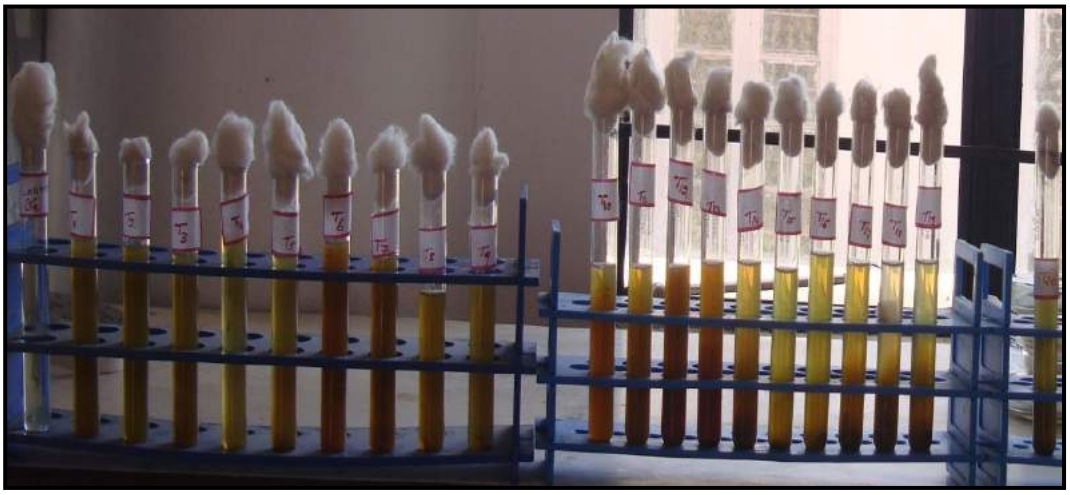
Table 4.8 Qualitative assay of ammonia production by different native biocontrol agents (BCAs

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

Whereas

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

b) Qualitative assay of ammonia production by *Pseudomonas* isolates (Pf-1 to Pf-12) and T_0 (Control)



a) Qualitative assay of ammonia production by *Trichoderma* isolates (T-1 to T-20) and T_0 (Control)

Plate 22 Qualitative assay of ammonia production by native rhizospheric BCAs

The results of present investigation are in agreement with the results of workers like Dixit *et al.* (2015) who reported that isolates (11 isolates) of fluorescent *Pseudomonas* showed positive result for ammonia production. Lalngaihawmi and Bhattacharyya (2019) also evaluated *Trichoderma* spp. for ammonia production and results revealed that all the *Trichoderma* spp. showed positive result. The findings of present work are also in harmony with the findings of earlier workers Bhakthavatchalu *et al.,* 2013; Prasad *et al.,* 2017 and Rai, 2017 which suggest that the production of ammonia by both *Trichoderma* and *Pseudomonas* isolates have positive impact on thegrowth of plant.

4.5.4 Qualitative assay of 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 color indicated IAA production. The results of qualitative assay of IAA production by different native BCAs are presented in Table 4.9. The results revealed that *Pseudomonas* isolates (Pf-2, Pf-3, Pf-4, Pf-11 and Pf-12) showed strong production. In *Trichoderma* isolates (T-2, T-6, T-8, T-9, T-11 and T-20) exhibited medium IAA production as evidenced by qualitative analysis of culture supernatant (Plate 23a and b).

In the present investigation, 5 isolate of *Pseudomonas* and 6 isolates of *Trichoderma* were observed to produce IAA at varying intensity. This occurrence may be described to the heterogeneous nature of the source and the strains of the antagonists.

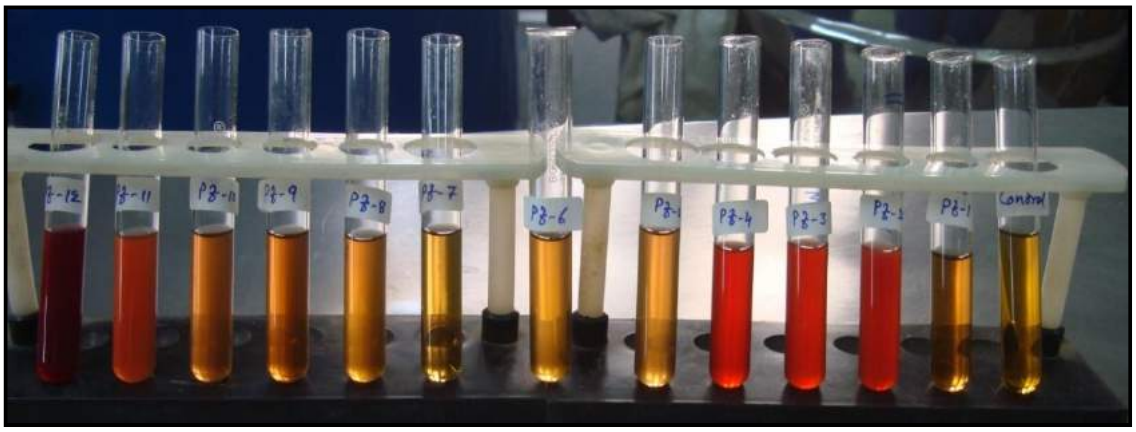
The above findings are in accordance with the result of several workers. Bhakthavatchalu *et al.* (2013) reported thatthe isolate *P. aeruginosa* FP6 was found positive for the production of IAA. Dixit *et al.* (2015) also reported that all 20 *Trichoderma* spp. Isolates tested elucidated positive results for IAA

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

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

Whereas

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



a) Qualitative assay of IAA production by different native *Pseudomonas* isolates (Pf-1 to Pf-12) and T_0 (Control)

b) Qualitative assay of IAA production by different native *Trichoderma* isolates (T_1 to T_{20}), T_0 (Control)

Plate 23 Qualitative assay of IAA production by different native BCAs

production. Similarly, Prasad *et al.* (2017) also reported that, 24 isolates of fungal BCAs particularly *Trichoderma* spp. and 12 bacterial isolates showed positive resultsfor IAA production.

4.5.5 Qualitative assay of Phosphate solubility

The results of qualitative assay of phosphate solubilizationby different native BCAs are presented in Table 4.10. The results revealed that *Pseudomonas* isolates (Pf-2, Pf-8 and Pf-12) and *Trichoderma* isolates (T-8, T-10, and T-20) elucidated medium production of phosphate solubilization (Plates 24 and 25).The results of the present investigation suggest that the phosphate solubilization by both *Trichoderma* and *Pseudomonas* isolates have obvious influence on the plant growth.

The present findings confirm the observation made by Prasad *et al.* (2017) who reported that eleven bacterial isolates showed solubilization zone except *P. fluorescens*-4. The isolate *P. fluorescens*-2 recorded highest solubilization zone followed by *P. fluorescens*-1. All *Trichoderma* isolates did not show any phosphate solubilization zone. Lal *et al*. (2022) also reported thephosphate solubilisation potential of bacterial isolates Pf14 (5.65) showing maximum phosphate solubilization, followed by Pf10 (4.50) and Pf11 (4.50). Similarly, highest SE wasobserved by Pf14 (82.29%), followed by Pf10 (77.78%) and Pf11 (77.78%).

The findings of present work are in confirmation with the results of many workers like Kapri and Tewari, 2010; Gangwar*et al.*, 2012; Bhakthavatchalu*et al.*, 2013; Tiwari *et al.*, 2016; Rai, 2017; Lalngaihawmi and Bhattacharyya, 2019 and Thi *et al.* (2020).

Table 4.10 Qualitative assay of phosphate solubilization by different native biocontrol agents (BCAs)

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

Whereas

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

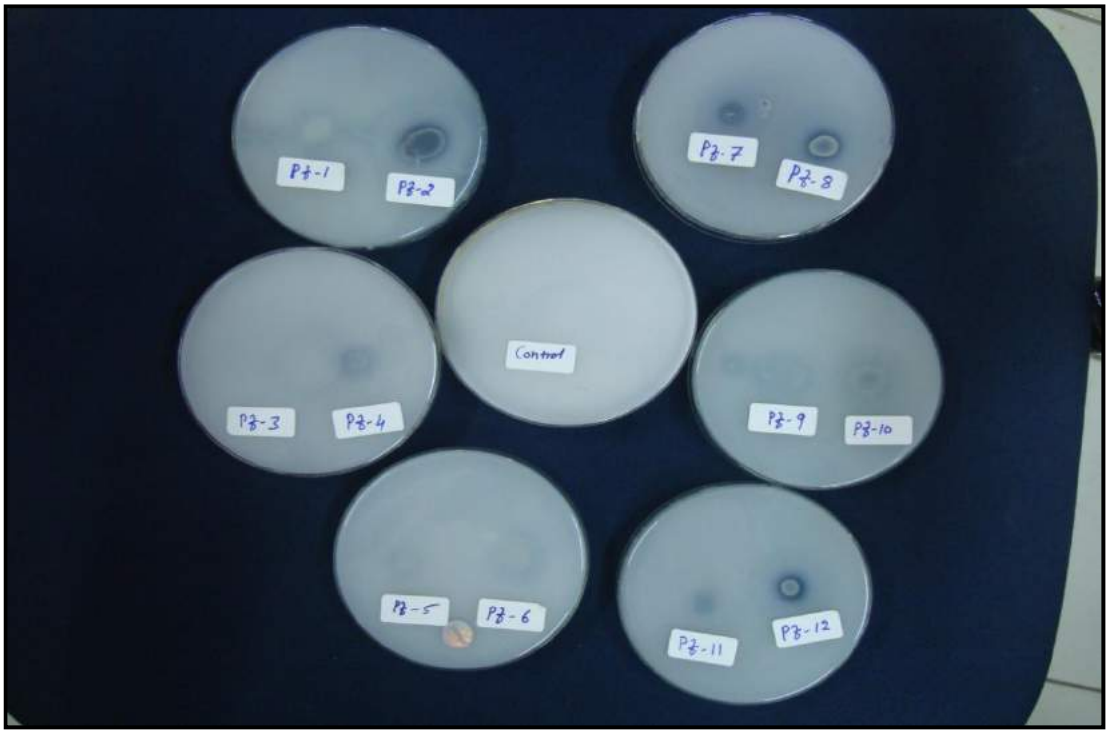


Plate 24 Qualitative Phosphate production by bacterial native BCAs

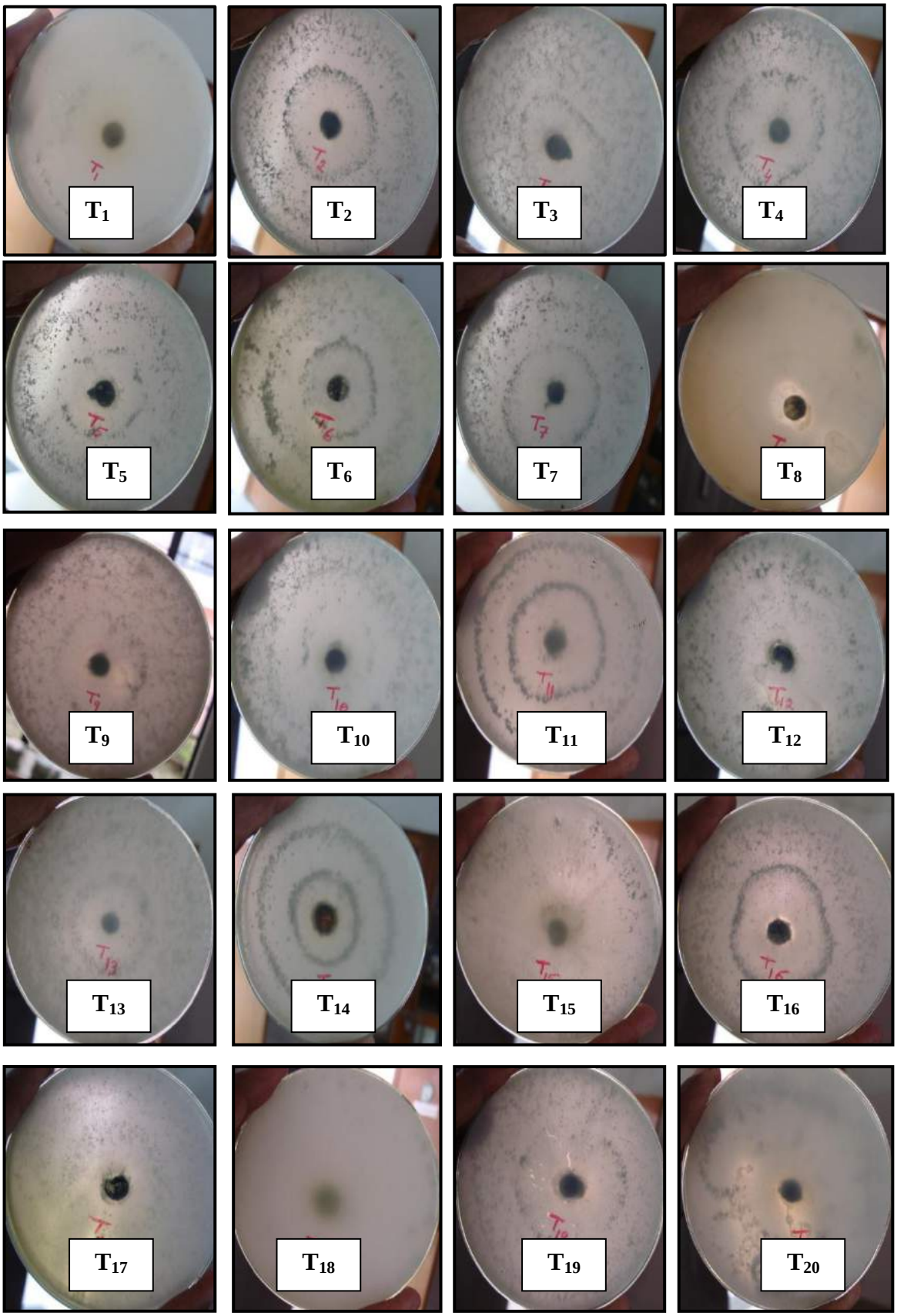


Plate 25 Qualitative assay of phosphate solubilisation by native *Trichoderma* isolates (T_1- T_{20})

4.5.6 Qualitative assay of Siderophore production

Chrome azurol Sulfonate (CAS) assay was used to detect the production of siderophore of 20 isolates of *Trichoderma* and 12isolates of *Pseudomonas* following the procedure given by Schwyn and Neilands, 1987.The results of qualitative assay of siderophore productionby different native BCAs are presented in Table 4.11. In this present study, strong siderophore production was exhibited by *Pseudomonas* isolates Pf-2, Pf-6, Pf-10 and Pf-12 (Plate 27b). And among the tested *Trichoderma* isolates, isolate T-7, T-8, T-11, T-14, T-15, T-16, T-18, T-19 and T-20 exhibited medium siderophore production by pink and orange halo colour development (Plates 26 and 27a).

Similar work done by Bhakthavatchalu *et al.* (2013) recorded change in the color of the CAS agar from blue to orange red by the isolate confirmed the ability of *P. aeruginosa* FP6 to produce siderophore. Further Dixit *et al.* (2015) reported that amongst20 isolates of *Trichoderma* spp. and 11 isolates of fluorescent *Pseudomonas* spp.evaluated for siderophore production in CAS solid medium, *Trichoderma* spp. isolates T5, T6, T8 and T15 showed positive production of pink halo colour in the medium. And among the*Pseudomonas* isolates, maximum siderophore production was observed in Pf12 followed by Pf27 and Pf28 except Pf25.

The findings of present work are also in conformity with the works done by earlier workers (Demanage*et al.*, 1987; Kamensky *et al*. (2003); Mahesh (2007); Manikandan *et al.*, 2010; Gangwar*et al.*, 2012; Prasad *et al.*, 2017; Rai, 2017 and Lalngaihawmi and Bhattacharyya, 2019).

Table 4.11 Qualitative assay of siderophore production by different native biocontrol agents (BCAs)

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

Whereas

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

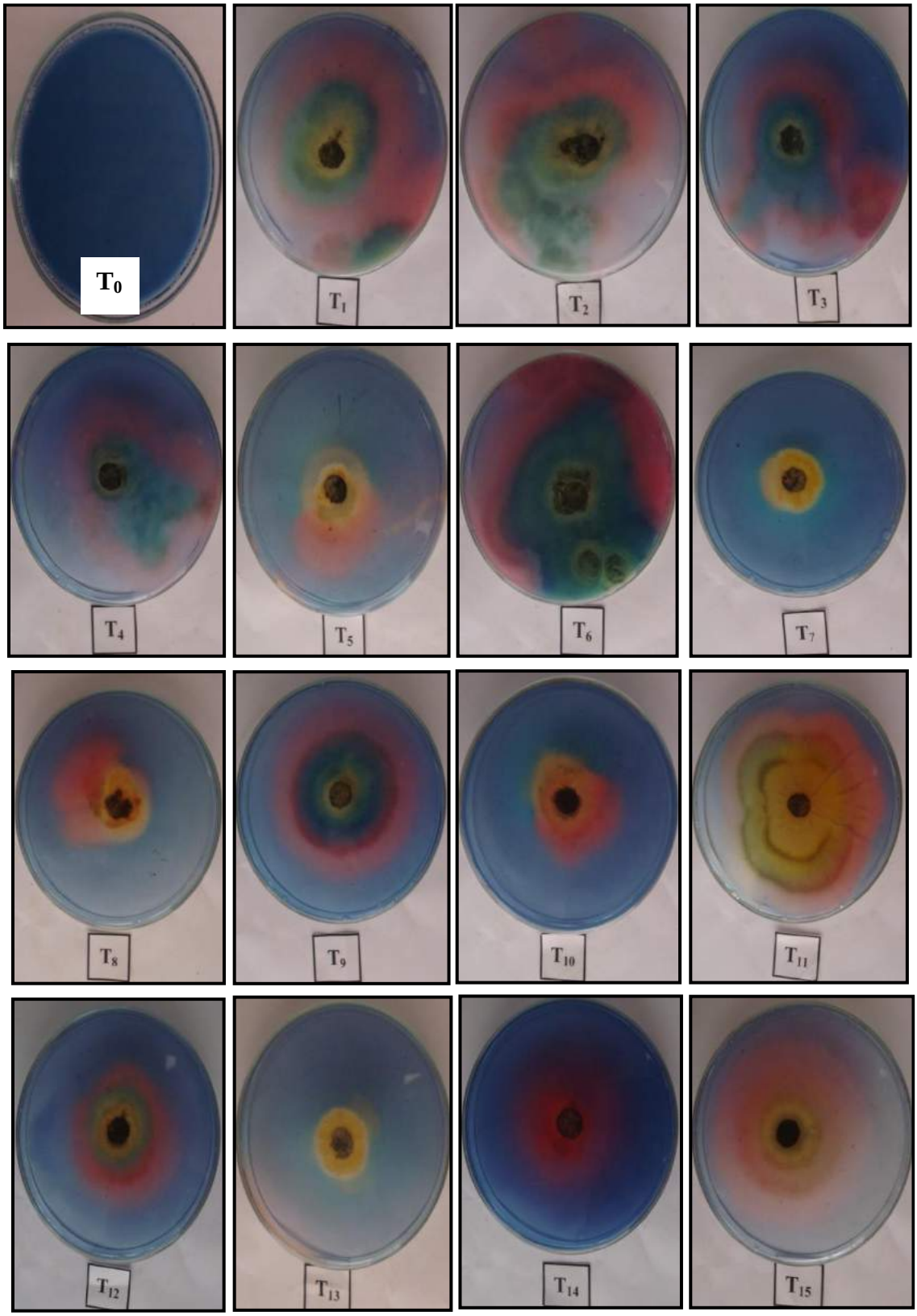
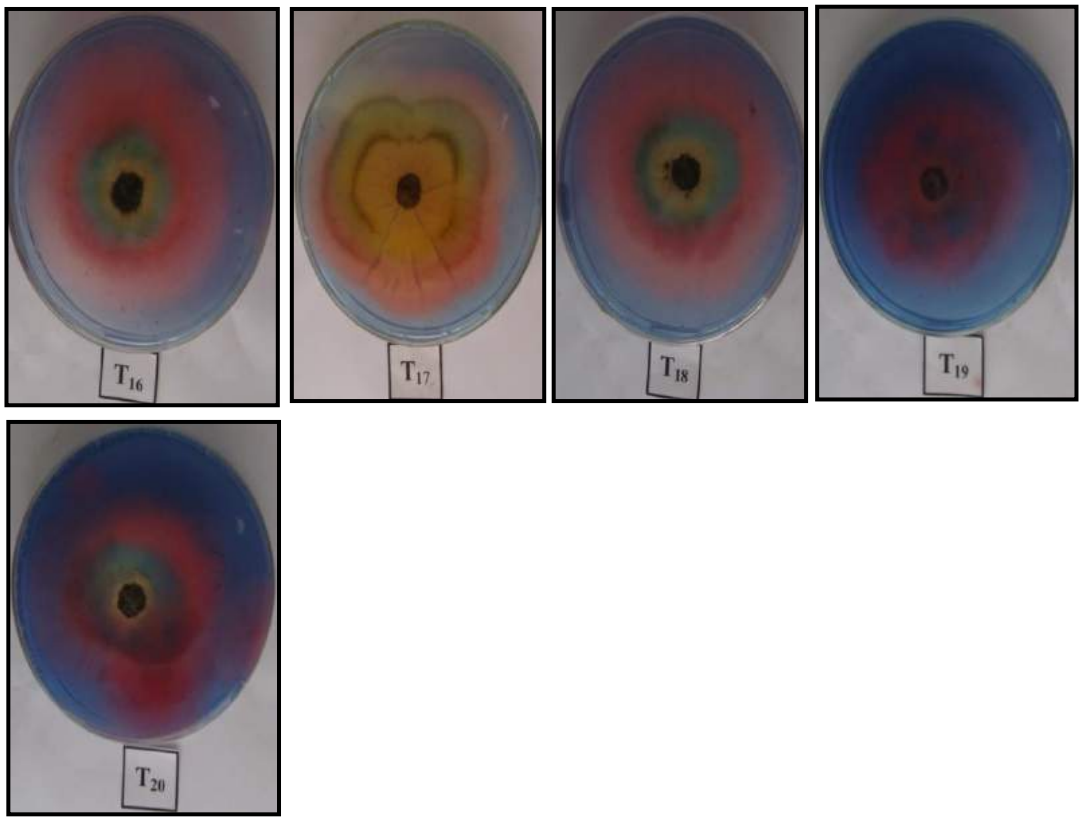


Plate 26 Qualitative assay of siderophore production by native *Trichoderma* isolates (T_1-T_{15})



a) Qualitative assay of siderophore production by native *Trichoderma* isolates (T_{16}- T_{20})

b) Qualitative assay of siderophore production by native *Pseudomonas* isolates (T_1- T_{12})

Plate 27 Qualitative assay of siderophore production by native rhizospheric BCA isolates

4.5.7 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). Results were recorded and are depicted in Table 4.12. Reactions to HCN production were recorded as weak (yellow to light brown), moderate (brown) and strong (reddish brown). The results revealed that only 3 *Pseudomonas* isolates (Pf-2, Pf-7 and Pf-12) elucidated positive results for HCN production (Plate 28a).The results of the present investigation on the production of HCN by *Pseudomonas* isolates suggest to have an absolute influence on the high degree of inhibition of *S.rolfsii.*

Works akin to the present findings were also reported by Manikandan *et al*. (2010).They recorded the HCN production by *P. fluorescens* Pf1 and observed that Pf1 cultures of different ages changed the yellow colour of the filter paper to dark brown as compared to the control treatment. Further Dixit *et al.* (2015) also reported that amongst 11 isolates of fluorescent *Pseudomonas*tested for HCN production, colour change from yellow to dark brown was found in most of the isolates, except Pf4, Pf6, Pf25. While, Pf20, Pf27 and Pf28 were highly HCN producing isolates as compared to others.The findings of Thi *et al* (2020) who reported that among the 15 bacterial isolates subjected to HCN production assay, only TK1and SAK6 were found to be efficient hydrogen cyanide producers, supports the present investigation.

The findings of present work are in agreementwith the findings of earlier workers like Ramette *et al.,* 2003; Bhakthavatchalu *et al.,* 2013; Tiwari *et al*. (2016) and Prasad *et al.*, 2017 who were reported the production of HCN by rhizospheric BCAs.

Table 4.12 Qualitative assay of HCN production by *Pseudomonas* isolates

***Pseudomonas* isolate**	**HCN production**	***Pseudomonas* isolate**	**HCN production**
Control	**-**	**Pf-7**	**+**
Pf-1	**-**	**Pf-8**	**-**
Pf-2	**+**	**Pf-9**	**-**
Pf-3	**-**	**Pf-10**	**-**
Pf-4	**-**	**Pf-11**	**-**
Pf-5	**-**	**Pf-12**	**+**
Pf-6	**-**		

Whereas

1) **-** = Negative.

2) + = Positive.

4.5.8 Qualitative screening of enzymes production by rhizospheric native BCAs

4.5.8.1 Qualitative screening of pectolytic enzyme production

Trichoderma spp.and *Pseudomona*s spp were screened for secretion of pectolytic enzyme.The isolates showing range of pectolytic production were designated as *viz.,* no enzyme activity, + isolates showing very low enzyme activity, ++ isolates showing medium enzyme activity, +++ isolates showing strong enzyme activity, ++++ isolates showing very high enzyme activity.The results of qualitative assay of pectolytic enzyme production by the fungal and bacterial isolates are depicted in Table 4.13. The results revealed that *Pseudomonas* isolates Pf-2, Pf-5, Pf-6, Pf-10 and Pf-12 elucidated medium results for pectolytic production (Plate 28b). And among the *Trichoderma* isolates, T-2, T-8, T-9, T-10, T-11, T-16, T-17 and T-20 showed strong production of pectolytic enzymes (Plate 29).

Similar work done by Maria *et al.* (2001) confirms the present investigation. They reported that *T. harzianum*, *T. viride*and *T. koningii* produced extracellular pectinases. Further Thi *et al* (2020) reported that 15 rhizobacterial isolates were subjected to pectolytic activity test and found out that 5 isolates could not produce pectinase while the other 10strains could produce a positive amount of pectolytic enzyme. The present results are similar with earlier work done by Marcia *et al.* (1999); Kashyap *et al*., 2001; Qualhato *et al.,* 2013; Yannam *et al*., 2014; Tepe and Dursun 2014) and Tsegaye *et al* (2019).

Table 4.13 Qualitative assay of pectolytic enzyme production by different native biocontrol agents (BCAs)

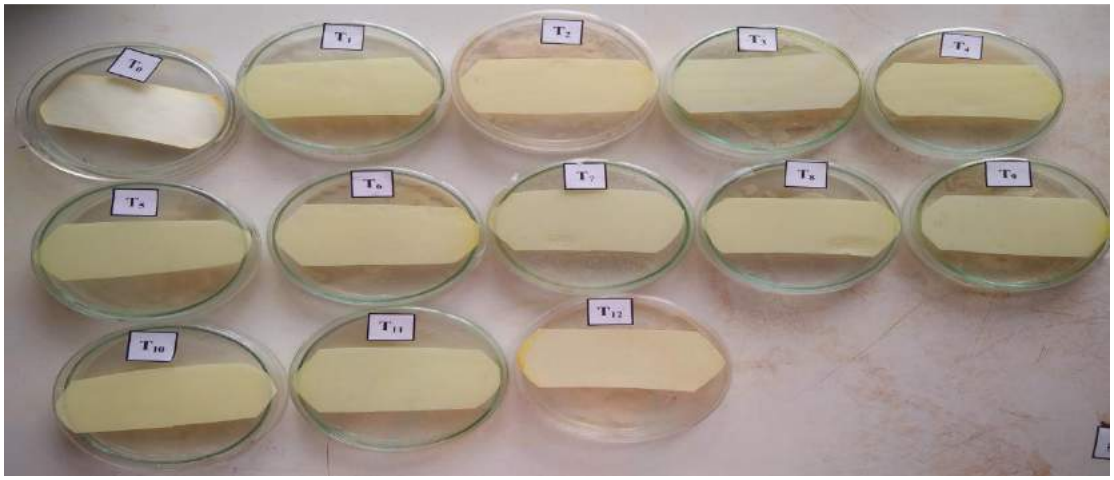
BCAs	Pectolytic production	BCAs	Pectolytic production
(A)*Pseudomonas* isolates-		**(B) *Trichoderma* isolates**	
Control		**Control**	
Pf-1	+	**T-1**	-
Pf-2	++	**T-2**	+++
Pf-3	+	**T-3**	++
Pf-4	+	**T-4**	++
Pf-5	++	**T-5**	++
Pf-6	++	**T-6**	++
Pf-7	+	**T-7**	-
Pf-8	+	**T-8**	**+++**
Pf-9	-	**T-9**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

1) **-** = Negative.

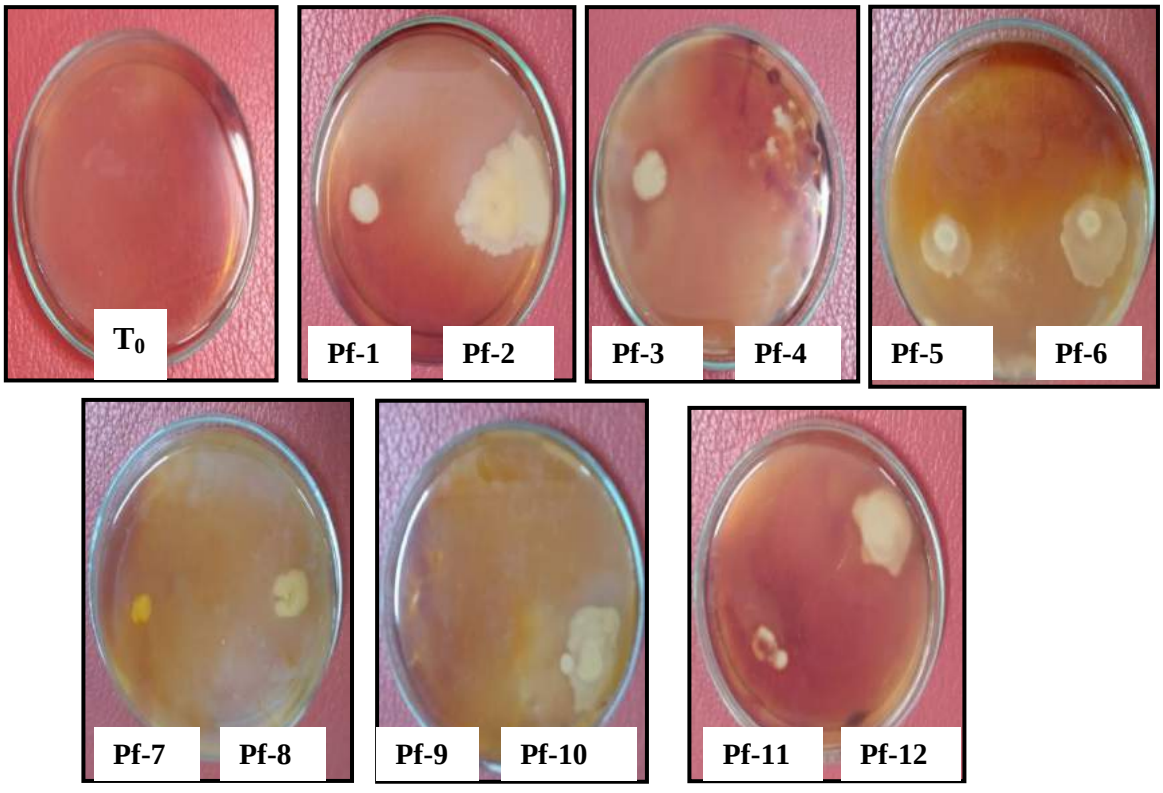
2) + = Low

3) ++=Medium

4) +++=Strong



a) Qualitative HCN production by native bacterial isolates (Pf -1to Pf -12) and control



b) Qualitative pectolytic enzyme production by native bacterial isolates (Pf -1to Pf -12) and control

Plate 28 Qualitative assay of HCN and pectolytic enzymes production by native bacterial isolates (Pf -1 to Pf -12) and T_0 (control)

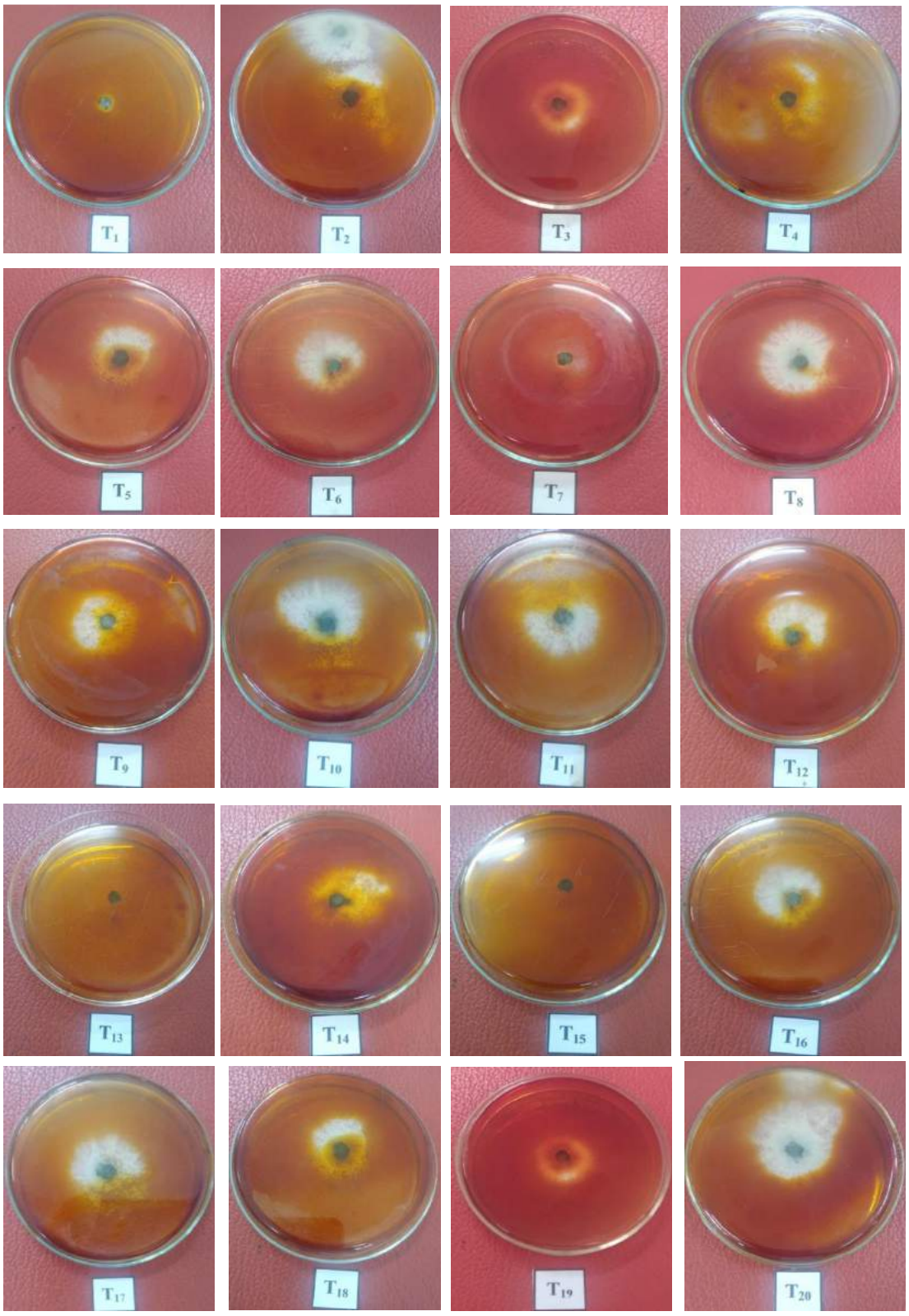


Plate 29 Qualitative pectolytic enzyme production by native *Trichoderma* isolates T_1- T_{20}

4.5.8.2 Qualitative screening of cellulose enzyme production

Trichoderma spp.and *Pseudomona*s spp were screened for secretion of cellulose enzyme.The appearance of the clear zone around the colony after the flooding of iodine solution shows the production of cellulose. The isolates showing showing various range of cellulose production were designated as *viz;*- no enzyme activity, + isolates showing very low enzyme activity, ++ isolates showing medium enzyme activity, +++ isolates showing strong enzyme activity, ++++ isolates showing very high enzyme activity.The results of qualitative assay of cellulose production by the fungal and bacterial isolates are depicted in Table 4.14.

Perusal of the data revealed that amongst the 12 *Pseudomonas* isolates tested, 10 *Pseudomonas* isolates Pf-1, Pf-2, Pf-3, Pf-5, Pf-6, Pf-7, Pf-8, Pf-9, Pf-10 and Pf-12 elucidated strong results for cellulose production (Plate 30) whereas, Pf-4 and Pf-11 showed very low cellulose activity.

In case of *Trichoderma* isolates, 9 isolates T-1, T-4, T-5, T-6, T-8, T-10, T-12, T-17 and T-20 produced strong cellulose enzyme production and the remaining isolates showed low to medium enzyme activity (Plate 31).

Similar work done by Jayaraj *et al*. (2005) reported the production of extracellular lytic enzymes by bacterial antagonists against fungal plant pathogens was well documented. Further, similar findings of Mishra (2010) who observed that the *Trichoderma* spp exhibited highest cellulase activity of 3.6µ/ml and it was found to suppress the mycelial growth of *P. aphanidermatum.* Likewise, El-Komy*et al.* (2015) also reported the production of extracellular enzymes by *Trichoderma* isolates.

The present results are in similar with earlier work done by Kumar and Gupta (1999); Khalid *et al.* (2006);Jain and Dhawan (2008); Kamala and Indira(2014); Parmar *et al*.(2015); Tsegaye *et al* (2019) and Thi*et al*. (2020).

Table 4.14 Qualitative assay of cellulose production by different native biocontrol agents (BCAs)

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

1) - = Negative.

2) + = Low

3) ++=Medium

4) +++=Strong

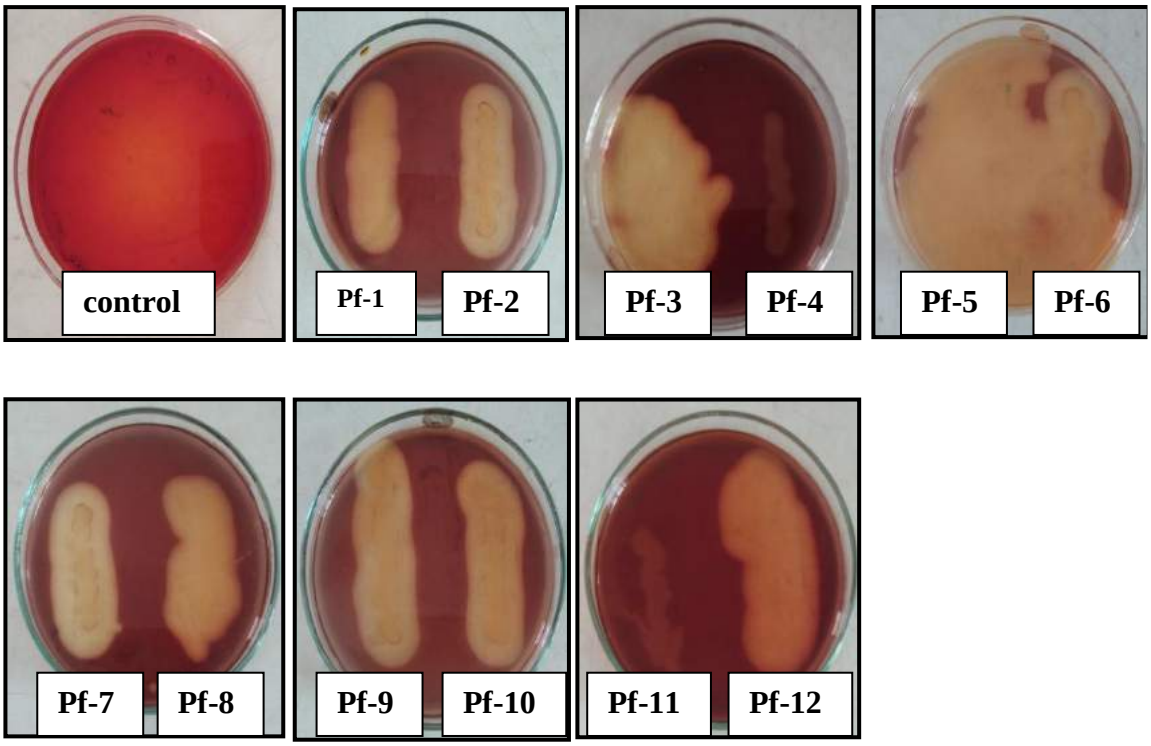


Plate 30 Qualitative assay of cellulose production by native bacterial BCA isolates (Pf-1 to Pf-12) and control

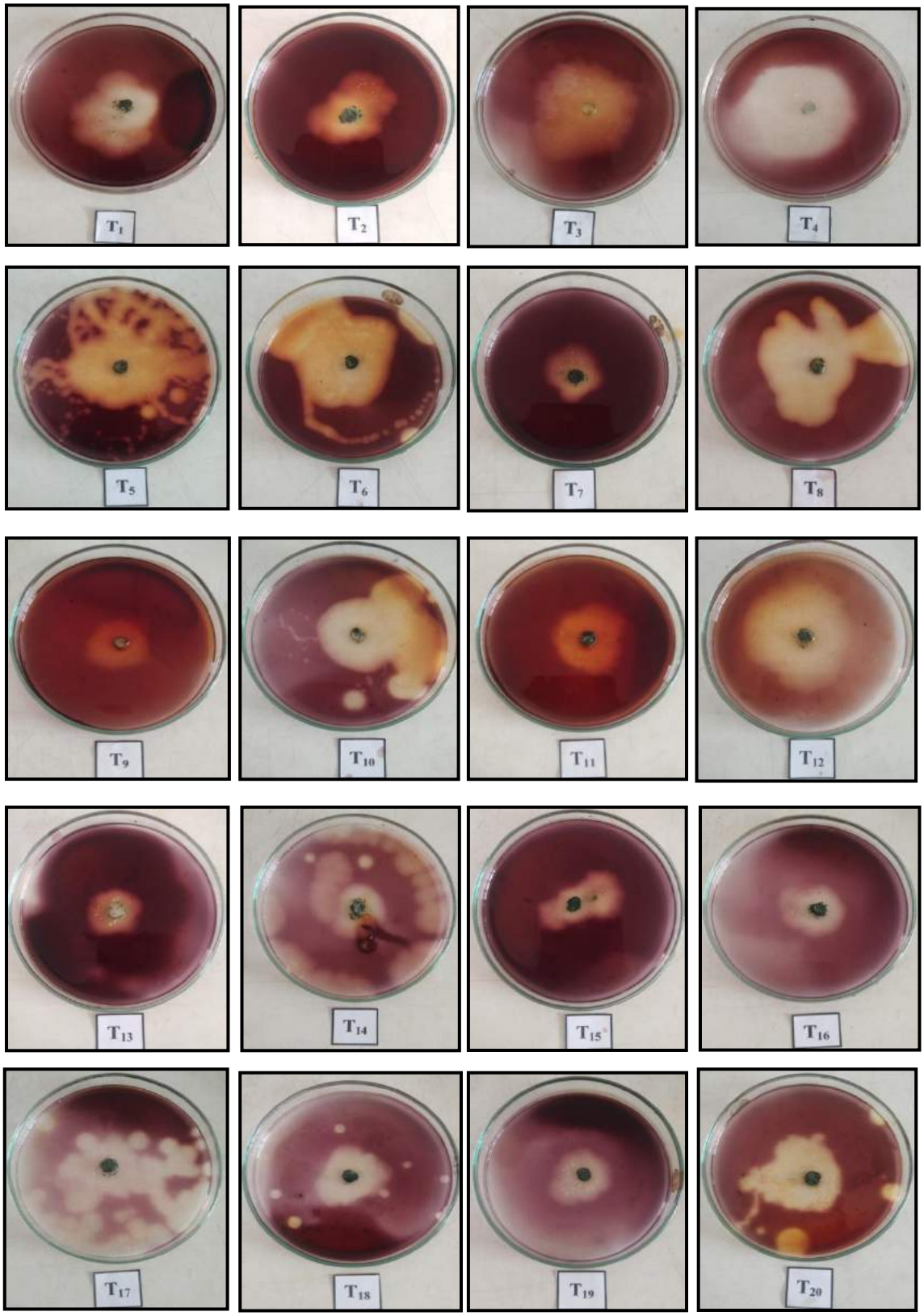


Plate 31 Qualitative assay of cellulose production by native *Trichoderma* isolates ($T_1 – T_{20}$)

4.5.8.3 Qualitative screening of Amylase production

Trichoderma spp.and *Pseudomona*s spp were screened for secretion of amylase enzyme.The isolates were showing in varying range of amylase production *viz.*- no enzyme activity, + isolates showing very low enzyme activity, ++ isolates showing medium enzyme activity, +++ isolates showing strong enzyme activity, ++++ isolates showing very high enzyme activityThe results of qualitative assay of amylase production by the fungal and bacterial isolates are depicted in Table 4.15.

The results revealed that *Trichoderma* isolates T-2, T-4, T-7, T-8, T-11, T-12, T-13, T-14, T-17 and T-20 showed strong production of amylase (Plate 32). But Pf-2 of *Pseudomonas* isolate elucidated strong results for amylase production (Plate 33a).

The present findings are in agreement with Maria *et al.,* (2001) who reported that *T. harzianum, T. viride*and *T. koningii* produced extracellular cellulose, amylase and pectinases. Amylases are also employed in the starch processing industries for the hydrolysis of polysaccharides. Similarly,Tsegaye *et al* (2019) reported that 95 isolates of rhizobacteriaexhibited positive results for amylase production. The present results are also similar with earlier work doneby Malleswari *et al* (2013); Verma and Shahi (2015) and Thi*et al*. (2020).

4.5.8.4 Qualitative screening of Catalase production

The production ofcatalase by *Pseudomonas* isolates were determined as per the protocol given by Aneja (2003). The evolution of oxygen in the form of bubble indicates positive result of catalase poduction. Results were recorded and are depicted in Table 4.16. From the table it is clear that all 12 *Pseudomonas* isolates elucidated positive results for catalase production (Plate 33b).

Table 4.15 Qualitative assay of amylase production by different native biocontrol agents (BCAs)

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

1) - = Negative.

2) + = Low

3) ++=Medium

4) +++=Strong

Table 4.16 Qualitative assay of catalase production by *Pseudomonas* isolates

***Pseudomonas* isolate**	**Catalase production**	***Pseudomonas* isolate**	**Catalase production**
Pf-1	**+**	**Pf-7**	**+**
Pf-2	**+**	**Pf-8**	**+**
Pf-3	**+**	**Pf-9**	**+**
Pf-4	**+**	**Pf-10**	**+**
Pf-5	**+**	**Pf-11**	**+**
Pf-6	**+**	**Pf-12**	**+**

1) = Negative.

2) + = Positive

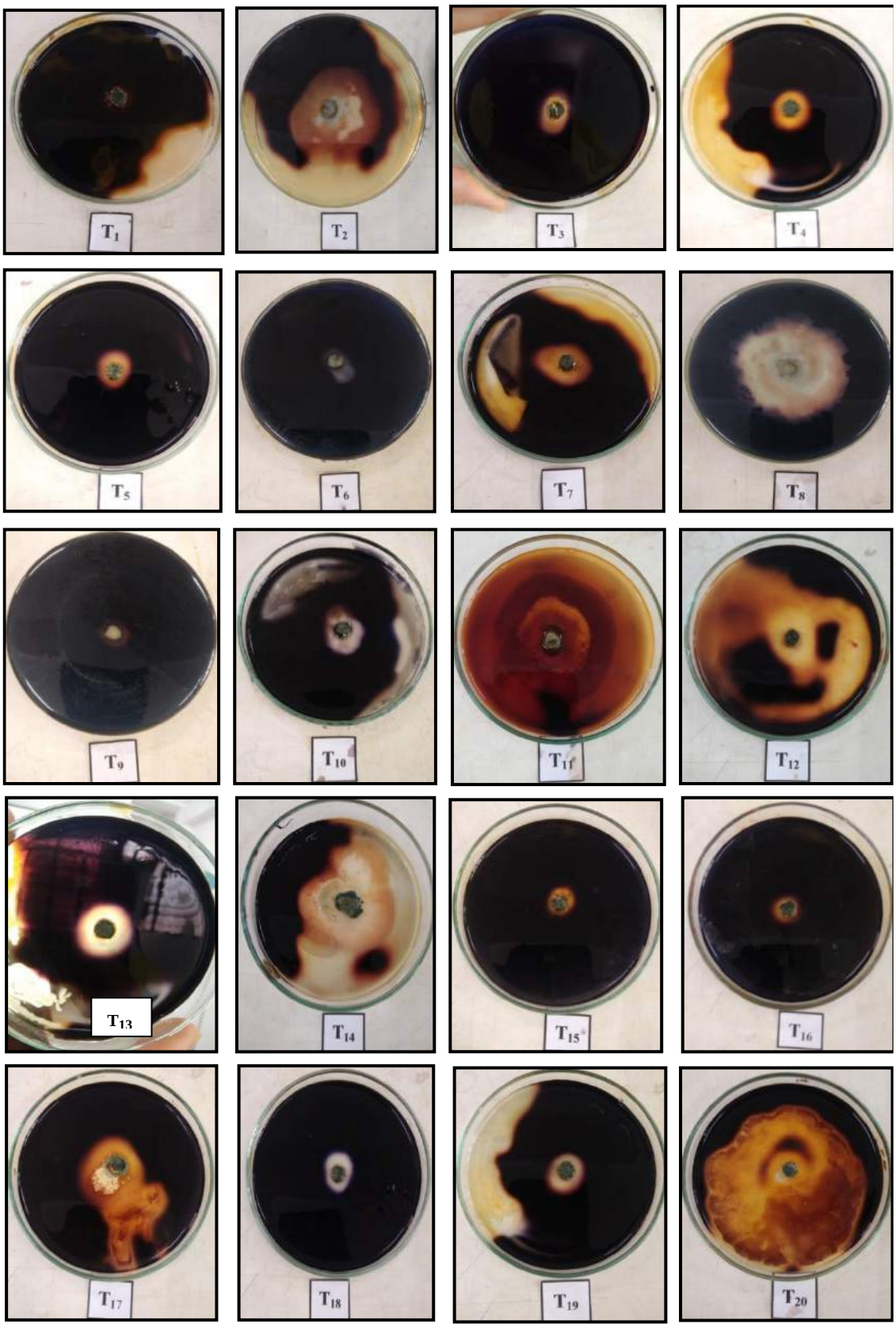
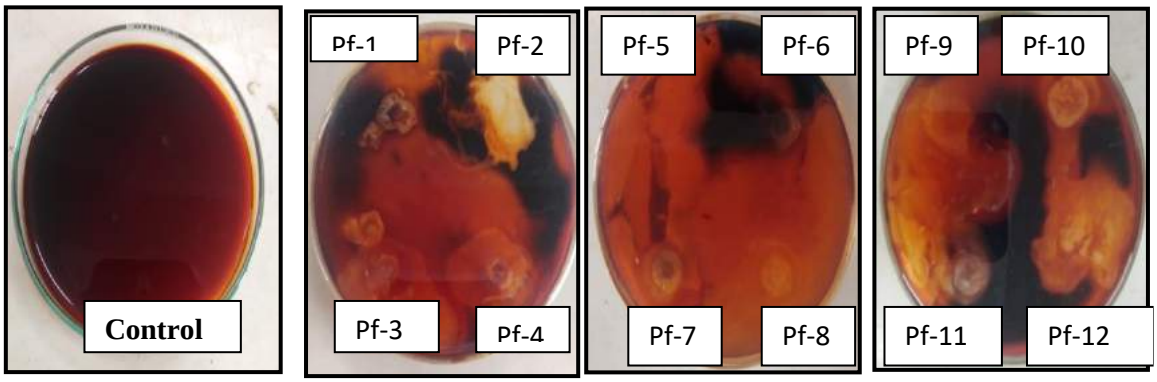
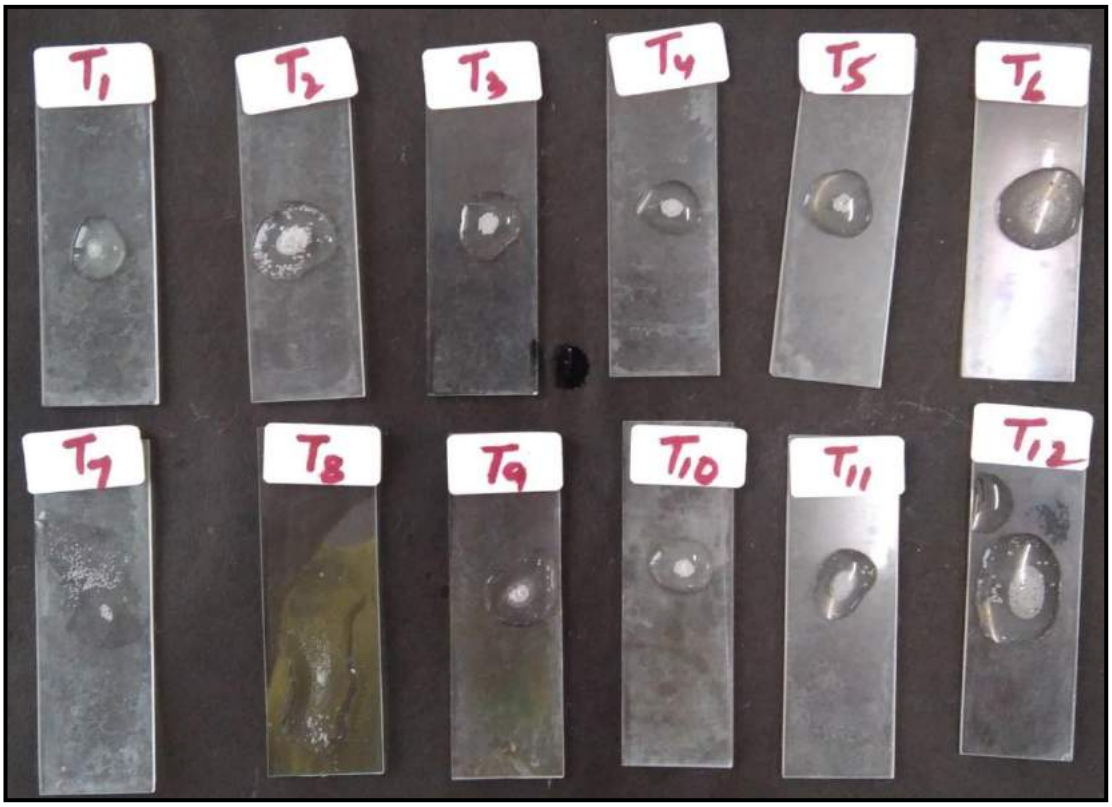


Plate 32 Qualitative assay of amylase production by native *Trichoderma* isolates (T_1- T_{20})



a) Qualitative assay of amylase production by bacterial isolates (Pf-1 to Pf -12) and control



b) Qualitative assay of catalase production by native bacterial isolates (Pf -1 to Pf -12)

Plate 33 Qualitative assay of amylase and catalase production by native bacterial isolates

The present results are in agreement with Malleswari *et al.* (2013) who reported the production of catalase which was exhibited by all the 219 isolatesof rhizobacteria. Catalase activity was detected in all the bacterial strains that may be potentially very advantageous for plant growth promotion activities. Similarly Verma and Shahi (2015) isolated 23 bacteria from rhizosphere soils and found out that 16 bacterial isolates were positive for catalase activity. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress.

4.5.9 Mycoparasitism activity of native *Trichoderma* isolates

All 20 isolates of *Trichoderma* under the study were tested for mycoparasitism activity against *S. rolfsii*. The presence or absence of coiling (Plate 34) was observed under compound microscope and results are presented in Table 4.16. All, 20 isolates showed the presence of coiling as hyphal interactions between *Trichoderma* isolates and *S. rolfsii.*

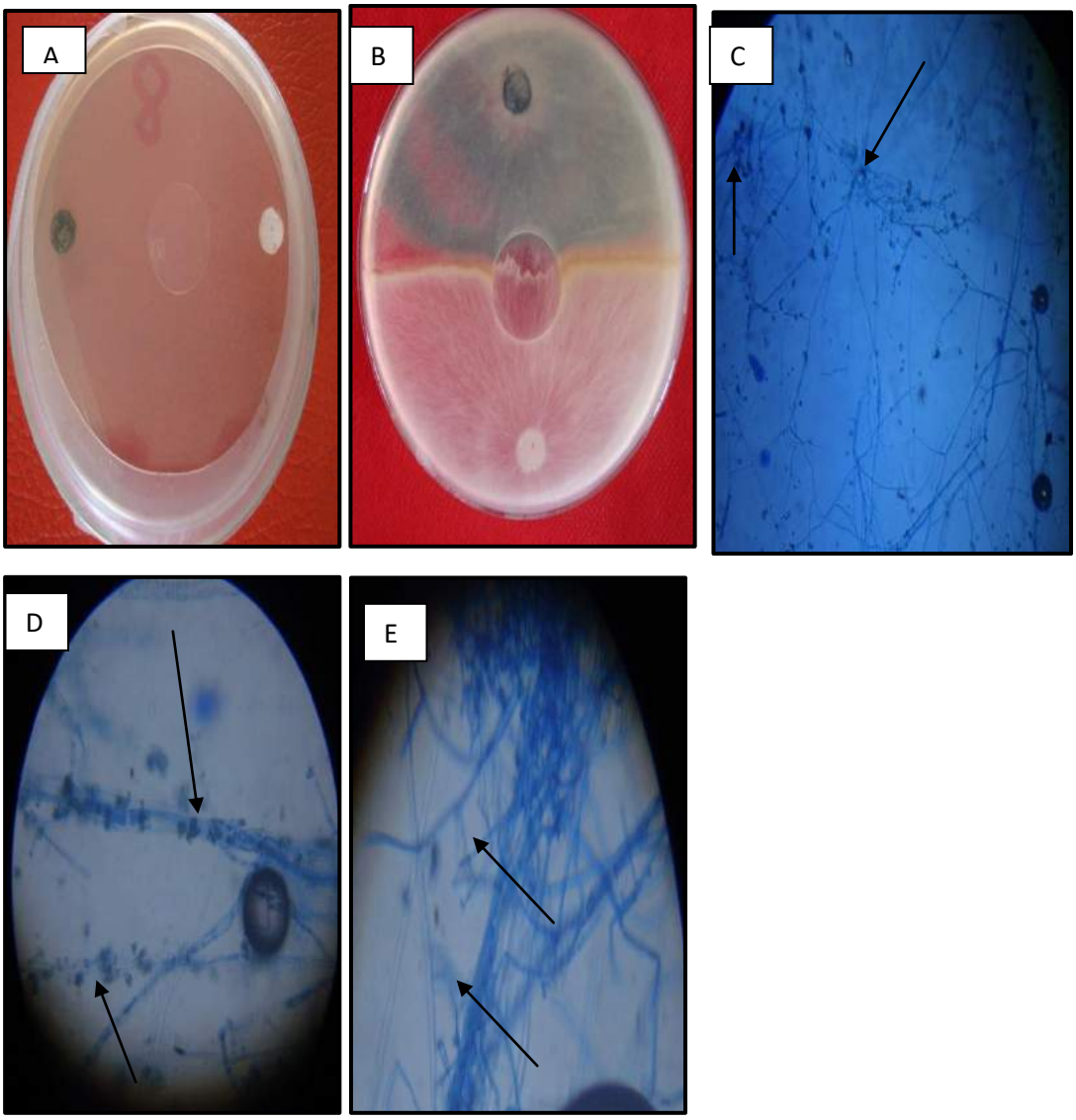
In the present investigation characteristic envelopment and coiling around of the hyphae by all isolates of *Trichoderma* spp. was observed. The hyphae of *Trichoderma* spp. were also observed to grow in close proximity to the hyphae of *S. rolfsii* before coagulation and disintegration occurred.

Similar work done by Saravanakumar (2002) confirms the present findings. They observed the zone of interaction between *Trichoderma* spp and *S. rolfsii* which revealed the hyperparasitic activity of antagonist on the test pathogen. This may result in disorganization or digestion of protoplasm contents or directly penetrates the hypae of *S. rolfsii*. The results of mycoparasitic behaviours of *Trichoderma* spp. followed almost the same pattern against *S.rolfsii* was compared with the earlier workers Coley-Smith and Cooke (1971); Elad *et al.* (1980) and Elad and Chet (1983).

Table 4.17 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-9	+		
T-10	+		
T-11	+		
T-12	+		
T-13	+		

+ = the presence of coiling.



(A) View of inoculated assembly

(B) Assembly after 7 days of incubation

(C) Microscopic view of coiling hyphae (black arrows) of *Trichoderma*sp. under 10 x

(D) Lysis of pathogen hypha

(E) Microscopic view of coiling hyphae (black arrows) of *Trichoderma*sp. under 45 x

Plate 34 Mycoparasitism interaction between *S. rolfsii* and *Trichoderma* isolate

4.6 Identification of potential BCAs

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

Molecular identification of potential *Trichoderma* isolates (T-8 and T-20) was performed by using Internal Transcribed Spacer (ITS) region of 18S rRNA. Sequence analyses of two isolates (T-8 and T-20) were done to confirm species identity (Table 4.18). 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.

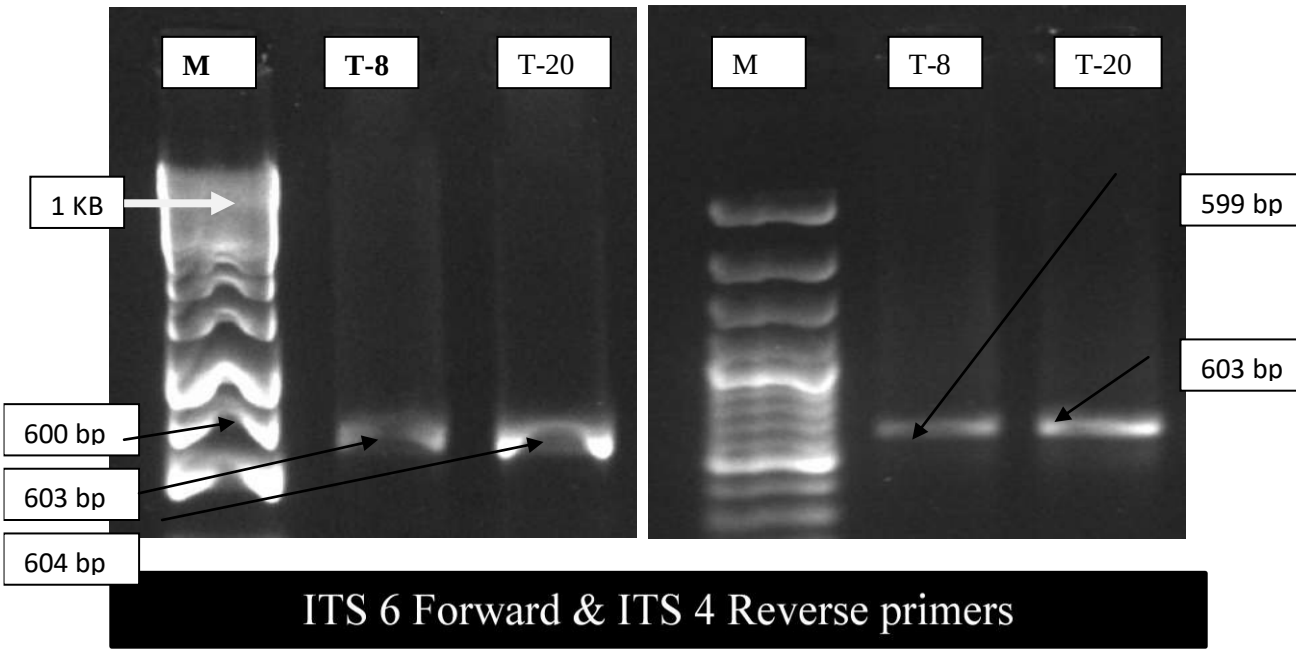
The ITS forward (ITS6*;* 5'-GAAGGTGAAGTCGTAACAAGG-3') and reverse (ITS4*;* 5'-TCCTCCG CTTATTGATATGC-3') oligonucleotide pairs amplified a single DNA fragment of approximately 601 and 599 bp amplicon size in both the isolates (Plate 35). ITS sequences of both the isolates were submitted to NCBI Gene Bank (OK147762 and OK147763), 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 findings of present results are in conformity with earlier workers Kullnig-Gradinger *et al*. (2002); Sun *et al.* (2012); Gherbawy *et al*. (2014) and Rai (2017).

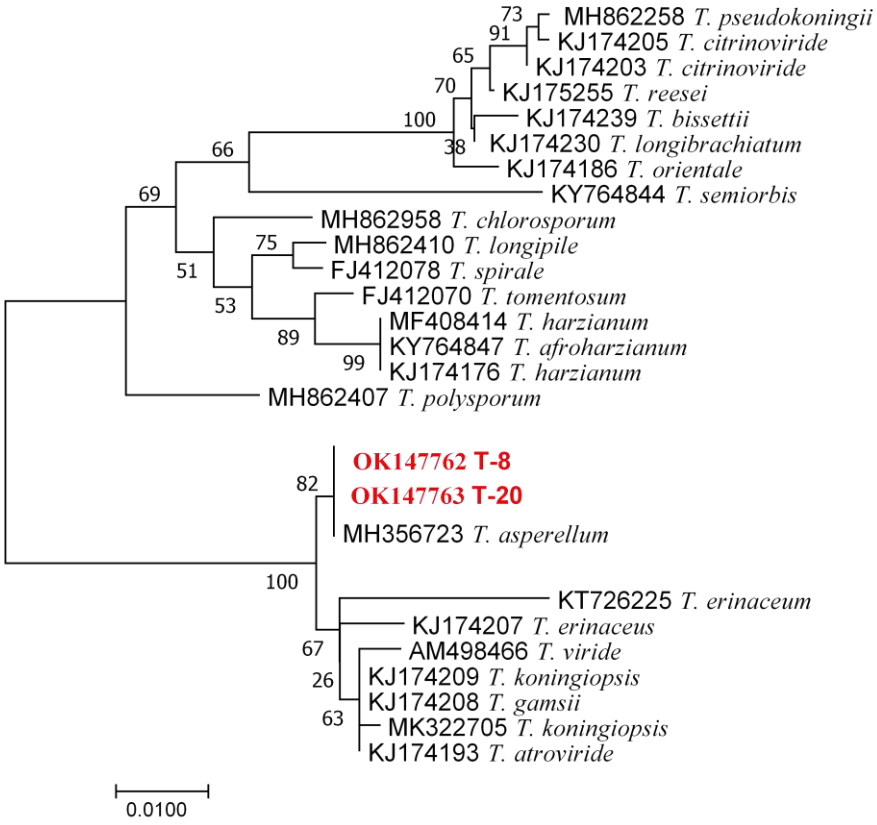
Table 4.18 Internal Transcribed Spacer (ITS) region of rDNA sequence of potential *Trichoderma* isolates

IsolateNo.	Accession No.	Primer & their sequence	Sequence	Base pair	Similarity
T-8	OK147762	**ITS6 Forward:** 5'-GAAGGTGAAGTCGTAACAAGG-3'	GGCCTGGGGAAGCGGAGGGACATTACCGAGTTTACACTCCCAAAC CCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCC CGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACC AACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGC TCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATC TCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA CATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCA TTTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGGGATCGGG ACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGC CGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGC GCGGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAATGTTG ACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT AAAGCCGGAGGAAAA	601	*T. asperellum*
		ITS 4 Reverse: 5'-TCCTCCGCTTATTGATATGC-3'	GGGGCGGGATCCAACCTGATCCGAGGTCACATTTCAGAAAGTTGG GTGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTG CAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATT TCGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGAT CCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATG CCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGAT GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGC GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTT GATTCATTTTGAATTTTTGCTCAGAGCTGTAAGAAATACGTCCGCG AGGGGACTACAGAAAGAGTTTGGTTGGTTCCTCCGGCGGGCGCCT GGTTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCGAGGCA ACAGTTTGGTAACGTTCACATTGGGTTTGGGAGTTGTAAACTCGGT AATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACCCT TCACCTTCAAA	599	*T. asperellum*

T-20	OK147763	**ITS6 Forward:** 5'-GAAGGTGAAGTCGTAACAAGG-3'	GGCCTGGGGAAGAGGAGGGACATTACCGAGTTTACAACTCCCAAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCATAAAAGCCGGGAAGAAAAT	604	*T. asperellum*,
		ITS 4 Reverse: 5'-TCCTCCGCTTATTGATATGC-3'	GGGCGTTTTTTTTTGAGGGGGGGGCATCATACTGATCGAGGTCACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTTGAATTTTTGCTCAGAGCTGTAAGAAATACGTCCGCGAGGGGACTACAGAAAGAGTTTGGTTGGTTCCTCCGGCGGGCGCCTGGTTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCGAGGCAACAGTTTGGTAACGTTCACATTGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTTTCACCTTACAAAA	603	*T. asperellum*



a) PCR amplification of ITS6 and ITS6 (600 bp) gene of rDNAin *Trichoderma* isolates (T-8 and T-20), M is 1 KB



b) Phylogenetic tree based on ITS region of rDNA sequences

Plate 35 PCR amplification and phylogenetic analysis of potential *Trichoderma* isolates

Identification of potential *Pseudomonas* isolates

4.6.2.1 Cultural and morphological characteristic of selected bacterial isolates

The potential *Pseudomonas* isolates (Pf-2 and Pf-12) were identified as *P. fluorescens* based on the cultural and morphological characteristics. The results found out the two isolates were gram negative, rod shaped cells, creamy mucoid colony with smooth edges and yellow-green fluorescent pigmentation produced under ultraviolet (UV) light are presented in Table 4.19 and Plate 36.

Similar work done reported by Garrity *et al.* (2005); Verma *et al* (2015) who were confirmed the identity of fluorescent pseudomonads by Gram's staining technique, colony morphology, fluorescens and cell shape.

4.6.2.2 Molecular characterization of selected bacterial isolates.

Molecular identification of potential *Pseudomonas* isolates (Pf-2 and Pf-12) was performed by using Internal Transcribed Spacer (ITS) region of 16S rRNA gene sequences. ITS sequences of both the isolates were submitted to NCBI Gene Bank (MN783298 and MN783297), which showed 92.66 % and 89.75 % similarity with *Pseudomonas fluorescens* during BLAST analysis (Table 4.20). This was used for construction of phylogeny tree and subsequently, these isolates were identified as *P. fluorescens* (Plate 37).

It is inferred from the present study, that taxonomical knowledge on *Pseudomonas* isolates is important for molecular 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. Similar work done reported by Verma *et al* (2015) and Singh *et al*. (2022) who were confirmed the identity of fluorescent pseudomonads by molecular analysis.

Table 4.19 Morphological and cultural characteristics of bacterial isolates collected from rhizosphere soils

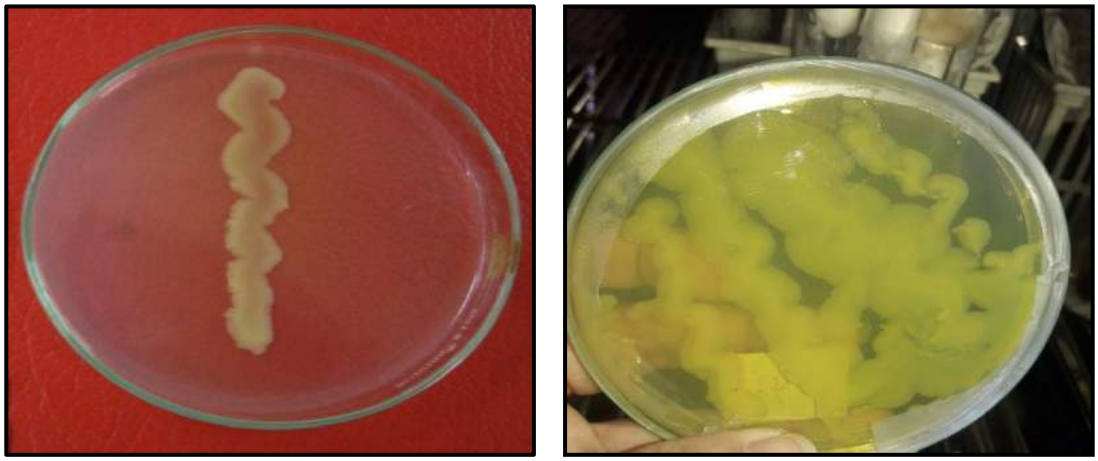
Sl. No	**Isolates**	**Shape**	**Surface**	**Colour**	**Flourescent under UV light**	**Gram Staining**
1	Pf-2	Rod	Creamy, Smooth edges	Greenish yellow	+ve	**-ve**
2	Pf-12	Rod	Creamy, Smooth edges	Greenish yellow	+ve	**-ve**

+ ve =positive

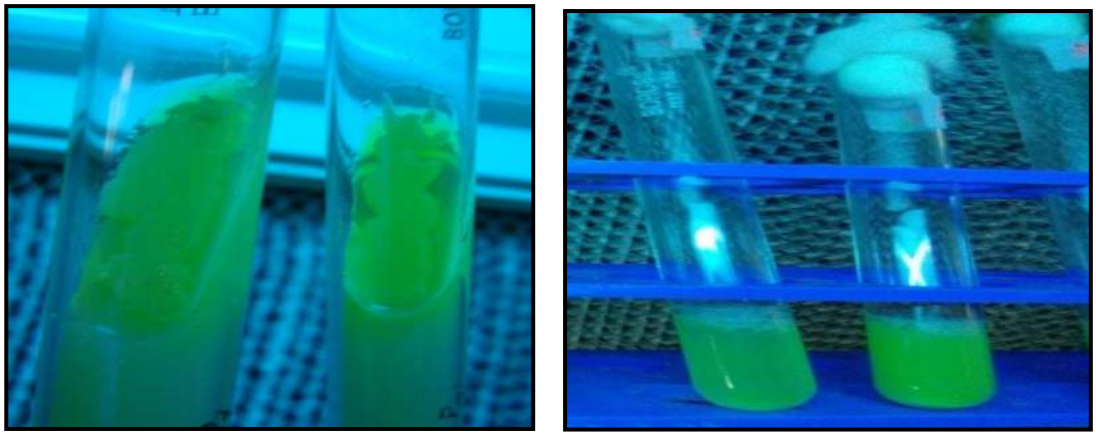
-ve = negative

Table 4.20. Internal Transcribed Spacer (ITS) region of rDNA sequence of potential native *Pseudomonas* isolates

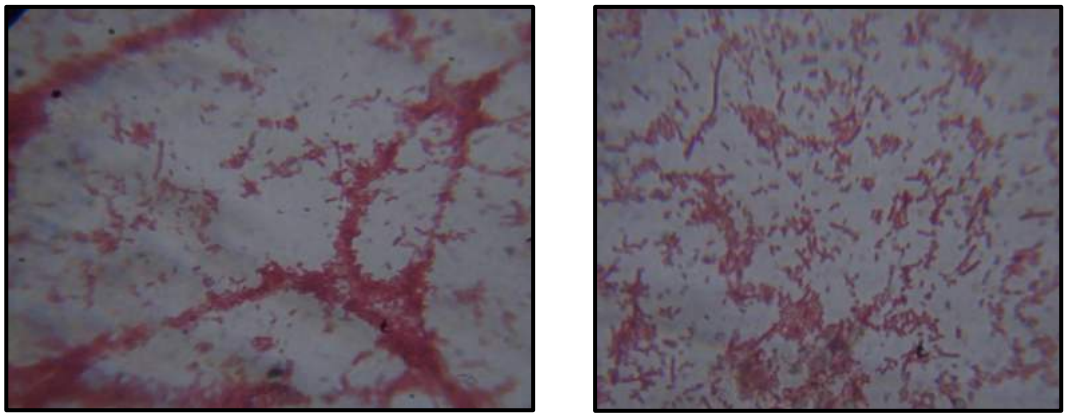
Isolate No.	Accession No.	Primer & their sequence	Sequence	Base pair	Similarity (%)
Pf-2	MN783298	**Forward (ITS1):** 5'-AAGT CGTAACAAGGT AG-3' **Reverse (ITS2):** 5'-GACCATA TATAACCCCAA G-3'	TGTGGAACCTGGTGGGGGCGACATTCGCCGGAAAATTCGATAA TACTGCAATTTGAGTAACTCACAAATTTTACCTTAGCCTGAATGG ACCAGTGAAAGTAACGTTCAGTCTATCTTCTATCACATACCCAAA TTTTTAAAGAACGAACTAGTCAAAGACTAGAAATCAACATTCACC ATCGCATGGATGGAATGCTCATTTCTAAGCTTAATACAAACAGA AGCAGTAGTGGTGGAGCCAAACGGGATCCAACCGCTGACCTCC GGCGGGCAAGGCAGGCGCTATCCCTGCTGACCTATGGCCCCT GATTTCTACAGGCGTTTCCCACACAAAATTGGTGGGTCTGGGCA GATTGCAACTGCCCACCCCACCCTTATCAGGGGTGCGCTCTAA CCAACTGAGCTCCAGACCCAATTTCGGGGTGCTTCTTTCGTGTT CTTCTTTGAATCTTGCAAGTCGTGTGGGAACTTAGGGGGCACCT CATGTGGTCCATATATGAGGTGATAAAGCCGTAAGTTCACCTAC GGCTACCTTGTTTAATACTTGTAGCATCTTATATTACCTGGGGG AGAGTGGTTAATTGTTATGAACAGTTGGGAGGGGGGGGGGGGG GGGGGGGGA	621	*P. fluorescens* (92.66%)
Pf-20	MN783297	**Forward (ITS1):** 5'-AAGT CGTAACAAGGT AG-3' **Reverse (ITS2):** 5'-GACCATA TATAACCCCAA G-3'	GGGGGGAAGGCGGCTGGATCCCTCCTTATCGACGACTCAGCT GCGGCCATAAGTTCCCACACGAATTGCTTGATTCATTGAAGAAG ACGAAGGAAGCAGCCCGAAGTTGGGTCTGTAGCTCTCTTGGTT AAAGCGCACCCCTGAAAATGGTGACGTCGGCTGTTCTAATCTG CCCGCACCCACCAATTTGGTGTGGGAAACGCCTTCGAAAATAC CGGGCCATAGCTTTTCTGGGAGAGCGCCTGCCTTGCATGGGGA GGTCAGCGGTTCGATCCCGCTTGGCTCCACCACTACTGCTTCT GTTTGTATAAAGCTTAAAATTGAGCATTCCAACTTTGCGATGGTG AATGTTGATTTCTAGTCTTTGACTAGTTCATTCTTGGAAATTTGG GTATGTGATGAAAGATAGACTGAAGTTACTTTCACTGGTGACGG ATTGGTTATGGTAAAAGTTCTGAGTGGCTAGATGGAGCTCAAAG GAGTTCGGCGAAAGTCGTCTTCACGTAGGACCCCTGCTTGGGG TTAGAATGGTC	533	*P. fluorescens* (89.75%)



a) Creamy mucoid and smooth edges on King's B agar plate

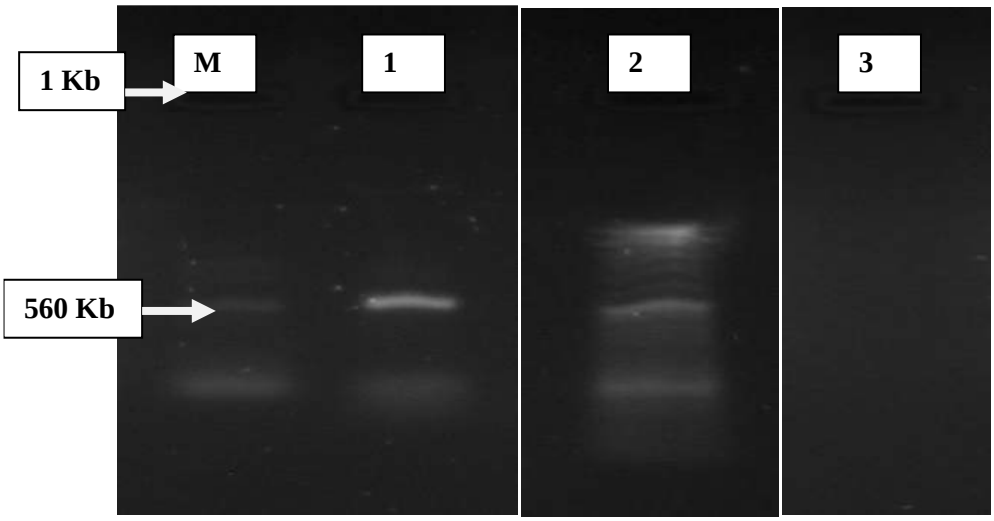


b) A yellow-green, fluorescent pigmentation in King's B agar slant (Pf-2 and Pf-12) and King's B broth (Pf-2 and Pf-12) under UV light

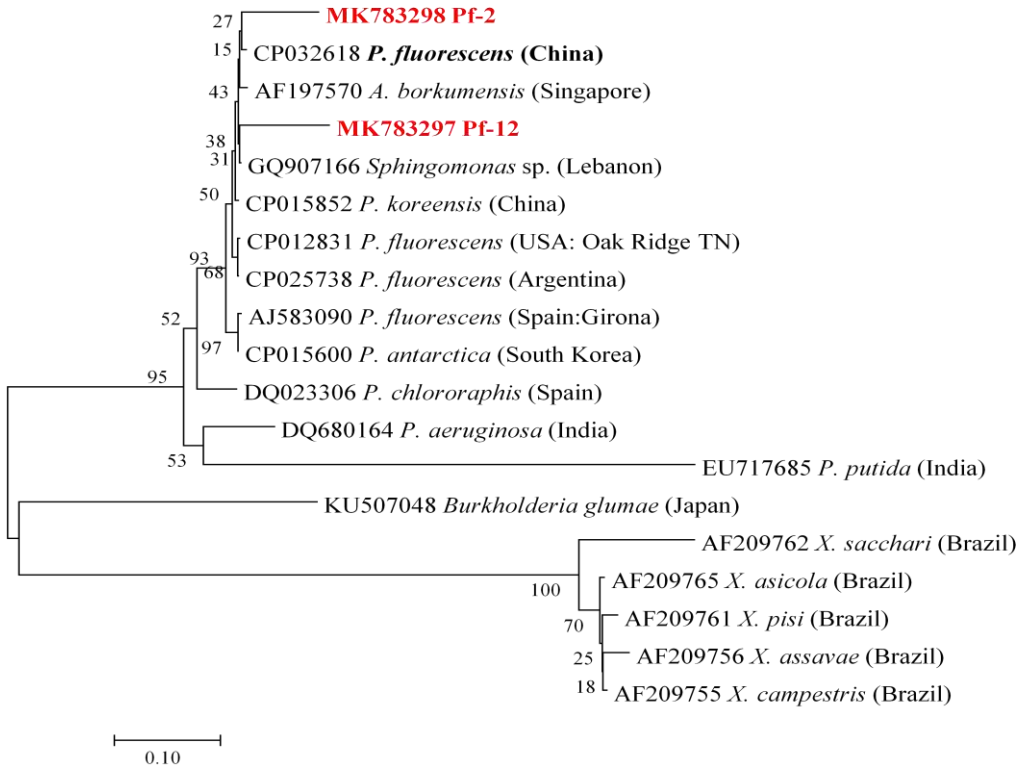


c) Microscopic view of rod shaped and gram negative

Plate 36 Morphological and cultural characterization of potential *Pseudomonas* isolates



a) PCR amplification, M= 1 Kb DNA ladder; lane 1 template from Pf-2 (560 Kb); lane 2 template from Pf-20 (560 Kb) and lane 3 template from negative control



b) Phylogenetic tree based on rDNA sequences of *Pseudomonas i*solates

Plate 37 PCR amplification and phylogenetic analysis of potential *Pseudomonas* isolates

4.7 *In vitro* compatibility of potential rhizospheric antagonists

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. The microorganisms showing positive compatibility among them were recorded. The data showed compatibility among all the treatment combinations of the four bioactive microorganisms *in vitro* (Table 4.21). No clear inhibition zone was observed between the tested microbial consortia (Plate 38 and 39). Absence of inhibition zone indicated that the potential isolates of *Trichoderma* and *Pseudomonas* were compatible with each other.

In this present investigation, all the potent bio control agents were compatible with each other. None of the organisms were found to inhibit each other and hence concluded that all the organisms taken are compatible with each other and can be used to develop consortia..

Similar work was observed by Zegeye *et al.* (2011) who 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. Further Mishra *et al.* (2013) also reported that isolates PBAP-10, PBAP-15, PBAP-17, PBAP-27 of fluorescent pseudomonads and isolates PBAT-1, PBAT-6, PBAT-38 and PBAT-43 of *Trichoderma* exhibited no or very little antagonism against each other. Fungal isolate PBAT-43 (*T. harzianum*) and bacterial isolate PBAP-27 (*P. fluorescens*) emerged as most compatible and efficient and therefore were used for development of mixed formulation. Harshita *et al*. (2018) was also of the view that reported the compatibility among the fungal (BCAs *in vitro.* Absence of inhibition zone indicated that the BCAs (*T. harzianum*, *B. subtilis* and *P. fluorescens*) were compatible with each other. The findings of present work are also in conformity with the findings of

Table 4.21 *In vitro* analysis of compatibility amongst microbial consortia

Treatment	Treatment combination	Compatible/Non-compatible (+/-)
T_1	Pf-2 + Pf-12	**+ve**
T_2	Pf-12 + T-8	**+ve**
T_3	Pf-12+ T-20	**+ve**
T_4	Pf-2 + T-8	**+ve**
T_5	Pf-2 + T-20	**+ve**
T_6	T-8 + T-20	**+ve**
T_7	Pf-2 + Pf-12 + T-8	**+ve**
T_8	Pf-2 + Pf-12+ T-20-	**+ve**
T_9	Pf-12 + T-8 + T-20	**+ve**
T_{10}	Pf-2 + T-8 + T-20	**+ve**
T_{11}	Pf-2 + Pf-12 + T-8 + T-20	**+ve**

+= compatible

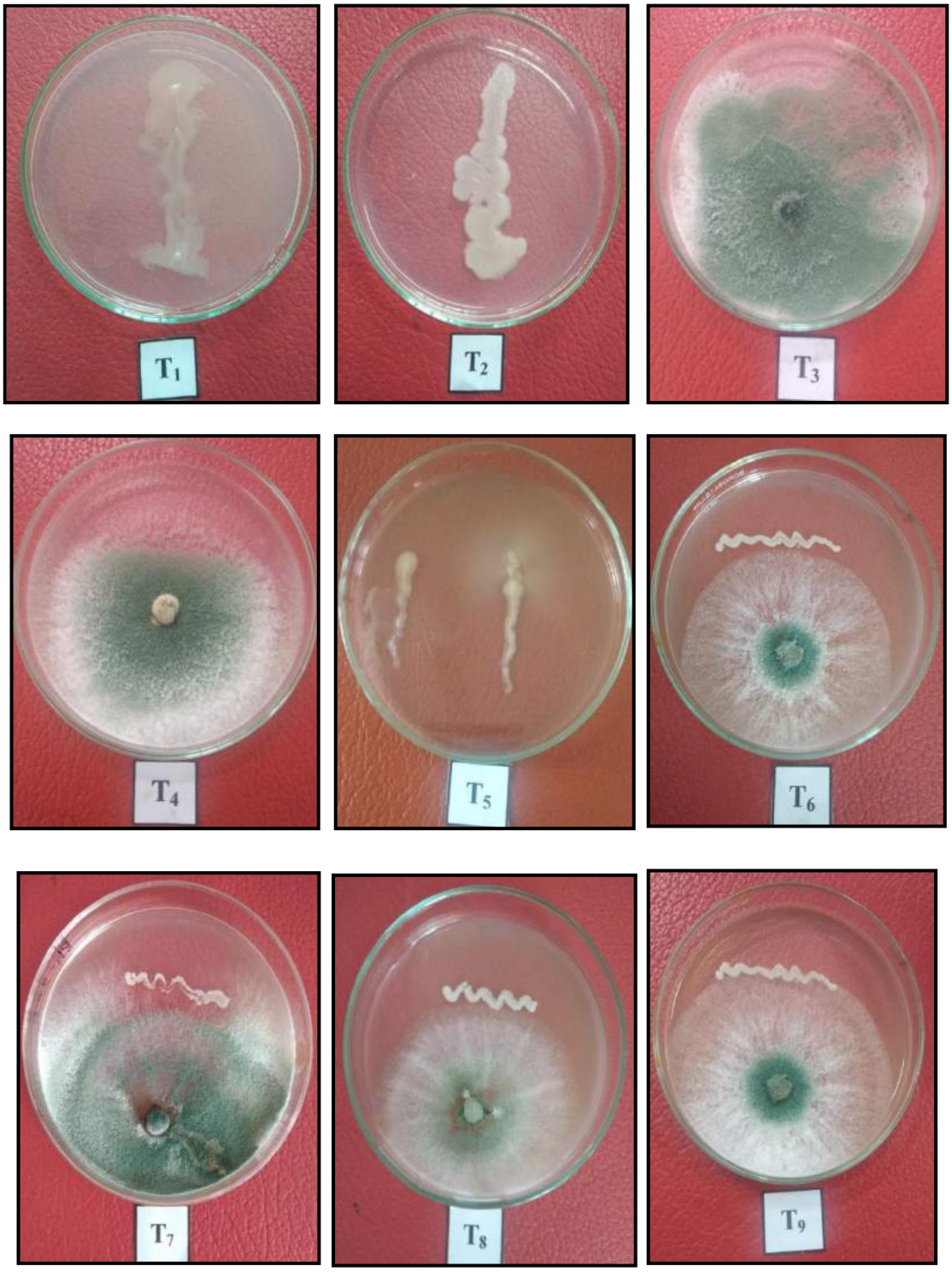
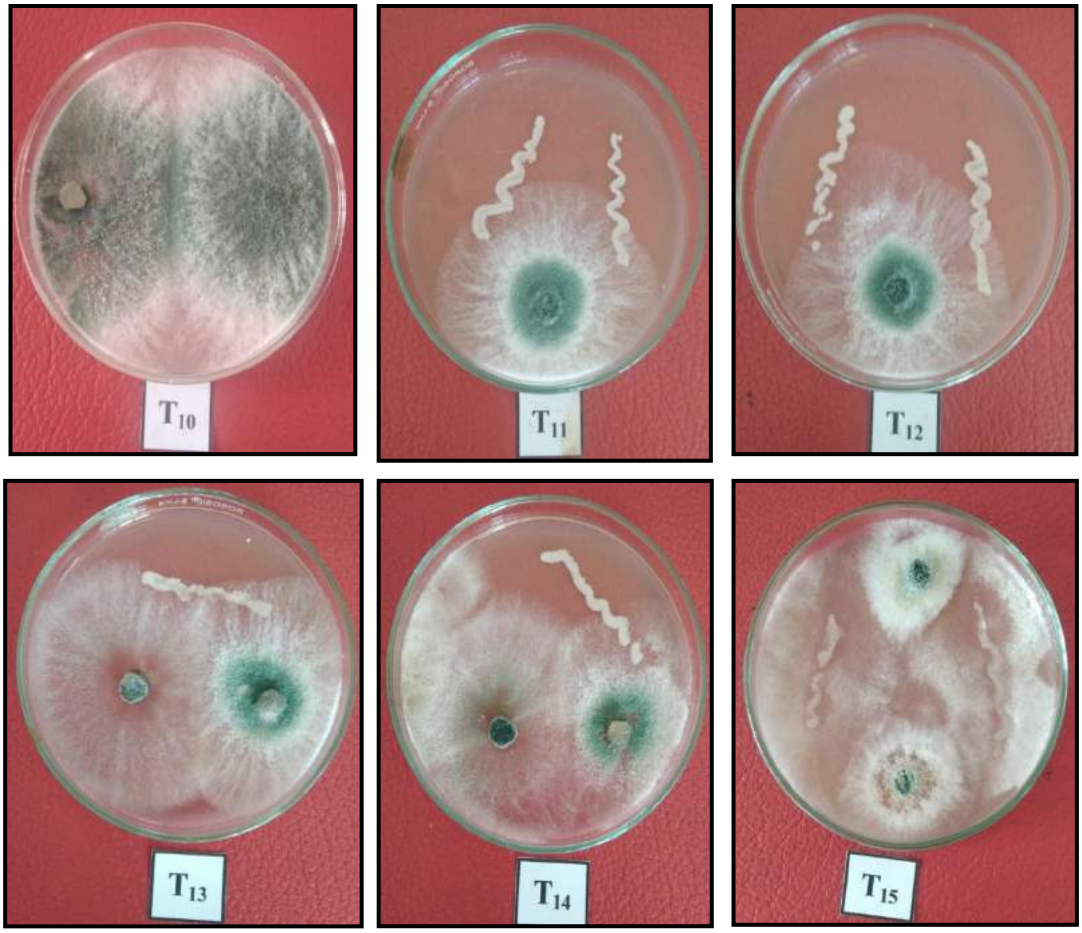


Plate 38 *In vitro* interactions amongst microbial consortia



T_1 (Control-I, Pf-2 alone)
T_2 (Control-II, Pf-12 alone)
T_3(Control-III, T-8 alone)
T_4 (Control-IV, T-20 alone)
T_5 (Pf-2 + Pf-12)
T_6 (Pf-12 + T-8)
T_7 (Pf-12 + T-20)
T_8 (Pf-2 + T-8)
T_9 (Pf-2 + T-20)
T_{10} (T-8 +T-20)
T_{11}(Pf-2 + Pf-12 + T-8)
T_{12}(Pf-2 + Pf-12 +T-20)
T_{13}(Pf-12+ T-8 +T-20)
T_{14}(Pf-2 + T-8+T-20)
T_{15} (Pf-2 + Pf-12 + T-8 +T-20)

Plate 39 *In vitro*interactions amongst microbial consortia

earlier workers (Siddiqui and Shaukat, 2003; Kumar *et al.,* 2014; Nath *et al.,* 2016 and Lalngaihawmi and Bhattacharyya, 2019).

4.8 *In vitro* antagonistic efficacy of compatible microbial consortia (CMC) against *S. rolfsii*

The inhibitory effects of compatible microbial consortia (CMC) were tested *in vitro* against *S. rolfsii* adopting dual culture bioassay technique. A total of 12 treatment combinations were compared and produced varying inhibitions (%) *in vitro* against *S. rolfsii* (Table 4.22; Fig 4.7). All consortia tested against *S. rolfsii* were found to significantly inhibit the mycelial growth of *S. rolfsii* (Plate 40).

Among the different consortia sets tested *in vitro* the highest inhibition of pathogen was recorded in T_{11} [Pf-2 + Pf-12 + T-9 +T-20 (83.75 %)] followed by T_7 (76.40 %) at 5 days after incubation at 25 ±2 °C.

In this present study, the reason behind the use of consortia against *S. rolfsii* is that multiple potent isolates allow the deployment of several different biocontrol mechanisms simultaneously and has more significant effect than single bioagent used. Hence, based on the results obtained from this study the best microbial consortia CMC (Pf-2 + Pf-12 + T-8+ T-20) inhibited the pathogen significantly and was found to be the most effective consortia and used for further pot studies.

The results of present findings are similar with the report of Nath *et al.* (2016) that the antagonistic potential of CMC of *T. parareesei*, *P. fluorescens*, *B. subtilis* and *Azotobacter chroococcum* against soil-borne pathogen *R. solanacearum.* The highest inhibition of test pathogen was recorded from

Table 4.22 *In vitro* antagonistic effect of compatible microbial consortia on radial growth and per cent inhibition of *S. rolfsii*

Treat.	Treatment combination	Inhibition of *S. rolfsii* growth		
		Radial growth (cm)	**Radial growth (cm) inhibited**	**Combine Inhibition (%)**
T_0	Control *S.rolfsii* alone	3.90	0.00	00.00 (4.05)
T_1	Pf-2 + Pf-12	0.96	2.93	75.20 (60.29)
T_2	Pf-12 + T-8	1.05	2.85	73.07 (58.74)
T_3	Pf-12+ T-20	1.20	2.70	69.22 (56.32)
T_4	Pf-2 + T-8	0.95	2.95	75.63 (60.42)
T_5	Pf-2 + T-20	1.13	2.70	69.53 (57.60)
T_6	T-8 + T-20	1.08	2.80	72.21 (58.21)
T_7	**Pf-2 + Pf-12 + T-8**	**0.94**	**2.93**	**76.40 (60.94)**
T_8	Pf-2 + Pf-12+ T-20-	1.14	2.70	70.76 (57.26)
T_9	Pf-12 + T-8 + T-20	0.96	2.91	75.29 (60.20)
T_{10}	Pf-2 + T-8 + T-20	1.13	2.73	71.02 (57.43)
T_{11}	**Pf-2 + Pf-12 + T-8 + T-20**	**0.63**	**3.26**	**83.75 (62.24)**
SEm±		0.00	0.00	0.25
C.V. (%)		6.78	4.84	4.47
CD (*p*= 0.01)		0.19	0.22	6.92

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

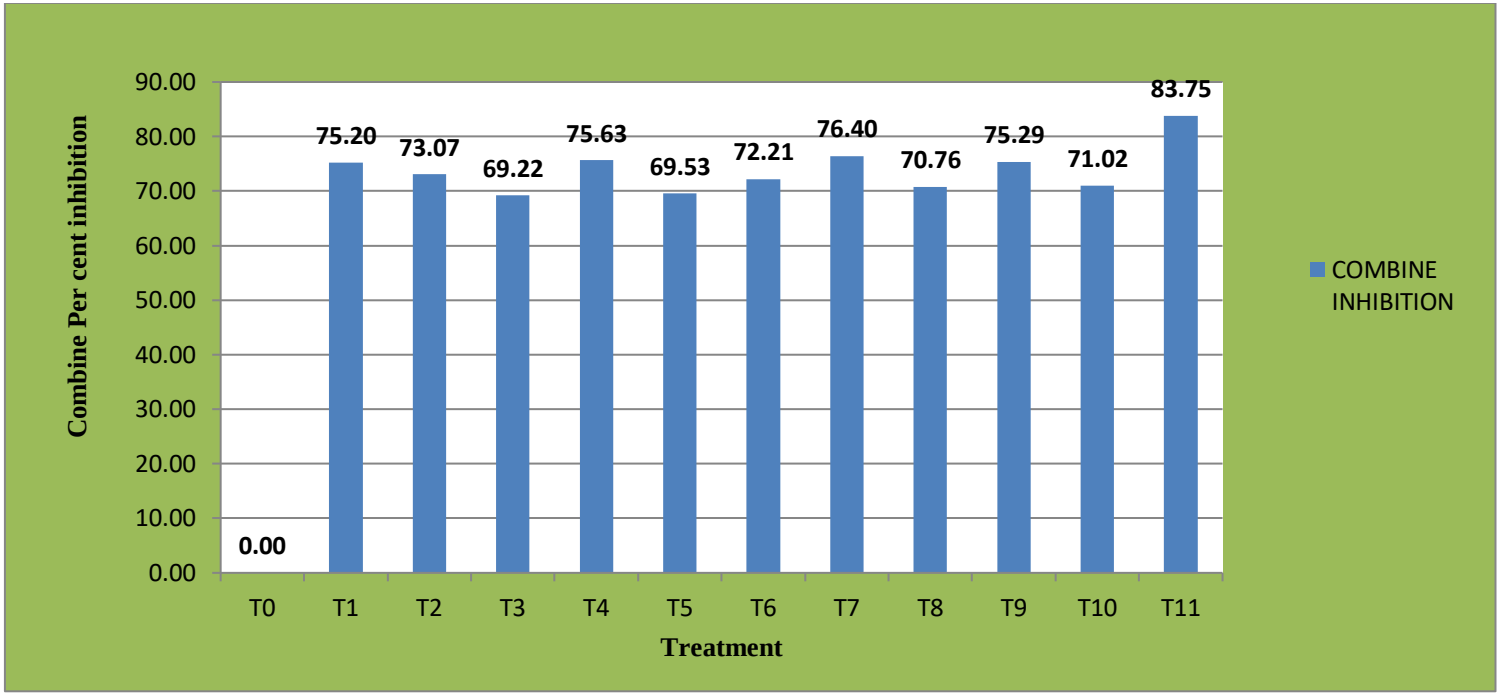
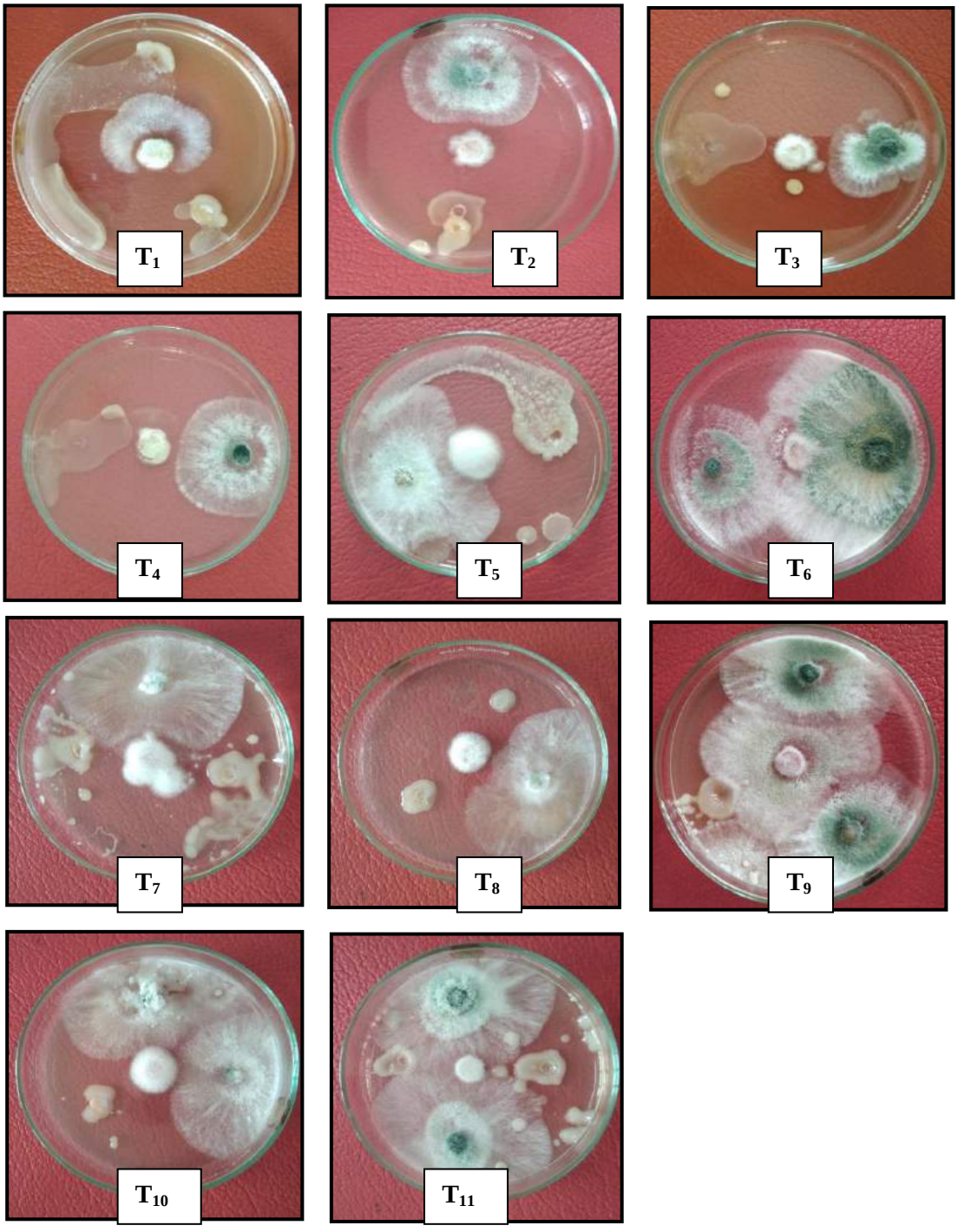


Fig 4.7 *In vitro* antagonistic effect of compatible microbial consortia on per cent inhibition of *S.rolfsii*



T_0 (Control,*S. rolfsii* alone)
T_1 (Pf-2 + Pf-12)
T_2 (Pf-12 + T-8)
T_3 (Pf-12 + T-20)
T_4 (Pf-2 + T-8)
T_5(Pf-2 + T-20)
T_6 (T-8 +T-20)
T_7 (Pf-2 + Pf-12 + T-8)
T_8 (Pf-2 + Pf-12 +T-20)
T_9 (Pf-12+ T-8 +T-20)
T_{10} (Pf-2 + T-8+T-20)
T_{11} (Pf-2 + Pf-12 + T-8 +T-20)

Plate 40 *In vitro* antagonistic effect of CMC on radial growth of *S. rolfsii*

consortia of *T. parareesei* + *P. fluorescens* + *B. subtilis* + *A. chroococcum* (91.10 %) followed by *T. parareesei* + *P. fluorescens* + *B. subtilis* (81.10 %) and *P. fluorescens* + *B. subtilis* + *A. chroococcum* (68.14 %). Further Lalngaihawmi and Bhattacharyya (2019) reported that the antagonistic potential of the three promising *Trichoderma* spp. individually as well as in combinations against *F. oxysporum* f.sp. *cubense.* The result revealed that the efficacy of all the treatments differed significantly with that of control 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. These results are also similar to those findings of earlier workers Sundaramoorthy and Balabaskar, 2013; Khan *et al.,* 2018; Vrieze *et al.*, 2018 and Mahendra *et al*., 2022.

4.9 *In vitro* evaluation of plant growth promoting ability of microbial isolates

In vitro study conducted to check out the efficacy of selected best antagonists individually *viz.*, Pf-2, Pf-12, T-8 and T-20 and their consortia (Pf-2+Pf-12+T-8+T-20) on seed germination, seedling vigour index, seedlings shoot length, root length, fresh and dry weight of shoot and fresh and dry weight of root was carried out (Srinivasan and Mathivanan, 2009). The results obtained are presented in Table 4.23 and illustrated in Fig 4.8 and 4.9.

The result observation on per cent germination of different treatments was recorded on 10 DAS. French bean seed germination per cent was significantly higher in T_5 [CMC (89.50 %)] followed by T_3 [Pf-2] with 81.75

Table 4.23 *In vitro* effects of CMC on French bean seed germination (%), seedling shoot length, root length 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(T-8)	80.25 (63.79)	5.92	991.7	237.0	5.05	173.2	43.00	883.05
T_2(T-20)	79.75 (63.25)	5.85	990.5	233.5	5.00	173.0	43.00	865.27
T_3(Pf-2)	81.75 (64.71)	6.07	1003.2	239.2	5.10	177.5	45.50	913.10
T_4(Pf-12)	81.50 (64.53)	6.02	997.5	237.7	5.02	176.0	44.50	889.95
T_5(CMC)	**89.50 (71.09)**	**7.02**	**1228.2**	**294.0**	**6.42**	**223.0**	**67.50**	**1201.05**
T_6(Chemical control)	70.25 (56.09)	4.77	679.5	160.7	4.35	105.2	15.25	640.65
T_7(Control)	66.75 (54.79)	4.37	646.0	151.5	4.15	99.75	14.00	568.65
SEm±	0.15	0.03	1.388	1.29	0.02	0.774	0.18	1.12
C.V. (%)	1.36	4.53	1.04	4.07	3.38	3.36	3.34	1.16
CD (*p*= 0.01)	2.14	0.51	19.4	18.10	0.33	10.85	2.61	19.91

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

Table 4.24 *In vitro* effects of CMC on per cent increase of French bean seed germination (%), shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and seedling vigour index at 10 DAS

Treatment	Per cent increase 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(T-8)	20.22	35.46	53.51	56.43	21.68	73.63	182.0	55.30
T_2(T-20)	19.46	33.86	53.32	54.12	20.48	73.43	182.0	52.16
T_3(Pf-2)	22.47	38.90	55.29	57.88	22.89	77.94	225.0	60.58
T_4(Pf-12)	22.09	37.75	54.41	56.89	20.96	76.44	217.8	56.50
T_5(CMC)	34.08	60.64	90.12	94.05	54.69	123.6	383.5	111.2
T_6(Chemical control)	5.24	7.78	5.18	6.07	4.81	5.46	7.894	12.67
T_7(Control)	-	-	-	-	-	-	-	-

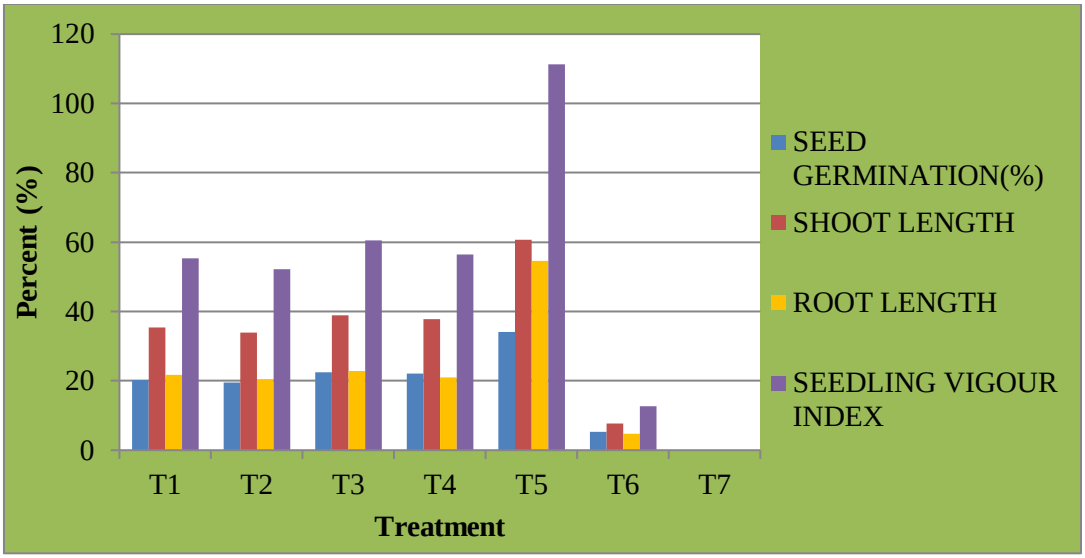


Fig 4.8 *In vitro* effects of per cent increase of French bean seed germination (%), shoot length, root length and seedling vigour index at 10 DAS

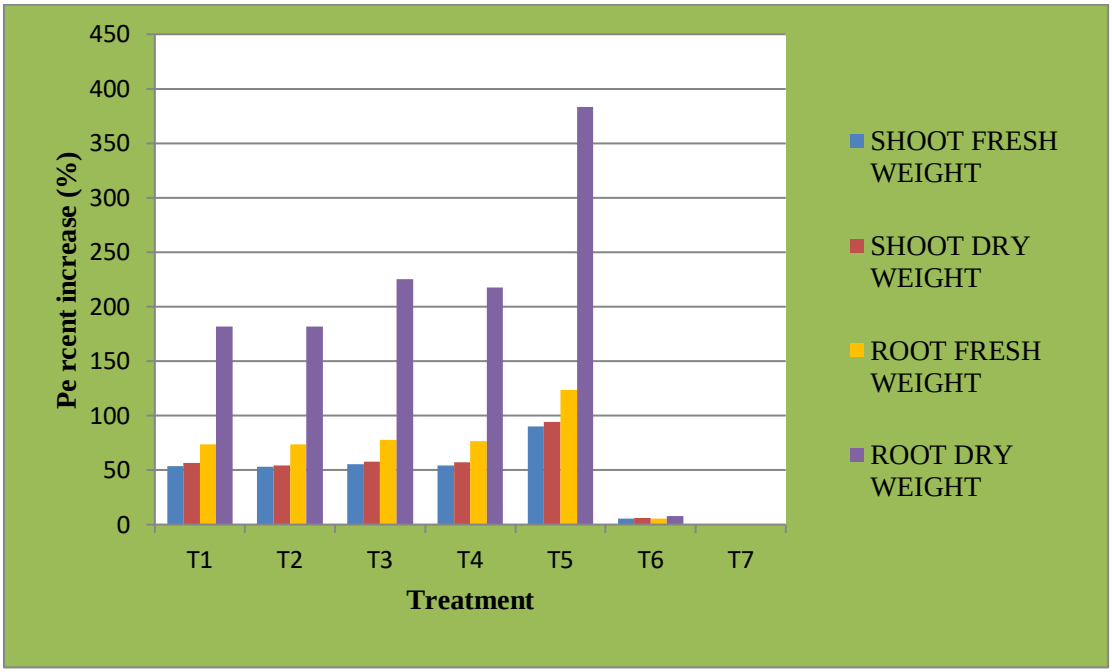
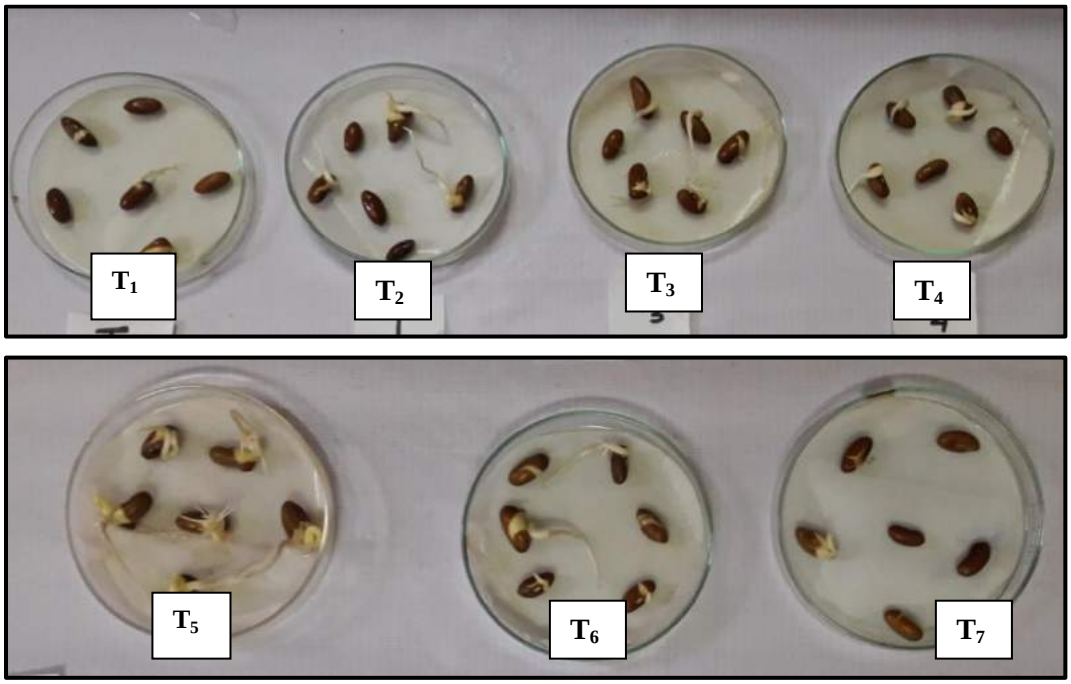
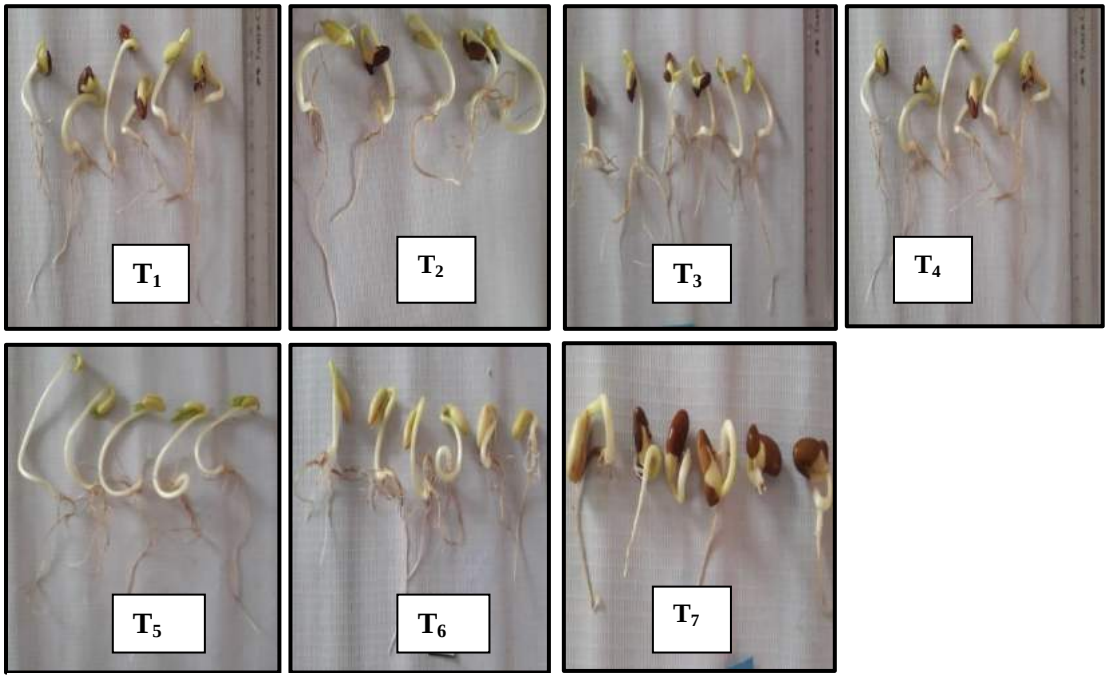


Fig 4.9 *In vitro* effects of per cent increase of French bean shoot fresh and dry weight, root fresh and dry weight at 10 DAS



a) Effect of compatible microbial consortia (CMC) on seed germination of French bean at 7 DAS



b) Effect of compatible microbial consortia (CMC) on seedlings length and root length of French bean at 10 DAS

Plate 41 Effect of compatible microbial consortia (CMC) on French bean seed germination and seedling length

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% which is statistically at par with T_4 [Pf-12 (81.50 %)], T_1 [T-8 (80.25 %)] and T_2 [T-20 (79.75 %)]. The lowest seed germination per cent was observed in T_7 [control plate (66.75 %)] (Plate 41a). The experimental result revealed that T_5 increased seed germination per cent over control treatment with 34.08 %.

The above results are in conformity with the works done by Murthy *et al*. 2013 who reported that *Bacillus* spp. have the rapid multiplication ability in the immediate proximity of germinating seedlings thus increasing the probability of establishment of antagonist in individual roots. Nazir *et al*. (2011) also observes an increased seed germination of tomato and chilli by application of *T. viride* and *T. harzianum*. Further Eutesari *et al*. (2013) also reported that seed germination per cent and seedling growth indices showed improvement when *T. harzianum*, *T. viride*, *T. viride* and *P. fluorescens* were applied on soybean seedlings

Root length and shoot length of individual French bean seedlings (60 seedlings/ treatment) were measured. The data on shoot length is depicted in Table 4.23. Shoot length was longer in all the treated treatments as compared to the control (Plate 41b). Among these treatments, significantly longer shoot was recorded in T_5 (7.02 cm) which is followed by T_3 with 6.07 cm which is at par with T_4 (6.02 cm). Minimum shoot length was observed in T_7 (3.02 cm). This experimental result revealed that T_5 increased shoot length (60.64 %) over control treatment.

Root length was also longer in all the treatments as compared to the control. Among these treatments, significantly higher root was recorded in T_5 (6.42 cm) than the other treatments tested. This was followed by T_3 (5.10 cm) which was found to be statistically at par with T_1 (5.05 cm). Minimum root length was observed in control (4.35 cm). This experimental result revealed that the T_5 increased root length over control treatment with 54.69 %.

The above results are in conformity with findings of Murthy *et al.* 2013 that maximum shoot length (5.76 cm) and root length (8.55 cm) was obtained from seeds treated with*T. harzianum*+ *T. asperellum* + *T. viride.* Kabir *et al.* (2013) also reported that PBGR isolate B110 showed the highest shoot and root growth with 26% and 35% increments respectively. Similar findings were also reported by Eutesari *et al.* 2013.

The vigour index of French bean seedlings was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in number. Seedling vigour index was higher in all the treatments as compared to the control (Table 4.23). Among these, significantly higher seedling vigour index was recorded in T_5 (1201.05) followed by T_3 (913.10). Minimum seedling vigour index was observed in control (568.65). This experimental result revealed that the T_5 increased vigour index over control treatment with 111.2 %.

The above findings are in harmony with the observation of Sudharani *et al.* (2014) who reported that combination of *Azotobacter chroococcum* + *B. megaterium* + *P fluorescens* + *B. subtilis* + *T. harzianum* showed enhanced vigour of cabbage sedlings. Bakthavatchalu *et al.* (2013) also recorded higher seed germination (92.0%) with an overall better seed vigour index when cowpea seeds were treated with *P. aeruginosa* FP6.

The fresh weight of shoot and root of individual French bean seedlings (60 seedlings/ treatment) were measured. Fresh weight of seedling shoot was higher in all the treatments as compared to the control. Among these treatments, significantly higher fresh weight of shoot was recorded in T_5 (1228.2 mg) than the other treatments. This was followed by T_3 (1003.2 mg) which is statistically at par with T_4 (997.5 mg), T_1 (991.7 mg) and T_2 with 990.5 mg. Minimum fresh weight of shoot was observed in T_7-control (646.0

mg). This experimental result also revealed that the T_5 increased fresh weight of shoot (90.12 %) over control treatment.

Root fresh weight was also higher in all the treated treatments as compared to the control. Among these treatments, significantly higher fresh weight of root was recorded in T_5 (223.0 mg) than the other treatments which was followed by T_3 (177.5 mg) statistically at par with T_4 (176.0 mg), T_1 (173.2 mg) and T_2 (173.0 mg). Minimum root fresh weight was observed in control (0.10 mg). This experimental result revealed that the T_5 increase root fresh weight over control treatment with 123.6 %.

The findings of the present work are in aggrement with the work done by Murthy *et al.* 2013 who reported 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).

The dry weight of root and shoot of individual seedlings (60 seedlings /treatment) were measured after oven drying at 60° C for 24 hrs and expressed in mg. Dry weight of seedling shoot was also higher in all the treated treatments as compared to the control. Among these treatments, significantly higher dry weight of shoot was recorded in T_5 (294.0 mg) than the other treatments. Next followed by T_3 (239.2 mg) which is statistically at par with T_4 (237.7 mg). Minimum shoot dry weight was observed in control (151.5 mg). This experimental result revealed that the T_5 increased shoot dry weight (94.05 %) over control treatment

Root dry weight was also higher in all the treated treatments as compared to the control. Among these treatments, significantly higher dry weight of root was recorded in seed treated with T_5 (67.50 mg) than the other treatment tested which was followed by T_3 (45.50 mg), T_4 (44.50 mg),

T_1(43.00 mg), T_2 (43.00 mg) which were found to be statistically at par with each other. Minimum root dry weight was observed in control (14.00 mg). This experimental result also revealed that the T_5 increased root dry weight (383.5 %) over control treatment.

The above findings confirm the work of Eutesari *et al.* (2013) who reported that seedling growth indices namely root length, seedling length and dry weight of root showed improvement when *T. harzianum, T. viride, T. atroviride* and *P. fluorescens* were applied on soybean seedlings. Also increased seedlings and leaf area per plant and total chlorophyll amount was reported. The findings of present work are in harmony with the findings of earlier workers (Zaidi and Singh (2004); Raja *et al*. (2006); Kumar *et al.,* 2010; Sudharani *et al*. (2014); Verma *et al* (2015) and Singh *et al*. (2019).

4.10 *In vivo* evaluation of microbial compatible consortia (CMC) against collar rot of French bean under pot conditions

The study was conducted to test the efficacy of selected best consortia through seed treatment and soil drenching in checking plant growth promoting traits and controlling collar rot of French bean under pot conditions during 2018 and 2019. In the present study, the application of microbial consortia (Pf-2+Pf-12+T-8 +T-20) at regular interval (0, 15 and 30 DAS) had effectively increased on seed germination, seedling vigour index, seedlings shoot length, root length, fresh and dry weight of shoot and fresh and dry weight of root.

4.11.1 Per cent germination at 10 DAS

Pooled data of the seed germination per cent was significantly higher in all CMC treated treatments. Datas are presented in Table 4.25 and illustrated in Fig 4.10. Highest per cent germination was found in treatment T_1 (85.12 %), T_2 (85.00 %); T_3 (84.87%) and T_4 (80.75 %) which were statistically at par with

Table 4.25 Effects of CMC on French bean seed germination (%) at 10 DAS

Treatment	Seed Germination(%) at 10 DAS			Per cent increase over positive control
	2018	2019	Pooled	
T_1	**86.25(68.43)**	**84.00(66.67)**	**85.12(67.49)**	**42.46**
T_2	86.25(68.51)	83.75(66.50)	85.00(67.39)	42.26
T_3	85.75(67.96)	84.00(66.71)	84.87(67.18)	42.04
T_4	82.50(65.29)	79.00(62.83)	80.75(64.02)	35.14
T_5	72.50(58.53)	71.50 (57.78)	72.12(58.14)	20.70
T_6	61.50(51.66)	58.00(49.61)	59.75(50.63)	-
T_7	72.00(58.05)	72.00(58.12)	72.00(58.06)	20.50
SEm±	0.50	0.77	0.48	
C.V. (%)	4.49	7.16	4.38	
CD (*p*=0.05)	5.16	8.01	4.97	

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

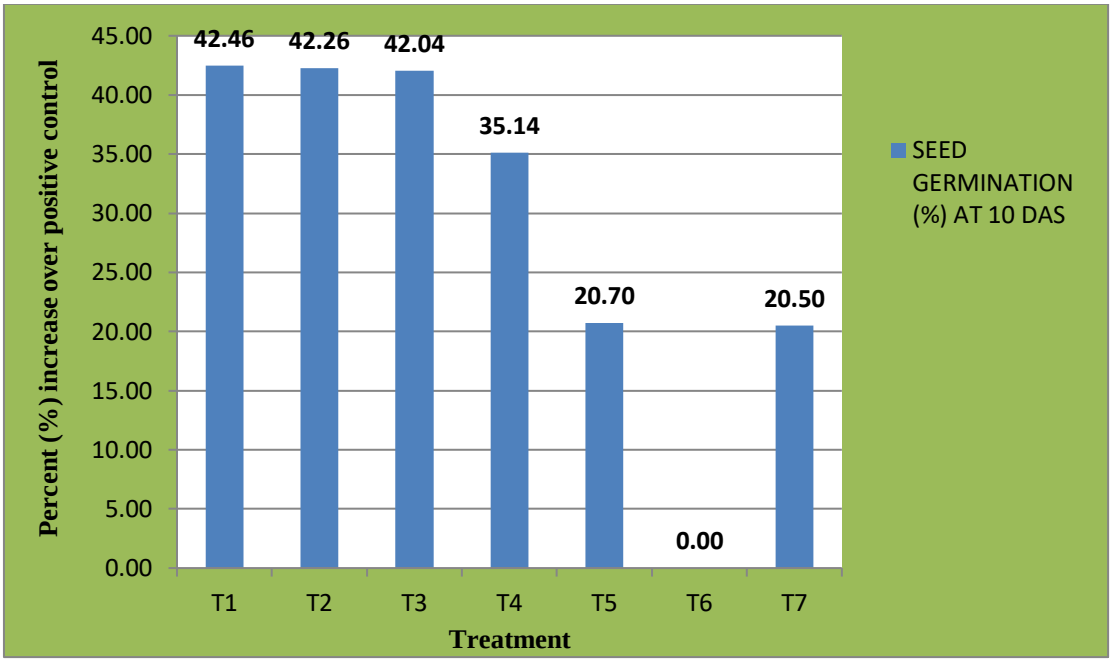

Fig 4.10 Effects of CMC on French bean seed germination (%) at 10 DAS

each other. The lowest seed germination per cent was observed in T_6 [positive control (59.75 %)] treatment. This experimental result also revealed that the per cent increase of seed germination over control was maximum on T_1 with 42.46 %.

The improvement in French bean seed germination might be due to food reserve mobilization by bioagents. The results of the present findings confirm the work of Maiyappan *et al*. (2010) who reported the efficacy of microbial consortium of four rhizobacteria against *S. rolfsii*, *F. oxysporum* and *R. solani* recorded with high per cent seed germination of green gram under pot condition.

4.11.2 Shoot length and root length (cm) at 20 DAS and 60 DAS

The shoot and root length of individual French bean (20 plants/treatment) were measured at 20 DAS and 60 DAS. The data on shoot length are depicted in Table 4.26. Shoot length was longer in all the treated treatments as compared to control. At 20 DAS pooled data revealed that there was not much significant difference recorded among the CMC treated treatments *i.e*., T_1, T_2, T_3 and T_4 (15.85 cm, 15.75 cm, 15.47 cm and 14.45 cm) which were statistically at par with each other. Interestingly at 60 DAS pooled data recorded that, T_1 (26.9 cm) showed highest shoot length which was statistically at par withT_2 (25.5). Minimum shoot length was observed in T_6 control at 20 and 60 DAS (7.77 cm and12.8 cm). This experimental result revealed that the T_1 increased shoot length with 104.5 % at 20 DAS and 110.5% at 60 DAS over control treatment (Table 4.27and Fig 4.11).

Similarly, root length of individual French bean (20 plants/ treatment) was measured at 20 DAS and 60 DAS. The result of root length is presented in Table 4.25 and illustrated in Fig 4.11.

Table 4.26 Effects of CMC on shoot and root length of French bean plant at 20 and 60 DAS

Treatment	Shoot length (cm)at 20 DAS			Shoot length (cm)at 60 DAS			Root length (cm) at 20 DAS			Root length (cm) at 60 DAS		
	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled
T_1	**15.95**	**15.80**	**15.85**	**27.37**	**26.50**	**26.95**	**9.17**	**9.12**	**9.15**	**17.12**	**16.25**	**16.68**
T_2	15.80	15.72	15.75	25.15	25.10	25.12	9.15	9.15	9.15	13.90	14.25	14.06
T_3	15.50	15.47	15.47	25.50	25.50	25.52	9.15	9.02	9.08	13.95	14.32	14.13
T_4	14.52	14.40	14.45	24.17	23.20	23.70	8.37	8.05	8.21	13.17	14.02	13.58
T_5	9.87	9.75	9.80	16.22	15.52	15.87	5.75	5.77	5.76	10.87	10.92	10.88
T_6	8.00	7.60	7.77	13.12	12.50	12.80	4.80	4.72	4.76	8.80	8.55	8.66
T_7	9.95	9.82	9.87	16.30	16.05	16.15	5.77	5.80	5.78	10.95	11.15	11.02
SEm±	0.07	0.08	0.08	0.12	0.21	0.13	0.05	0.06	0.06	0.15	0.19	0.16
C.V. (%)	4.13	4.93	4.40	4.04	6.97	4.63	5.14	6.56	6.33	8.34	10.5	8.90
CD (*p*=0.05)	0.77	0.91	0.84	1.25	2.11	1.42	0.56	0.71	0.68	1.15	1.97	1.66

Table 4.27 Effects of CMC on per cent increase of shoot and root length of French bean plant at 20 and 60 DAS over positive control

Treatment	Per cent increase of shoot and root length over positive control			
	Shoot length		Root length	
	20 DAS	60 DAS	20 DAS	60 DAS
T_1	**104.5**	**110.54**	**93.1**	**92.60**
T_2	103.2	96.25	92.6	62.35
T_3	99.6	99.37	89.8	63.15
T_4	86.4	85.15	69.8	56.91
T_5	26.4	23.98	21.1	25.63
T_6	-	-	-	-
T_7	27.3	26.17	21.4	27.25

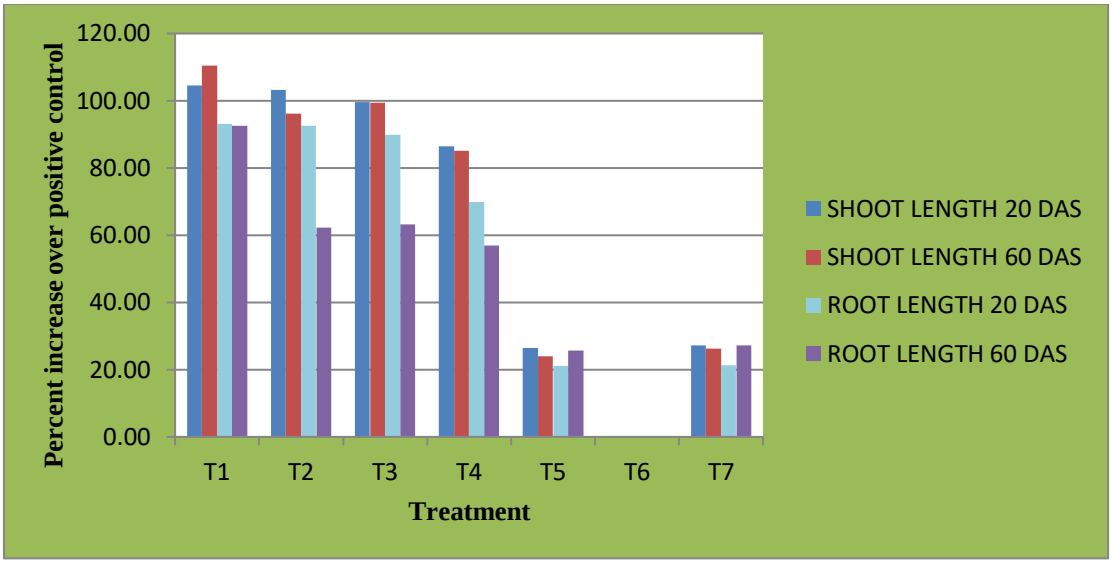

Fig 4.11 Effects of CMC on per cent increase of shoot and root length of French bean plant at 20 and 60 DAS over positive control

At 20 DAS, pooled data of the results revealed that T_1, T_2 and T_3 did not differ significantly with 9.17cm, 9.15 cm and 9.02cm respectively. Interestingly at 60 DAS, pooled data revealed that T_1 (16.68 cm) gave highest root length. This was followed by T_3 (14.13 cm) which is statistically at par with T_2 (14.06 cm) and T_4 (13.58 cm). Minimum root length was observed in control (4.75 cm at 20 and 8.6 cm at 60 DAS). This experimental result revealed that the T_1 increase root length with 93.1 % at 20 and 92.60 % at 60 DAS over positive control treatment.

The increase in plant shoot and root length may be due to increase in the levels of growth hormones *viz.*, IAA, gibberellic acid and defense enzymes *viz.*, peroxidase, polyphenol oxidase and superoxide dismutase as reported by Biam and Majumdar, 2019. Indole-3 acetic acid (IAA) is one of the most physiologically active auxins which is a common product of L-tryptophane metabolism produced by several microorganism PGPR. Microgranism isolated from rhizosphere region of various crop have an ability to produce IAA as secondary metabolites due to rich suppy of substarates. IAA helps in production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uotake (Lynch, 1985)

The results of the present findings are in agreement with the findings of earlier worker Kumar *et al.* (2010) who reported that combined application of *T. harzianum* and *P. fluorescens* resulted in significant growth of seedling (18.38 cm at 30 DAS) in sweet pepper. Similarly 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. They further added that there was significant increased in root and shoot 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) and root length (27.75 cm) was recorded when

lettuce plants was treated with application of consortia of *T. viride* + *B. thuringiensis* + *P. fluorescens*.

4.11.3 Effect of CMC on plant vigour index (VI) at 20 and 60 DAS

The vigour index of French bean plant was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in number. Plant vigour index was higher in all the CMC treated treatments as compared to positive control treatment (T_6) at 20 and 60 DAS as shown in Tables 4.28 and illustrated in Fig 4.12. At 20 DAS, higher plant vigour index was recorded from T_1 (2154.1) which is statistically at par with T_2 (2112.2) and T_3 (2079). Minimum seedling vigour index was observed in T_6 [positive control (750.3 at 20 DAS)]. But at 60 DAS, significantly higher plant vigour index was recorded from T_1 (3695.2) than other treatment. Also, percent increase in vigour index over positive control was observed to be highest at T_1 at both 20 DAS and 60 DAS (187.1 % and 183.5 %).

The increase in plant vigour index upon microbial consortia treated treatments may be due to increased seed germination percentage, shoot length and root length of French bean plants. The findings of present work are in conformity with the findings of earlier worker Jhumishree *et al*. (2018) who reported that significant enhancement in shoot, root length and vigour index of plants was observed on seed treatment with *Trichoderma* isolates and *P. fluorescens* as compared untreated plant. They found maximum plant vigor index was recorded on treatment with Tr-7, *i.e*., 3383.3 with 26.5cm shoot length and 7.3cm root length followed by 3296.7, 3066.7 and 2791.2 with Tr-6, Tr-2 and Tr-1, respectively, in variety JG14 as compared to 1589 and 2149.3 in pathogen treated control and untreated control, respectively. Similarly Bhakthavatchalu *et al.* (2013); Eutesari *et al.* (2013); Sudharani *et al*. (2014) and Singh *et al*. (2022) also reported the plant growth promoting abilities of BCAs enhanced seedling vigour over control.

Table 4.28 Effects of CMC on French bean plant vigour index and per cent increased over control at 20 and 60 DAS

Treatment	Vigour index at 20DAS			Per cent increase over positive control	Vigour index at 60DAS			Per cent increase over positive control
	2018	2019	Pooled		2018	2019	Pooled	
T_1	**2216.6**	**2091.6**	**2154.1**	**187.1**	3803.6	3586.8	3695.2	183.5
T_2	2147.6	2077.0	2112.2	181.5	3363.7	3291.3	3327.5	155.2
T_3	2109.4	2049.6	2079.5	177.2	3378.5	3343.2	3360.8	157.8
T_4	1864.5	1769.6	1817.0	142.1	3069.0	2938.8	3003.9	130.4
T_5	1134.9	1108.2	1121.5	49.4	1964.2	1887.6	1925.9	47.7
T_6	787.0	713.4	750.3	-	1346.8	1218.0	1303.4	-
T_7	1130.4	1123.2	1126.7	50.1	1958.4	1951.2	1954.7	49.9
SEm±	11.77	16.91	10.97	-	19.20	28.0	18.01	-
C.V. (%)	5.06	7.58	4.81	-	4.98	7.53	4.753	-
CD (*p*=0.05)	121.1	174.1	147.6	-	197.6	288.3	191.5	-

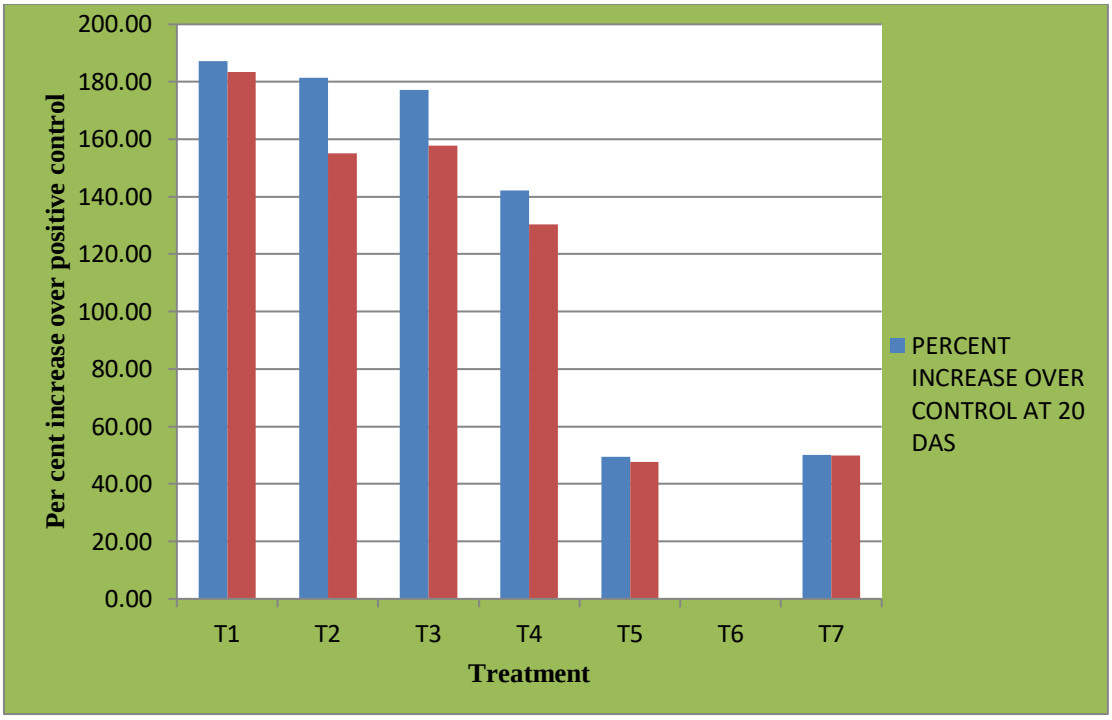

Fig 4.12 Effects of CMC on French bean plant vigour index and per cent increased over control at 20 and 60 DAS

4.11.4 Effect of CMC on fresh and dry weight (g) of shoot at 20 DAS and 60 DAS

The fresh and dry weight of shoot of individual French bean (20 plants/ treatment) was measured at 20 and 60 DAS. The pooled data on fresh shoot weight is depicted in Tables 4.29 and illustrated in Fig 4.13. Fresh shoot weight was higher in all the CMC treated treatments as compared to positive control. At 20 DAS pooled data of fresh shoot weight revealed that CMC treated treatments *i.e.*, T_1, T_2 and T_3with9.22 g; 9.07 g and 9.09 g respectivelydid not show significant difference among them. Similarly at 60 DAS, T_1 (42.23 g) showed highest fresh shoot weight which is statistically at par with T_2, T_3 and $T_4$with 40.72 g, 41.02 g, and 40.20 grespectively. Minimum fresh shoot weight was observed in T_6[positive control (4.88 g and18.85 g)at 20 and 60 DAS].Results also revealed that T_1recorded highest increase in fresh shoot weight over positive control with 88.9 % (20 DAS) and 127.1 % (60 DAS) (Table 4.30 and Fig 4.13).

Perusal of the pooled data depicted in Tables 4.29 and illustrated in Fig 4.12 revealed that dry shoot weight was higher in all the CMC treated treatments as compared to positive control. At 20 DAS, CMC treated treatments *viz.*, T_1, T_2 and T_3 (0.53 g; 0.52 g) found highest dry weight of shoot which are statistically at par with each other. Similarly at 60 DAS, T_1 (12.03 g) showed highest dry shoot weight which is statistically at par with T_2, T_3 and T_4 (11.45 and11.50 g and 11.36 g) respectively. Minimum dry shoot weight was observed in positive control (0.26 g and 2.78 g) at 20 and 60 DAS. Increase in dry shoot weight over positive control was observed highest in T_1 which was 103.05 % at 20 DAS and 332.73 % at 60 DAS (Table 4.30 and Fig 4.12).

Table 4.29 Effects of CMC on French bean fresh and dry weight (g) of shoot at 20 and 60 DAS

Treatment	Fresh weight (g) of shoot 20 DAS			Fresh weight (g) of shoot 60 DAS			Dry weight (g) of shoot at 20 DAS			Dry weight (g) of shoot at 60 DAS		
	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled
T_1	**8.97**	**9.47**	**9.22**	**42.65**	**41.82**	**42.23**	**0.51**	**0.54**	**0.53**	**12.20**	**11.90**	**12.03**
T_2	8.73	9.42	9.07	41.65	40.25	40.72	0.50	0.54	0.52	11.27	11.65	11.45
T_3	8.72	9.48	9.09	41.85	40.47	41.02	0.51	0.54	0.52	11.45	11.57	11.50
T_4	8.15	8.90	8.39	41.20	39.75	40.20	0.48	0.50	0.49	11.32	11.42	11.36
T_5	6.27	6.39	6.33	25.30	24.32	24.80	0.37	0.37	0.37	8.30	7.92	8.11
T_6	4.78	4.98	4.88	19.47	18.25	18.85	0.23	0.28	0.26	2.97	2.55	2.78
T_7	6.29	6.40	6.35	25.90	24.42	25.15	0.37	0.37	0.37	8.37	7.90	8.13
SEm±	0.03	0.10	0.04	0.45	0.28	0.34	0.01	0.00	0.01	0.14	0.11	0.10
C.V. (%)	3.28	9.34	4.11	9.33	6.09	7.23	9.4	4.40	5.61	10.24	8.18	7.56
CD (*p*=0.05)	0.35	1.08	0.46	4.67	2.93	3.55	0.01	0.02	0.03	1.41	1.11	1.04

Table 4.30 Effects of CMC on French bean plant per cent increase of fresh and dry weight of shoot at 20 and 60 DAS over control

Treatment	Per cent increase of fresh and dry shoot weight over positive control			
	Fresh shoot weight		Dry shoot weight	
	20 DAS	60 DAS	20 DAS	60 DAS
T_1	**88.93**	**127.16**	**103.05**	**332.73**
T_2	85.86	116.02	101.14	311.87
T_3	86.27	117.61	99.23	313.66
T_4	71.92	113.26	88.93	308.63
T_5	29.91	31.56	43.89	191.72
T_6	-	-	-	-
T_7	29.71	33.42	42.74	192.44

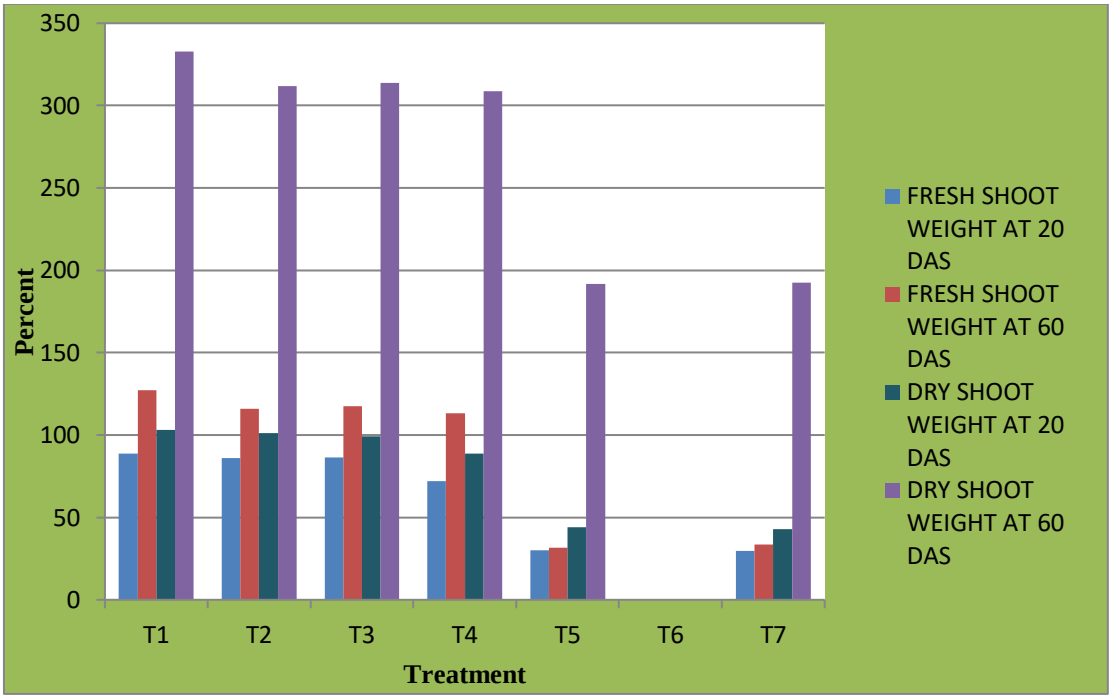

Fig 4.13 Effects of CMC on French bean plant per cent increase of fresh and dry weight of shoot at 20 and 60 DAS over control

The increase in mean plant fresh and dry weight upon microbial consortia treated treatments may be due to higher metabolic activity that leads to the better mobilization efficiency of reserved food by bioagents that might contribute for the better growth of plants. Works akin to the present findings were also reported by Shandeep *et al*. (2013). They reported that combined inoculation of*T. Harzianum* and *P. fluorescens* on vanilla plants registered the maximum length of vine (82.88 cm), highest number of leaves (26.67/plant), recorded the highest fresh weight of shoots (61.54 g plant^{-1}), 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.* Lamsal *et al.* (2013) also reported that, *in vivo* assay of all the bacterial isolates were found to be capable of enhancing different growth parameters (shoot/root length, fresh biomass and dry matter) in comparison with non-inoculated control plants.

4.11.5 Effect of CMC on fresh and dry weight (g) of root at 20 DAS and 60 DAS

Data onfresh and dry root weight arepresented in Table 4.31 and illustrated in Fig 4.14. At 20 DAS, pooled data of fresh root weight was found to be highest in T_1, T_2, T_3 and T_4 with 0.56 gand 0.54 g respectively which is statistically at par with each other. Similarly at 60 DAS, T_1 recorded highest fresh root weight (5.05 g) which was statistically at par with T_2, T_3 and T_4 (4.68 g, 4.72 g, and 4.62 g) respectively. Minimum fresh root weight was observed in T_6 [positive control (0.31 and 1.27 g)] at 20 and 60 DAS.Data on per cent increase in fresh root weight over positive controlalso revealed that the T_1recorded maximum with 81.08 % at 20 DAS and 297.6 %at 60 DAS (Table 4.32 and Fig 4.13).

Table 4.31 Effects of CMC on French bean fresh and dry weight (g) of root at 20 and 60 DAS

Treatment	Fresh root weight at 20 DAS			Fresh root weight at 60 DAS			Dry root weight at 20 DAS			Dry root weight at 60 DAS		
	2018	**2019**	**Pooled**	**2018**	**2019**	**Pooled**	**2018**	**2019**	**Pooled**	**2018**	**2019**	**Pooled**
T_1	0.55	0.58	0.56	**5.12**	**4.97**	**5.05**	0.10	0.11	0.11	**1.17**	**1.10**	**1.13**
T_2	0.54	0.58	0.56	4.72	4.62	4.68	0.10	0.11	0.11	1.02	0.97	1.00
T_3	0.54	0.58	0.56	4.77	4.67	4.72	0.09	0.10	0.10	1.07	1.00	1.03
T_4	0.51	0.56	0.54	4.67	4.57	4.62	0.09	0.10	0.09	1.00	0.92	0.96
T_5	0.38	0.37	0.37	2.40	2.32	2.36	0.07	0.07	0.07	0.72	0.70	0.71
T_6	0.31	0.30	0.31	1.27	1.27	1.27	0.06	0.05	0.05	0.27	0.32	0.30
T_7	0.38	0.38	0.38	2.47	2.42	2.45	0.07	0.06	0.07	0.75	0.72	0.73
SEm±	0.01	0.01	0.00	0.05	0.05	0.05	0.17	0.24	0.39	0.02	0.02	0.05
C.V. (%)	3.94	3.60	2.72	11.14	10.65	10.88	4.82	6.51	3.98	14.51	13.39	13.14
CD (*p*=0.05)	0.02	0.03	0.01	0.59	0.55	0.57	0.01	0.01	0.01	0.18	0.16	0.17

Table 4.32 Effects of CMC on French bean plant per cent increase of fresh and dry weight of root at 20 and 60 DAS over positive control

Treatment	Per cent increase of fresh and dry root weight over positive control			
	Fresh root weight		Dry root weight	
	20 DAS	60 DAS	20 DAS	60 DAS
T_1	**81.08**	**297.63**	**120.00**	**276.67**
T_2	81.01	268.50	120.00	233.33
T_3	80.02	271.65	100.00	243.33
T_4	74.67	263.77	80.00	220.00
T_5	19.23	85.82	40.00	136.67
T_6	-	-	-	-
T_7	22.23	92.91	40.00	143.33

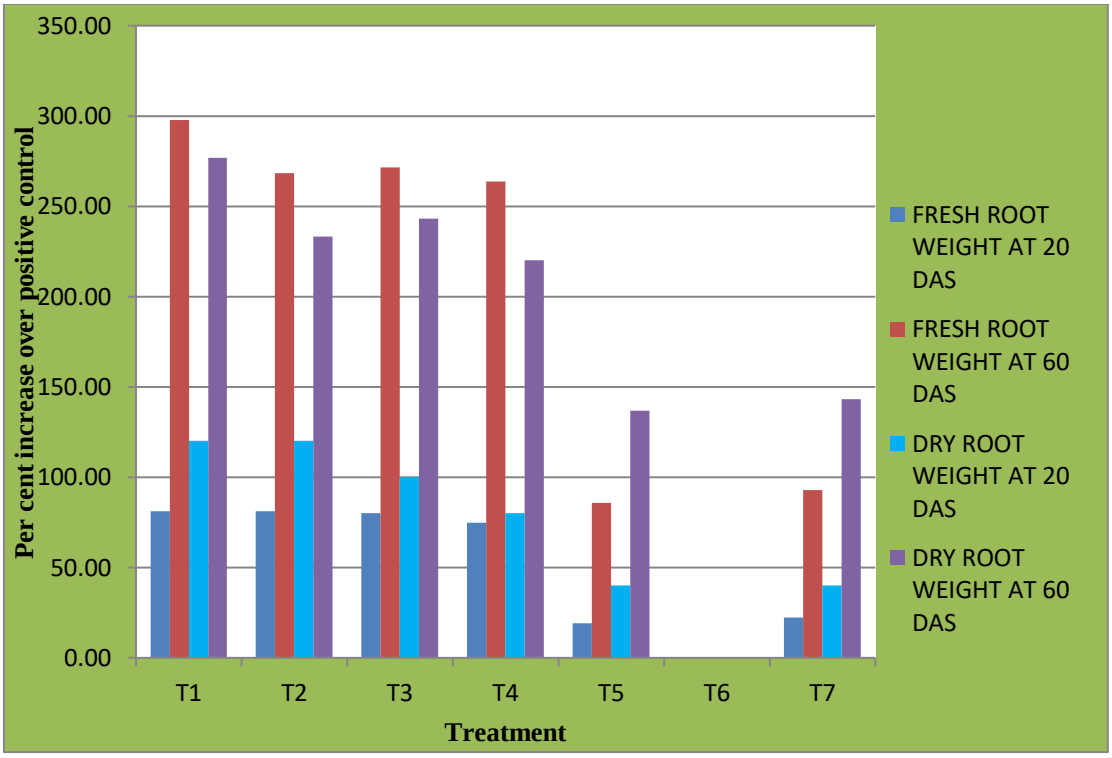

Fig 4.14 Effects of CMC on French bean plantper cent increase of fresh and dry weight of root at 20 and 60 DAS over positive control

Perusal of the data presented in Table 4.31 revealed that at 20 DAS, dry root weight was higher in all the CMC treated treatments *viz.* T_1, T_2, and T_3 with 0.11 g; 0.10 and 0.09 g respectively which were statistically at par with ach other. Similarly at 60 DAS, T_1 (1.13 g) showed highest dry root weight which is statistically at par with T_2, T_3 and T_4 with 1.00 g, 1.03 g, and 0.96 g respectively. Minimum dry root weight was observed in T_6 control with 0.02 and 0.30 g at 20 and 60 DAS. Increase in dry root weight over positive control was observed highest in T_1 which was 120.00 % at 20 DAS and 276.67 % at 60 DAS (Table 4.32 and Fig 4.14).

In these present studies, an attempt was made to study the effect of compatible microbial consortia on French bean plant growth and developmental traits in which effective results were obtained with microbial consortia treated treatments under *in vivo* conditions. The improvement in French bean seed germination might be due to food reserve mobilization. Also the increase in mean plant fresh and dry weight upon microbial consortia treated treatments may be due to higher metabolic activity that leads to the better mobilization efficiency of reserved food that might contribute for the better growth of plants which might helps in increased seed germination, shoot length and root length and hence increase in the mean plant fresh and dry weight. Moreover, the increase in plant vigour index upon microbial consortia treated treatments may be due to increased seed germination percentage, shoot length, root length and dry weight of French bean plants.

The investigation of present works are in conformity with the findings of earlier worker like Khan *et al.* (2018) who reported there was significant increase in shoot dry weight and root dry weight of lettuce plants in treatments with bioformulations of *T. viride* + *B. thuringiensis* + *P. fluorescens* 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 treated with application of consortia of *T. viride* + *B. thuringiensis* + *P. fluorescens.*

Similarly, Eutesari *et al.* (2013); Kabir *et al.*(2013); Lamsal *et al.* (2013); Sandeep *et al*. (2013) and Sharma *et al.* (2015) reported that the application of BCAs increased fresh and dry weight of plant over control.

4.11.6 Effect of CMC on number of leaves and branches per plant

The pooled data concerning the effect of various treatments in relation to number of leaves and branches per plant is depicted in Table. 4.33 and illustrated in Fig 4.15. The results indicated that microbial consortia treated plants have more number of leaves than control. Highest number of leaves per plant was recorded in T_1 (26.57) which is statistically at par with T_2 (25.45), T_3 (25.25). Least number of leaves per plant was recorded in T_6 positive control with 10.51. The experimental result also revealed that the highest per cent increase in number of leaves per plant over positive control was observed from $T_1$with 152 %.

Similarly number of branches per plant (40 tagged plants per treatment) were counted and recorded. The pooled data concerning to effect of various treatments in relation to number of branches per plant is depicted in Table. 4.33 and illustrated in Fig 4.15. The results indicated that microbial consortia treated plants have more number of branches than control treatments. Highest number of branches was recorded in T_1 (8.57) which is statistically at par with T_3 (7.95), T_2 (7.92) and T_4 (7.82). Least number of branches per plant was recorded in T_6 positive control (4.70). The experimental result also revealed that per cent increase in number of branches per plantover positive controlwas highest in $T_1$with 82.44 %.

Table 4.33 *In vivo* effects of CMC on French bean number of leaves and branches per plant after harvesting

Treatment	No. of leaves per plant				No. of branches per plant			
	2018	2019	Pooled	per cent increase over positive control	2018	2019	Pooled	per cent increase over positive control
T_1	**26.40**	**26.70**	**26.57**	**152.80**	**8.55**	**8.60**	**8.57**	**82.44**
T_2	25.35	25.50	25.45	142.15	7.90	7.95	7.92	68.93
T_3	25.10	25.35	25.25	140.24	7.85	7.90	7.95	69.14
T_4	25.00	25.45	25.22	139.96	7.85	7.80	7.82	66.38
T_5	19.52	18.80	19.27	83.34	6.30	6.30	6.25	32.97
T_6	10.27	10.75	10.51	-	4.75	4.65	4.70	-
T_7	19.87	18.95	19.55	86.01	6.30	6.45	6.40	36.17
SEm±	0.72	0.66	0.64	-	0.50	0.12	0.11	-
C.V. (%)	8.83	8.24	7.96	-	4.93	4.59	4.50	-
CD (p=0.05)	2.81	2.58	2.51	-	0.50	0.47	0.46	-

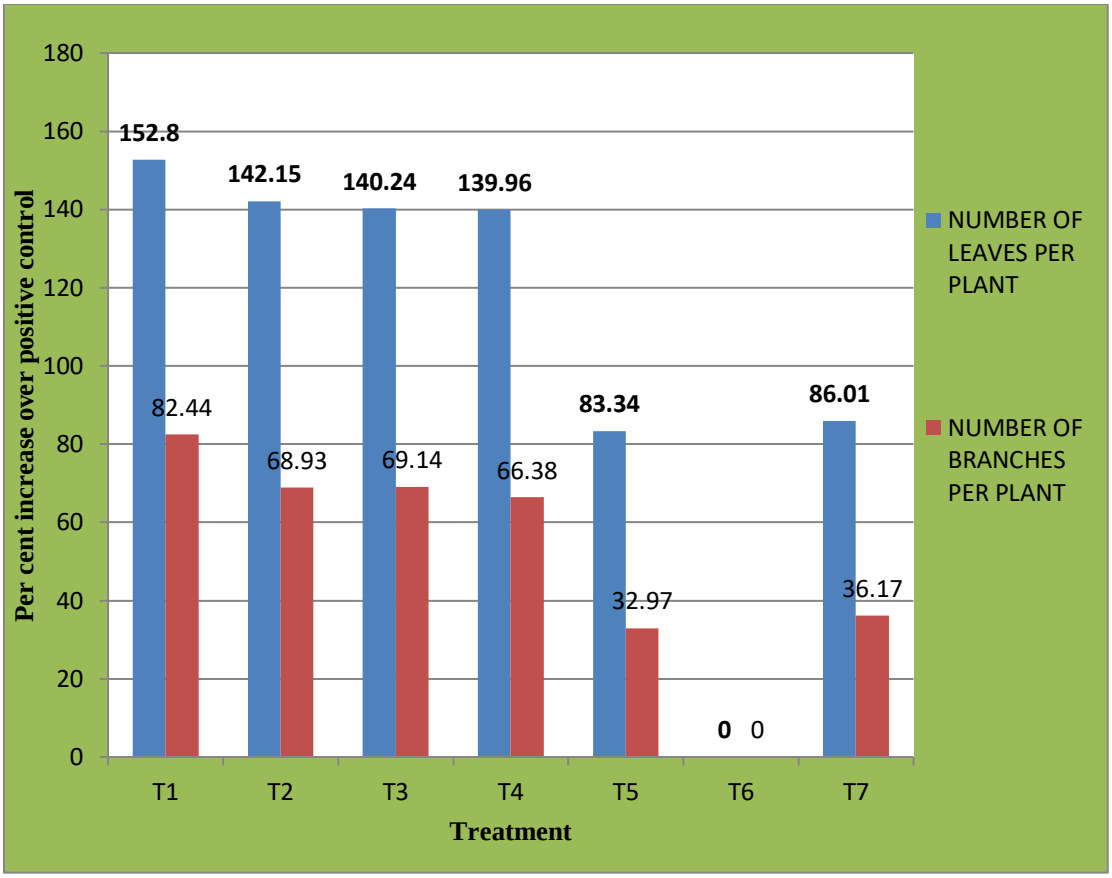

Fig 15 Effects of CMC on French bean number of leaves and branches per plant after harvesting

In the present investigation, the increase in number of branches and leaves per plant in microbial consortia treated treatments might be due to effects of applied microbial consortia which are known to enhance plant growth and development of various plant parts and higher growth leads to more branching and more number of leaves. 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 other defense enzymes, resistance to abiotic stresses and uptake of nutrients.

The findings of present work are in agreement with the findings of earlier worker Biam and Majumder (2019) who reported that the influence of *Trichoderma* isolates (*T. hamatum* strain CEN693 (TR 55), *T. hamatum* strain US10 (TR 66), *T. hamatum* strain DIS 326F (TR 122) and *T. harzianum* (TR 136) on the growth parameters such as numbers of leaves and branches under greenhouse conditions. 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).

4.11.7 Effect of CMC on length of pod, number of green pod and Fresh weight of pod (g pod^{-1})

The length of harvested pod (40 pods per treatment) were counted and recorded. The pooled data concerning to effect of various treatments in relation to length of pod, number of pod per plant and weight of pod is depicted in Table. 4.34 and illustrated in Fig 4.16.

The results indicated that healthier and higher pod lengths were observed frommicrobial consortia treated plants than in control. However, there was no significant difference among the CMC treated treatments *viz*., T_1, T_2, T_3 and T_4 with 15.43 cm; 15.37 cm; 15.41 cm and 15.37 cm respectively. Reduced length of bean pod was recorded in T_6 positive control with 10.05cm

Table 4.34 *In vivo* effects of CMC on length of French bean pod, number of green pod per plant and fresh weight of pod

Treatment	Length of bean pod (g)				No. of green pod per plant				Fresh weight of green pod (g)			
	2018	2019	Pooled	per cent increase over control	2018	2019	Pooled	per cent increase over control	2018	2019	Pooled	per cent increase over control
T_1	**15.47**	**15.40**	**15.43**	**53.53**	**22.05**	**22.00**	**21.90**	**146.89**	**6.45**	**6.44**	**6.45**	**51.23**
T_2	15.32	15.40	15.37	52.93	20.37	20.40	20.40	129.98	6.42	6.44	6.43	50.93
T_3	15.35	15.47	15.41	53.33	20.50	20.25	20.37	129.65	6.46	6.41	6.45	51.23
T_4	15.15	15.40	15.37	52.93	20.55	19.70	20.12	126.88	6.32	6.30	6.32	48.35
T_5	13.95	13.35	13.65	35.82	13.85	13.85	13.85	56.14	5.92	5.92	5.92	38.96
T_6	10.02	10.02	10.05	-	9.00	8.75	8.87	-	4.12	4.02	4.26	-
T_7	13.87	13.55	13.75	36.81	14.25	14.35	14.32	61.14	5.95	5.94	5.95	39.67
SEm±	0.26	0.07	0.08	-	0.11	0.12	0.11	-	0.06	0.05	0.07	-
C.V. (%)	4.96	3.92	4.19	-	4.77	5.25	4.86	-	3.15	2.88	3.21	-
CD (*p*=0.05)	1.03	0.81	0.87	-	1.19	1.31	1.21	-	0.27	0.25	0.28	-

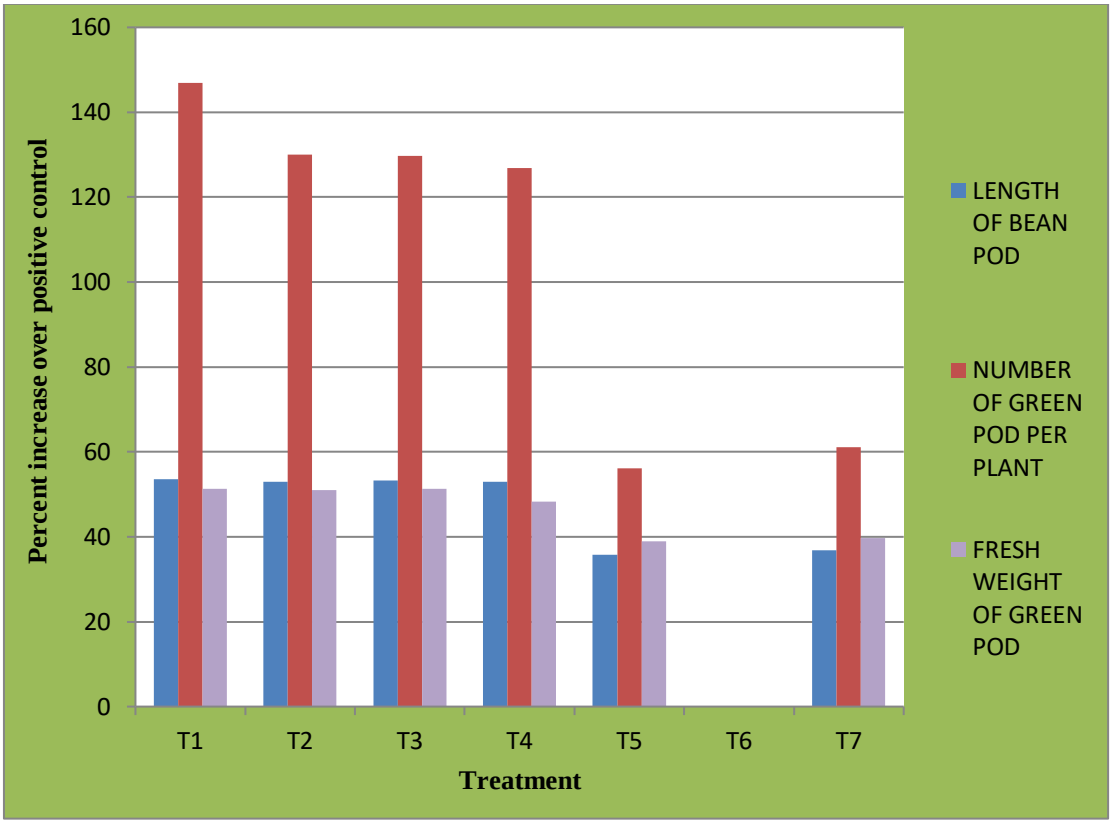

Fig 4.16 *In vivo* effects of CMC on per cent increase in length of French bean pod, number of green pod per plant and fresh weight of pod

Percent increase inlength of pod over positive control was recorded to be highest from T_1 with 53.33 %.

Perusal of the data recorded in Table 4.34, Fig 4.16 revealed that microbial consortia treated plants have a greater number of pod per plant than in control. It was observed that T_1 recorded the highest number of pod per plant (21.90) which was statiscally at par with T_2 (20.40), T_3 (20.37) and T_4 (20.12) respectively. Least number of pods per plant was recorded in T_6 control (8.87). It was also observed that increase in the number of pod per plant over positive control was highest in T_1 with 146.89 %.

From the pooled data recorded in Table 4.34, Fig 4.16 it indicated that microbial consortia treated plants recorded higher fresh weight of pods than in positive control. It was recorded that T_1 and T_3 recorded the highest fresh weight of pod with 6.45 g which was statistically at par with T_2 and T_4 with 6.43 g and 6.32 g respectively. Least fresh weight of pod was recorded in T_6 (positive control) with 4.26 g. The result also revealed that on the treated treatments *viz*., T_1, T_2, T_3, and T_4 there was an increase in fresh weight of pod over positive control treatment (51.23 %; 50.93 %; 48.35 %) respectively.

In the present investigation, the increase in length of pod, number of pod and increase weight of pod in microbial consortia treated treatments might be due to effects of applied microbial consortia in supplying required plants nutrients which attributed high vegetative growth and development. Production of IAA, siderophore and other enzymes which might induce flowering and pod activity.

The results of the present work are in agreement with the findings of earlier worker like Manikandan *et al*. (2010) who reported the liquid formulation of PGPR as seed treatment + seedling dip + soil drenching of liquid formulation of Pf1 significantly increased the fresh weight of tomato fruit (38.96 g) under glasshouse and 43.67 g under field conditions over

untreated control. Similarly, Singh *et al*. (2022) reported the used of microbial consortia of *Trichoderma* species and *Pseudomonas fluorescens* species. *In vivo* results also revealed that, CMC-1 significantly increased tomato number of leaves per plant (116.48 %), number of branches per plant (146.57 %), number of fruits per plant (185.52 %), fresh weight of fruit (42.59 %) and marketable fruit yield (313.02 %) over control treatment.

4.11.8 Effect of CMC on marketable pod yield per plant and treatment

The data concerning the effect of various treatments in relation to yield of French bean pod are depicted in Table. 4.35 and illustrated in Fig.4.17. The pooled data of pod yield per plant revealed that T_1 (122.9 g) recorded highest yield followed by T_2 (116.2 g) which was statistically at par withT_3 and $T_4$with 115.1 g and 113.7 g respectively. The lowest yield was recorded from positive control with 52.4 g plant^{-1}. It was also observed that the per cent increase of French bean pod yield per plant over positive control was highest in T_1 with 144.3 %.

The actual mean yield per treatment was recorded and the data concerning to effect of various treatment in relation to calculated marketable pod yield per treatment are depicted in Table. 4.35. Perusal of the data revealed that T_1 recorded highest calculated marketable pod yield (2517.7 g treatment^{-1}) followed by T_3 (2396 g) which was statistically at par with T_2 and T_4 with 2382.0 g and 2315.0 g respectively. The lowest yield per treatment was recorded in control with 1170.0 g. Per cent increase of marketable pod yield per treatmentover control was also recorded to be highest inT_1 with 1145.1 %.

In the present study, the increase in high marketable pod yield per plant and per treatment might be due to effects of applied microbial consortia in supplying required plants nutrients which attributed high vegetative growth and developement and other production of hormones, enzymes and their

Table 4.35 ***In vivo*** **effects of CMC on French bean marketable pod yield per plant and per treatment**

Treatment	Marketable pod yield (g plant^{-1})				Marketable pod yield (g treatment^{-1})			
	2018	2019	Pooled	Per cent increase over positive control	2018	2019	Pooled	Per cent increase over positive control
T_1	**121.8**	**124.0**	**122.9**	**144.3**	**2505.4**	**2530.1**	**2517.7**	**115.1**
T_2	117.6	114.8	116.2	129.0	2392.4	2371.7	2382.0	103.5
T_3	116.2	113.9	115.1	128.8	2399.0	2394.0	2396.5	104.8
T_4	112.9	112.2	113.5	125.64	2304.4	2325.7	2315.0	97.86
T_5	76.4	74.0	75.2	49.50	1652.4	1615.7	1640.5	40.21
T_6	49.9	50.7	50.3	-	1155.3	1184.7	1170.0	
T_7	77.2	75.8	76.7	52.4	1691.2	1633.0	1662.1	42.05
SEm±	0.63	0.51	0.37	-	11.76	14.13	11.17	-
C.V. (%)	4.68	3.78	2.77	-	4.08	4.92	3.87	
CD (*p*=0.05)	6.58	5.29	3.89	-	121.1	145.5	114.5	-

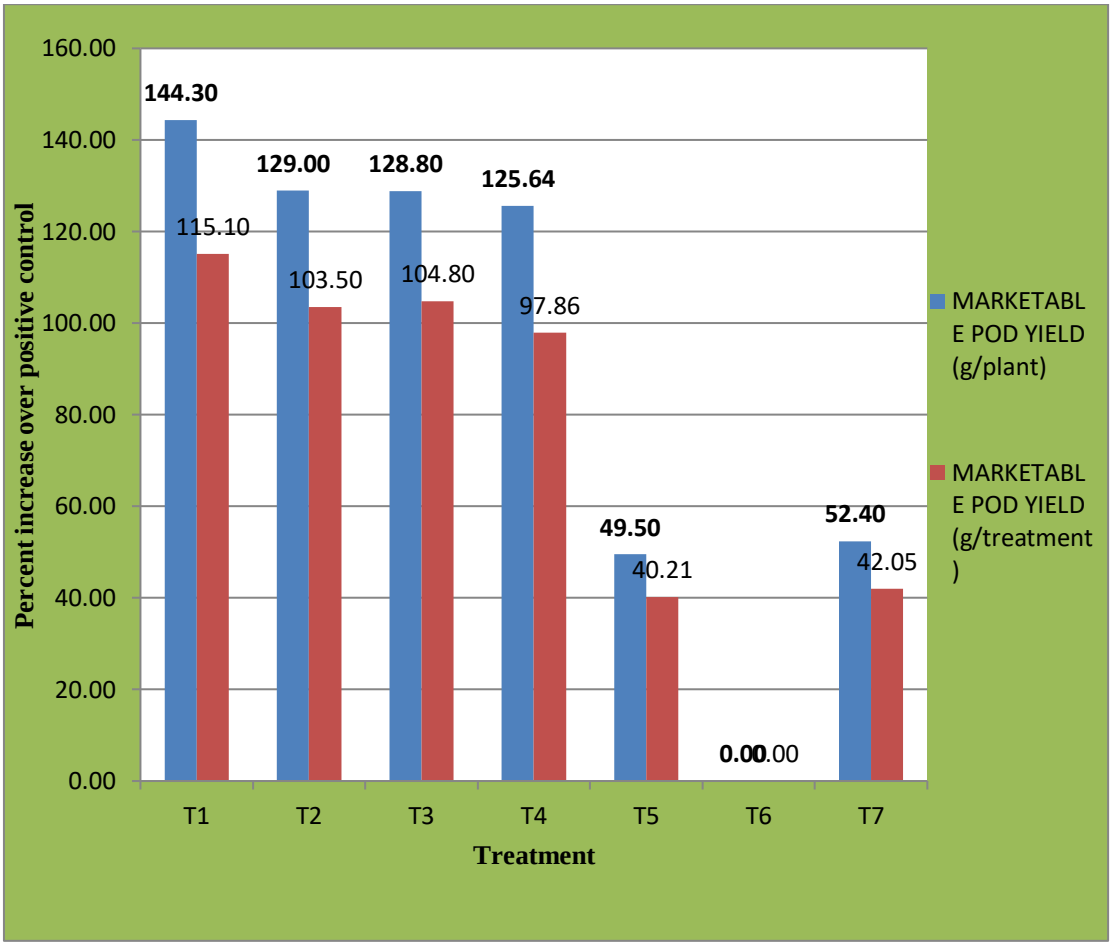

Fig 4.17 *In vivo* effects of CMC on French bean per cent increase marketable pod yield per plant and per treatment over positive control

synergistic action. The results of present investigation is in conformity with the findings of Srivastava *et al*. (2010) who reported the consortium of fluorescent *Pseudomonas* and *T. harzianum* formulation against *F. oxysporum* f. sp. *lycopersici* and found that combination of all three bioagents significantly enhanced the yield. Biam and Majumder (2019) also reported the influence of *Trichoderma* isolates enhanced yield increased in all the treatments compared to control (1.4 kg). The highest yield was recorded in TR 55 (2.25 kg). Similar findings were also reported by Manikandan *et al*. (2010), Hema and Selvaraj (2011); Khan *et al.* (2018) and Singh *et al* (2022).

4.11.9 Effect of CMC on plant biomass and number of sclerotia per infected plant

The pooled data concerning the effect of various treatments in relation plant biomass and number of sclerotia count per infected plant are depicted in Table. 4.36 and illustrated in Fig 4. 18. The results indicated that microbial consortia treated treatments showed maximum plant biomass *viz*., T_1, T_2, T_3 and T_4 with 72.08, 71.85, 71.95 and 71.87 % respectively which were statistically at par with each. Least plant biomass was recorded in T_6 [positive control (43.74 %)]. Perusal of the data recorded in Table 4.36 also revealed that the T_1 (64.83 %) recorded the maximum increase in biomass over positive control.

Pooled data on the number of sclerotia on infected plant indicatesthat microbial consortia treated plants suppressed 100 % sclerotial development over control. However, in T_4 pooled data revealed the presence of 2 numbers of sclerotia.

In the present study, the increase in plant biomass and suppression of sclerotial production might be due to effects of applied microbial consortia which might help in supplying required plants nutreints resulting high.

Table 4.36 ***In vivo*** **effects of CMC on French bean plant biomass after harvesting and number of sclerotia development per infected plant**

Treatment	Plant biomass				Number of sclerotia per infected plant			
	2018	2019	Pooled	Per cent increase over control	2018	2019	Pooled	Per cent decrease over control
T_1	72.68	71.56	72.10	64.83	0.00	0.00	0.00	100.0
T_2	72.63	71.06	71.85	64.27	0.00	0.00	0.00	100.0
T_3	72.68	71.27	71.97	64.54	0.00	0.00	0.00	100.0
T_4	72.58	71.07	71.83	64.22	4.00	0.00	2.00	98.68
T_5	59.05	57.48	57.02	30.36	0.00	0.00	0.00	100.0
T_6	42.93	44.55	43.74	-	190.50	113.25	151.625	-
T_7	59.45	57.45	57.21	30.80	0.00	0.00	0.00	100.0
SEm±	0.57	0.16	0.23	-	0.71	0.44	0.34	-
C.V. (%)	6.14	1.77	2.56	-	17.78	19.30	10.76	-
CD (*p*=0.05)	5.83	1.65	2.40	-	7.26	4.59	3.47	-

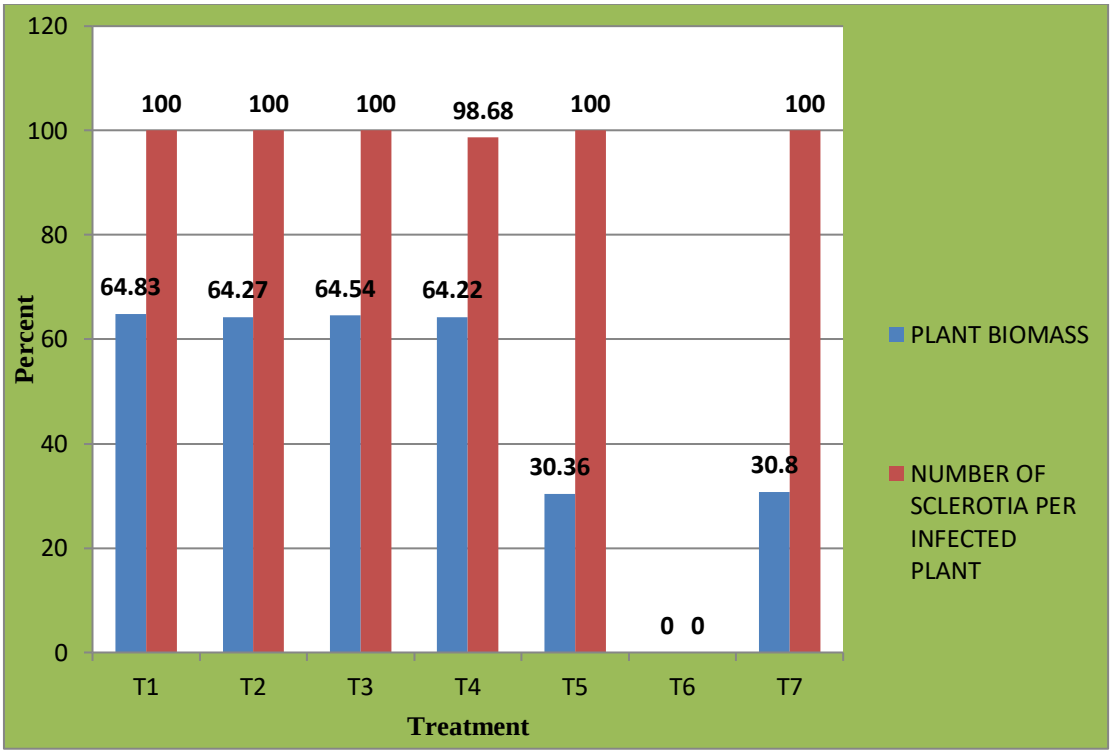

Fig4.18 *In vivo* effects of CMC on French bean plant biomass and number of sclerotia development per infected

vegetative growth, developement and other production of antibiotic compounds, enzymes etc. and their antagonistic action The result of present investigation is in conformity with Sudharani *et al*. (2014) who reported the plant growth promoting and disease suppressing abilities of BCAs and PGPRs under pot conditions. The application of *Azotobacter chroococcum* + *B. megaterium* + *P. fluorescens* + *B. subtilis* + *T. harzianum* enhanced cabbage total biomass, least disease incidence and more biocontrol efficiency. Further Contreras-Cornejo *et al*., 2014) reported an increase in total biomass, chlorophyll content and acceleration of flowering by isobutyl alcohol, isopentyl alcohol and 3-methylbutanal by application of *T. viride.* The similar trend was observed by Kotasthane *et al.* (2014) while testing 5 potential isolates of rhizobacterial species. against *S. rolfsii* and found effective with highest reduction in number of sclerotia produced (84.32%) over control treatment.

4.11.10 Effect of CMC on disease incidence of collar rot at 30 DAS

The collar rot disease incidence was assessed and converted into per cent disease index (PDI) for analysis. The disease incidence was recorded at 30 DAS and it is depicted in Table 4.37 and illustrated in Fig.4.19

Pooled dataon disease incidence revealed that T_5 chemical control recorded the lowest disease incidence with 5.50 %. This was followed by T_1 (11.50 %) which was statistically at par with T_2, T_3 and $T_4$with 18% and 19 % respectively. The experiment also revealed that T_5 [chemical checked (91.09 %)] showed highest per cent reduction of disease over positive control. Amongst the CMC treated treatments, T_1showed the highest per cent decrease over positive control with 81.38 %.

Table 4.37.*In vivo* effects of CMC on French bean disease incidence at 30 DAS

Treatment	Percent disease incidence (PDI)			
	2018	**2019**	**Pooled**	**per cent decrease over positive control**
T_1	**12.00 (20.14)**	**11.00 (19.30)**	**11.50 (19.78)**	**81.38**
T_2	18.00 (24.93)	18.00 (25.01)	18.00 (24.81)	70.85
T_3	19.00 (25.77)	19.00 (25.77)	19.00 (25.03)	69.23
T_4	19.50 (26.10)	18.00 (24.99)	18.00 (25.03)	70.85
T_5	**5.00 (12.76)**	**6.00 (13.98)**	**5.50 (13.51)**	**91.09**
T_6	62.00 (51.97)	61.50 (51.69)	61.75 (51.79)	-
T_7	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100
SEm±	0.52	0.51	0.39	-
C.V. (%)	18.96	18.97	14.49	-
CD (*p*=0.05)	5.39	5.32	4.04	-

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

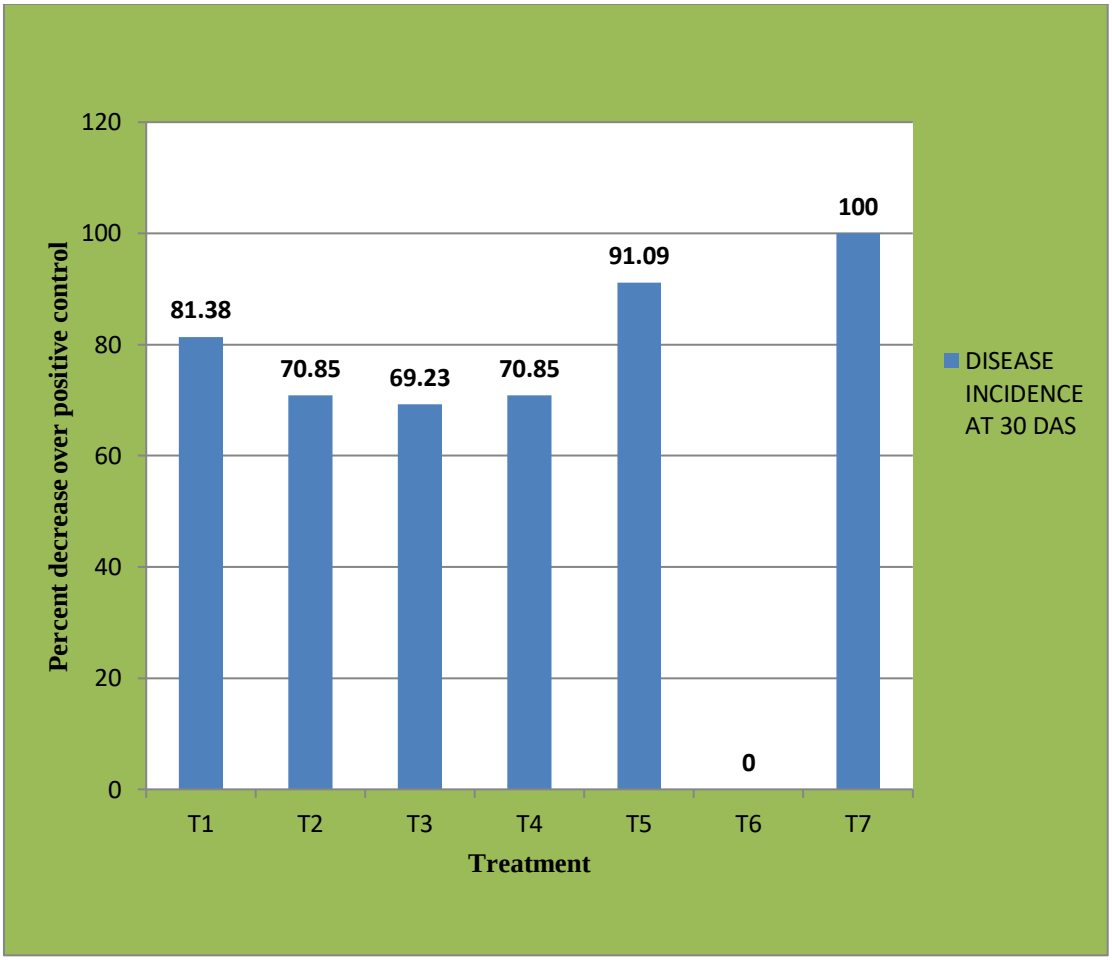

Table 4.19.*In vivo* effects of CMC on per cent decrease disease incidence at 30 DAS over positive control

In the present study, the application of microbial consortia through seed treatment followed by soil drenching at 0, 15 and 30 DAS had effectively checked disease incidence of collar rot under pot conditions. This reason behind this might be due to direct effects of the applied bioagents on suppression of pathogen growth in the soil.

The results of present investigation is in conformity with Khan *et al.* (2018) who reported the efficacy of three compatible potential microbial bioagents, *viz. T. viride, B. thuringiensis* and *P. fluorescens* against bacterial wilt of lettuce. 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 %). 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. Similarly Sudharani *et al*. (2014) who reported 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 least disease incidence and more biocontrol efficiency.

4.11.11 Effect of CMC on pre-emergence and post-emergence mortality percent

Observations on mortality per cent recorded as pre-emergence and post emergence of all plants in each replication and it is depicted in Table 4.38 and illustrated in 4.20. The application of microbial consortia differed in mortality of plant by differing in interval of application in French bean plants induced by *S. rolfsii* under pot conditions.

Table 4.38.*In vivo* effects of CMC on French bean pre emergence and post emergence disease mortality per cent

Treatment	Pre-emergence mortality per cent				Post emergence mortality per cent			
	2018	**2019**	**Pooled**	**Per cent decrease over positive control**	**2018**	**2019**	**Pooled**	**Per cent decrease over positive control**
T_1	**8.00 (16.42)**	**7.00 (15.30)**	**7.50 (15.88)**	**79.59**	**0.00(4.05)**	**0.00(4.05)**	**0.00(4.05)**	**100.00**
T_2	8.00 (16.42)	7.50 (15.86)	7.50 (15.88)	**79.59**	2.37(8.83)	2.00(8.13)	2.18(8.49)	87.26
T_3	8.50 (16.93)	7.00 (15.30)	7.75 (15.90)	**78.91**	2.37(8.83)	1.75(7.53)	1.87(7.89)	89.07
T_4	11.00 (19.17)	11.00 (19.27)	11.00 (19.23)	70.06	2.00(8.13)	2.00(8.13)	2.18(8.49)	87.26
T_5	**3.50 (10.32)**	**3.00 (9.66)**	**3.25 (10.29)**	**91.15**	**0.00(4.05)**	**0.00(4.05)**	**0.00(4.05)**	**100.00**
T_6	37.50 (44.50)	36.00 (43.83)	36.75 (44.30)	-	18.50(25.4)	16.25(23.7)	17.12(24.4)	-
T_7	**0.00 (4.05)**	**0.00 (4.05)**	**0.00 (4.05)**	**100**	**0.00(4.05)**	**0.00(4.05)**	**0.00(4.05)**	**100.00**
SEm±	0.28	0.33	0.25	-	0.06	0.07	0.05	
C.V. (%)	18.19	22.91	17.31	-	12.65	16.28	10.77	
CD (*p*=0.05)	2.92	3.44	2.65	-	0.67	0.75	0.52	

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

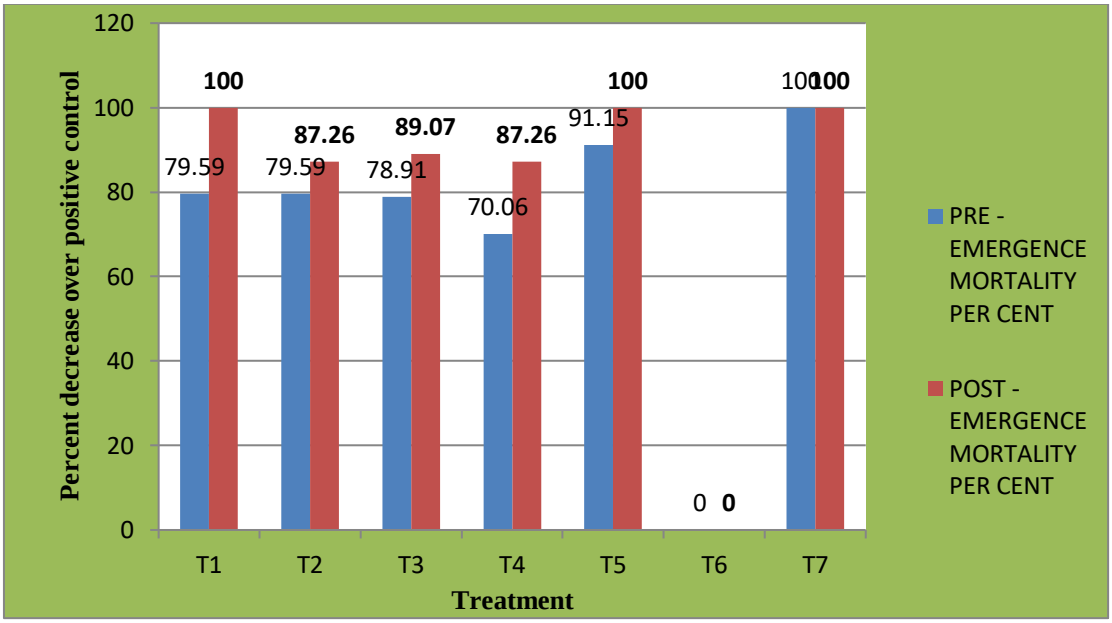

Fig 4.20 *In vivo* effects of CMC on French bean per cent decrease pre emergence and post emergence mortality over positive control

Pooled data of pre-emergence mortality per cent revealed that least mortality per cent was recorded fromT_5 [chemical checked (3.25 %)] followed by CMC treated treatments *viz*., T_1 and T_2 with 7.50 %. The experiment also revealed that T_5 recorded the highest per cent decrease over positive control with 91.15 %. Interestingly in post emergence mortality per cent, T_1, T_5 got zero mortality. CMC treated treatment T_1 completely checked the disease mortality at post emergence which is equal with chemical control. The progress of mortality in all treatments reduced as plant matured.

In the present study, the application of microbial consortia through seed treatment followed by soil drenching at 0, 15 and 30 DAS had effectively checked the French bean plant mortality under pot conditions. This reason behind this might be due to direct effects of the applied compatible microbial consortia (CMC) on suppression of pathogen growth in the soil. The results clearly indicate that the compatible microbial consortia could reduce the collar rot as well as enhance the growth promotion of French bean plants. The significant decrease of collar rot disease incidence and higher yield might have occurred due to direct effects of the bioagents on suppression of the pathogen population in the plant rhizosphere.

The results are in conformity with Manoranjitham and Prakasam (1999) who reported the seed treatment with *T. viride* and *P. fluorescens* showed a reduction in pre and post emergence damping off when compared to control. Similarly application of bioagents through different deliver methods used also reported by Nandakumar *et al.* (2001); Raj *et al*. (2004); Saravanakumar (2006); Sharma *et al.* (2009); Srivastava *et al*. (2010); Sudharani *et al*. (2014) in control of various soil borne fungal pathogens. Further similar findings also reported by Sharma *et al.* (2015) who evaluated consortial application of four strains of bacterial species (S_{21} + S_{25} + B_6 + A_{10}) showed significant effect (66.7 %) reduction of *S. rolfsii* disease mortality of chickpea under pot condition.

SUMMARY AND CONCLUSIONS

The present investigations on "**Biological remediation in the management of collar rot of French bean (*Phaseolus vulgaris* L..**)" was carried out under *in vitro* as well as in pot condition in the experimental site 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 collar rot of French bean 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 early visible symptoms of collar rot of French bean were recorded as progressive yellowing and wilting of leaves shown as initial symptoms. Leaves turn brown, dry and subsequently followed by producing abundant white fluffy mycelium at the collar region. In advanced stage, compactly development of mycelia with round white fuzzy mycelia begin to turn mustard-seed like structures called sclerotia which is smooth and light tan, brown in colour. The affected lesions rapidly develop woody tissue and gradually die.
- Symptoms also appeared on pod only at or near soil surface. Pod becomes soft, water-soaked, sunken lesions and shortly covered with white mycelium, eventually developing sclerotia spreads over the infected pod surface.
- The pathogen causing collar rot of French bean was isolated from infected stems and roots of French bean showing typical symptoms and pathogenicity test was carried out to establish the ability of fungal isolate to

produce typical symptoms of pathogen under artificial condition. The pathogen was re-isolated for confirmation.

- The mycelium of isolated fungus, *Sclerotium rolfsii* was grown on potato dextrose agar medium and incubated at 25±2 °C. *S.rolfsii* produced white cottony mycelial growth on potato dextrose agar medium and the fluffy colony. Initially, the pathogen produced white colored sclerotia and then their color was changed from white to off-white, light brown and tan when they attained maturity. The sclerotia were mostly globose and sometimes sub-spherical in shape.
- The pathogen was identified as *S. rolfsii* based on the nature of disease observed, cultural and morphological characters seen under the microscope.
- For obtaining better insight in the antagonistic potential of native BCAs, 32 isolates were evaluated against *S. rolfsii* by dual culture technique.
- Highest growth inhibition of pathogen recorded amongst the *Pseudomonas* isolates [Pf-2 (87.55 %), Pf-12 (87.11 %) and Pf-10 (84.00 %)]. And amongst *Trichoderma* isolates highest inhibition per cent was found in T_8 (85.77 %) which is statistically at par with T_{20} (84.88%).
- The effects of volatile metabolites of 20 isolates of *Trichoderma* and 12 isolates of *Pseudomonas* were assessed against *S. rolfsii*. Amongst the *Trichoderma* isolates, T_8 and T_{20} were found to be most promising in production of volatile compounds with 90.00 % and 89 % per cent inhibition which is at par with each other. Amongst the *Pseudomonas* isolates, maximum per cent inhibition was observed in T_2 (85.18 %) which is at par with T_{12} (83.70 %) and were significantly superior to all other treatments. Further followed by T_{11} (75.56 %), T_6 (63.33 %) and T_8 (45.93 %) respectively.

- The effect of non volatile production by 20 potential isolates of *Trichoderma* sp. and results revealed that isolates T_1, T_6, T_8, T_{13}, T_{14}, T_{15}, T_{16}, T_{18} and T_{20} were found total inhibition 100.00 % of the growth of *S. rolfsii*.
- And in case of *Pseudomonas* isolates maximum per cent inhibition was observed in Pf_{12} (88.15 %) which is at par with Pf_2 (88.1.4 %) and were significantly superior to all other treatments followed by Pf_3 (74.80 %), Pf_{10} (72.90 %) and Pf_9 (71.15 %) respectively.
- All 32 isolates showed positive results for ammonia production. *Pseudomonas* isolates Pf-1, Pf-2, Pf-3, Pf-4, Pf-5, Pf-6, Pf-7, Pf-8, Pf-9 and Pf-12 and *Trichoderma* isolates T-1, T-2, T-3, T-6, T-7,T-8, T-11, T-12, T-13 and T-20 exhibited strong ammonia production
- The qualitative assay of IAA production by different native BCAs are revealed that *Pseudomonas* isolates Pf-2, Pf-3, Pf-4, Pf-11 and Pf-12 showed strong production. In *Trichoderma* isolates T-2, T-6, T-8, T-9, T-11 and T-20 exhibited medium IAA production.
- Phosphate solubility test was conducted and the results revealed that *Pseudomonas* isolates (Pf-2, Pf-8 and Pf-12) and *Trichoderma* isolates (T-8, T-10, and T-20) elucidated medium production of phosphate solubilisation.
- Strong siderophore production was exhibited by *Pseudomonas* isolates Pf-2, Pf-6, Pf-10 and Pf-12. And among *Trichoderma* isolates, isolate T-7, T-8, T-11, T-14, T-15, T-16, T-18, T-19 and T-20 exhibited medium siderophore production.
- The production of HCN by *Pseudomonas* isolates revealed that only 3 *Pseudomonas* isolates (Pf-2, Pf-7 and Pf-12) elucidated positive results for HCN production.
- Qualitative pectinolytic enzyme production assay was carried out and the results revealed that *Pseudomonas* isolates Pf-2, Pf-5, Pf-6, Pf-10 and Pf-12

elucidated medium results for pectolytic production. And among the *Trichoderma* isolates, T-2, T-8, T-9, T-10, T-11, T-16, T-17 and T-20 showed strong production of pectolytic enzymes.

- The results of qualitative cellulose production revealed that 10 *Pseudomonas* isolates Pf-1, Pf-2, Pf-3, Pf-5, Pf-6, Pf-7, Pf-8, Pf-9, Pf-10 and Pf-12 elucidated strong results for cellulose production. In case of *Trichoderma* isolates, 9 isolates T-1, T-4, T-5, T-6, T-8, T-10, T-12, T-17 and T-20 produced strong cellulose enzyme production.
- Qualitative assay of amylase production by the fungal and bacterial isolates and the results revealed that *Trichoderma* isolates T-2, T-4, T-7, T-8, T-11, T-12, T-13, T-14, T-17 and T-20 showed strong production of amylase. But Pf-2 of *Pseudomonas* isolate elucidated strong results for amylase production
- Qualitative assay of catalase production by bacterial isolates revealed that all 12 *Pseudomonas* isolates elucidated positive results for catalase production
- All 20 isolates of *Trichoderma* isolates showed the presence of coiling as hyphal interactions between *Trichoderma* isolates and *S. rolfsii*..
- All promising native microbial isolates were able to release inorganic phosphorus from tri-calcium phosphate and showed consistent ability to produce siderophore, HCN, ammonia, IAA, volatile and non volatile metabolites, pectolytic enzymes, cellulose, amylase and mycoparasitism ability.
- Based on *in vitro* antagonistic capabilities of *Trichoderma* and *Pseudomonas* isolates against *S. rolfsii* and their elucidation for various biocontrol mechanisms, the potent isolates were selected as *Pseudomonas* isolates (Pf-2 and Pf-12) and *Trichoderma* isolates (T-8 and T-20) for further studies.

- Molecular identification of potential *Trichoderma* isolates (T-8 and T-20) was performed by using Internal Transcribed Spacer (ITS) region of 18S rRNA.
- Sequence analyses of two isolates (T-8 and T-20) were done to confirm species identity. ITS sequences of both the isolates were submitted to NCBI GenBank (OK147762 and OK147763), 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-12) were identified as *P. fluorescens* based on the following morphological characteristics, gram negative, rod shaped cells, creamy mucoid colony with smooth edges and yellow-green fluorescent pigmentation produced under ultraviolet (UV) light.
- Molecular identification of potential *Pseudomonas* isolates (Pf-2 and Pf-12) was done by using Internal Transcribed Spacer (ITS) region of 16S rRNA gene sequences. ITS sequences of both the isolates were submitted to NCBI Gene Bank (MN783298 and MN783297), which showed 92.66 % and 89.75 % similarity with *Pseudomonas fluorescens* during BLAST analysis
- *In vitro* compatibility test amongst microbial consortia of potent isolates of *Trichoderma* and *Pseudomonas* conducted showed the absence of inhibition zone indicating that the potential isolates of *Trichoderma* and *Pseudomonas* were compatible with each other.
- The inhibitory effects of compatible microbial consortia (CMC) showed significant highest inhibition of pathogen recorded in T_{11} [Pf-2 + Pf-12 + T-9 +T-20 (83.75 %)] followed by T_7 [Pf-2 + Pf-12 + T-8 (76.40 %)].

- *In vitro* result showed that, CMC increased vigour index of French bean seedlings (55.30 %) including germination per cent (20.22%), shoot length (35.46 %) and root length (21.68 %) over control at 10 DAS.
- In pot experimental results also revealed that, T_1 (seed treatment + soil drenching at 0, 15 and 30 DAS) increased vigour index (187.1 % and 183.5% at 20 and 60 DAS), germination per cent (42.46 % at 10 DAS), shoot length (104.5 % and 110.5 % at 20 and 60 DAS) and root length (93.1 % and 92.60 % at 20 and 60 DAS) over positive control.
- CMC treated treatment T_1 also significantly promoted the French bean number of leaves per plant (152.80 %), number of branches per plant (82.44 %), length of pod (53.53 %), number of pod per plant (146.89 %), fresh weight of pod (51.23 %) and marketable yield per treatment (115.1 %) over positive control treatment.
- Highest reduction of collar rot disease incidence was recorded in chemical control treatment (91.09 %) followed by CMC treated treatment T_1 (81.38 %).
- Least mortality per cent of pre emergence recorded in T_5 (chemical treatment) by reducing 91.15 %. CMC treated treatments *viz.* T_1, T_2 and T_3 recorded reducing mortality per cent of 79.59 %., 79.59 % and 80.27 % over positive control. Interestingly in post emergence mortality per cent, CMC treated treatment T_1 and T_5 got total checked of mortality (100 %).

Based on *in vitro* experiment the combined application of microbial consortia (CMC) was superior to the individual application of T-8, T-20, Pf-2 and Pf-12 isolates. Further in pot study, the best CMC was applied and found out the best effective treatment. The treatment T_1(seed treatment + soil drenching at 0, 15 and 30 DAS) was found effective in controlling the collar rot of French bean as well as induced substantial amount of growth and yield attributing parameters in French bean under pot conditions. This promising indigenous consortium enhanced the quality of the French bean plants from the

point of health and vigour of the plant. The quality of the plant in turn determined improved the yield and reduced the losses due to collar rot disease in an eco-friendly manner. Hence, microbial consortia have potential of exhibiting tremendous potential for its commercial exploitation.

Conclusions

Collar rot of French bean caused by *Sclerotium rolfsii* is destructive soil borne disease. It is an economically important disease of bean occurring worldwide including India. An indigenous liquid bioformulation of compatible microbial consortium was developed in order to combat collar rot of French bean. The *S. rolfsii* is known to suppress the polyphenol oxidase (PPO) in host during pathogenesis hence there is a need to elucidate the exact mechanism of suppression. Some isolates are resistant to commonly used fungicide. In order to overcome this trait, it is crucial needs to explore and find durable resistance. Several commercial French bean grown in Nagaland are highly susceptible to collar rot 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 effectiveness of native rhizospheric bioagents and its possible sustainaibility against collar rot of French bean. On the other hand, native bioagents (*P. fluorescens* Pf-2 + *P. fluorescens* Pf-12 + *T. asperellum* T-8 + *T. asperellum* T-20) based liquid compatible microbial consortium is sustainable, eco-friendly, enhances French bean plant growth and protect the plants from collar rot disease because of these obvious high level of antagonistic ability against *S. rolfsii*.

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. A collaborative study involving Department of Plant Pathology, Nagaland University and commercial French bean growers may be elucidated to popularize the use of microbial consortia which in turn help in designing management strategy against the *S. rolfsii* for efficient disease management. This study will not only help in benefiting the French bean growers but it will also reduce the dependence on fungicides and problem of environmental pollution.

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APPENDIX

ANOVA TABLE FOR 4.2 *IN VITRO* EXP.01 (Radial growth of pathogen)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	102.488	5.124	896.777	7.683
Error	42	0.240	0.007	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.2 *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	20	102.581	5.122	769.389	1.894
Error	42	0.280	0.006	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.2 *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	20	18171.949	908.592	749.014	3.328
Error	42	50.945	1.210	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.3 *IN VITRO* EXP.02 (Radial growth of pathogen)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	116.113	9.671	3144.768	6.679
Error	26	0.080	0.000	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.3 *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	12	116.113	9.671	3144.768	6.679
Error	26	0.079	0.000	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.3 *IN VITRO* EXP.02 (Inhibition %)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	20643.113	1720.255	3156.662	6.356
Error	26	14.169	0.549	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.4 EXP.03 (*In vitro* test for radial growth –Fungal isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	462.094	23.108	3308.197	1.009
Error	42	0.293	0.009	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.4 EXP.03 (*In vitro* test for radial growth inhibited –Fungal isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	456.477	22.825	3423.539	4.903
Error	42	0.279	0.006	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.4 EXP.03 (*In vitro* test for inhibition %–Fungal isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	56418.798	2820.936	3432.825	4.632
Error	42	34.516	0.827	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.5 EXP.03 (*In vitro* test for radial growth – Bacterial isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	237.594	19.797	3217.462	4.953
Error	26	0.159	0.001	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.5 EXP.03 (*In vitro* test for radial growth inhibited –Bacterial isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	237.594	19.797	3217.462	4.953
Error	26	0.160	0.001	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.5 EXP.03 (*In vitro* test for inhibition % -Bacterial isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	29333.882	2444.496	3215.535	4.991
Error	26	19.765	0.762	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.7 EXP.04 (*In vitro* test for radial growth – Bacterial isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	139.260	11.602	1810.420	8.662
Error	26	0.166	0.004	-	-
Total	38	-	-	-	

ANOVA TABLE FOR 4.7 EXP.04 (*In vitro* test for radial growth inhibited –Bacterial isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	170.059	14.174	2210.739	6.485
Error	26	0.166	0.004	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.7 EXP.04 (*In vitro* test for inhibition % – Bacterial isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	12	17193.494	1432.796	1811.488	8.603
Error	26	20.566	0.799	-	-
Total	38	-	-	-	-

ANOVA TABLE FOR 4.6 EXP.04 (*In vitro* test for radial growth –Fungal isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	305.041	15.251	2745.385	5.039
Error	42	0.233	0.005	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.6 EXP.04 (*In vitro* test for radial growth inhibited –Fungal isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	310.749	15.530	3058.852	5.201
Error	42	0.213	0.000	-	-
Total	62	-	-	-	-

ANOVA TABLE FOR 4.6 EXP.04 (*In vitro* test for inhibition %–Fungal isolates)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	20	38365.193	1918.256	3056.180	5.303
Error	42	26.368	0.626	-	-
Total	62	-	-	-	-

ANOVA TABLE EXP.05 (Combined inhibition %)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	11	15509.006	1409.907	153.331	1.877
Error	24	220.678	9.199	-	-
Total	35	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Germination %)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	1408.851	234.805	205.453	1.567
Error	21	24.000	1.148	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Shoot length)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	18.731	3.120	46.339	4.872
Error	21	1.415	0.063	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Root length)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	12.742	2.128	73.751	5.124
Error	21	0.605	0.028	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Seedling vigour index)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	1012419.795	168736.635	1705.289	4.216
Error	21	2077.935	98.944	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Fresh weight of shoot)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	998608.851	166434.805	1762.329	2.987
Error	21	1983.250	94.444	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Dry weight of shoot)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	59234.712	9872.453	120.655	3.628
Error	21	1718.250	81.824	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Fresh weight of root)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	45979.425	7663.230	260.716	1.341
Error	21	617.250	29.398	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VITRO* EXP.06 (Dry weight of root)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	8423.212	1403.860	824.653	8.422
Error	21	35.750	1.703	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Germination % at 10DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	2208.425	368.074	29.846	3.096
Error	21	259.000	12.333	-	-
Total	27	-	-	-	-

ANOVA TABLE *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	6	275.625	45.935	163.640	1.629
Error	21	5.895	0.287	-	-
Total	27	-	-	-	-

ANOVA TABLE *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	6	89.311	14.881	101.084	2.179
Error	21	3.095	0.142	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Plant 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	6	8408983.722	1401497.282	206.447	1.499
Error	21	142559.845	6788.560	-	-
Total	27	-	-	-	-

ANOVA TABLE *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	6	63.523	10.588	177.857	6.901
Error	21	1.250	0.055	-	-
Total	27	-	-	-	-

ANOVA TABLE *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	6	0.274	0.044	27.261	7.079
Error	21	0.039	0.006	-	-
Total	27	-	-	-	-

ANOVA TABLE *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	6	0.234	0.035	118.276	4.445
Error	21	0.000	0.003	-	-
Total	27	-	-	-	-

ANOVA TABLE *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	6	0.006	0.002	88.145	8.637
Error	21	0.003	1.442	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Shoot length at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	779.981	129.998	178.194	6.777
Error	21	15.320	0.725	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Root length at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	177.635	29.604	26.459	9.309
Error	21	23.505	1.111	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Plant vigour index at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	20709688.785	3451614.795	191.053	3.318
Error	21	379396.805	18066.516	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Fresh shoot weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	2396.331	399.386	39.606	2.187
Error	21	211.775	10.086	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Dry shoot weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	0.274	0.044	27.261	7.079
Error	21	0.039	0.006	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Fresh root weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	56.912	9.485	57.820	5.656
Error	21	3.445	0.160	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Dry root weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	2.252	0.375	24.140	2.117
Error	21	0.325	0.015	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* 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**
Treatments	6	582.131	97.028	26.531	9.037
Error	21	76.775	3.658	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Number of primary branches per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	38.905	6.487	54.912	9.383
Error	21	2.480	0.110	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Length of pod)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	92.321	15.388	31.019	2.169
Error	21	10.420	0.491	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Number of pod per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	548.335	91.389	137.403	9.676
Error	21	13.965	0.661	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01(Weight of green pod)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	17.975	2.998	102.943	1.812
Error	21	0.611	0.021	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Yield of pod per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	18259.125	3043.182	151.755	3.504
Error	21	421.115	20.052	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Yield of pod per treatment)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	6358454.622	1059742.433	156.299	2.595
Error	21	142390.265	6780.486	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Plant biomass)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	3142.145	523.695	33.330	1.108
Error	21	329.915	15.713	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Number of Sclerotia per infected plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	123607.712	20601.287	843.324	6.675
Error	21	513.000	24.425	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01(Disease incidence at 30 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	9608.851	1601.471	122.295	3.168
Error	21	275.000	13.092	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Pre emergence Disease mortality)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	3614.851	602.471	152.437	3.350
Error	21	83.000	3.953	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.01 (Post emergence disease mortality)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	1065.805	177.639	852.648	5.941
Error	21	4.375	0.203	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Germination % at 10DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	2229.212	371.537	12.506	5.379
Error	21	623.750	29.703	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (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	6	289.251	48.205	123.878	2.778
Error	21	8.175	0.381	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (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	6	85.811	14.308	60.929	3.387
Error	21	4.930	0.237	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Plant 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	6	7780435.52	1296739.25	92.463	5.345
Error	21	294498.75	14023.75	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (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	6	85.055	14.177	26.230	1.008
Error	21	11.347	0.542	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (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	6	0.264	0.042	109.951	9.309
Error	21	0.004	0.004	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (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	6	0.353	0.058	197.101	2.403
Error	21	0.003	0.003	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (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	6	0.014	0.007	59.403	4.346
Error	21	0.006	2.930	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Shoot length at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	793.535	132.255	63.892	2.127
Error	21	43.465	2.068	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Root length at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	168.515	28.084	15.501	9.584
Error	21	38.045	1.815	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Plant vigour index at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	19828697.8	3304782.9	86.001	1.106
Error	21	806914.5	38424.5	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Fresh shoot weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	2391.265	398.547	100.06	2.417
Error	21	83.640	3.988	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Dry shoot weight at 60DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	128.531	21.428	36.040	5.329
Error	21	12.480	0.592	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Fresh root weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	53.771	8.968	62.476	2.646
Error	21	3.015	0.144	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Dry root weight at 60 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	1.602	0.263	22.460	4.027
Error	21	0.250	0.019	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Number of leaves per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	763.505	127.254	41.064	1.559
Error	21	65.080	3.090	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Number of primary branches per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	41.752	6.950	67.654	1.202
Error	21	2.160	0.108	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Length of Pod)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	98.805	16.465	53.740	1.155
Error	21	6.435	0.304	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Number of pod per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	557.625	92.930	116.034	5.390
Error	21	16.820	0.809	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Weight of green pod)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	17.097	2.849	80.704	2.087
Error	21	0.743	0.033	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Yield of pod per plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	18793.892	3132.317	241.672	2.945
Error	21	272.180	12.969	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Yield of pod per treatment)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	6508315.085	1084719.186	110.793	8.619
Error	21	205598.115	9790.385	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Plant biomass)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	2687.504	447.915	356.771	5.212
Error	21	26.364	1.254	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Number of sclerotia per infected plant)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	43973.351	7328.898	751.683	2.215
Error	21	204.750	9.750	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Disease incidence at 30 DAS)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	5161.282	860.212	120.706	3.617
Error	21	149.663	7.127	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Pre emergence mortality)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	3399.712	566.610	103.465	1.727
Error	21	115.000	5.471	-	-
Total	27	-	-	-	-

ANOVA TABLE *IN VIVO* EXP.02 (Post emergence mortality)

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	6	823.925	137.324	524.311	9.505
Error	21	5.500	0.269	-	-
Total	27	-	-	-	-