

**MOLECULAR DIVERSITY ANALYSIS AND ANTAGONISTIC
POTENTIAL OF PLANT GROWTH PROMOTING
MICROORGANISMS FOR THE MANAGEMENT OF
EMERGING DRY ROOT ROT OF CHICKPEA CAUSED BY
Rhizoctonia bataticola (Taub.) Butler.**

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RAICHUR - 584 104**

JULY, 2022

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in

PLANT PATHOLOGY

By

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CERTIFICATE

This is to certify that the thesis entitled “**MOLECULAR DIVERSITY ANALYSIS AND ANTAGONISTIC POTENTIAL OF PLANT GROWTH PROMOTING MICROORGANISMS FOR THE MANAGEMENT OF EMERGING DRY ROOT ROT OF CHICKPEA CAUSED BY *Rhizoctonia bataticola* (TAUB.) BUTLER.**” submitted by **Ms. MEGHANA S. PATIL, ID. No. PHD19AGR9020.**, for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY**, to the University of Agricultural Sciences, Raichur is a record of bonafide research work carried out by her during the period of her study in this University, under my guidance and supervision, and the thesis has no previously formed the basis of the award of any other degree, diploma, associateship, fellowship or other similar titles.

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4. _____
(NAGARAJ, M. NAIK)

Affectionately dedicated to
MY Parents and sisters
My husband
My guide dr. Gururaj sunkad AND
Farming community

LIST OF SYMBOLS/ABBREVIATIONS

| Abbreviations | Descriptions |
|----------------------|---|
| % | : Per cent |
| > | : More than |
| ± | : Plus or minus |
| °C | : Degree centigrade |
| µl | : Micro litre |
| Anon. | : Anonymous |
| ANOVA | : Analysis of Variance |
| AUDPC | : Area Under Disease Progress Curve |
| BA | : Bacillus Agar |
| BLAST | : Basic Local Alignment Searching Tool |
| BOD | : Biological Oxygen Demand |
| bp | : Base pairs |
| CD | : Critical Difference |
| Cfu | : Colony forming unit |
| CRD | : Completely Randomized Design |
| CTAB | : Cetyltrimethylammoniumbromide |
| DAI | : Days after incubation |
| DAS | : Days after sowing |
| DMRT | : Duncans Multiple Range Test |
| dNTPs | : Deoxy nucleotide triphosphate |
| EC | : Emulsifiable Concentrate |
| <i>et al.</i> , | : And others |
| <i>etc.</i> | : Things |
| Fig | : Figure/s |
| FYM | : Farm Yard Manure |
| g | : Gram/s |
| GC-MS/MS | : Gas chromatography mass tandem spectrometry |
| h | : Hour/s |
| ha | : Hectare |
| <i>i.e.</i> , | : That is |
| kg | : Kilogram/s |
| l | : Litre/s |
| m | : Meter |

| | | |
|---------------|---|---|
| M | : | Molar |
| m/z | : | Mass to charge ratio |
| min | : | Minute/s |
| ml | : | Mililitre |
| mt | : | Million ton/s |
| NA | : | Nutrient Agar |
| NB | : | Nutrient Broth |
| NCBI | : | National Center for Biotechnology Information |
| ng | : | Nano grams |
| PCR | : | Polymerase Chain Reaction |
| PDA | : | Potato Dextrose Agar |
| PDB | : | Potato Dextrose Broth |
| PDI | : | Per cent disease index |
| pH | : | Potential of hydrogen |
| ppm | : | Parts per million |
| PVP | : | Polyvinylpyrrolidone |
| RCRD | : | Randomized Completely Randomized Design |
| rDNA | : | Ribosomal DNA |
| RH | : | Relative humidity |
| rpm | : | Revolution per minute |
| s | : | Seconds |
| Rs | : | Rupees |
| RT | : | Retention time |
| SC | : | Suspension Concentrate |
| Sl. No. | : | Serial number |
| t | : | Tonnes |
| T10 E1 buffer | : | 10mM TrisHCl base and 1 mM EDTA buffer |
| TAE Buffer | : | Tris acetate EDTA factor |
| TSM | : | Trichoderma specific media |
| <i>viz.</i> , | : | Namely |
| w/v | : | Weight/volume |
| WS | : | Water dispersible fine powder |
| μmol | : | Micromoles |
| mm | : | Millimeter/s |

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PHD19AGR9020**

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I. INTRODUCTION

Chickpea (*Cicer arietinum* L.), also known as gram, bengal gram, Egyptian peas, ceci beans and kabuli chana in all over the world. It is one of the earliest cultivated annual legumes of the family Fabaceae (Zohary and Hopf, 2000). It's probable place of origin lies in South East Turkey. This versatile legume is grown in 57 countries around the world (Merga and Haji, 2019) and it had a lion's share of 49.3 per cent in the total pulse production during 2020-21. It is the third most important pulse in the world after common bean and field pea. The crop is mainly rainfed and is often grown on residual soil moisture during winter in the tropics and spring in the temperate and Mediterranean regions (Pooran, 2020).

India is the largest producer of chickpea in the world followed by Australia, Myanmar and Ethiopia (Anon., 2020). Globally, chickpea is grown in an area of 137 lakh ha with a production of 142.4 lakh tonnes and productivity of 1038 kg/ha (Anon., 2020). In India, 11911.18 thousand tonnes of chickpea is cultivated under 9995.92 thousand ha of area with productivity of 1192 kg/ha (Anon., 2021) and it accounts for nearly 70 per cent of pulse export from India. Among the chickpea producing states in India, Madhya Pradesh ranks first in production followed by Rajasthan, Maharashtra, Uttar Pradesh and Karnataka. In Karnataka, the major chickpea growing districts are Gulbarga, Bidar, Dharwad, Gadag, Ballari, Koppal, Vijayanagar and Raichur. Karnataka alone produces the chickpea of 445.63 thousand tones in an area of 713 thousand ha with 625 kg/ha productivity (Anon., 2021).

Chickpea comes in a variety of different types and colour, not just the beige variety that we use in the diet; it can also be black, green, red and brown. Two distinct market type classes in chickpea are desi and kabuli. Desi chickpeas are the most prominent by far than kabuli, accounting for 80 per cent of global production (Vikram, 2021). However, chickpea also an essential crop for protein nutrition (Senthil-Kumar *et al.*, 2022). They contain a huge number of nutrients, proteins, minerals, vitamins, antioxidants, and also a wide range of essential amino acids. Additionally, chickpea is a great source of both soluble and dietary fibre, important for maintaining a healthy digestive system. Soluble fiber may assist with reducing the absorption of cholesterol into the bloodstream and helps to maintain blood sugar levels, which may help to reduce the risk of developing heart disease and also aid in managing diabetes. A cup of chickpeas,

weighing 164 g, can provide almost one-third trusted source of an adult's daily diet *i.e.*, 267 calories of energy, 14.4 g of protein, 4.2 g of fat, 44.7 g of carbohydrates, 7.8 g of sugar, 12.5 g of fiber, 80.4 mg of calcium, 4.7 mg of iron, 78.7 mg of magnesium, 274 mg of phosphorous, 474 mg of potassium. Hence chickpeas are fantastic alternative to meat for vegetarians (Ahmad *et al.*, 2005).

However, chickpea cultivation is affected due to multiple abiotic and biotic stresses. It is affected by 172 plant pathogens which cause seed, soil borne and foliar diseases *viz.*, 67 fungi, 3 bacteria, 22 viruses and mycoplasma and 80 nematodes reported from 55 countries (Manjunatha *et al.* 2011). Yield losses due to damage to the root architecture are attributed to root diseases (Gaur *et al.* 2013). Among root diseases, in addition to Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *ciceri*), chickpea is affected by dry root rot, caused by the necrotrophic phytopathogenic fungus *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid]. Other root rots in chickpea include wet root rot (caused by *Thanatephorus cucumeris*, formerly known as *Rhizoctonia solani*), collar rot (caused by *Athelia rolfsii*), and black root rot (caused by *F. solani*) (Sharma *et al.*, 2015 and Rai *et al.*, 2022).

Dry root rot in chickpea was first reported from India by Mitra in 1931. It is widespread in chickpea growing regions such as South Asia, Africa, Iran, Mexico, the United States, and Spain. The recent rise in global temperature and worsening of drought spells has aggravated dry root rot outbreaks in chickpea. The disease causes yield loss up to 100 per cent in susceptible varieties of chickpea under favorable conditions (Gupta and Sharma, 2015). The disease is more prevalent between flowering and podding stages, especially in rainfed chickpea cultivation areas, where soil moisture recedes as the crop growth advances. Soil moisture deficit and hot temperature (30-35 °C) are the two known factors to predispose the crop to dry root rot disease (Sharma and Pande, 2013).

Typical root symptoms of dry root rot disease include in the taproots and lateral roots. Initial symptoms are scattered necrotic brownish to black lesions in roots, progressing to rotting and withering of lateral roots, accompanied by prematurely dried, straw-colored foliage. Many black microsclerotia of *M. phaseolina* are visible in the root bark, cortex and pith regions. It is plausible that premature drying occurs due to the blockage of the stele by fungal microsclerotia and mycelia (Singh *et al.*, 1990),

compromising the transport of water and nutrients. Foliar symptoms start with gradual yellowing from the base to the top leaves (Sharma *et al.* 2012). Under mild infection by the pathogen, patches of diseased plants are relatively small, corresponding with yield losses up to 5-10 per cent. However, under moderate infection, infected patches can expand and coalesce, corresponding to yield losses of 30-50 per cent. Under severe infection, the entire chickpea field shows premature dried plants, accounting for 60-80 per cent yield losses (Rai *et al.*, 2022).

Rhizoctonia bataticola is a soil borne, necrotrophic and polyphagous fungal pathogen. The genus *Rhizoctonia* (“Root killer”) was described by the French mycologist Augustin Pyramus de Candolle in 1815 for plant pathogenic fungi that produce both hyphae and sclerotia. A comprehensive survey and redistribution of species name “*Rhizoctonia*” was published in 1994 by Anderson and Stalpus. According to this published information, *R. bataticola* is used as a synonym to *M. phaseolina* (Tassi) Goid. Currently, *M. phaseolina* is officially recognised as the correct taxonomic name (CMI description of pathogenic fungi No.275) with the sclerotial stage known as *R. bataticola* (Holliday and Punithalingam, 1970).

The characteristic morphological features of *R. bataticola* are right-angle mycelial branching, multinucleate septate mycelia, cross-wall formation at the beginning of new branching mycelia and partial hyphal fusion. The fungus *R. bataticola* exists in an anamorph (sclerotial) stage in soil and on crop residues (Sharma and Pande, 2013). Hence, microsclerotia of this fungus, which are capable of withstanding harsh environmental conditions, serve as primary inoculum in the disease cycle (Rai *et al.*, 2022).

At present, boosting agricultural productivity relies heavily on the use of chemicals, which are economically unavailable to many farmers throughout the world and can cause negative environmental impacts. In addition, environmental stresses may also be a major constraint to plant growth and yield, causing low crop productivity, affecting global food security (Lopes *et al.*, 2021). Therefore, to increase global agricultural production in a more economically and environmentally sustainable way, there is need to use lesser chemicals and increase plant tolerance to biotic stresses. The use of plant growth-promoting microorganisms (PGPMs) is potentially advantageous for improving

crop productivity, food quality and security in more sustainable and eco-friendly manner (Etesami, 2020).

The rhizosphere and endophytic fungal and bacterial community can harbor beneficial organisms known as PGPMs. Based on the interaction of roots with plants, PGPMs includes organisms present in the soil *i.e.*, Plant Growth Promoting Rhizobacteria (PGPR) as well as Plant Growth Promoting Fungi (PGPF) and also organisms present inside the plant *i.e.*, endophytes (Mitra *et al.*, 2019).

PGPMs improve plant growth by enhancing the availability of nutrients, as phyto-stimulators, by regulation of phytohormones and by increasing plant tolerance against biotic stresses (Lopes *et al.*, 2021). PGPMs also act as bio-pesticides or bio-control agents against plant pathogens, through competition for nutrients, antagonism and induce systemic resistance (Khan *et al.*, 2013). Based on their effects mentioned above, PGPMs increase auxin, gibberellin, cytokinin and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase concentrations. These beneficial microbes can also secrete volatile metabolites, which can induce disease resistance (Khan *et al.*, 2020). Thus, there is an urge to understand better and move forward with knowledge that PGPMs hold great promise in promoting productivity through synergistic interactions with host plants and can play role in disease management (Msimbira and Smith, 2020).

Kloepper and Schroth (1981) termed the beneficial rhizobacteria as plant growth-promoting rhizobacteria. These encompass all bacteria that inhabit the plant roots and exert a positive effect by various mechanisms, ranging from a direct influence (Increased P solubilization and uptake of nutrients or production of plant growth regulators), to an indirect effect (Suppression of pathogen by producing siderophore or antibiosis). Some of these PGPR can also enter root interior and establish as endophytic populations. Antibiotic, anti-fungal metabolites, enzyme, phenolic compound, signaling compound and other determinants of defense in response to pathogen attack are produced by PGPR as their plant growth promoting activity indirectly (Kloepper and Schroth, 1978). In agriculture many rhizospheric microbial genera are being used. They are *Pseudomonas* sp., *Azotobacter* sp., *Bacillus* sp., *Rhizobium* sp., *Klebsiella* sp., *Azospirillum* sp., *Enterobacter* sp., *Caulobacter*, sp. *Micrococcous* sp., *Chromobacterium* sp., *Alcaligenes* sp., *Burkholderia* sp., *Serratia* sp., *Arthrobacter* sp., *etc.*, Out of all these species, *Pseudomonas* sp. and *Bacillus* sp. are most reported PGPRs (Mitra *et al.*, 2019).

Non-pathogenic soil borne filamentous fungi are classified under plant growth promoting fungi. PGPF have their economical and beneficial effects on plants growth and sustainable agriculture. Based on the research articles and reviews, PGPF belongs to the genus like *Fusarium*, *Penicillium*, *Mycorrhiza*, *Phoma*, *Gliocladium*, *Metarhizium*, *Trichoderma*, *Pythium*, *Aspergillus* etc., (Mitra *et al.*, 2019).

Endophytes are those microorganisms which live inside the plant tissues for part of their life cycle or for their entire lifespan without showing their presence as the plants do not express any visible symptoms. At the most basic level, 'endophyte' simply refers to the location of the organism: 'endo' means within and 'phyte' means plant, therefore describing all organisms that live inside a plant. Hence an endophyte can be fungi, bacteria or an actinomycete. Numerous studies suggested that endophytic PGPMs colonization may result in enhanced phosphorus, improved drought tolerance, improved plant growth and provides tolerance against various biotic and abiotic stresses (Suman *et al.*, 2013). Therefore, they can be used as bio-regulators to induce resistance against diseases and as biological control agents against certain pathogens as well as weeds. These endophytes may prove to be a major source of new bioactive compounds used in pharmaceutical, agricultural as well as food industry. Most reported endophytes are *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp., *Streptomyces* sp., *Actinoplanes* sp., *Alternaria* sp., *Trichoderma* sp., *Fusarium* sp., etc., (Mitra *et al.*, 2019).

However, despite the importance of the PGPMs-plant relationship, the knowledge on the interactions between PGPMs and pathogens under hostile environmental conditions is still rather limited in case of dry root rot of chickpea. Hence, there is a need to explore PGPMs now for the purpose of improving plant growth and as well as management of chickpea dry root rot. Keeping this in view, it is essential to collect and isolate the PGPMs from different geographic regions and to identify the antagonistic PGPMs against *R. bataticola*.

PGPMs stimulate growth promoting activity of the host plant directly by changing and producing the plant growth regulators. They reported to produce certain growth promoting hormones such as indole acetic acid, phosphate, zinc and other micro and macro nutrients mobilization and solubilization, production of siderophores and enzymes such as β -1-3-glucanase and chitinase. These are called as plant growth promoting traits

of PGPMs. Very less work is carried on plant growth promoting traits of PGPMs is done in case of chickpea. Hence, it is necessary to study on this aspects in chickpea.

Recently, molecular tools and techniques gained much importance in detection, characterization and to study the diversity of microbial population. Therefore, molecular characterization of the potential PGPMs using universal primers may help in identification as well as to study the diversity of PGPMs. In case of chickpea, limited literature is available on molecular aspects. It is essential to make an effort to characterize and analyze the diversity of PGPMs isolated from chickpea rhizosphere and plant tissues (Endophytes).

Microbes are a good source for extraction of biologically active compounds due to their ease of isolation, growth and inability to impact negatively on the environment. The bio-active compounds may be growth promoters and resistance inducers. The extraction and identification of bio-active compounds play an important role in the current scenario. Hence, the studies on extraction, identification, screening of bio-active compounds and quantitative estimation of bio-active compounds responsible for inhibition of *R. bataticola* may be useful for scientific community.

Many PGPMs have been reported to improve the growth as well as disease management in several crops. However, the information on exploitation of PGPMs for the management of dry root rot of chickpea is very meager. Therefore, it is imperative to decipher how these PGPMs are cost-effective and eco-friendly measures for disease management. Hence, the studies related to use of PGMS alone and in combination under pot as well as field conditions for management of chickpea dry root rot are necessary in the era of organic farming.

Hence, keeping the above facts in view, the present investigation was carried out with following objectives.

Objectives:

1. Collection, isolation, identification of PGPMs and their antagonistic potential against *R. bataticola*.
2. Studies on plant growth promoting traits of potential PGPMs.
3. Molecular characterization and diversity analysis of potential PGPMs and their screening for bio-active compound production against *R. bataticola*.
4. Extraction, identification of bio-active compounds and screening of crude extract from potential PGPMs responsible for inhibition of *R. bataticola*.
5. Management of dry root rot using potential PGPMs in pot and field experiments.

II. REVIEW OF LITERATURE

Dry root rot of chickpea caused by necrotrophic phytopathogenic fungus *Rhizoctonia bataticola* is gaining importance in the changed scenario of climate when growing crop is predisposed to high temperature and moisture stress. Being mainly a soil inhabiting pathogen, high temperature and soil moisture stress are responsible factors for the development of disease. The disease is distributed in most chickpea-growing areas in India and even in other countries. However, limited literature is available on molecular characterization, information on bio-active compounds and use of PGPMs (endophytic and rhizospheric) for the management of diseases. The literature pertaining to occurrence and distribution of dry root rot disease, symptomatology, causal organism of the disease, extraction, identification, screening of bio-active compounds and management of dry root rot using potential PGPMs presented in this chapter will help us to understand the existing research. Wherever the literature on particular aspect of PGPMs was scanty, it was amply supplemented with and supported by other pathogens and crops.

2.1 Dry root rot

2.1.1 Occurrence and distribution of disease

The dry root rot of chickpea was first reported from India by Mitra (1931). Later, the disease was reported from major chickpea growing areas in India and other countries like Iran (Kaiser *et al.*, 1968), USA (Westerlund *et al.*, 1974) and several countries in Asia and Africa (Nene *et al.*, 1996). Dry root rot is widespread in chickpea-growing regions such as South Asia, Africa, Iran, Mexico, the United States, and Spain (Gupta and Sharma, 2015).

Tripathi and Sharma (1983) reported the appearance of *Rhizoctonia* root rot in pulses. An association of *Fusarium* sp. and *Rhizoctonia* sp. with wilted chickpea was first reported by Narasimhan (1929). Subsequently, the disease was reported from Punjab (Luthra, 1938), Madhya Pradesh (Sharma and Khare, 1969), West Bengal (Biswas and Guptha, 1981) and Haryana (Tripathi and Sharma, 1983). The disease was also reported from Egypt, Iran, Kenya, Lebanon, Mexico, Myanmar, Pakistan, Spain, Sri Lanka, Sudan, Syria, Tanzania, Turkey, Uganda, USA and Zambia. It causes considerable yield losses

that vary from 5-50 per cent and may cause 100 per cent losses in susceptible cultivars under favourable conditions (Nene *et al.*, 1996).

Manjunatha *et al.* (2011) reported the dry root rot disease incidence in the farmer's field at Raichur, Gulbarga and Bidar districts of Karnataka. The maximum incidence of disease was noticed in Gulbarga (9.8 %) followed by Raichur (7.6 %) and the least (6.18 %) in Bidar. The overall incidence of dry root rot ranged from 1-19 per cent across the districts.

The occurrence and severity of dry root rot of chickpea in the year 2010-11, was accessed in Jammu and Kashmir. The maximum disease incidence was up to 40 per cent in Shangus and minimum disease incidence was up to 4.11 per cent in Naina village of Jammu and Kashmir. The disease occurrence was observed in late October to mid November. The intensity of the disease was high in the month of February and March during late flowering and podding stages (Khan *et al.*, 2012).

Ghosh *et al.* (2013) reported the distribution and incidence of chickpea disease in Central and Southern parts of India such as Andhra Pradesh, Karnataka, Madhya Pradesh and Chhattisgarh in 2010-2011. The disease was found in all the sites surveyed and its incidence ranged from 8.9-10.3 per cent irrespective of cultivar type and location.

Dry root rot of chickpea is more prevalent between flowering and podding stages, especially in rainfed areas of chickpea cultivation, where soil moisture recedes as crop growth advances. The disease incidence is also influenced by soil pH, with the highest dry root rot incidence occurring at pH 5.0. Similarly, soil type has an impact on disease incidence with clay and sandy soil characteristics supporting dry root rot occurrence (Sharma and Pande, 2013).

Srinivas (2016) recorded the status of dry root rot incidence in chickpea in sixty eight different chickpea growing locations of central (Madhya Pradesh and Maharashtra) and southern (Andhra Pradesh, Telangana and Karnataka) India. Mean maximum dry root rot incidence was observed in Telangana (18.28 %) followed by Madhya Pradesh (18.10 %), Karnataka (7.85 %), Andhra Pradesh (5.40 %) and the least in Maharashtra (5.38 %). They also studied that the maximum severity of dry root rot disease was at the temperature of 35°C with disease rating of 8.5 irrespective of the isolate. This was followed by 30°C with disease severity rating of 7.9 followed by 25°C with disease

severity rating of 7.0. It was observed that, at the temperature of 20°C and 15°C, there was development of the lesions but the disease could not develop further. The disease severity was very low at 15°C with 1.4 rating while it was 3.1 at 20°C.

Deepa *et al.* (2018) recorded the occurrence and distribution of dry root rot of chickpea in eight districts of the major chickpea growing areas in North Karnataka viz., Bidar, Kalaburagi, Yadagiri, Raichur, Koppal, Ballari, Bijapur and Dharwad during 2015-16 and 2016-17. Among the districts, mean disease incidence was maximum in Kalaburagi (32.93 %) followed by Raichur (29.87 %) and Vijayapur district (24.84 %). The least disease incidence was recorded in Yadgiri district (3.35 %). The overall mean incidence of dry root rot (38.72 %) was more during *rabi*, 2015-16 compared to *rabi*, 2016-17 (32.73 %) and the North Karnataka recorded overall mean incidence of 35.72 per cent.

2.1.2 Symptomatology

Singh *et al.* (1990) reported that roots infected with *R. bataticola* caused the disintegration of cortical tissues with mycelial and sclerotial bodies plugging the xylem vessels. The dried plants due to disease generally appeared during flowering and podding time and were scattered in the field. The seedlings were also got infected. Drooping of petioles and leaflets was confined to the top of plant. Leaves and stem of affected plant usually straw coloured but in some cases, the lower portion of the stem was brown. When the plant was uprooted, lower portion of the tap root remained in the soil. It was devoid of lateral and finer roots. Dark minute sclerotial bodies were seen on the roots exposed inside the wood.

According to Dastur (1935) only mature plants were affected by the dry root rot pathogen, showing bronzing of the leaves on one or more of the lower branches. The colour of leaves later changed to yellow and then brown. The affected branches and leaf stalks of diseased plants were stiff and turned upwards and leaflets stand more or less vertically and shed prematurely. The terminal part of the tap root was black or brown and shriveled.

Among the several soil-borne fungal diseases, dry root rot caused by *R. bataticola* Butler is the most severe disease of chickpea especially where, the crop is mostly grown in *rabi* season under rainfed conditions. Predominantly, disease appears around flowering

and podding stage. The first symptom is yellowing and sudden drying of the plants. The tap root becomes dark brown quite brittle in dry soil and shows extensive rotting resulting in the loss of lateral roots. The lower portion of the tap root is often left in the soil when plant is uprooted (Nene *et al.*, 2012).

Sharma *et al.* (2015) described that dry root rot symptoms were commonly observed during post-flowering stage of chickpea which include drooping and chlorosis of petioles and leaflets, initially confined to top leaves of the plant. Leaves and stems of affected plants were usually straw coloured and in some cases, the lower leaves and stems were brown. The tap root turns black with signs of rotting and is devoid of most of the lateral and finer roots. The dead roots were quite brittle and showed shredding of the bark. The tip of the root were easily broken leaving the lower portion of the tap root in the soil when plants were uprooted. Dark minute sclerotial bodies were seen on the roots exposed and inner side of the bark or when split open at the collar region vertically.

Khaliq *et al.* (2020) described that dry root rot, caused by *Rhizoctonia bataticola* is a serious and widespread disease of chickpea. Pod setting and late-flowering are generally the stages where the plant is most vulnerable to dry root rot. Infected plants appear completely dried. Destruction of lateral roots and extensive rotting are the symptoms commonly associated with the disease on rotten roots and minute sclerotial bodies appear mainly on the outer surface of the tap root and in the pith cavity.

2.1.3 Pathogen

The *R. bataticola* as a plant pathogen was recognized by Halsted (1890). Taubenhau (1913) gave the name of the genus as Sclerotium because of absence of spores and the species name as *bataticola* because it was pathogenic to *Ipomea batatus* (L.) Lam. Briton Jones (1925) transferred the fungus to the genus *Rhizoctonia* based on the identification of cultures by Butler (1918).

Ashby (1927) accepted *Macrophomina* and rejected the binomial *M. phaseoli* and proposed a new binomial *M. phaseolina* as the pycnidial stage of *R. bataticola* on the basis that *M. phaseoli* was the earliest applicable binomial. He was not aware that Tassi had earlier described *M. phaseolina*. Haigh (1930) suggested that *R. bataticola* can be

used for sclerotial isolate and pycnidial strains should be called *M. phaseolina*. Goidanich (1947) examined the original material of Tassi and compared it with *M. phaseoli*, *M. corchori*, *M. cajani*, *M. sesami*, *M. philippinensis*, *Dothiorella cajani* and *D. phaseoli*. He confirmed all of them were identical. He corrected the mistake made by Ashby (1927). According to the International Code of Botanical Nomenclature, the binomial *M. phaseolina* was the valid name for the pycnidial stage of *R. bataticola*.

Sharma *et al.* (2012) reported that the fungus grows rapidly on potato dextrose agar and produces brown to grey coloured mycelium that become darker with age. The young hyphae were thin, hyaline, aseptate and dichotomously branched and later produce typical black sclerotia. Reverse side of the colony color of most of the isolates showed black color. The fungus produce abundant aerial mycelium, as high as to touch the cover of the culture plates, or mycelium is found to be completely or partially suppressed. Most of the isolates grew very fast and covered the plate within 96 h.

Dry root rot is caused by a chickpea-specific strain of *M. phaseolina*. In earlier literature, the causal agent was referred to as *R. bataticola*. *M. phaseolina* (Tassi) Goid. is a soil borne necrotrophic fungal phytopathogen. Based on the morphology and sequence data, it is classified under division Ascomycota. Mycelia and microsclerotia of the fungus are present in soil and chickpea plants. Neither pycnidia nor a sexual stage have been reported to date in chickpea. The fungus reproduces by undergoing fragmentation (Ghosh *et al.*, 2013; Sharma and Pande, 2013 and Rai *et al.*, 2022).

The genus *Rhizoctonia* (“Root killer”) was described in 1815 by the French mycologist Augustin Pyramus de Candolle for plant pathogenic fungi that produce both hyphae and sclerotia (Sharma *et al.*, 2015).

Srinivas (2016) used five isolates (Rb 2, Rb 13, Rb 22, Rb 40 and Rb 63) to know the effect of temperature on colony growth of *R. bataticola*. All the isolates were grown at seven different temperatures *viz.*, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C. The optimum growth of the fungal isolates was found at 35°C (79.3, 90.0 and 90.0 mm at 48, 72 and 96h after incubation respectively). The next best temperature was 30°C with radial growth of 71.3, 88.1 and 90.0 mm at 48, 72 and 96 h after incubation respectively) followed by 25°C, 20°C and 15°C. There was very meager growth at 40°C (10.4, 15.0 and

17.5 mm at 48, 72 and 96 h after incubation respectively) and no growth was observed at 45°C.

The *R. bataticola* produced grey cottony mycelial growth on potato dextrose agar medium and the colony morphology was compact or fluffy or appressed. The characteristic features of *R. bataticola* are right angle branching of the mycelium and constriction of the branch near the point of origin. The young hyphae are thin, hyaline, septate and dichotomously branched and the sclerotia formed are black, smooth, varying from spherical through oblong to irregular shapes (Deepa *et al.* 2018).

Gade *et al.* (2018) analyzed 40 isolates of *R. bataticola* from different agro climatic regions of India for morphological variability. The sclerotial size was measured by preparing slides from 7- day-old pure cultures of *R. bataticola* isolates and examined under a microscope by using ocular micrometer. Sizes of fifty randomly selected sclerotia were measured and their means were calculated. The isolates were classified as large (>80 µm), medium (60-80 µm), and small. The results indicated the significant variations among the isolates regarding the size of their sclerotia. Maximum sclerotial size was 120.11 µm while the smallest size of sclerotia was 42.03 µm. The individual average sclerotial size of isolates ranged from 42.03-120.11 µm.

Chiranjeevi *et al.* (2020) collected the different isolates of *R. bataticola* and were isolated from the samples collected from major chickpea growing areas of Andhra Pradesh. The isolates were purified by single sclerotial isolation technique and were identified as *R. bataticola* based on morphological and cultural characters using the descriptions given by Barnett and Barry (1972). The mycelium was initially light brown in colour which was later converted to dark brown to black in colour. Production of aerial mycelium was also observed in some isolates. The vegetative mycelium was characterized by the formation of barrel shaped cells and the formation of septum near the origin of branch of the mycelium. Branching occurred mostly at right angle to parent hyphae, but branching at acute angles was also observed.

Rhizoctonia is a genus of anamorphic fungi in the order Cantharellales and family Ceratobasidiaceae. *Rhizoctonia* species do not produce spores, but are composed of hyphae and sclerotia (hyphal propagules). *Rhizoctonia* species are saprophytic, but some act as facultative plant pathogens causing commercially important crop diseases. It is well

known to cause diseases viz., leaf blight, stem blight, wilt, seedling decay, root rot, seedling blight, charcoal rot, stalk rot and fruit rot in crop plants. *Rhizoctonia* mainly associated to roots as a soil borne fungi. If host plant is absent then they behave like saprophytes and grow on dead and decay organic materials. Therefore, this fungus has reported in forest as well as agricultural soil throughout the world and majorly involved in economic loss of crop yield (Sonawane *et al.*, 2021).

Microsclerotia are quiescent structures by which *M. phaseolina* maintains its viability under harsh conditions such as winter, drought, and flood. This quiescent state can often last for 10 months under dry storage. Scanning electron micrographs demonstrated that it produces a rindless type of microsclerotium, in which all of the cells have a thick cell wall. Because the microsclerotia are cemented together by a dark-pigmented gelatinous matrix, they appear rigid and dark in color. The pigmentation is most intense at the center of a microsclerotium and least at the periphery. The diameter of microsclerotia of *M. phaseolina* ranges from 50 to 150 μm (Rai *et al.*, 2022).

2.1.4 Pathogenisity test

Basbagci *et al.* (2022) conducted pathogenicity test for 19 isolates using susceptible chickpea seeds (cv. ILC 482) in pots with the one of the most virulent isolate (D33). Inoculum of *R. bataticola* was prepared on the sterile wheat grains in test tubes. The mixture of perlite: silt loam + fine sand (2: 1) was autoclaved and filled in 13 cm diameter plastic pots. Five disinfected ILC 482 seeds were placed on the sand for each pots, and then five pathogen-colonized wheat grains were placed near the seeds to serve as inoculum. Five inoculated pots were used. For the control treatment, the seeds were sown in pots without inoculum. All the pots were incubated at 25 ± 1 °C 12 h photoperiod for 7 weeks in a growth chamber. Re-isolation was made from ungerminated seeds and roots of diseased plants, fulfilling Koch's postulates.

Lekhraj *et al.* (2012) conducted pathogenisity test of *M. phaseolina* isolates of infected chickpea collected from 9 various parts of Rajasthan, Hissar and New Delhi. Pathogenicity of these isolates tested on 40 day old chickpea cultivar C-235, but there was no direct relationship between cultural characters and virulence of the isolates. The isolate BKN was highly pathogenic. The isolates SGN, NHR, DUR, HAU, DEL and HNG having different types of colony growth were moderately pathogenic and the

isolates CHR and UPR with different mycelium characters were less pathogenic. Similarly, there was no apparent correlation between the virulence and growth rate of the fungal colonies.

Fifty isolates of *R. bataticola* were collected on chickpea from different agro climatic zones in India and evaluated for the degree of variability in pathogenicity test. All the isolates proved pathogenic on chickpea cultivar BG 212. The dry root rot disease severity ranged from 4 to 9 (1-9 severity rating scale). The effect of isolates on severity was significant. The pathogenic and non pathogenic isolates were not concentrated in any one particular state/region. Among the 94 isolates of *R. bataticola* collected from different agro ecological zones, 8 isolates from Andhra Pradesh and Madhya Pradesh showed less aggressive on chickpea cultivar BG 212 (Sharma *et al.*, 2012).

Deepa *et al.* (2018) conducted pathogenicity test in sick pot by growing susceptible variety (A-1) of chickpea. The first symptom of yellowing of one or more branches started on 20 day after sowing. Later, drooping of branches was observed on 32th to 35th day after sowing. Drying and shredding of leaves were observed near the soil surface and wilting of plants took place on 45 to 50 day after sowing. Uprooting the affected plants was easy and root portion showed black rotted as well as shredding of roots. Symptoms due to dry root rot in the pots inoculated with *R. bataticola* culture were similar to dry root rot affected plants in the field. Reisolation of the fungus from root region of the plants was made and pathogenic cultures obtained were compared with original culture of *R. bataticola*. The culture was found similar with regard to all morphological characters on PDA and thus pathogen was identified as *R. bataticola*.

Lakhran *et al.* (2018) tested for its pathogenicity to ascertain ability of the organism to cause dry root rot in chickpea plant. The pathogenicity of *M. phaseolina* was tested under pot conditions by seed and soil inoculation techniques to prove pathogenicity by following two methods of inoculation *i.e.* seed and soil inoculation techniques. Among these, soil inoculation method was proved highly effective. *M. phaseolina* isolated from infected roots of chickpea plant found pathogenic when seed and soil was inoculated artificially to chickpea plant under pot house conditions. The characteristic symptoms of root rot appeared after 20 days. The most recognizable symptoms were sudden death of chickpea seedlings. First leaves start withering and drying and the root rot was followed gradually killing whole plant. Roots of the infected plant showed brown to blackish

lesions. Isolation from artificially inoculated plant yielded *M. phaseolina* which was identical to original one.

2.2 Plant growth promoting microorganisms

PGPMs have shown potential in the management of various agricultural problems and especially their use in the form of biofertilizers and biopesticides has resulted in lesser reliance on synthetic agrochemicals. However, the role of PGPMs in the remediation of ecosystems through removal of recalcitrant compounds and as alleviators of abiotic stresses, helping to combat the impact of climate changes (Mishra *et al.*, 2017).

The main interaction between rhizosphere microbe's and plants mainly bacteria, fungus and arbuscular mycorrhiza fungi play an important role in obtaining water and nutrients from the rhizospheric zone and soil (Mitra *et al.*, 2019). PGPMs have proved themselves of great importance when it comes to sustainable agriculture. PGPMs help in the plant growth promotion by different mechanisms. PGPMs *viz.*, Rhizobacteria, actinomycete, fungai, arbuscular mycorrhizal fungi and endophytes, they employ different mechanisms to restore plant growth and nutrition. PGPMs can be symbiotic showing direct beneficial interaction with plant or non-symbiotic action PGPMs also help in protection from phytopathogens and also help in growth promotion. PGPMs also show promising results in the field (Mitra *et al.* 2019).

The inoculation of PGPMs in agricultural crops is considered an eco-friendly alternative to chemicals. Microbial inoculants are mainly inoculated onto seeds, roots and soil. PGPMs improve plant growth by enhancing the availability of nutrients, the regulation of phytohormones, and by increasing plant tolerance against biotic and abiotic stresses (Lopes *et al.* 2021).

2.3 Collection, isolation, identification of PGPMs and their antagonistic potential against *R. bataticola*.

Rangeshwaran *et al.* (2008) isolated five endophytic and two rhizospheric bacteria from healthy chickpea plants and identified them based on morphological, physiological and biochemical tests. *Erwinia herbicola* and *Enterobacter agglomerans*, which had nitrate-solubilizing ability, were isolated from the root endosphere and *Bacillus megaterium*, *B. circulans* and one unidentified species of *Bacillus* were isolated from the

leaf and stem tissues. Higher growth promotion was noticed in chickpea plants treated with *B. megaterium*, *E. agglomerans* and *Bacillus* sp. and the plants treated with endophytes survived well in the presence of the pathogen, *Rhizoctonia solani*. Hence, they concluded endophytic bacteria from healthy plant tissue could play a useful role in plant protection.

Reddy (2010) isolated 23 antagonistic microflora (8 fungi and 15 bacteria) from rhizosphere soil and root endophytes of chickpea. The potential antagonistic isolates were identified based on their ability to inhibit the growth of *Rhizoctonia bataticola* in dual culture technique. Among the eight fungal antagonistic isolates, *Trichoderma* isolate-3 (T₃) was superior with highest per cent (57.83 %) inhibition of the growth of *R. bataticola* followed by *Trichoderma* isolate-4 (T₄) (54.21 %). Regarding root endophytes, the isolate REB-8 showed maximum inhibition (76.47 %) of growth of *R. bataticola* followed by RB-1 (74.11 %) and REB-9 (71.76 %).

Amrutha *et al.* (2014) isolated total of 40 antagonistic bacteria, out of which 20 bacteria were obtained from rhizosphere soil and 20 bacteria from root as root endophytes in chickpea against dry root rot. The bacterial isolates from rhizosphere soil were designated as CRB-1 to CRB-20 followed by root endophytic bacteria as CREB-1 to CREB-20. Among the 20 rhizospheric bacterial antagonists tested, the isolate CRB-13 showed the maximum inhibition (86.66 %) and among the 20-root endophytic bacterial antagonists tested, the isolate CREB-13 showed the maximum inhibition (95.55 %) of growth of *R. bataticola*.

Ganga *et al.* (2015) isolated endophytic bacteria from the roots of dry root rot infected chickpea plants using phosphate buffer. Individual colonies were streaked on plates of nutrient agar media and tryptic soy agar media. Anti-microbial assay was performed by dual culture method. Twenty three isolates were tested against *R. bataticola* among them five isolates were showing good inhibition. Maximum (81 %) inhibition was recorded by the isolate B5. The five isolates, B5 with 81 per cent, K1 with 77 per cent, K2 with 75 per cent, C1 and A5 with 74 per cent were most promising results and this shows that these bacteria may involve in controlling the pathogen

Sreevidya *et al.* (2016) isolated 89 isolates from the soil samples and screened for their antagonistic potential against major pathogens of chickpea such as *Fusarium*

oxysporum f. sp. *ciceri*, *M. phaseolina* and *S. rolfsii* by dual culture assay, four actinomycetes viz. SAI-13, SAI-29, VAI-7 and VAI-40 were found most promising.

Gindi *et al.* (2018) characterized five strains of *Streptomyces* (AC-5, AC-6, AC-10, AC-18 and AC-19) and another five strains of *Bacillus* (BS-10, BS-15, BS-17, BS19 and BS-20) for their antagonistic potential against dry root rot of chickpea, caused by *R. bataticola*, by dual culture assay and blotter paper assay. These strains were earlier reported by them as biological control agents against Fusarium wilt of chickpea, caused by *F. oxysporum* f. sp. *ciceri* and plant growth-promoting agents in chickpea. In their present study, both the *Streptomyces* and *Bacillus* strains inhibited three strains of *R. bataticola* (RB6, RB24 and RB115) in both dual culture and blotter paper assays.

Shirasangi and Hegde (2018) isolated total of 82 (28 from leaf, 33 from stem and 21 from root) fungal endophytes from apparently healthy groundnut plant parts and evaluated these endophytes against *R. solani* by dual culture technique. Among 28 leaf endophytes, the endophyte LFDwAC-7 (48.63 %) showed the maximum mycelial inhibition which was on par with LFDwAC-9 (46.67 %), LFBaBa-26 (46.67 %) and LFBaAV-21 (45.88 %). The endophyte LFDwAC-4 (3.14 %) showed the least mycelial inhibition. Among the 33 stem endophytes, the endophyte SFDwAC-8 showed the maximum mycelial inhibition of 49.63 per cent which was on par with SFDwAC-7 (48.15 %) and SFDwBi-33 (47.78 %). The endophyte SFDwAC-15 was less effective with the least mycelial inhibition (22.22 %) followed by SFBeKh-24 (25.56 %). Among 21 root endophytes, the endophyte RFDwSo-33 (49.80 %) showed the maximum mycelial inhibition which was on par with RFDwAC-8 (48.24 %), RFDwSo-34 (47.45 %) and RFDwAC-9 (46.27 %). The endophyte RFBaAv-31 (25.49 %) showed the least mycelial inhibition and it was less effective as compared to other endophytes.

Thirty plant growth promoting rhizobacteria and 20 endophytic bacteria were isolated from different varieties of turmeric (*Curcuma longa* L.) from South India and were identified based on biochemical assays and 16S rDNA sequence analysis. The isolates were screened for antagonistic activity against *P. aphanidermatum* and *R. solani* causing rhizome rot and leaf blight diseases in turmeric, by dual culture and liquid culture assays. Results revealed that only five isolates of PGPR and four endophytic bacteria

showed more than 70 per cent suppression of test pathogens in both assays. Selected isolates showed multiple Plant growth promoting traits (Vinayarani and Prakash, 2018).

Veenashri *et al.* (2019) evaluated four bioagents for their efficacy in inhibiting mycelial growth of *R. bataticola* causing dry root rot of chickpea by dual culture technique. The results indicated that *T. harzianum* is highly antagonistic to *R. bataticola* by suppressing its growth by 78.67 per cent. Paper towel method was also followed to test the efficacy of bioagents in suppressing the seed borne infections. Among the four bioagents with three concentrations *viz.*, 0.4, 0.8 and 1.2 per cent tested, as the concentration increased the percent seed infection was reduced, percent germination and vigour index were increased. *T. harzianum* with the concentration of 1.2 per cent has shown the best result. Seed treatment with *T. harzianum* showed least mean seed infection (13.73 %), higher mean per cent germination (78.03 %) and high mean vigour index (1461.47).

2.4 Studies on plant growth promoting traits of potential PGPMs

Akhtar and Siddiqui (2007) isolated non - rhizobial endophytic bacteria from the root nodules of chickpea to assess their ability to improve plant growth and symbiotic performance and to control root rot in chickpea under saline soil conditions. A total of 40 bacterial isolates from internal root tissues of chickpea grown in salinated soil. Four bacterial isolates, namely *B. cereus* NUU1, *Achromobacter xylosoxidans* NUU2, *B. thuringiensis* NUU3 and *B. subtilis* NUU4 colonizing root tissue demonstrated plant beneficial traits and antagonistic activity against *F. solani*. The strain *B. subtilis* NUU4 proved significant plant growth promotion capabilities, improved symbiotic performance of host plant with rhizobia and promoted yield under saline soil as compared to untreated control plants under field conditions.

A total of 150 strains (endophytic and rhizospheric isolates) isolated from canola were characterized for plant growth promoting (PGP) traits. Among them, 100 isolates produced indole-3-acetic acid (IAA), whereas 17 isolates solubilized phosphate, 44 isolates produced siderophore, 34 produced 1-aminocyclopropane- 1-carboxylate (ACC) deaminase and five produced hydrocyanic acid (HCN). All of the screened isolates with different growth promoting traits were separately inoculated as different mixtures on two rice cultivars under abiotic conditions. All of the IAA producing strains were consistently

more active and more competitive in colonizing rice seedlings as compared with other isolates (Etesami *et al.*, 2014).

Vinayarani and Prakash (2018) isolated 30 plant growth promoting rhizobacteria and 20 endophytic bacteria from different varieties of turmeric from South India. All the isolates were screened for antagonistic activity against *P. aphanidermatum* and *R. solani*, causing rhizome rot and leaf blight diseases in turmeric, by dual culture and liquid culture assays. Only five rhizospheric and four endophytic bacterial isolates which were proved best in dual culture assay, tested for their plant growth promoting traits. All the five rhizospheric PGPR isolates *viz.*, RBac-DOB-S4, RBacDOB-S16, RBacDOB-S21, RBacDOB-S24, RBacDOB-S70 and four endophytic bacterial isolates BacDOB-E12, BacDOB-E15, BacDOB-E19, BacDOB-E21 were able to produce IAA with l-tryptophan as a precursor. Except BacDOB-E12, the other isolates exhibited positive results for HCN production, while three isolates solubilized inorganic phosphate $\text{Ca}_3(\text{PO}_4)$ and four isolates were positive to cellulase activity. PGPR isolates RBacDOB-S21, RBacDOB-S24 and endophytic bacterial isolates BacDOB-E19, BacDOB-E21 were positive for multiple PGP traits *viz.*, IAA, HCN, Siderophore production, inorganic phosphate solubilization, production of cellulase and protease.

Navarro *et al.* (2019) screened a total of 137 bacterial isolates from surface sterilized root, stem and nodule tissues of soybean for their antifungal activity against major phytopathogens like *R. bataticola*, *M. phaseolina*, *F. udam* and *S. rolfsii*. Eight isolates belonged to *Bacillus* and one to *Paenibacillus*. The selected isolates were screened for biocontrol traits like production of hydrogen cyanide (HCN), siderophore, hydrolytic enzymes, antibiotics and plant growth promoting traits like indole 3-acetic acid production, phosphate solubilization, and nitrogen fixation. Their results indicated the most efficient biocontrol isolates are *Paenibacillus* sp. (HKA-15) and *Bacillus* sp. (HKA-121) for biocontrol of charcoal rot as well as soybean plant growth promotion.

Mukherjee *et al.* (2020) conducted a study to identify culturable endophytes of the germinating and dry seeds of chickpea (*Cicer arietinum* L.) and their functional attributes. They isolated 29 bacterial strains from chickpea seeds (8 strains from dry and 21 strains from germinating seeds). Phylogenetic analysis based on 16S rDNA showed that the seed endophytic bacteria belong to *Enterobacter* sp., *Bacillus* sp., *Pseudomonas* sp.,

Staphylococcus sp., *Pantoea* sp. and *Mixta* sp. Isolates produced significant amount of Indole-3-acetic acid (IAA) (*Enterobacter hormaechei* BHUJPCS-15; 58.91 µg/ml), solubilised phosphate (*B. subtilis* BHUJPCS-24; 999.85 µg/ml) and potassium, ammonia (*B. subtilis* BHUJPCS-12; 148.73 µg/ml).

2.4.1 Indole acetic acid production

Auxin is one of the plant hormones that influence the process of forming plant tissues, namely growth, division, and cell differentiation and protein synthesis. The results showed that there is a type of auxin which has a considerable role, namely Indole Acetic Acid (IAA) (Restu and Payangan, 2019). Indole-3-acetic acid (IAA, 3-IAA) is the most common, naturally occurring, plant hormone of the auxin class. IAA is produced in young leaves, stems and seeds from transamination and decarboxylation reactions of tryptophan (Sarker and Rashid, 2013).

Ghadbane *et al.* (2015) evaluated the antagonistic activity of *Streptomyces* spp. against the chickpea wilt caused by *F. oxysporum* by streak assay method and screened for their ability to produce extracellular enzymes and indole acetic acid (IAA). The four *Streptomyces* spp., produces relatively high levels of IAA, solubilize phosphate and produce extracellular fungal cell-wall degrading enzymes (chitinase and protease). The biocontrol assays revealed that the *Streptomyces* strains significantly promoted the growth of the chickpea plants and showed greater suppression of wilt disease.

Restu and Payangan (2019) aimed to identify rhizosphere fungi and evaluate the ability of IAA production. From the result, they obtained five fungus genera (*Aspergillus*, *Trichoderma*, *Rhizopus*, *Penicillium* and *Fusarium*). IAA production ability test showed that *Fusarium* has the highest concentration, which was 38,611 ppm. *Fusarium* isolates have the potency to be developed as biological fertilizers.

2.4.2 Siderophore production

Siderophore production by endophytes helps the plants in iron sequestration and increases the concentration of available iron to inner tissues (Dimpka, 2016). In addition

to iron acquisition, they also give nutritional competition to the pathogens by limiting the iron availability to them and inhibit their growth (Ahmed and Holmstrom, 2015).

Maheshwari *et al.* (2019) isolated and screened the siderophore producing endophytes from nodules and roots of *Cicer arietinum* and *Pisum sativum* plants. Out of total 84 isolates, only 14 endophytes produced siderophore and their quantitative analysis was also done. Ten best siderophore producers (Above 65 per cent siderophore units) were characterized for the type of siderophore produced. Most of them were producing hydroxamate and carboxylate type of siderophores. These 10 isolates were evaluated for other plant growth promoting traits *in vitro*. All of them produced ammonia and indole-3-acetic acid (IAA). Isolate CPFR10 was found to be positive for all the PGP traits *viz.* ammonia, organic acid, HCN and IAA production.

2.4.3 Ammonium production

Hassan (2017) identified plant growth promoting endophytes (PGPE) of *Teucrium polium* and characterized plant growth promoting properties of endophytes. Seven bacterial endophytes were isolated and identified as *B. cereus* and *B. subtilis*, where five endophytic fungi were obtained and assigned to *Penicillium chrysogenum* and *P. crustosum*. The isolated endophytes differentially produced indole acetic acid, ammonia and phosphate solubilization. In order to investigate the effect of endophytes on plant growth. Four representative endophytes and their consortiums were selected concerning to their potential ability to promote plant growth. The results indicated that microbial endophytes possessing a vital role to improve plant growth and could be used as inoculants to establish a sustainable crop production system.

2.4.4 Phosphate solubilization

Phosphorus is a macronutrient required for the proper functioning of plants. Because phosphorus plays vital role in every aspect of plant growth and development. Phosphate solubilizing microbes are group of beneficial microorganisms capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble P form that can easily be assimilated by plants. Application PSM by inoculating in soil appears to be an efficient way to convert the insoluble phosphorus compounds to plant available P

form, resulting in better plant growth, crop yield and quality. *Bacillus*, *Pseudomonas*, *Rhizobium*, *Aspergillus* and *Penicillium* are the most efficient P solubilizers for increasing bioavailability of P in soil (Kalayu, 2019).

Five highly efficient phosphate solubilizing bacterial strains, H22, Y11, Y14, Y34 and S32 were screened and isolated from an alfalfa rhizosphere in heavy metal-contaminated reclamation area in Shanxi Province, China. Based on morphological observations, 16S rRNA sequencing and cellular fatty acid composition analysis, H22, Y11 and Y34 were identified as *Pseudomonas* sp., while Y14 and S32 were identified as *Pantoea* sp. Among them, S32 showed the highest P-solubilizing efficiency in culture medium containing $\text{Ca}_3(\text{PO}_4)_2$, lecithin, and powdered phosphate rock. In addition, the acid and alkali phosphatase activities of S32 were tested as 6.94 U/100 mL and 4.12 U/100 mL, respectively. The soil inoculation experiment indicated that inoculation with S32 resulted in significant increase in the plant height, biomass, root growth and P uptake of rice in both experimental and reclaimed soil (Chen and Liu, 2019).

2.4.5 HCN production

Reetha *et al.* (2014) evaluated 2 strains of *B. subtilis* and 2 strains of *P. fluorescens* (obtained from rhizosphere soil from sunflower plants) for the capability in cyanide synthesis and also the potential of super strains on reduction in the fungal growth of *M. phaseolina*. Among these, *Pf1* and *CPf5* isolates indicated a strong production of HCN. Isolates of *B. subtilis* have not produced HCN. In quantitative estimation, *Pf1* and *CPf5* recorded the maximum OD value of 0.094 and 0.085 respectively but *Bs10* recorded least OD value (0.015). The antibiotics produced by all the four effective bacterial isolates were effective against *M. phaseolina* and recorded reduction in growth of the pathogen ranged between 61.13 to 69.62 per cent reduction over control. Antibiotics produced by *Pf1* were maximum per cent reduction in the fungal growth (69.92 %) followed by *CPf5* (65.59 %). The *Bacillus* isolates *Bs10* and *CBs4* were showing efficacy of 63.36 per cent and 61.13 per cent reduction over control.

2.5 Molecular characterization and diversity analysis of the potential PGPMs and their screening for bio-active compound production against *R. bataticola*

2.5.1 Molecular characterization and diversity analysis of the potential PGPMs

Hernández *et al.* (2014) reported that many species of *Bacillus* including *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. cereus*, *B. mycoides* and *B. thuringiensis*, are known to suppress growth of several fungal pathogens such as *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Sclerotium*, *Gaeummanomyces*, *Nectria*, *Pythium*, *Phytophthora* and *Verticillium*. The main property of antagonist bacterial strains is production of antifungal antibiotics, which seem to play a major role in biological control of plant pathogens.

Sang *et al.* (2014) isolated 576 endophytic bacteria from the leaves, stems and roots of 10 rice cultivars and identified 12 of them as diazotrophic bacteria using a specific primer set of *nif* genes. Through 16S rDNA sequence analysis, *nif H* genes were confirmed in the two species of *Penibacillus*, three species of *Microbacterium*, three *Bacillus* species and four species of *Klebsiella*. Rice seeds treated with this plant growth promoting rhizobacteria (PGPR) showed improved plant growth, increased height and dry weight and antagonistic effects against fungal pathogens. These results strongly suggest that the endophytic diazotrophic bacteria characterized in this study could be successfully used to promote plant growth and inducing fungal resistance in plants.

Endophytic bacteria from roots (12 isolates) and nodules (76 isolates) of chickpea legume grown in Haryana Agricultural University farm were isolated. Further selected 62 isolates were used to determine molecular diversity by RFLP of PCR amplified 16S rDNA. Endophytes from roots formed five separate clusters and nodule endophytes formed 13 clusters. Isolate CNE215 from nodules and CRE1 from roots possessed multiple beneficial traits and belonged two different clusters. These two isolates were identified after amplification and sequencing of 16S rRNA gene. Isolate CNE215 showed more than 98 per cent similarity with partial sequence of 16S rRNA gene of *B. subtilis*, whereas CRE1 showed more than 98 per cent similarity with *B. licheniformi* (Singh *et al.* 2015).

Osman and Yin (2018) isolated the PGPRs from pea plant (SE-7, LE-26, SQ-7 and SQ-9), which could promote growth through genes that encode for the synthesis of

specific growth stimuli or other growth-promoting traits such as vitamins, antibiotics, and secondary metabolites. Sequencing of 16S ribosomal RNA showed that these strains were mostly similar to *Bacillus* sp. (99 per cent similarity). Using the EzBioCloud 16S rRNA database, it was found that one strain was likely to be *B. paramycooides* based on 100 per cent similarity, two strains were *B. wiedmannii* based on 99.05 and 100 per cent similarity, and the remaining strain was *B. amyloliquefaciens* based on 99.64 per cent similarity.

Taoufiq *et al.* (2018) used faba bean (*Vicia faba*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and common bean (*Phaseolus vulgaris*) to harbor the endophytic bacteria. A total of seventy-two isolates were obtained and characterized using PCR-ARDRA (Amplified Ribosomal DNA Restriction Analysis) of 16S rDNA. The identification was done by using primers 16Sa (5'-CGCTGGCGGCAGGCTTAACA-3') and 16Sb (5'-CCAGCCGCAGGTTCCCCT-3'). The findings showed a high molecular biodiversity of among isolates. 40.9 per cent of the isolated endophytic bacteria were *Ensifer meliloti* (*Sinorhizobium meliloti*) isolated in nodules of chickpea and common bean, 31.8 per cent were *Rhizobium* sp. isolated in nodules and roots of common bean and lentil and 27.3 per cent were *Enterobacter* sp. isolated in roots of faba bean, common bean, chickpea and lentil.

Hyder *et al.* (2020) reported that out of fifteen plant growth promoting rhizobacterial (PGPR) strains recovered from chilli rhizosphere, eight were found potential antagonists to *Phytophthora capsici* *in vitro*. Bacterial strains with strong antifungal potential were subjected to molecular analysis. The 16S rRNA sequence analysis of tested bacterial strains showed 98-100 per cent identity with *P. putida*, *P. libanensis*, *P. aeruginosa*, *B. subtilis*, *B. megaterium* and *B. cereus* sequences available in the National Center for Biotechnology Information (NCBI) GenBank nucleotide database. All sequences of identified bacteria were submitted to GenBank for accessions numbers (MH796347-50, MH796355-56, MH801129 and MH801071). They have concluded that all the tested bacterial strains significantly suppressed the *P. capsici* infections (52.3-63 %) and enhanced the plant growth characters in chilli pepper under greenhouse conditions.

Mukherjee *et al.* (2020) isolated 29 bacterial strains from chickpea seeds (8 strains from dry and 21 strains from germinating seeds) for enhancing plant growth attributes

and bio-control activity against *Fusarium* sp. Molecular characterization of isolates was carried out by amplifying of 16 S rRNA genes by colony polymerase chain reaction (PCR) method. PCR was performed by using 16 s rRNA gene primers such as forward primer as 27 F (5'-AGAGTTTGATCCTGGCTCAG-3', and reverse primer as 939R (5'-TACGGTTACCTTGTTACGACTT-3'). Phylogenetic analysis based on 16S rDNA showed that the seed endophytic bacteria belong to *Enterobacter* sp., *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Pantoea* sp. and *Mixta* sp. Isolates produced significant amount of Indole-3-acetic acid (IAA) (*Enterobacter hormaechei* BHUJPCS-15; 58.91 µg/ml), solubilised phosphate (*B. subtilis* BHUJPCS-24; 999.85 µg/ml) and potassium, ammonia (*B. subtilis* BHUJPCS-12; 148.73 µg/ml), and also inhibited the growth of chickpea pathogen (*P. aeruginosa* BHUJPCS-7 against *F. oxysporum* f. sp. *ciceris*) under laboratory conditions.

Dong *et al.* (2021) who isolated total of 53 bacterial strains from the rhizosphere soil, nine of which exhibited good bio-control effect against the pathogenic bacteria of soft rot disease caused by *Pectobacterium carotovorum* sub sp. *carotovorum*. Four strains such as CAB-L005, CAB-L012, CAB-L014 and CAB-L022 exhibited strong antagonistic effects. On the basis of the sequence homology of 16SrRNA genes, the similarity between strain CAB-L005 and *B. tropicus* was 100 per cent, that between strain CAB-L012 and *B. subtilis* was 99 per cent, and that between strain CAB-L014 and *B. tequilensis* was 100 per cent and similarity between strain CAB-L022 and *Bacillus cereus* was 100 per cent. The isolated bacteria demonstrated good biocontrol effects in field experiments.

Noveriza *et al.* (2022) isolated endophytic fungi by direct planting method, purification and characterization of fifty isolates were conducted on potato dextrose agar media. Based on ITS sequence analysis, the DNA sequences of isolate A-TKR1 were identical to *Aspergillus flavus*, isolate ETKR3 to *Penicillium citrinum* and isolate ECT-TKR7 to *Neofusicoccum parvum*. Phylogenetic analysis was constructed using the UPGMA method based on ITS DNA sequences of three endophytic fungi from *Eucalyptus* leaves and their similarity with fungal isolates from the Gene Bank. The similarity of those isolates was 100, 99.81 and 99.82 per cent, respectively.

2.5.2 Screening for bio-active compound production against *R. bataticola*

Out of 19 *Pseudomonas* isolates two isolates viz., Ps-14 and Ps-17 exhibited antagonistic effect on growth of *F. oxysporum* f. sp. *ciceris* under *in vitro* conditions. Fungal growth inhibition due to production of volatile metabolites was exhibited by Ps-17 (13.80 %) and Ps-14 (9.20 %) (Poonam *et al.*, 2013).

Hannane *et al.* (2016) evaluated the ability of several isolates belonging to rhizobacteria (*Pseudomonas* and *Bacillus*) to control the mycelium growth of *Fusarium oxysporum* f. sp. *ciceris*. The results revealed that B61 (*B. aneurinilyticus*) and P39 (*P. luteola*) and P70 (*P. fluorescens*) were found to be most efficient with 77 and 55.5 per cent respectively, while B39 (*B. firmus*) and P41 (*P. luteola*) were the most efficient by volatile compounds with 70.5 and 77.5 per cent, respectively.

Nagamani *et al.* (2017) showed that volatile metabolites produced by *T. asperellum* were found most efficacious in reducing the mycelial growth of *F. oxysporum* f. sp. *ciceris* by 86.70 per cent. It was also proved that volatile compounds produced by *T. harzianum* showed strong inhibitory effect on the mycelial growth *F. oxysporum* f. sp. *ciceris* (79.25 %) followed by the *T. viride* (64.16 %). Higher concentration of volatile metabolites was produced in isolates Pf-2, which inhibited *F. oxysporum* f. sp. *ciceris* by 100 per cent. Pf-15 and Pf-28 inhibited by 78.20 and 74.40 per cent, respectively (Architha, 2018).

Ranjana (2020) tested twenty native isolates of fluorescent Pseudomonads for volatile compounds production in inverted plate technique. The results showed that the isolates produced considerable amount of volatile compounds which varied within the isolates. Higher concentration of volatile compounds was produced by isolate PF-19 (66.67 %) followed by PF-14 (64.44 %), PF-6 (56.67 %) and lower concentration of volatile compounds was produced by PF-9 (4.44 %). All the isolates have shown significant difference in mycelial growth inhibition when compared to the control.

Ranjana (2020) tested twenty native *Trichoderma* isolates were for volatile compounds production in inverted plate technique. The results showed that all the isolates produced considerable amount of volatile compounds which varied among isolates. Higher concentration of volatile compounds was produced by isolate TR-14 (72.22 %) followed by TR-19 (66.67 %), TR-9 (65.93 %) and lower concentration of volatile

compounds was produced by TR-18 (2.59 %). All the isolates have shown significant difference in mycelial growth inhibition when compared to the control.

2.6 Extraction, identification of bio-active compounds and screening of crude extract from potential PGPMs responsible for inhibition of *R. bataticola*.

Dubey *et al.* (2011) reported that the metabolites which are obtained by the culture filtrate of *T. harzianum* caused maximum growth inhibition (91.10 %) followed by *T. viride* (90.30 %) and *T. virens* (88.00 %) against *R. bataticola*. During bio-control experiments, the four *P. fluorescens* strains were able to protect *Medicago truncatula* plants from *Botrytis cinerea* infection by reducing stem disease symptoms and root browning. The volatile organic compounds's emitted by all four *Pseudomonas* strains were similar and sulfur-containing compounds were among the most abundant, including dimethyl disulfide. Interestingly, the strain UM270 was the only one that produced dimethyl hexadecylamine, a compound with antifungal and PGP activities.

Padmanabhan *et al.* (2016) conducted an experiment to determine the potential bioactive components of metabolites of *Pseudomonas* sp. using polar and non-polar solvent by GC-MS analysis. The chemical compositions of ethanol and chloroform extracts of bacterial secondary metabolites were investigated using Perkin-Elmer instrument. The GC-MS analysis provided nine compound with different retention time and peak area namely 2,6,10- Trimethyl-12-oxatricyclo [7.3.1.0 (1,6)] tridec-2-ene; Nicotinic acid,1,6-dihydro-4-hydroxy-6-oxo-2-propylethyl ester; Benzoic acid,2-amino-6- chloro-methyl ester and Heptadecanoicacid, 9-methyl-,methylester; 2-(2,6,6-Trimethylcyclohex-1-enyl) cyclopropanecarboxylic acid, methyl ester; 9-Octadecenoic acid; Pentadecanoic acid,13methyl-methyl ester. Out of nine compounds Heptadecanoic acid, 9- methyl-, methyl ester was found as similar in both extracts. Five dissimilar and two similar compounds were obtained from the two solvents.

Ahsan *et al.* (2017) reported that *Streptomyces* strain KX852460 is has antifungal activity against *R. solani* AG-3 that is the causal agent of target spot disease in tobacco leaf. The aim of the study was to identify bioactive antifungal compounds produced by *Streptomyces* strain KX852460 against *R. solani* AG-3. Ethyl acetate was used to extract the culture filtrate. The active fractions were purified by silica gel column chromatography having 52 mm zone of inhibition against *R. solani* AG-3. The purified

fractions were identified by gas chromatography–mass spectrometry technique. Twenty seven compounds were identified and most of them were derivatives of aromatic compounds. Eicosane (C₂₀H₄₂) and dibutyl phthalate (C₁₆H₂₂O₄) were found antifungal compounds in this study. While morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyloxy)-], cyclononasiloxane, octadecamethyl-(C₁₈H₅₄O₉Si₉) and benzoic acid, 2,5-bis (trimethylsiloxy) (C₁₆H₃₀O₄Si₃) were the major compounds with highest peak number. These results suggested that *Streptomyces* strain KX852460 had good general antifungal activity and might have potential biocontrol antagonist against *R. solani* AG-3 to cure the target spot in tobacco leaf.

Avinash (2017) screened thirty five isolates of *Trichoderma* species for production of volatile compounds, two isolates viz., Tri-4 (*T. viridae*) and Tri-12 (*T. hamatum*) performed better under *in vitro* against major fungal pathogens viz., *F. oxysporum* f. sp. *udum*, *R. bataticola*, *S. rolfsii* and *A. alternate*. Further, secondary metabolites were extracted and characterized from isolate Tri-4 (*T. viridae*). LCMS result confirmed the presence of antifungal metabolites viz., ferulic acid (C₁₀H₁₀O₄) at 195.11m/z, harzianic acid (C₁₉H₂₇NO₆) at 366.31 and viridofungin A (C₃₁H₄₆O₁₀N) at 592.31 with retention time of 1.94, 3.46 and 3.15 min respectively.

Nagamani *et al.* (2017) isolated twenty *Trichoderma* isolates from chickpea rhizosphere and screened for their efficacy against soil borne plant pathogens namely *R. bataticola*, *F. oxysporum* f. sp. *ciceri* and *S. rolfsii*. In chickpea the isolates ATPU 1, ATPP 6, KNN 4, KNO 2, KNPG 3 and KNP 1 were most efficient in the production of volatile and non-volatile compounds.

Ten isolates of *T. asperellum* were used for characterization of secondary metabolites through gas chromatography-mass spectrometry (GC-MS) analysis to establish valid correlation between the production of antifungal metabolites and their bio-efficacy as bio control agents. The investigation revealed that the culture filtrate of *T. asperellum* isolates showed the presence of 673 secondary metabolites at different retention time with a range of 39 (Ta-20) to 101 (Ta-12) with GC-MS. Out 673 volatile metabolites, 55 metabolites were found to be most abundant from which seven metabolites from Ta-14 and Ta-20, six metabolites from Ta-8, Ta-17 and Ta-29, five metabolites from Ta-45, Ta-15, Ta-10 and Ta-12 and remaining three metabolites from Ta-2 isolate, respectively (Srinivasa *et al.*, 2017).

Awad *et al.* (2018) evaluated different biological activities of *T. viride* fungus. *T. viride* was isolated from the cucumber rhizosphere. *T. viride* inhibited the mycelial growth of *F. solani*, *R. solani* and *S. rolfii*. Also, the alcoholic extract of the fungal mycelia proved a potent antibacterial activity against *B. subtilis*, *E. coli* and *P. fluorescens*. In addition, it exhibited a significant antifungal activity against *C. albicans*, *F. solani*, *F. oxysporium*, *R. solani* and *P. ultimum* at 100 µg/disc. Furthermore, the volatile constituents were isolated from fresh mycelia of *T. viride* and subjected to GCMS/MS analysis. Total protein (10 %), carbohydrate (19.57 %), steroidal (13.95 %) and triterpenoidal content (38.34 %) were determined in the alcoholic extract of *T. viride* mycelia.

Hameed *et al.* (2018) conducted the study to identify the volatile metabolite products of *P. fluorescens* for their antifungal activity against fungal pathogens. The results showed that the volatile compounds were highly effective to suppress the growth of fungal pathogens. Nagwa *et al.* (2018) isolated volatile constituents from the fresh fungus *T. viride* and evaluated as antimicrobial agent. The volatile constituents showed remarkable antibacterial and antifungal effects *i.e.*, 29.62, 63.12 and 70.37 per cent at 10, 50 and 100 µg/disc, respectively.

Mishra *et al.* (2018) evaluated four *Trichoderma* isolates (CA-03, CA-06, CA-07 and CA-08) for the production of volatile and non volatile metabolites. GC-MS analysis was carried out in order to identify active secondary metabolites of potential *Trichoderma* isolates CA-07 and CA-06. A total of 19 and 15 peaks were identified for CA-07 and CA-06, respectively. Those compounds were mainly carboxylic acid and fatty acid esters. Gupta *et al.* (2019) reported that the GCMSMS analysis of ethyl acetate extract of *T. harzianum* revealed the presence of 12 compounds. These compounds were determined as four volatile alcohols (1-4) and fatty acid esters (5-12). The mixtures of secondary metabolites were further evaluated against the pathogenic strains for determination of antimicrobial spectrum.

The experiment was conducted to assess the potential of volatile and non-volatile metabolites released from three selected species of *Trichoderma* viz. *T. viride*, *T. harzianum* and *T. konigii* isolates against three isolates of *F. oxysporum* f. sp. *ciceri* which caused wilting of chickpea. Data proved that *T. harzianum* produced maximum inhibition zone (76.90 %) against FOC strain of *F. oxysporum* f. sp. *ciceri* followed by *T.*

viridae (70.10 %). *In vitro* studies have demonstrated that volatile compounds produced by *T. harzianum* showed strong inhibitory effect on the mycelial growth of all isolates of test pathogen but maximum against FOC2 isolate *i.e.*, 79.25 per cent followed by the *T. viride i.e.*, 64.16 per cent against FOC1 isolate (Kumar *et al.*, 2019).

Ranjana (2020) made an attempt to identify the bio-active volatile compounds produced by efficient *T. asperellum* (TR-14) which was selected based on both dual culture and inverted plate technique. The compounds were extracted by using standard solvent extraction method into ethyl acetate and identified by GC-MS/MS analysis. The ethyl acetate extract of *T. asperellum* (TR-14) dissolved in acetone was subjected to gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis. The GC-MS/MS analysis revealed that the extract showed the presence of 29 compounds at different retention time ranging from 4.910 to 21.868 min. The mass to charge (m/z) ratio of compounds ranged from 43 to 190. Further, the author reported that the volatile constituents of *T. asperellum* (TR-14) significantly inhibited the growth of pathogen at all the concentrations tested. Maximum inhibition of pathogen was observed at 15 per cent concentration (75.93 %) followed by 64.07 per cent and 44.44 per cent at 10 and 5 per cent concentrations, respectively. Maximum inhibition of pathogen observed at 15 per cent concentration (73.33 %) followed by 62.96 per cent and 31.48 per cent at 10 and 5 per cent concentrations, respectively in case of volatile constituents of *P. fluorescens* (PF-19).

2.7 Management of dry root rot using potential PGPMs in pot and field experiments

Prasanthi *et al.* (2000) evaluated fungal and bacterial antagonists as seed and soil application against safflower root rot caused by *R. bataticola*. Both seed treatment and soil drenching with antagonists increased safflower seedling percentage survival being seed treatment being more effective than soil drenching with highest survival rate 83.33 per cent by *T. viride* and 86.66 per cent by *P. fluorescens*.

Rhizosphere microflora of chickpea was enumerated under irrigated conditions and found that carbendazim as seed treatment and soil drench coupled with soil application of *T. harzianum* reduced maximum fungal population of *Fusarium* sp. and *M. phaseolina*. FYM also reduced pathogen population and stimulated all other microflora

more than other treatment there by showing antagonism against *Fusarium* sp. and *M. phaseolina* (Patel and Anahosur, 2001).

Thilagavathi *et al.* (2007) tested the biocontrol agents *T. viride* (strains *Tv1* and *Tv13*), *P. fluorescens* (*Pf1* and *Py15*) and *B. subtilis* (*Bs16*) individually and in combination for their effectiveness against root rot of greengram caused by *M. phaseolina*. The biocontrol agents tested *in vitro* against *M. phaseolina*, combinations of *P. fluorescens* + *T. viride* (*Pf1+Tv1*, *Pf1+Tv13* and *Py15+Tv1*) inhibited mycelial growth of the pathogen and they also promoted the growth of the greengram seedlings. A combination of *Pf1+Tv1* was most effective in reducing root rot incidence under glass-house and field conditions as compared with other single or combined treatments or the untreated control. Moreover, a combination of *Pf1+Tv1* followed by *Pf1+Tv13* and *Py15+Tv1* significantly increased yield under glass house and field condition.

Karumuri and Singh (2016) screened total 16 strains of *Trichoderma* among the 25 strains isolated from different crop rhizospheric soil samples collected from different districts of Madhya Pradesh. The dual culture studies of these isolates against three fungal soil borne pathogens of chickpea viz., *S. rolfisii*, *F. oxysporum* f. sp. *ciceri* and *R. bataticola* revealed that all the strains exhibited control over three pathogens to a different extent. Two strains TsA2 and Ts11 were effective against all the three pathogens. Studies on the volatile compounds emanating from the growing cultures of *Trichoderma* strains revealed that they had inhibitory effect on the growth of three pathogens. The strain Ts3 was effective against all the three pathogens. The pot experiment showed similar result with the dual culture experiment. The strains Ts3, Ts5 and Ts4 showed minimum percent of collar rot incidence, the strains TsA2, Ts11 and Ts3 showed minimum percent of dry root rot incidence and Ts4, Ts5 and TsA3 showed minimum incidence of wilt in chick pea at the observed stages of the crop.

T. harzianum and plant growth promoting rhizobacteria (PGPR) have shown high efficacy against the Collar rot (*S. rolfisii*) of chickpea (*Cicer arietinum*) *in vitro* as well as in the field. They used *T. harzianum* (104, 106 and 108 spore/ml) and two PGPRs (*P. fluorescens* strain 4 and *P. aeruginosa*) as foliar spray with the fresh and heat inactivated microorganisms. Foliar application of *T. harzianum* (108 spore/ml) and *P. fluorescens* strain 4 (108 cfu/ml) showed maximum efficacy in reducing plant mortality as compared to the control. Foliar application of fresh-and heat-inactivated (121°C for 10 min) *P.*

fluorescens strain 4, and *T. harzianum* reduced 15-25 per cent plant mortality but *P. aeruginosa* showed very little disease control of 10–15 per cent (Maurya *et al.*, 2008).

Patel *et al.* (2020) screened endophytes of leaf and stem isolates under dual culture technique. The 12 isolates were significant but three isolates are EBS-2, EBS-3 and EBS-4 showed higher suppression towards *R. bataticola*. These three isolates used further for roll towel method (*in vitro*) and net house (pot cultivation). The observations of roll towel method indicated that the isolates *EBL-3* and *EBL-4* showed highest germination percentage. Root length was maximum in *EBL-2*, 3. The shoot length and vigour index was highest in *EBL-3*. The observations on effect of endophytic bacteria on *R. bataticola* under pot culture were also recorded. The cohabitation of *T8EBL* (2+3+4) and *T7EBL* (4+2) were significant on four genotypes JG14, JG16, JG62 and JG315 of pathogen. The *EBL-3* isolate was most significant in field and lab conditions.

III. MATERIAL AND METHODS

The present investigation on “Molecular diversity analysis and antagonistic potential of plant growth promoting microorganisms for the management of emerging dry root rot of chickpea caused by *Rhizoctonia bataticola* (Taub.) Butler” was carried out during 2020-2022 at Bio-input Entrepreneurship Centre, College of Agriculture, Raichur and Organic Farming Research Institute and Pesticide Residue and Food Quality Analysis Laboratory, University of Agricultural Sciences, Raichur, Karnataka. The molecular work on characterization and diversity analysis of potential PGPMs was carried out at Rice Pathology Laboratory, All India Co-ordinated Research Programme on Rice, Agricultural Research Station, Gangavathi. Raichur is situated in North Eastern Dry Zone (Region-1 and Zone-2) of Karnataka state at 16° 12' N longitude, 77° 21' E latitude with an altitude of 389.37 m above mean sea level. The details of the methodology followed during the investigation are presented below.

3.1 General laboratory procedures

3.1.1 Glassware and cleaning

Laboratory experiments were conducted using Borosil, Qualigens and Technico glassware. The glassware were kept in the cleaning solution containing potassium dichromate ($K_2Cr_2O_7$), concentrated sulphuric acid (H_2SO_4) at 60 g and 60 ml respectively in one litre of water. Later, they were washed with detergent powder followed by two washings in running tap water and then rinsed with distilled water and kept in a hot air oven for few min for complete drying of moisture.

3.1.2 Sterilization

All glassware, solid and liquid culture media were sterilized by autoclaving at 1.1 kg pressure per square cm (121 °C) for 20 min. Soil used for experiments was sterilized for two h at 1.33 kg pressure per square cm in an autoclave. All cultural studies were conducted in aseptic condition under laminar air flow. The surface of working area was sterilized by swabbing with 70 per cent alcohol before working. The blades, scissors, inoculation loop, needle, cork borer and forceps were sterilized by autoclaving and surface sterilization was done by 70 per cent alcohol followed by heating under the flame.

3.1.3 Media used in the experiments

Potato dextrose agar (PDA)

The composition of the PDA is as follows

| | |
|---|--------------|
| Agar-A gar | : 20.00 g. |
| Dextrose (C ₆ H ₁₂ O ₆) | : 20.00 g. |
| Potato | : 20.00 g. |
| Distilled water (Volume to make up) | : 1000.00 ml |

Two hundred grams of peeled potatoes were cut into small pieces and boiled in distilled water and then extract was collected by filtering through muslin cloth. Dextrose and agar (20.0 g each) were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water and sterilized as described earlier and preserved for further use. Finally, the pH was adjusted to 5.6 ± 0.2 .

Preparation of PDA slants

PDA slants were prepared by transferring 3 ml of the medium to culture tubes. The tubes were plugged with non-absorbent cotton and sterilized in an autoclave. After sterilization, the tubes were removed from the autoclave when they were still in hot condition (*i.e.* approximately 40 °C), kept in a slanting position for the medium to solidify. After solidification, the slants were kept in refrigerator for further use.

Plating of medium

The sterilized medium was melted and distributed in Petri plates (9 mm diameter) at the rate of 20 ml per plate aseptically in a laminar air flow chamber and allowed to solidify. The plates containing the medium were used for culturing and maintenance of fungus in the laboratory.

Potato dextrose broth

Potato dextrose broth was prepared same as that of potato dextrose agar except the addition of solidifying agent agar agar. Two hundred grams of peeled potatoes were cut

into small bits, boiled in distilled water and then extract was collected by filtering through muslin cloth. Dextrose 20 g was dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water and sterilized as described earlier and preserved for further use.

Nutrient Agar (NA)

| | |
|-----------------|--------------|
| Beef extract | : 3.0 gm |
| Peptone | : 5.0 gm |
| NaCl | : 0.5 gm |
| Agar | : 15.0 gm |
| Distilled water | : 1000.00 ml |

For the preparation of nutrient agar medium, the above constituents were weighed and dissolved completely to form a homogeneous solution by heating in a micro-oven. Later, the pH of the media was adjusted to 7.0 by adding sodium hydroxide/hydrochloric acid and was subjected for autoclaving.

Nutrient Broth (NB)

| | |
|-----------------|--------------|
| Beef extract | : 3.0 gm |
| Peptone | : 5.0 gm |
| NaCl | : 0.5 gm |
| Distilled water | : 1000.00 ml |

For the preparation of nutrient broth medium, the above constituents were weighed and dissolved to form a homogeneous solution by heating in a micro-oven. Later, the pH of the medium was adjusted to 6.5 by adding sodium hydroxide/hydrochloric acid and was subjected for autoclaving.

***Trichoderma* specific medium (TSM)**

| | |
|---|--------------|
| MgSO ₄ · 7H ₂ O | : 0.20 gm |
| K ₂ HPO ₄ | : 0.90 gm |
| KCL | : 0.15 gm |
| NH ₄ NO ₃ | : 1.00 gm |
| Glucose | : 3.00 gm |
| Rose Bengal | : 0.15 gm |
| p-dimethylaminobenzenediazosodium sulfonate | : 0.30 gm |
| Chloramphenicol | : 0.25 gm |
| Pentachloronitrobenzene | : 0.20 gm |
| Agar | : 20.00 gm |
| Distilled water | : 1000.00 ml |

For the preparation of *Trichoderma* Specific Medium, the above constituents were weighed and dissolved completely to form a homogeneous solution by heating in a micro-oven and was subjected for autoclaving.

King's B agar medium

| | |
|---------------------------------|--------------|
| Peptone | : 15.00 gm |
| MgSo ₄ | : 1.50 gm |
| K ₂ HPO ₄ | : 1.50 gm |
| Glycerol | : 10.00 ml |
| Agar | : 20.00 gm |
| Distilled water | : 1000.00 ml |
| Adjust pH to | : 7.0 |

For the preparation of King's B agar medium, the above constituents were weighed and dissolved to form a homogeneous solution by heating in a micro-oven. Later, the pH of the medium was adjusted to 7.0 by adding sodium hydroxide/hydrochloric acid and was subjected for autoclaving.

Pseudomonas Agar (For Fluorescence)

This medium is recommended for the detection of fluorescent production by *Pseudomonas fluorescens*.

| | |
|----------------------------|--------------|
| Casein enzymic hydrolysate | : 10.00 gm |
| Proteose peptone | : 10.00 gm |
| Dipotassium phosphate | : 1.5gm |
| Magnesium sulphate | : 1.5gm |
| Agar | : 15.00 gm |
| Distilled water | : 1000.00 ml |
| Final pH (at 25 °C) | : 7.0±0.2 |

Thirty eight grams of Pseudomonas agar was suspended in 1000 ml distilled water containing 10 ml glycerol. Then medium was boiled to dissolve the ingredients completely and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.

Bacillus Agar medium

| | |
|---------------------|-------------|
| Peptone | : 10.00 gm |
| HM extract | : 1.00 gm |
| D-Mannitol | : 10.00 gm |
| Sodium chloride | : 10.00 gm |
| Chromogenic mixture | : 3.2 gm |
| Phenol red | : 0.025 gm |
| Agar | : 15.00 gm |
| Final pH (at 25 °C) | : 7.1 ± 0.2 |

For the preparation of *Bacillus* agar medium, the above constituents were weighed and dissolved to form a homogeneous solution by heating in a micro-oven. Later, the pH of the medium was adjusted to 7.1 by adding sodium hydroxide/hydrochloric acid and was subjected for autoclaving.

3.1.4 Isolation and purification of the pathogen

Hyphal tip isolation method was used for obtaining pure culture of the pathogen. Chickpea plants showing typical dry root rot symptoms were washed under running tap water and blot dried. Infected roots were cut into pieces of 5-6 mm size and surface sterilized by dipping in 1 per cent sodium hypochlorite for 1 min and washed thoroughly thrice in sterile distilled water to remove the traces of sodium hypochlorite. Then, the pieces were transferred by using forceps on to sterilized PDA medium in Petri dishes and incubated at 25 ± 2 °C to obtain mycelial growth. After 48 h of incubation, hyphal tips of the growing mycelium were marked on the underside of the Petri dish with a glass marker by viewing through a light microscope. The hyphal tips from margins of colonies were cut with the help of sterilized 5 mm cork borer and transferred to Petri dish containing PDA. On the basis of morphological characters of mycelium and sclerotia, the pathogen was identified as *R. bataticola*. The pure culture of pathogen was grown on PDA slant and was stored at 4 °C for further studies.

3.1.5 Mass multiplication of *R. bataticola*

The mass multiplication of *R. bataticola* was carried out on sorghum grains. About 200 gm of sorghum grains were dispensed in a conical flask (500 ml) and soaked in water for overnight and excess water was drained off. The flasks containing sorghum grains were autoclaved twice at 15 pounds per inch square pressure (121 °C) for 15 min. Each flask were inoculated with a mycelial disc (1 cm) from a 10 days old culture of *R. bataticola* grown on PDA under aseptic conditions and incubated at 28 ± 2 °C for 20 days. The flasks were shaken on alternate days to get uniform growth. The above impregnated sorghum grains were allowed for drying by spreading them on a clean paper sheet in shade at room temperature. After drying, the giant culture so obtained was used for preparing sick pots in glass house.

3.1.6 Proving the pathogenicity

The pathogenicity test was carried out to prove Koch's postulates. The giant culture developed on sorghum grain medium was used as inoculum to conduct pathogenicity test. The inoculum @ 50 g/kg of soil was mixed in sterilized soil and kept for incubation. Untreated control pots were kept without the inoculation of the inoculum. After 10 days of incubation, chickpea seeds of the susceptible genotype Annigeri-1 were sown in the pots. Observations on root rot infection were recorded. Reinoculation was made from plant tissue showing characteristic symptoms to compare with that of original isolate for confirmity.

3.2 Collection, isolation, identification of PGPMs and their antagonistic potential against *R. bataticola*.

3.2.1 Collection of PGPMs

Plant parts of healthy chickpea plants and soil samples from rhizosphere were collected during *Rabi*, 2020 for isolation of isolates of PGPMs, and endophytes, respectively. The isolates were collected from 11 different districts of northern Karnataka viz., Bagalkot, Bellary, Bidar, Dharwad, Gadag, Haveri, Kalaburagi, Koppal, Raichur, Vijayapura and Yadgir wherever chickpea is grown.

For the collection of endophytes, all plant parts including roots were placed in sterile polyethylene bags and transported to the laboratory under 4 °C for further work. For collection of rhizospheric PGPMs, soil sampling was done by scraping the top 2-3 cm soil with the kurpie at a depth of 20-40 cm and distance of 25-50 cm from the base of the plant. Soil samples were collected from 3-4 places in a field randomly. About 250 gm of rhizospheric soil was collected in polythene bags with proper labeling. Later, these rhizospheric soil samples were air dried and packed, then carried to laboratory for further work.

3.2.2 Isolation of endophytic and rhizospheric PGPMs

3.2.2.1 Isolation of endophytic PGPMs:

Isolation of seventy endophytic PGPMs (40 fungal and 30 bacterial) from plant samples was carried out by randomly excising different parts (leaf, shoot and root of 0.5

cm length each) using sterile scissors. For isolation of both fungal and bacterial endophytes, surface sterilization of selected plant tissues was done by dipping in 1 per cent sodium hypochlorite for 1 min. and washed thoroughly thrice in sterile distilled water to remove the traces of sodium hypochlorite. After that, the pieces were then transferred by using forceps on to 70 per cent alcohol for few seconds followed by rinsing in sterile double distilled water and later they were dried in laminar air flow before placing it on nutrient medium.

For isolation of 40 endophytic fungal PGPMs, the sterilized plant pieces were transferred by using forceps on to the PDA medium which was previously added with 1 per cent streptomycin to suppress bacterial growth. Later, Petri dishes were incubated at 25 ± 2 °C to obtain mycelial growth and required cultures were purified in PDA media for further studies. The mycelial growth for each isolate and cultural characters such as colony pigmentation, colony morphology, colony surface, margin, sectoring and zonations were observed after the incubation.

For isolation of 30 endophytic bacterial PGPMs, spread plate technique was used. The sterilized plant tissues were ground using a sterile pestle and mortar. The tissue extract was subsequently incubated at 28 °C for 30 min. to allow the complete release of endophytic microorganisms from the host tissue. Later, the tissue extract was spread on the nutrient agar medium plate by using sterilized spreader. Then, the plates were incubated for up to 2-3 days at 28 °C-30 °C. Colonies with respect to color, size and shape were observed after the incubation period and purified in specific media for further studies. The pure bacterial cultures were used for the observations on staining reaction and cell shape by using stereo binocular microscope.

3.2.2.2 Isolation of rhizospheric PGPMs:

Isolation of 56 rhizospheric PGPMs (26 fungal and 30 bacterial) was carried out by serial dilution technique (Waksman, 1922). Soil dilutions were made by suspending 1g of soil of each sample in 10 ml of sterile distilled water. Dilutions of 10^{-3} , 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid over-crowding of the fungal cultures. Whereas, dilutions of 10^{-6} , 10^{-7} and 10^{-8} were used to isolate bacteria. 1 ml of the suspension of each dilution was added to sterile Petri dishes in triplicates containing sterile PDA and NA medium for fungi and bacteria, respectively. 1 per cent streptomycin

solution was added to the PDA medium for preventing bacterial growth before pouring into Petri plates. The plates were then incubated at 25 ± 2 °C for 4-10 and 28- 37 °C for 2-3 days for growth of fungal and bacterial colonies, respectively. After successful growth of PGPMs, the pure cultures of fungi were sub-cultured in the potato dextrose agar and cultural characters were observed. In case of bacteria, the colony characters with respect to color, size and shape were observed after the incubation period and purified in nutrient agar media for further studies. The pure bacterial cultures were used for the observations on characters staining reaction and cell shape by Gram staining technique using stereo binocular microscope and also the isolates were tested for their fluorescence under UV spectrometer.

3.2.3 Staining reaction of endophytic and rhizospheric bacterial PGPMs.

Staining reaction was carried out by Gram staining technique according to the standard procedure as mentioned in Laboratory Guide for Identification of Plant Pathogenic Bacteria published by the American Phytopathological Society (Schaad, 1992). A loopful of colony of 24 h old bacterial culture was taken and smeared on to the glass slide and passed over flame for two min to heat fix and then stained with crystal violet (primary stain). The slide was washed with distilled water after one minute and further rinsed with iodine solution for one minute. Thereafter, slide was washed with gram's decolourizer and subsequently drained with distilled water. Later, stained with counter stain safranin for one min and washed with distilled water, dried with tissue paper and observed the bacterial cells under stereo binocular microscope.

Source and designation of chickpea fungal endophytic PGPM isolates

| Sl. No. | District | Village | No. of fungal isolates obtained | Isolate code |
|-------------|------------|----------------------|---------------------------------|--------------|
| 1 | Koppal | Yelburga | 4 | FEPGPM - 1 |
| | | | | FEPGPM - 2 |
| | | | | FEPGPM - 3 |
| | | | | FEPGPM - 4 |
| | | Hanmanhatti | 6 | FEPGPM - 5 |
| | | | | FEPGPM - 6 |
| | | | | FEPGPM - 7 |
| | | | | FEPGPM - 8 |
| | | | | FEPGPM - 9 |
| | | | | FEPGPM - 10 |
| | | | | FEPGPM - 11 |
| | | | | FEPGPM - 12 |
| | | Kushtagi | 3 | FEPGPM - 13 |
| | | | | FEPGPM - 14 |
| | | | | FEPGPM - 15 |
| | | Ganganhal | 3 | FEPGPM - 16 |
| | | | | FEPGPM - 17 |
| | | Hiresindogi | 1 | FEPGPM - 18 |
| | | Koppal | 2 | FEPGPM - 19 |
| FEPGPM - 20 | | | | |
| 2 | Raichur | Raichur (UAS campus) | 1 | FEPGPM - 21 |
| | | Sindhanur | 1 | FEPGPM - 22 |
| | | Rampur | 1 | FEPGPM - 23 |
| 3 | Haveri | Chandapura | 1 | FEPGPM - 24 |
| 4 | Kalaburagi | ZARS, Kalaburagi | 1 | FEPGPM - 25 |
| | | Bheemalli | 1 | FEPGPM - 26 |
| | | Malagatti | 1 | FEPGPM - 27 |
| | | | 1 | FEPGPM - 28 |
| 5 | Bidar | Humanabad | 1 | FEPGPM - 29 |
| | | Basavakalyan | 1 | FEPGPM - 30 |
| 6 | Bagalkot | Bagalkot | 1 | FEPGPM - 31 |
| | | Kodihal | 1 | FEPGPM - 32 |
| 7 | Vijayapura | Vijayapura | 2 | FEPGPM - 33 |
| | | | | FEPGPM - 34 |
| | | Basavana Bagewadi | 1 | FEPGPM - 35 |
| 8 | Ballari | Ballari | 2 | FEPGPM - 36 |
| | | | | FEPGPM - 37 |
| | | Kampali | 1 | FEPGPM - 38 |
| 9 | Dharwad | Garag | 1 | FEPGPM - 39 |
| 10 | Gadag | Gajendrigad | 1 | FEPGPM - 40 |

Source and designation of chickpea bacterial endophytic PGPM isolates

| Sl. No. | District | Village | No. of bacterial isolates obtained | Isolate code |
|----------|------------|-----------------------|------------------------------------|--------------|
| 1 | Dharwad | Garag | 1 | BEPGPM - 1 |
| | | UAS, Dharwad | 1 | BEPGPM - 2 |
| | | Narendra | 1 | BEPGPM - 3 |
| 2 | Gadag | Gajendrigad | 1 | BEPGPM - 4 |
| | | Naregal | 1 | BEPGPM - 5 |
| 3 | Haveri | Motebennur | 2 | BEPGPM - 6 |
| | | | | BEPGPM - 7 |
| | | Mugalikatti | 1 | BEPGPM - 8 |
| 4 | Kalaburagi | ZARS, Kalaburagi | 1 | BEPGPM - 9 |
| 5 | Yadgiri | Bheemrayanagudi | 1 | BEPGPM - 10 |
| | | Saidapur | 1 | BEPGPM - 11 |
| 6 | Bidar | Dubalgundi | 2 | BEPGPM - 12 |
| | | | | BEPGPM - 13 |
| | | Hudugi | 1 | BEPGPM - 14 |
| 7 | Vijayapura | Basavana Bagewadi | 1 | BEPGPM - 15 |
| 8 | Bagalkot | Bagalkot | 1 | BEPGPM - 16 |
| | | Karadi | 1 | BEPGPM - 17 |
| 9 | Ballari | Ballari | 2 | BEPGPM - 18 |
| | | | | BEPGPM - 19 |
| 10 | Kushtagi | Kushtagi | 4 | BEPGPM - 20 |
| | | | | BEPGPM - 21 |
| | | | | BEPGPM - 22 |
| | | | | BEPGPM - 23 |
| | | Ganganal | 1 | BEPGPM - 24 |
| | | Hiresindhogi | 1 | BEPGPM - 25 |
| Yalburga | 1 | BEPGPM - 26 | | |
| 11 | Raichur | New area (UAS campus) | 1 | BEPGPM - 27 |
| | | Siddanabhavi camp | 1 | BEPGPM - 28 |
| | | Askihal | 1 | BEPGPM - 29 |
| | | Janakirao camp | 1 | BEPGPM - 30 |

Source and designation of chickpea rhizospheric fungal PGPM isolates

| Sl. No. | District | Village | No. of bacterial isolates obtained | Isolate code |
|---------|------------|--------------------------|------------------------------------|--------------|
| 1 | Gadag | Naregal | 2 | SFPGPM- 1 |
| | | | | SFPGPM- 2 |
| 2 | Haveri | Chandapura | 2 | SFPGPM- 3 |
| | | | | SFPGPM- 4 |
| 3 | Dharwad | Garag | 1 | SFPGPM- 5 |
| | | Dharwad | 1 | SFPGPM- 6 |
| | | Narendra | 1 | SFPGPM- 7 |
| 4 | Kalaburagi | ZARS, Kalaburagi | 1 | SFPGPM- 8 |
| 5 | Bagalkot | Kodihal | 2 | SFPGPM- 9 |
| | | | | SFPGPM- 10 |
| 6 | Bidar | Basavakalyan | 3 | SFPGPM- 11 |
| | | | | SFPGPM- 12 |
| | | | | SFPGPM- 13 |
| 7 | Vijayapura | Vijayapura | 1 | SFPGPM- 14 |
| 8 | Yadgiri | Saidapur | 2 | SFPGPM- 15 |
| | | | | SFPGPM- 16 |
| 9 | Ballari | Ballari | 2 | SFPGPM- 17 |
| | | | | SFPGPM- 18 |
| 10 | Raichur | Askihal | 1 | SFPGPM- 19 |
| | | Janakirao camp | 1 | SFPGPM- 20 |
| | | New area (UAS campus) | 1 | SFPGPM- 21 |
| | | Siddanabhavi camp | 1 | SFPGPM- 22 |
| 11 | Kushtagi | Kushtagi | 4 | SFPGPM- 23 |
| | | | | SFPGPM- 24 |
| | | | | SFPGPM- 25 |
| | | | | SFPGPM- 26 |

Source and designation of chickpea rhizospheric bacterial PGPM isolates

| Sl. No. | District | Village | No. of bacterial isolates obtained | Isolate code |
|----------|------------|--------------------------|------------------------------------|--------------|
| 1 | Dharwad | Garag | 1 | SBPGPM- 1 |
| | | Thadakoda | 1 | SBPGPM- 2 |
| | | Narendra | 1 | SBPGPM- 3 |
| 2 | Gadag | Gajendri gad | 2 | SBPGPM- 4 |
| | | | | SBPGPM- 5 |
| 3 | Haveri | Chandapura | 3 | SBPGPM- 6 |
| | | | | SBPGPM- 7 |
| | | | | SBPGPM- 8 |
| 4 | Kalaburagi | ZARS, Kalaburagi | 1 | SBPGPM- 9 |
| 5 | Yad giri | Bheemar ay ana gudi | 2 | SBPGPM- 10 |
| | | | | SBPGPM- 11 |
| 6 | Bidar | Basavakaly an | 1 | SBPGPM- 12 |
| | | Dubalgundi | 1 | SBPGPM- 13 |
| | | Hudugi | 1 | SBPGPM- 14 |
| 7 | Vijayapura | Hittinhalli | 1 | SBPGPM- 15 |
| 8 | Bagalkot | Bagalkot | 2 | SBPGPM- 16 |
| | | | | SBPGPM- 17 |
| 9 | Ballari | Ballari | 2 | SBPGPM- 18 |
| | | | | SBPGPM- 19 |
| 10 | Kushtagi | Kushtagi | 4 | SBPGPM- 20 |
| | | | | SBPGPM- 21 |
| | | | | SBPGPM- 22 |
| | | | | SBPGPM- 23 |
| | | Ganganal | 1 | SBPGPM- 24 |
| | | Hiresindhogi | 1 | SBPGPM- 25 |
| Yalburga | 1 | SBPGPM- 26 | | |
| 11 | Raichur | New area (UAS campus) | 2 | SBPGPM- 27 |
| | | | | SBPGPM- 28 |
| | | Askihal | 1 | SBPGPM- 29 |
| | | Rampur | 1 | SBPGPM- 30 |

3.2.4 Antagonistic potential of PGPMs against *R. bataticola*

To identify the potential PGPMs against *R. bataticola*, the efficacy of the antagonistic activity of rhizospheric and endophytic PGPMs was tested by dual culture technique (Xu and Kim, 2014) under *in vitro* conditions.

In the dual culture technique, test antagonist PGPM (fungi or bacteria) was placed at one side and on the opposite side, the mycelial disc of test pathogen measuring 5 mm diameter of five days old culture was placed aseptically on sterile Petri dish containing PDA medium for fungi and NA medium for bacteria. In the control plate, a mycelial disc of pathogen was placed aseptically alone without test PGPM. The Petri plates were then incubated at $28 \pm 2^\circ$ C. Three replications were maintained for each test PGPM. The observations on growth of pathogen in test plate as well as in control were measured in all the replications. Later, per cent inhibition of mycelial growth of test pathogen was calculated by the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition in growth of test pathogen.

C = Radial growth of pathogen (mm) in control.

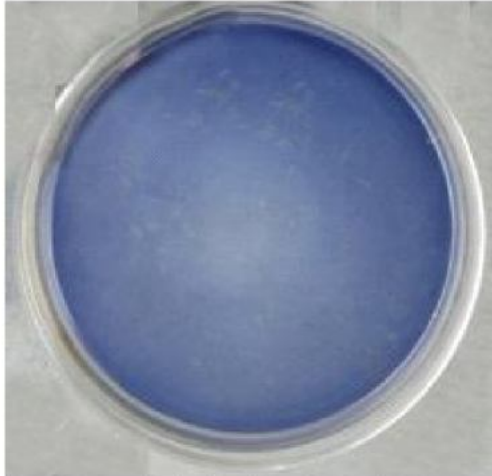
T = Radial growth of pathogen (mm) in treatment.

3.3 Studies on plant growth promoting traits of potential PGPMs

Based on the dual culture assay results, the potential rhizospheric and endophytic PGPMs isolates were tested for their plant growth promoting traits. Out of 40 fungal and 30 bacterial endophytic PGPMs, 8 fungi and 8 bacteria were selected for the studies, respectively. Similarly, out of 26 fungal and 30 bacterial rhizospheric PGPMs, 3 fungal and 9 bacterial isolates were selected for plant growth promoting trait studies, respectively. The media and reagents used for studies on plant growth promoting traits are presented in Plate 1.



Salkowski reagent for IAA production test



Blue agar plates for siderophore production test



Peptone water for ammonium production



Pikovskaya medium for phosphate solubilization test



PDA + Glycine and NA + Glycine for HCN production

Plate 1: Media and reagents used for studying on plant growth promoting traits of potential PGPMs

3.3.1 Indole Acetic Acid (IAA) production

Indole acetic acid (IAA) production was detected as described by Brick *et al.* (1991). Culture media such as potato dextrose and nutrient broths were inoculated with the fungal and bacterial cultures, respectively. Then, the bacterial broth cultures were allowed to grow in shaking incubator (28 °C, 100 rpm) for 48 h for obtaining log phase in case of bacteria and for 4-5 days for fungi. Uninoculated broth cultures were kept as control for both fungi and bacteria. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent. Salkowski reagent (prepared for the colorimetric test) was prepared in the fume hood by mixing 2 ml of 0.5M FeCl₃, 49 ml of water and 49 ml of 70 per cent perchloric acid. Development of pink colour indicates positive reaction for IAA production. IAA helps plant directly for the root elongation and increase the absorption of nutrients from the surrounding habitats.

3.3.2 Siderophore production

The siderophore production by PGPMs helps the plants in iron sequestration and increases the concentration of available iron to inner tissues. In addition to iron acquisition, they also give nutritional competition to the pathogens by limiting the iron availability to them and inhibit their growth (Dimkpa, 2016). Siderophore production was detected by the method given by Schwyn and Neilands (1987). All the glassware were cleaned with 6M HCl to remove any trace elements and then rinsed with ddH₂O.

A. Preparation of blue dye:

- a. Solution 1: 0.06 g of CAS (Fluka chemicals) was dissolved in 50 ml of ddH₂O.
- b. Solution 2: 0.0027 g of FeCl₃-6 H₂O was dissolved in 10 ml of 10 mM HCl.
- c. Solution 3: 0.073 g of HDTMA was dissolved in 40 ml of ddH₂O.

Solution 1 was mixed with 9 ml of Solution 2 and then Solution 3 was mixed. Solution now turned to blue color. Later, the blue colored solution was autoclaved and stored in a plastic container/bottle.

B. Mixture solution:

- a. Minimal media 9 (MM9) salt solution stock: 15 g KH_2PO_4 , 25 g NaCl, and 50 g NH_4Cl was dissolved in 500 ml of ddH₂O.
- b. 20 per cent Glucose stock: 20 g glucose was dissolved in 100 ml of ddH₂O.
- c. NaOH stock: 25 g of NaOH was dissolved in 150 ml ddH₂O (pH ~12).
- d. Casamino acid solution: 3 g of casamino acid was dissolved in 27 ml of ddH₂O. Then, 3 per cent of 8-hydroxyquinoline was extracted in chloroform to remove any trace iron. Later, the solution was filtered and sterilized.

C. CAS agar preparation:

- a. 100 ml of MM9 salt solution was added to 750 ml of ddH₂O. To that, 32.24 g piperazine-N,N'-bis (2-ethanesulfonic acid) PIPES was dissolved. After that 15 g Agar-agar was added. Then the solution was autoclaved and cooled to 50 °C.

(PIPES will not dissolve below pH of 5. So pH was maintained up to 6 and PIPES was added slowly while stirring. While stirring, slowly pH was brought up to 6.8.)

Note: pH should not exceed 6.8 as solution will turn to the solution green.

- b. 30 ml of sterile Casamino acid solution and 10 ml of sterile 20 per cent glucose solution was added to MM9/PIPES mixture. Then, 100 ml of blue dye solution was slowly added along the glass wall with enough agitation to mix thoroughly. Finally, the CAS agar medium was blue in color.

The CAS agar medium was poured in to the Petri plates aseptically and allowed to solidify. The freshly grown fungal and bacterial PGPM cultures were placed in the centre of the plate and incubated at 28 °C for 48-72 h for bacteria and 25 °C for 3-5 days for fungi. Orange halos around the culture/colonies on blue agar plates were indicative for siderophore production.

3.3.3 Ammonium production

The selected endophytic and rhizospheric PGPM isolates were tested for the production of ammonium in peptone water. Freshly grown cultures of fungi and bacteria PGPMs were inoculated in 10 ml peptone water in each tube and incubated for 48-72 h at 36 ± 2 °C (for bacteria) and 4-5 days at 25 ± 3 °C (for fungi). Nessler's reagent (0.5 ml) was added to each culture tube. Development of brown to yellow colour indicated positive test for ammonium production (Cappuccino and Sherman, 1992). Ammonium protects plant through suppression of root pathogens.

3.3.4 Phosphate solubilization

The phosphate solubilization study was conducted by using Picovskaya medium (PVK) supplemented with tricalcium phosphate and 1.5 per cent Agar-agar (Picovskaya 1948; Nautiyal, 1999). Test fungal and bacterial PGPM isolates were stabbed on plate using sterile cork borer. The observation on halo zone was measured after 3 days of incubation at 28°C.

3.3.5 Production of Hydrogen Cyanide (HCN)

Production of HCN was determined in slants containing PDA and NA supplemented with 4.4 g/l of glycine (Lorck, 1948). The test fungal and bacterial cultures were inoculated on PDA and NA slants, respectively. Then, Whatman No.1 filter paper strips dipped in 0.5 per cent picric acid and in 2 per cent sodium carbonate solution were inserted from the top of each test tube. Then the test tubes were sealed with parafilm and incubated at 28- 30 °C for 4 days. Change in colour of inserted filter paper to brown or reddish-brown was recorded as positive reaction.

3.4 Molecular characterization and diversity analysis of potential PGPMs and their screening for bio-active compound production against *R. bataticola*

The endophytic and rhizospheric PGPMs which showed higher plant growth promoting traits were further selected for molecular characterization and diversity analysis. Out of 8 fungal and 8 bacterial endophytic PGPMs, 7 fungal and 5 bacterial isolates were selected for molecular studies. Similarly, out of 3 fungal and 9 bacterial rhizospheric PGPMs, all the 3 fungal and 4 bacterial isolates were chosen. Totally, 10

fungal and 9 bacterial endophytic and rhizospheric PGPMs which were proved to be promising were subjected to molecular characterization by DNA isolation and PCR analysis by using ITS and 16S r DNA universal primers.

3.4.1 Culture preparation

3.4.1.1 Culture preparation of endophytic and rhizospheric fungal PGPMs

Mycelial discs of actively growing 7 days old culture of isolates of all the 10 fungal PGPMs were grown separately on 30 ml potato dextrose broth in 100 ml flask and incubated for 5 days at 27 ± 2 °C. Well grown fungal mat was placed on the Whatman No. 1 filter paper for 30 min., which was fixed on a funnel in order to drain excess of medium and kept in the deep freezer for overnight before homogenizing it. Later, fungal mat was used for isolation of total genomic DNA.

3.4.1.2 Culture preparation of endophytic and rhizospheric bacterial PGPMs

Actively growing loopful culture (1 day old culture) of isolates of all the 9 bacterial PGPMs were grown separately on 30 ml nutrient broth in 100 ml flask and incubated in a shaker at 180 rpm, 28⁰C for 24 hrs. After the incubation, the bacterial broth cultures of isolates turned in to turbid and further these broth cultures were used for isolation of total genomic DNA.

3.4.2 DNA isolation

3.4.2.1. DNA isolation of endophytic and rhizospheric fungal PGPMs

- DNA of selected fungal PGPMs was extracted using Cetyl Trimethylammonium Bromide (CTAB) method from vegetative mycelia.
- The harvested mycelium was grounded to fine paste by using freshly prepared Lyses buffer in pestle and mortar.
- About 1 ml of finally crushed mycelia was taken in 2 ml eppendorf tube and 250 µl of freshly prepared CTAB buffer was added and incubated for 10 min at 60 °C in hot water bath (10010, Venchal scientific, USA).

- Then 10 μ l of Proteinase K (20 mg/ ml) was added and again incubated for 10 min at 60 $^{\circ}$ C in hot water bath (10010, Venchal scientific, USA).
- Later, equal volume of Chloroform: Isoamyl alcohol (24:1 solution) was added to eppendorf tubes. Tubes were vigorously shaken till solution become cloudy and turbid.
- Tubes were kept for centrifugation (Mikro 220R Hettich Zentrifugen, Germany) at 12000 \times g for 10 min. Later the clear liquid suspension (aqueous phase) floating on top of tube was separately taken out and transferred to another 2 ml eppendorf tube without mixing with other two layers of protein and plant debris using micro pipettes.
- Then 10 μ l of RNAase, 750 μ l of Isopropanol (chilled) and 250 μ l of 3M Sodium acetate was added to the tube and slowly tilted the tubes upside down for one minute and kept tubes under -20 $^{\circ}$ C for overnight to precipitation of the DNA.
- Tubes were taken out after overnight incubation and centrifuged at 10000 \times g for 10 min. Supernatant was discarded and creamy white coloured DNA pellet was retained.
- Later tubes were washed using 70 per cent chilled ethyl alcohol by centrifuging at 10000 \times g for 10 min and kept in laminar air flow for 15 min to remove the traces of ethyl alcohol.
- Then, 30 μ l of 1X T.E buffer was added to air dried pellet to dissolve the DNA pellet. Then DNA samples were stored at -20 $^{\circ}$ C for long term storage for further studies.

3.4.2.2 DNA isolation of endophytic and rhizospheric bacterial PGPMs

Genomic DNA from all the bacterial PGPM isolates was extracted using the sucrose lysis method by following the steps described below,

- 1.5 ml of culture was transferred to a microcentrifuge tube and was centrifuged (Mikro 220R Hettich Zentrifugen, Germany) at 12000 \times g for 5 min. The pellet was collected and it was repeated with 1.5 ml culture.

- The pellets were resuspended in a 400 μl sucrose buffer and subjected to vortex (MX-F, DLAB, Beijing, China).
- 30 μl of Lysozyme was added to it and incubated for at 60 $^{\circ}\text{C}$ for 10 min in a hot water bath (10010, Venchal scientific, USA).
- 45 μl of 10 per cent Sodium Dodecyl Sulphate (SDS) and 8 μl of Protienase K was added and incubated at 60 $^{\circ}\text{C}$ for 10 min in a hot water bath.
- The freshly prepared NaCl (5M) of about 280 μl and 140 μl of 10 per cent CTAB were added. Then it was incubated for 10 min at 60 $^{\circ}\text{C}$.
- Equal amount of chloroform: isoamyl alcohol (24:1) was added and was mixed well by inverting the tube until the phase was mixed completely.
- Then the mixture was centrifuged at 10000 $\times g$ for 10 min and the upper aqueous phase was transferred to a new tube.
- 50 μl of 3M sodium acetate (ice cold) was added and mixed well.
- Then 300 μl isopropanol (ice cold) was added and mixed gently to precipitate DNA. It was incubated overnight at -20 $^{\circ}\text{C}$.
- After overnight incubation, the mixture is spun at 12000 $\times g$ for 15 min to pellet down the DNA and supernatant was discarded.
- 1ml of 70 per cent ethanol (chilled) was added to the pellet and spun at 12000 rpm for 10 min (twice).
- The supernatant was discarded and allowed the pellet to air dry in the laminar air flow.
- DNA was resuspended in 40 μl of TE buffer.
- Then 3 μl RNase was added and incubated at 37 $^{\circ}\text{C}$ for 30 min.
- The obtained DNA is kept at 4 $^{\circ}\text{C}$ for short term storage and at -20 $^{\circ}\text{C}$ for long term storage.

3.4.3 Agarose gel electrophoresis for endophytic and rhizospheric PGPMs

After the isolation of DNA from the endophytic and rhizospheric PGPMs, in order to confirm the presence of DNA, agarose gel electrophoresis was carried out. It is a method by which DNA fragments were separated based on their charge and size when electric field is applied to the DNA. The negatively charged DNA migrates through the pores of an agarose gel towards the positively charged end of the gel when an electrical current is applied with smaller fragments migrating faster. The resulting bands were visualized using ultraviolet (UV) light.

3.4.3.1 Preparation of TAE buffer

About 9.31 gm of EDTA was dissolved in 40 ml water and volume was made to 100 ml. Then to this solution, 57.1 ml of glacial acetic acid and 242 gm of Tris-base was added and solution was made up to 1000 ml to get 50x (50 M) solution. From this 50x buffer, 1x working TAE buffer prepared by diluting with distilled water.

3.4.3.2 Preparation of agarose gel and electrophoresis

For the preparation of 1 per cent agarose gel, 1 g of high EEO (Electroendosmosis) agarose powder (SRL, Maharashtra) was dissolved in 100 ml of TAE buffer and heated to mix all the contents. After cooling, 3 μ l of ethidium bromide (0.5 μ g/ml) was added. Later, agarose was casted onto the casting tray with the comb intact. After solidification of gel, combs were removed to create wells and the tray was transferred to the electrophoresis system.

The 10X TAE loading buffer was added to gel tank. Later, 2 μ l of DNA was mixed with 2 μ l of loading dye (Bromo phenol blue) and loaded in to the wells and DNA ladder (100 bp) was loaded in the first well of the tray. Electrophoresis was carried out at 60 V/cm for 60 min. Later, the gel tray was removed and placed in the Gel documentation unit (Essential V6, UVITEC, Cambridge, UK) and observed for the presence of DNA bands.

3.4.4 Polymerase Chain Reaction (PCR) amplification

The universal primers such as ITS 1 and ITS 4 were used to amplify the ITS region of fungal PGPMs, whereas 16s rDNA F and 16s rDNA R primers were used to

amplify 16s rDNA genomic region of bacterial PGPM isolates by using PCR with following amplification conditions described by Praveen *et al.* (2019).

3.4.4.1 Primer synthesis

ITS primer synthesis

Universal primers ITS1-F (CTTGGTCAT-TTAGAGGAAGTAA) and ITS4-R (TCCTCCGCT-TATTGATATGC) were used for characterization of fungal isolates. Primer sequences were synthesized at commercial facilities (Eurofins, Bangalore, India).

16s rDNA primer synthesis

Universal Primers 16s rDNA F (GAG-TTT-GAT-CCT-GGC-TCA) and 16s rDNA R (AGA-AAG-GAG-GTG-ATC-CAG) were used for characterization of bacterial isolates. Primer sequences were synthesized at commercial facilities (Eurofins, Bangalore, India).

3.4.4.2 PCR reaction mixture

PCR reaction mixture for endophytic and rhizospheric fungal PGPMs

PCR was carried in a 10 μ l reaction mixture which comprised of following components

| Reaction mixture | Quantity |
|--|--------------------------------|
| Template DNA (25 ng/ μ l) | 1.00 μ l |
| Primer (5 pM/ μ l) | F-0.5 μ l |
| | R-0.5 μ l |
| dNTPs mix (2.5 mM each) | 1.00 μ l |
| 10X assay buffer with 15 mM MgCl ₂ | 1.00 μ l |
| <i>Taq</i> DNA polymerase (6 U μ l ⁻¹) | 1.00 μ l |
| Sterile distilled water | 5.00 μ l |
| Total | 10.00 μl |

PCR reaction mixture for bacterial PGPMs

PCR was carried in a 10 μ l reaction mixture which comprised of following components

| Reaction mixture | Quantity |
|--|--------------------------------|
| Template DNA (25 ng/ μ l) | 0.7 μ l |
| Primer (5 pM/ μ l) | F-0.5 μ l |
| | R-0.5 μ l |
| dNTPs mix (2.5 mM each) | 0.7 μ l |
| 10X assay buffer with 15 mM MgCl ₂ | 1.00 μ l |
| <i>Taq</i> DNA polymerase (6 U μ l ⁻¹) | 0.6 μ l |
| Sterile distilled water | 6.00 μ l |
| Total | 10.00 μl |

3.4.4.3. Thermo profile of PCR

Thermo profile of PCR for endophytic and rhizospheric fungal PGPMs

Amplifications were conducted in a thermal cycler (Veriti, Applied Biosystems, Singapore). The PCR programming was done following the standard protocol provided by Yugander *et al.* (2017) with some modifications. Initial denaturation was set for 3 min at 95⁰C for 1 cycle. Whereas, denaturation time was set for 30 sec at 95⁰C, annealing for 1 min at 58⁰C and extension for 8 min at 72⁰C for 35 cycles. The final extension was set for 8 min at 72⁰C for 1 cycle.

Thermo profile of PCR for endophytic and rhizospheric bacterial PGPMs

Amplifications were conducted in a thermal cycler (Veriti, Applied Biosystems, Singapore). The PCR programming was done following the standard protocol provided by Yugander *et al.* (2017) with some modifications. Initial denaturation was set for 1 min at 94⁰C for 1 cycle. Whereas, denaturation time was set for 1 min at 96⁰C, annealing for

1.5 min at 58 °C and extension for 1.5 min at 72 °C for 35 cycles. The final extension was set for 8 min at 72 °C for 1 cycle.

3.4.4.4. PCR amplification

The PCR mixture was added to the PCR tubes. Later, the PCR tubes were placed in thermocycler (Veriti, Singapore) and the PCR programme was run manually by setting the conditions as mentioned in section 3.4.4.3. On completion of the programme, in order to confirm the presence of DNA, 2 µl of PCR product along with 100 bp DNA ladder (Thermo Fisher scientific, USA) was loaded into the wells of gel casting tray. The electrophoresis was carried out at 60 V for 5 h. After the completion of the process, the agarose gel was visualized in gel documentation unit (UVITEC, UK) to confirm the amplification of the DNA. Later, the PCR products were stored at -20°C in deep freezer (Vestfrost, Denmark) for further studies.

3.4.5 Sequencing

Upon PCR amplification, about 50 µl of PCR product of all endophytic and rhizospheric PGPM isolates were sent to commercial facility (Eurofins, Bangalore, India) where they purify the PCR products and use them for sequencing. PCR amplicons of isolates were Sanger's dideoxy chain-termination method from both the ends using the same primers which were used for PCR amplification.

3.4.6 Identification of endophytic and rhizospheric PGPMs using NCBI BLAST

Primer sequences in raw reads were trimmed and processed sequences were subjected to BLAST search (<https://www.ncbi.nlm.nih.gov/BLAST/>) for query match against homologous sequences through NCBI. The species which showed highest per cent identity (90-100 %) with the deposited isolate was confirmed to be the identity of the isolate.

3.4.7 Diversity analysis using phylogenetic tree

The obtained ITS and 16S rDNA sequences were subjected to Basic Local Alignment Search Tool (BLAST) analysis against the sequence database at the National Centre for Biotechnology Information (NCBI) (available at <http://blast.ncbi.nlm.nih.gov>) to compare with the closest related fungal and bacterial species. The species with highest

similarity identity percentage (90-100 %) were selected for phylogenetic analysis. The determined sequences were aligned using CLUSTAL W in BioEdit Sequence Alignment Editor Version 7.2. and phylogenetic trees were constructed using MEGA 11 software. DNA substitutions were done according to the Maximum Likelihood Model. Phylogenetic relatedness of the PGPMs with other close relatives was clustered using Neighborhood Joining Method. Bootstrap replications of 1000 were used as the statistical confidence of the nodes in the phylogenetic trees. Outgroups were considered to support differences among species.

Sequence identity matrix was also constructed by aligning the determined sequences using CLUSTAL W in BioEdit Sequence Alignment Editor Version 7.2.

3.4.8 Screening for bio-active compounds production by potential PGPMs against *R. bataticola*

The 19 potential PGPMs which were identified by BLAST analysis were tested for production of bio-active compounds against *R. bataticola* by inverted plate technique (Dennis and Webster, 1971).

3.4.8.1 Fungal PGPMs

Two separate lids of sterilized Petri plates were taken and 20 ml of PDA medium was poured on each plate and allowed for solidification. Mycelial disc of 5 mm of test fungal PGPM and test pathogen was inoculated at the centre of Petri dish on each lid separately. Then Petri dish inoculated with pathogen were inverted over the Petri dish containing test fungal PGPM and both plates were sealed with the adhesive tape (parafilm) keeping antagonist in lower and pathogen in upper Petri dish lid. In the control, the Petri dish containing pathogen was inverted over the Petri dish containing PDA medium only. Three replications were maintained for each test PGPM and the plates were incubated at 28 ± 1 °C for five days. The colony diameter of the pathogen was measured in test pathogen plate as well as in control. The per cent inhibition was calculated by using formula given by Vincent (1947).

3.4.8.2 Bacterial PGPMs

The “Material and Methods” followed for screening fungal isolates were followed for bacterial PGPM also except the medium. For bacterial PGPMs, nutrient agar medium was used and the plates were incubated at 30 °C for five days.

3.5 Extraction, identification of bio-active compounds and screening of crude extract from potential PGPMs responsible for inhibition of *R. bataticola*.

3.5.1 Extraction of bio-active compounds from potential PGPMs

One endophytic and one rhizospheric fungal and bacterial PGPM each (totally 4 isolates) which showed highest per cent inhibition in inverted plate technique were selected for the extraction of bio-active compounds.

3.5.1.1. Extraction of bio-active compounds from fungal PGPMs

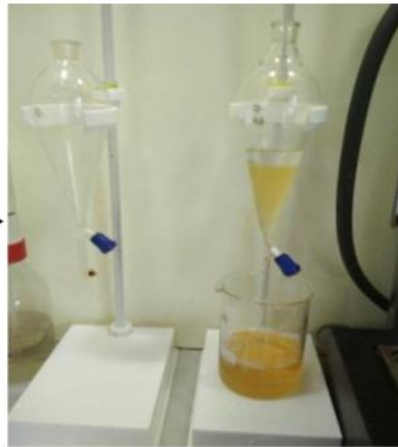
Two efficient fungal PGPMs were grown in 500 ml of potato dextrose broth and flasks were incubated at 28 ± 1 °C for 21 days. The culture filtrate was obtained by straining through the muslin cloth. Compounds were extracted by solvent extraction method into ethyl acetate (EtOAc) at the ratio of 1:1 (v/v). For this, 500 ml of fungal PGPM culture filtrate was added with 500 ml of ethyl acetate. Later, the contents were transferred into separatory funnel. The upper layer of solvent containing antifungal compounds was collected through separating funnel into conical flasks. Then, the ethyl acetate from the collected upper phase was evaporated by using rotary evaporator at 35 °C under reduced pressure. Finally, the residue obtained in the rotary evaporator was resuspended in acetone (solvent) for GC-MS/MS analysis (Plate 2).

3.5.1.2 Extraction of bio-active compounds from bacterial PGPMs

Two efficient cultures of bacterial PGPMs were inoculated in 500 ml of nutrient broth and flasks were incubated in the shaker at 28 ± 2 °C, 150 rpm for 96 h. After 96 h of incubation, harvested cultures were centrifuged at $10,000 \times g$ for 10 min at 4 °C. Later, supernatant was collected in separate flasks. All supernatants were acidified to pH 2 with 1 N HCl and pH was monitored with pH-meter. Acidified supernatants were extracted with equal volume of ethyl acetate. For this, in each flask with 500 ml of acidified supernatant, 500 ml of ethyl acetate were added. Later, flasks were put in a shaking



Culture filtrate of PGPM mixed with ethyl acetate



Separation of organic phase by using funnel separator



Pellet formation in rotary evaporator



Separation of compounds from solvent using rotary evaporator



Compounds in acetone



GC-MS/MS equipment

Plate 2: Schematic representation of extraction and identification of bio-active compounds from PGPMs

incubator for 10-15 min and then the content was transferred into separatory funnel. The upper organic phase containing bio-active compounds were collected through separating funnel into conical flasks. Later 20-30 g of anhydrous sodium sulphate were added to the flask and stirred with a glass stirrer. Then, the content was transferred to the rotary evaporator. Finally the ethyl acetate from the collected upper organic phase was evaporated and residue obtained in the rotary evaporator was dissolved in 2 ml methanol: 2 ml chloroform and stored at 4°C for GC-MS/MS analysis (Plate 2).

3.5.2 Identification of compounds by GC-MS/MS analysis

After the extraction of bioactive compounds from endophytic and rhizospheric PGPMs, their chemical characterization and qualitative estimation was carried out by GCMS/MS technique.

GC-MS/MS parameters were performed by using Agilent 7890B GC with 7000C MS system with oven temperature of 75 °C for 1 min and 30 °C to 300°C for 2 min. The inlet and transfer line temperature is programmed at 280 °C and 290 °C, respectively. The flow rate of helium gas was 1.0 ml/min. 1 µl samples were injected under split of 3:1. The ionization mass spectroscopic analysis was done with 70 eV. Interpretation of mass spectrum GC-MS/MS analysis was done by matching list of known compound's spectrum with Agilent's GC-MS/MS Mass Hunter, NIST MS Library and NIST's Automated Mass Spectral Deconvolution and Identification Software.

3.5.3 Screening for anti-microbial activity of crude extract of potential PGPMs

The effect of crude extract of four potential PGPMs on mycelial growth of *R. bataticola* was tested by using poisoned food technique (Shravelle, 1961). The residues of both fungal and bacterial PGPMs obtained after evaporation of organic phase were dissolved in acetone for fungal isolates and methanol: chloroform for bacterial isolates. Then the isolates were tested at different concentrations *viz.*, 500, 1000 and 2000 ppm. In poisoned food technique, the methanolic extract of fungal and bacterial PGPMs was added to the molten PDA medium and nutrient agar medium, respectively to obtain desired concentration. Later, 20 ml of molten nutrient medium mixed with methanolic extract was poured in to 90 mm diameter Petri plates. After solidification, 5 mm disc of pathogen was placed at the centre of the Petri dish. Suitable control plates were

maintained by adding water to the PDA and NA nutrient medium for fungi and bacterial PGPMs respectively. Each set of treatment was replicated thrice and plates were incubated at 28 ± 2 °C. Observations on radial growth of pathogen were recorded when maximum growth was observed in the control plate and per cent inhibition was calculated using the formula given by Vincent (1947).

3.6 Management of dry root rot using potential PGPMs in pot and field experiments

Before carrying the potential PGPMs for the management studies, they were tested for their influence on the growth parameters. Based on the *in vitro* studies, one highly efficient fungal endophytic PGPM and one rhizospheric fungal and bacterial PGPM each were used for the formulation of treatments under field and in pot culture experiments. The effective PGPM isolates were also tested for their compatibility with each other in the consortium before formulating the treatments for the management experiments. For this, test antagonist PGPMs were placed aseptically beside one another on sterilized Petri dish containing PDA medium and incubated at 28 ± 2 °C for 2-3 days.

3.6.1 Effect of potential PGPMs on growth parameters of chickpea

Plant growth promoting activity was assessed by using roll towel method (ISTA, 1999). Healthy seeds were first surface sterilized in sodium hypochlorite (1%) for 3 min and then in 70 per cent ethanol for 2 min followed by three washing in sterile water and allowed for air drying. After air-drying, the seeds were immersed overnight in mycelial and bacterial suspension of PGPMs which were mass multiplied in potato dextrose and nutrient broth respectively. Totally, three replications were maintained. For each replication fifty seeds were randomly counted and placed on blotter paper sheets and covered with a moistened blotter and rolled. Seeds which were soaked in PDB and NB alone served as control. Rolls were kept on a butter paper sheet and rolled as a single bundle and incubated in a growth chamber at 25 °C with 80 per cent relative humidity for 8 days. After incubation, germination percentage was noted along with root length and shoot length (Rangeshwaran *et al.*, 2002). Later, seedling vigour index was calculated by using the formula given below.

3.6.1.1 Observations to be recorded

a) Germination per cent

The germination counts were taken on fifth day for chickpea. Total germination percentage was calculated and expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds put for germination}} \times 100$$

b) Root length (cm)

Five normal seedlings in each replication were randomly selected for the measurement of root length on the day of count. The root length was measured from the collar region to the tip of the primary root. The average of five normal seedlings was expressed as root length in cm.

c) Shoot length (cm)

The seedlings used for measurement of root length were also taken for measuring shoot length. The shoot length was measured from collar region to the point of attachment of cotyledons. The average of five normal seedlings was expressed as shoot length in cm.

d) Seedling vigour index

The seedling vigour index was calculated by using the formula given by Abdul-Baki and Anderson (1973).

$$\text{Seedling vigour index} = (\text{Root length} + \text{Shoot length}) \times \text{Germination percentage}$$

3.6.2 Management of dry root rot using potential PGPMs in pot and field experiments

Pot and field experiments were carried out during *rabi* 2021-22 in order to evaluate the efficacy of rhizospheric and endophytic PGPMs for the management of disease. Observations were recorded on growth and yield parameters as well as total number of plants observed and number of plants infected. The treatment details were similar for the

management of the disease in both pot and field conditions. The PGPMs used for the formulation of the treatments were mass multiplied as given below.

3.6.2.1 Mass multiplication of fungal PGPMs

Potato dextrose broth medium was prepared and autoclaved at 15 lbs pressure for 15 min at 121 °C. Later it was allowed to cool down to room temperature. Later the discs of the fungal PGPMs were inoculated aseptically in the laminar air flow chamber and kept it for incubation for 25-30 days. The entire culture was mixed thoroughly in to a proper mixture. Talc powder was added to it in the ratio of 1:3 (for one litre of broth and 3 kg of talc powder). CMC (Carboxy methyl cellulose) (5 g/kg) was added and mixed with talc powder. Later it was kept for drying (for 2 days) and powdered it properly.

3.6.2.2 Mass multiplication of bacterial PGPMs

A loopful of bacterial culture was transferred to 250 ml in nutrient broth in a 500 ml of conical flask under aseptic conditions. Later it was incubated at room temperature on a rotary shaker for 48 h After the incubation, it was mixed with sterilized talc powder and calcium carbonate (1:2). The pH of the substrate (talc powder) was adjusted to 7.0 by addition of calcium carbonate. Later it was kept for drying (for 2 days) and powdered it properly.

3.6.2.2 Experimental details

| | |
|---------------------|--|
| Location | : Organic Farming Research Institute, UAS, Raichur |
| Season | : <i>Rabi</i> , 2021 |
| Design | : RCBD (Field experiment) / CRD (Pot experiment) |
| Variety | : JG - 11 |
| No. of treatments | : 9 |
| No. of replications | : 3 |
| Plot size | : 27 sq. m (Field experiment) and 3 pots / Treatment (Pot experiment) |
| Spacing | : 30 cm × 10 cm (Field experiment) |

The following treatments were imposed in both pot and field experiments.

3.6.2.3. Treatments Details:

| | |
|----------------|--|
| T ₁ | Seed treatment with <i>T. harzianum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>T. harzianum</i> at 2.5 kg /100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. |
| T ₂ | Seed treatment with <i>B. velezensis</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>B. velezensis</i> at 2.5 kg/100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. |
| T ₃ | Seed treatment with <i>T. asperellum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>T. asperellum</i> at 2.5 kg/100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. |
| T ₄ | Seed treatment with <i>T. harzianum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>T. harzianum</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>T. harzianum</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g/l. |
| T ₅ | Seed treatment with <i>B. velezensis</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>B. velezensis</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>B. velezensis</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g/l. |
| T ₆ | Seed treatment with <i>T. asperellum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>T. asperellum</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>T. asperellum</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g/l. |
| T ₇ | Seed treatment with <i>T. harzianum</i> (10 g/kg seed) + <i>B. velezensis</i> (10 g/kg seed) + <i>T. asperellum</i> (10 g/kg seed) followed by soil application of enriched vermicompost with <i>T. harzianum</i> , <i>B. velezensis</i> and each at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>T. harzianum</i> (10 g/l) + <i>B. velezensis</i> (10 g/l) + <i>T. asperellum</i> (10 g/l) and <i>Beauveria bassiana</i> at 2 g/l. |
| T ₈ | Seed treatment with <i>Trichoderma harzianum</i> at 5 g/kg seed followed by soil application of enriched FYM at 2.5 kg/100 kg per acre during sowing and foliar spray of 5 % NSKE + 10 % Cow urine 2 sprays (OF RPP). |
| T ₉ | Untreated control |

3.6.2.4. Observations recorded:

a) Disease incidence (%)

Per cent disease incidence was calculated using the following formula given by Wheeler (1969) in pot as well as field experiments.

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

b) Yield Parameters

Total yield (q/ha)

At the time of harvest, chickpea plants from all the treatments and replications were collected separately. After threshing and winnowing, the net plot yield was recorded and later converted to ha and expressed as q per ha in field experiment.

Test weight (g)

The test weight was also recorded by taking 100 seeds from each treatment and expressed as g. The data were subjected to statistical analysis by using standard statistical procedures.

c) Growth parameters

Plant height (cm)

The plant height was recorded of fifteen randomly selected plants and expressed in cm. The data were subjected to statistical analysis by using standard statistical procedures.

No. of branches per plant

Number of branches were recorded from fifteen randomly selected plants and expressed in numbers.

No. of pods per plant

Number of pods were recorded from fifteen randomly selected plants and expressed in numbers.

IV. RESULTS AND DISCUSSION

The present investigation was focused on the harnessing of PGPMs for the management of dry root rot disease of chickpea caused by *Rhizoctonia bataticola*. In order to explore the potential PGPMs against *R. bataticola*, the compilation of PGPMs from healthy chickpea plants and rhizosphere was undertaken from different districts of northern Karnataka and were categorized into endophytic and rhizospheric, fungal and bacterial isolates. Further, the PGPMs were assessed for their antagonistic potential by dual culture technique. The potential isolates which could inhibit the pathogen growth in dual culture technique, were screened for the exhibition of plant growth promoting traits. The PGPMs showing maximum number of plant growth promoting traits were subjected for molecular identification through DNA isolation and PCR analysis and further efforts were made to study the diversity analysis among them by constructing the phylogenetic tree. Subsequently, all these potential isolates were further screened for the production of bio-active compounds which could hinder the mycelial growth of pathogen by inverted plate technique. Moving forward, one fungal and bacterial isolate each from endophytic and rhizospheric isolates, which were dominant for inhibiting the pathogen were used for the extraction of bio-active compounds. The identification of the bio-active compounds produced by the potential PGPMs was analysed through GC-MS/MS. Furthermore, the potential PGPMs having higher antagonistic activity and plant growth promoting capacity were exploited for the management studies under pot and field experiments. Hence, in this chapter, all the findings obtained from the present investigation are presented and deliberated with the supporting previous reports for the discussion.

4.1 Disease symptomatology

The disease was initiated with yellowish discoloration of the foliage followed by premature drying and wilting at the seedling stage. The affected seedlings were failed to grow and produce the seeds. In the advanced stage of the disease, brownish to black lesions were found in the taproot and lateral roots. Later, these lesions lead to the necrosis of the lateral roots. Hence, when we uprooted the affected plant, the plant comes off easily with blackish brown taproot was intact, whereas the lateral and finer roots were destroyed and shredded. The affected plant and roots were brittle and easily broken. In the field, foliage of the infected plants became straw colored and were scattered in the patches (Plate 3).



Death of seedlings at early stage of infection



Yellowing of foliage



Infected plants in patches



Drying of whole plant



Infected tap root

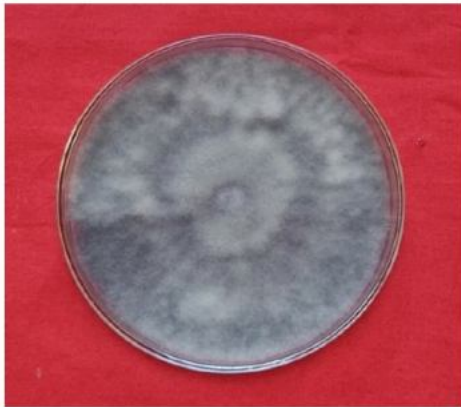
Plate 3: Disease symptoms observed under field conditions

The disease symptoms noticed in the present investigation are comparable to Sharma *et al.* (2015), who reported that the dry root rot symptoms were observed during post-flowering stage in chickpea include drooping and chlorosis of petioles and leaflets, initially confined to top leaves of the plant. Further, the affected leaves and stems were usually straw colored and in some cases, the lower leaves and stems were brown. The tap root turned to black with signs of rotting and was devoid of most of the lateral and finer roots. The dead roots were quite brittle and showed shredding of the bark. The tip of the root could easily break leaving the lower portion of the tap root in the soil when plants were uprooted. In some cases, dark minute sclerotial bodies were seen on the roots exposed and inner side of the bark or when split open at the collar region vertically.

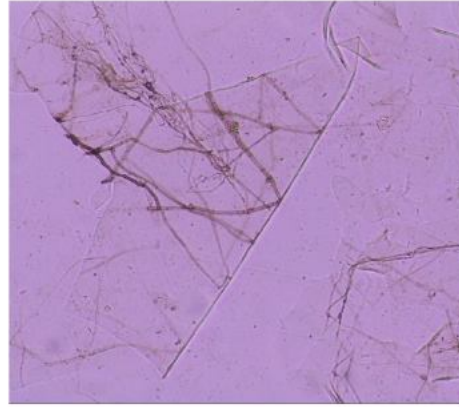
Similar results were reported by Deepa (2018) who observed that the first symptom of dry root rot was yellowing and partial or complete wilting of the stem or one or more branches. In advanced stage of the disease, affected plants became straw colored, but in some cases the lower leaves and stems showed brown discoloration. The tap root appeared black, rotten and devoid of most of the lateral and fine roots. The dead root became quite brittle and showed shredding of bark. The tip of the root was easily broken leaving the lower portion of the tap root in the soil when plants were uprooted, these symptoms were most commonly observed in chickpea in field condition. In severe cases there was no formation of the seeds.

4.1.2 Isolation and identification of the pathogen

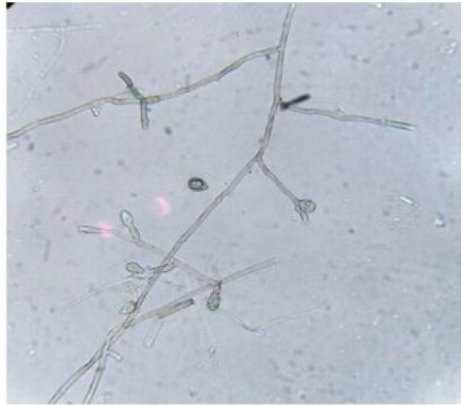
The results indicated that pathogen *R. bataticola* produced the white mycelial growth, later with growth; the color of mycelium was turned to greyish black. The characteristic features of *R. bataticola* were young hyphae were thin, hyaline, septate and dichotomously branched and later produced typical black sclerotia. The mycelium showed right angle branching and constriction of the branch near the point of origin. The sclerotia formed were black to brown in color and the shape varied from spherical, oblong to irregular shapes (Plate 4). Similarly, Chiranjeevi *et al.* (2020) isolated *R. bataticola* and observed morphological and cultural characters using the descriptions given by Barnett and Barry (1972). The mycelium was initially white in color which was later converted to dark brown to black in color. Production of aerial mycelium was also observed in some isolates. Branching occurred mostly at right angle to parent hyphae, but branching at acute angles was also observed. Similarly in the current study, the mycelium was white in



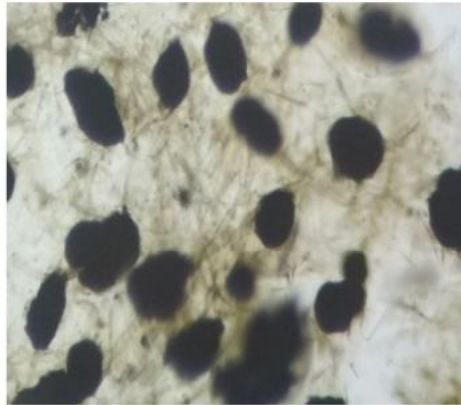
Pure culture of *Rhizoctonia bataticola*



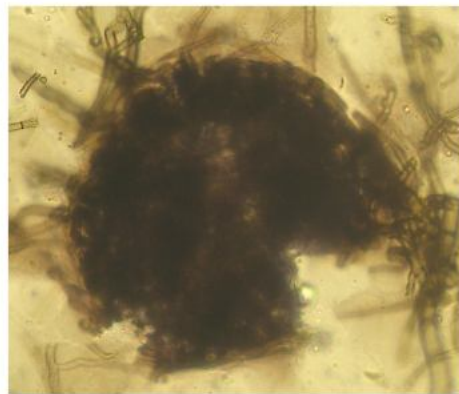
Mycelium (10X)



branching of mycelium at acute angle



Sclerotial bodies (40X)



Septate mycelium and sclerotial body (100X)

Plate 4: Cultural and morphological characters of *Rhizoctonia bataticola*

color, later converted to grayish black and with respect to branching, both acute and right angle branching were observed. Rai *et al.* (2022) observed the morphological features of *R. bataticola* such as micro sclerotia, right angle mycelial branching, multinucleate septate mycelia, cross-wall formation at the beginning of new branching mycelia and partial hyphal fusion. In the present investigation also, the presence of sclerotial bodies were observed which were black to brown in color and the shape varied from spherical, oblong to irregular shapes.

4.1.3 Pathogenicity test

The pathogenicity test is the main criteria for the identification of pathogen suspected being the etiology of a plant disease. Hence, in the present investigation, the pathogenicity test was conducted by following sick pot method. Chickpea seeds were sown in the sick soil which was previously mixed with the inoculum. The initiation of symptoms observed at 20 days after sowing with yellowing of foliage, one or more branches. Later, drying and shredding of leaves, drooping of branches were observed near the soil surface at 45 to 50 day after sowing. The affected plants came off easily upon uprooting with shredding of lateral roots. The root portion showed black rotted appearance as well as in some cases, the roots were brittle and easy to break. The disease symptoms of plants in the sick pots were similar to dry root rot affected plants in the field. Further, re-isolation of the pathogen was carried out from the root region of infected plants and obtained pathogenic cultures after incubation were compared with original culture of *R. bataticola*. The culture was found similar with regard to all morphological characters on potato dextrose agar and thus pathogen was identified as *R. bataticola*. Hence, with the pathogenicity test, it is confirmed that the culture obtained is *R. bataticola* which proved pathogenicity of the causal agent (Plate 5).

Likewise, soil inoculation method was used to prove the pathogenicity of *R. bataticola*. About 10 seeds of chickpea were sown in each pot with a diameter of 22.5 cm (sterilized) inoculated with pathogen @100g Kg⁻¹ soil. Pre-emergence rot of seedlings was observed and the survived plants showed stunted growth followed by wilting and drying of leaves and stems. When the infected plants were pulled out the tap root was blackened with devoid of lateral and finer roots. On re-isolation, the characters of the pathogen showed similarity with the original pathogen isolated from the field, thus Koch's postulates was proved (Reddy, 2010).



Mycelial growth of *R. bataticola* on sorghum grains



Initial yellowing of leaves



Drying of plants



Infected roots

Plate 5: Proving pathogenicity on Annigeri-1 variety

4.2 Collection, isolation, identification of PGPMs and their antagonistic potential against *R. bataticola*

The PGPMs have been the prime focus of research on increasing plant's ability to grow better in changing environmental conditions and are proving promising tools for environmental sustainability. Yet, more work needs to be carried out for establishing their firmness to manage the diseases in a sustainable manner. Hence, the study was undertaken to collect the PGPMs from different agro-ecosystems and used for further characterization and management of the disease.

4.2.1 Collection of PGPMs

Plant parts of healthy chickpea plants and soil samples from rhizosphere were collected during *rabi*, 2020 from eleven districts of northern Karnataka viz., Bagalkot Bellary, Bidar, Dharwad, Gadag, Haveri, Kalaburagi, Koppal, Raichur, Vijayapura and Yadgir wherever chickpea is grown (Fig. 1).

4.2.2 Isolation and identification of endophytic and rhizospheric PGPMs

Totally 126 PGPMs (70 endophytic and 56 rhizospheric) were isolated from the samples collected from eleven districts of north Karnataka by tissue isolation, spread plate technique and serial dilution method as described in 'Material and Methods'. Among 70 endophytic PGPMs, 40 isolates were fungal and 30 were bacteria while, 26 were fungal and 30 bacterial in case of rhizospheric PGPMs (Table 1). This indicated that the plant growth promoting micro organisms co-evolve with the host.

Variability is the universal phenomenon. Every isolate differ in their cultural and morphological characters. The knowledge on these key characteristics of the microorganisms will enable the identification of unknown PGPMs isolates. Hence, identification based on cultural as well as morphological characters of PGPMs was studied.

Table 1. List of endophytic and rhizospheric PGPMs collected from different districts of Karnataka during *rabi* 2020-21

| Sl. No. | Name of the district | Endophytic PGPMs | | Rhizospheric PGPMs | |
|--------------------|----------------------|------------------|-----------------|--------------------|-----------------|
| | | Fungal PGPMs | Bacterial PGPMs | Fungal PGPMs | Bacterial PGPMs |
| 1 | Bagalkot | 2 | 2 | 2 | 2 |
| 2 | Bidar | 2 | 3 | 3 | 3 |
| 3 | Ballari | 3 | 2 | 2 | 2 |
| 4 | Dharwad | 1 | 3 | 3 | 3 |
| 5 | Gadag | 1 | 2 | 2 | 2 |
| 6 | Haveri | 1 | 3 | 2 | 3 |
| 7 | Kalaburagi | 2 | 1 | 1 | 1 |
| 8 | Koppal | 19 | 7 | 4 | 7 |
| 9 | Raichur | 3 | 4 | 4 | 4 |
| 10 | Vijayapura | 4 | 1 | 1 | 1 |
| 11 | Yadgir | 2 | 2 | 2 | 2 |
| Total | | 40 | 30 | 26 | 30 |
| Grand total | | 70 | | 56 | |

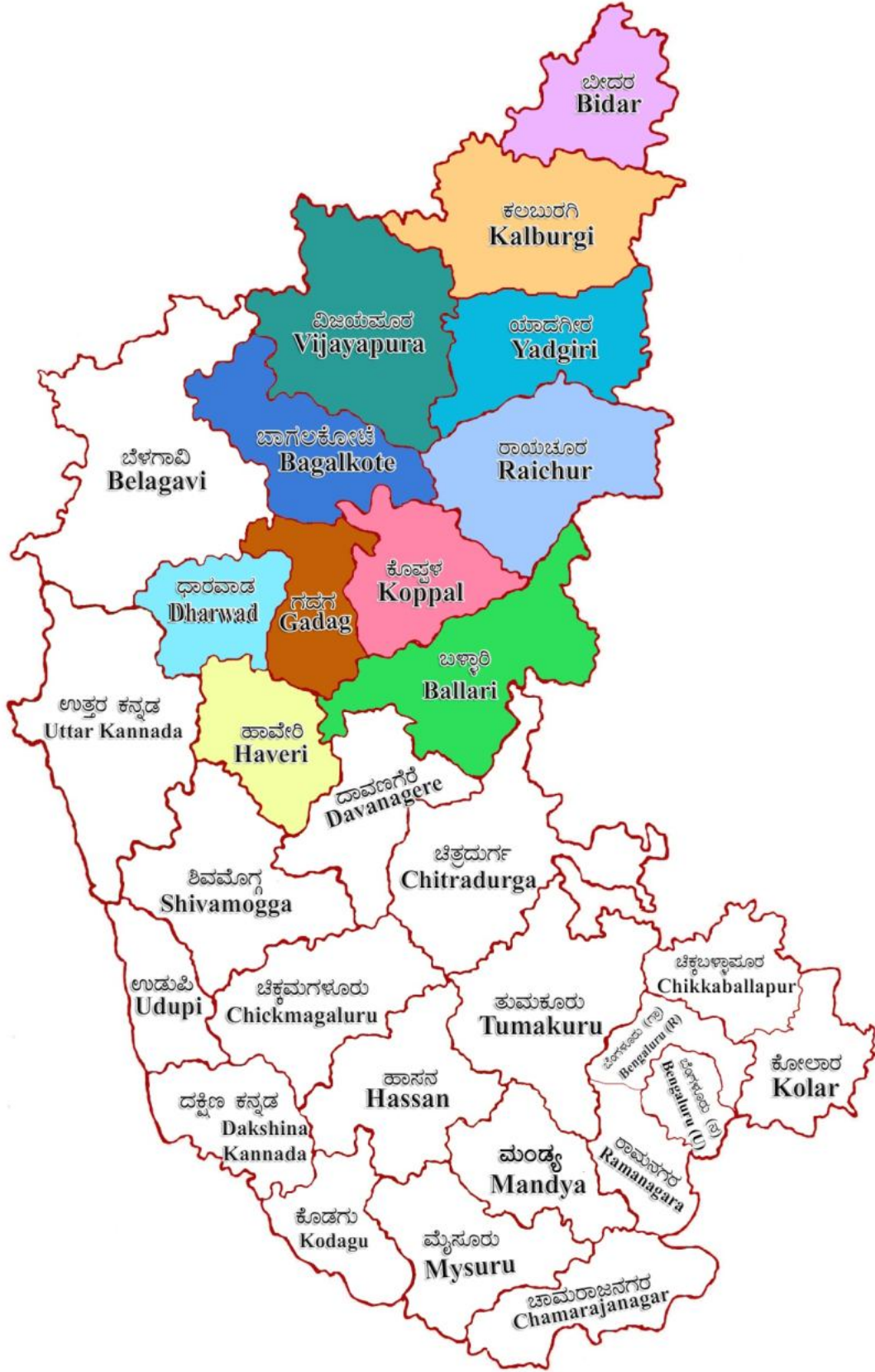


Fig. 1: Districts of Karnataka state from which PGPMs were collected during *rabi* 2020-21

4.2.2.1 Identification of endophytic PGPMs

The concern of present investigation was to discover the microorganisms called endophytes (either fungi or bacteria) living within plants tissues without causing any harm and produce the same or similar compounds like those produced by their plant hosts. Hence, seventy endophytic PGPMs (40 fungal and 30 bacterial) were isolated from plant tissues like leaf, shoot and root and were studied for their cultural and morphological characters.

4.2.2.1.1 Endophytic fungal PGPMs

Forty fungal isolates were isolated on PDA medium and incubated for 7 days at 25 ± 2 °C to obtain the mycelial growth. After the incubation, the observations on the cultural characters were recorded (Table 2, Plate 6) and results are presented as under.

Colony diameter

After 7 days of incubation, the mycelial growth of fungal PGPMs varied from 30.05- 90.00 mm in diameter. The maximum growth (75-90 mm) was recorded in twenty four isolates *viz.*, FEPGPM-1, FEPGPM-3, FEPGPM-5, FEPGPM-7, FEPGPM-10, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-18, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-33, FEPGPM-34, FEPGPM-36, FEPGPM-39, FEPGPM-40 which indicated that these isolates were fast growing. Nine isolates *viz.*, FEPGPM-2, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-17, FEPGPM-20, FEPGPM-21, FEPGPM-26 and FEPGPM-35 were moderate in growth (60-75mm) and only seven FEPGPM-4, FEPGPM-6, FEPGPM-12, FEPGPM-19, FEPGPM-22, FEPGPM-37, FEPGPM-38 were slow growing (30-60 mm) (Table 2a).

Colony color

The colony color of fungal endophytic PGPMs was very distinct. The varying colors were white, black, gray, maroon, green, golden brown, golden yellow, grayish black, grayish white, light maroon, brownish black color of mycelial growth. Among them, seven isolates (FEPGPM-2, FEPGPM-3, FEPGPM-5, FEPGPM-11, FEPGPM-15, FEPGPM-18, FEPGPM-32) were white, seven (FEPGPM-1, FEPGPM-7, FEPGPM-10,

Table 2: Cultural characters of endophytic fungal PGPMs on potato dextrose agar

| Sl. No. | Isolate code | Colony color | | Colony margin | Mycelial growth | Sectoring | Zonation | Colony surface | Colony diameter (mm) |
|---------|--------------|--|---------------------------|---------------|-----------------------|-----------|----------|----------------|----------------------|
| | | Front side of the plate | Reverse side of the plate | | | | | | |
| 1 | FEPGPM- 1 | Black | Black | Irregular | Uniform flat | Absent | Absent | Smooth | 89.0 |
| 2 | FEPGPM- 2 | White | White | Regular | Uniform light pluffy | Present | Absent | Smooth | 80.50 |
| 3 | FEPGPM- 3 | Creamish white | White | Regular | Uniform pluffy | Present | Absent | Smooth | 79.02 |
| 4 | FEPGPM- 4 | Greyish black | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 44.00 |
| 5 | FEPGPM- 5 | White | White | Regular | Uniform pluffy | Absent | Absent | Smooth | 81.02 |
| 6 | FEPGPM- 6 | Gray | Greyish black | Regular | Uniform light pluffy | Absent | Absent | Smooth | 59.32 |
| 7 | FEPGPM- 7 | Black | Black | Regular | Uniform flat | Present | Absent | Smooth | 90.00 |
| 8 | FEPGPM- 8 | Brown and black | Brown | Regular | Uniform pluffy | Absent | Absent | Smooth | 63.50 |
| 9 | FEPGPM- 9 | Gray | Greyish black | Regular | Uniform light pluffy | Absent | Absent | Smooth | 60.50 |
| 10 | FEPGPM- 10 | Black | Grey | Irregular | Uniform flat granular | Absent | Absent | Coarse | 90.00 |
| 11 | FEPGPM- 11 | White mycelial growth with dark green centre | Light green | Irregular | Centered pluffy | Absent | Absent | Smooth | 74.23 |
| 12 | FEPGPM- 12 | Green | Light green | Irregular | Uniform granular | Absent | Absent | Coarse | 29.00 |
| 13 | FEPGPM- 13 | Grayish black | Black | Regular | Uniform flat | Absent | Absent | Smooth | 90.00 |
| 14 | FEPGPM- 14 | Gray color with white border | Brown | Regular | Uniform pluffy | Absent | Absent | Smooth | 85.56 |

Contd.....

| Sl. No. | Isolate code | Colony color | | Colony margin | Mycelial growth | Sectoring | Zonation | Colony surface | Diameter (mm) |
|---------|--------------|--|---------------------------|---------------|------------------|-----------|----------|----------------|---------------|
| | | Front side of the plate | Reverse side of the plate | | | | | | |
| 15 | FEPGPM- 15 | White mycelial growth with purple centre | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 81.00 |
| 16 | FEPGPM- 16 | Golden brown | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 88.20 |
| 17 | FEPGPM- 17 | Grayish black | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 73.00 |
| 18 | FEPGPM- 18 | White | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 90.00 |
| 19 | FEPGPM- 19 | Black | Black with zonations | Irregular | Centered pluffy | Absent | Absent | Smooth | 40.30 |
| 20 | FEPGPM- 20 | Black centre with white border | Black | Regular | Centered pluffy | Absent | Present | Smooth | 69.00 |
| 21 | FEPGPM- 21 | Gray | Black | Regular | Uniform pluffy | Absent | Absent | Coarse | 71.00 |
| 22 | FEPGPM- 22 | Green | Light green | Irregular | Uniform granular | Absent | Absent | Coarse | 50.23 |
| 23 | FEPGPM- 23 | Grayish black | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 84.13 |
| 24 | FEPGPM- 24 | Golden yellow | Cream | Regular | Centered pluffy | Absent | Absent | Smooth | 80.00 |
| 25 | FEPGPM- 25 | Grayish black | Black | Irregular | Uniform pluffy | Absent | Present | Smooth | 80.00 |
| 26 | FEPGPM- 26 | Grayish black | Black and white | Regular | Uniform pluffy | Absent | Absent | Smooth | 70.35 |
| 27 | FEPGPM- 27 | Black | Black | Regular | Uniform flat | Absent | Absent | Smooth | 90.00 |

Contd.....

| Sl. No. | Isolate code | Colony color | | Colony margin | Mycelial growth | Sectoring | Zonation | Colony surface | Diameter (mm) |
|---------|--------------|------------------------------------|---------------------------------|---------------|-----------------------|-----------|----------|----------------|---------------|
| | | Front side of the plate | Reverse side of the plate | | | | | | |
| 28 | FEPGPM- 28 | Creamy golden with green at centre | Golden yellow | Regular | Uniform flat granular | Absent | Present | Coarse | 80.80 |
| 29 | FEPGPM- 29 | Grayish black | Black and white | Regular | Uniform pluffy | Absent | Present | Smooth | 90.00 |
| 30 | FEPGPM- 30 | Green | Light green | Regular | Uniform flat granular | Absent | Absent | Smooth | 80.45 |
| 31 | FEPGPM- 31 | Maroon | Black and maroon with zonations | Regular | Uniform pluffy | Absent | Present | Smooth | 80.98 |
| 32 | FEPGPM- 32 | White | Yellow | Regular | Centered pluffy | Absent | Absent | Smooth | 80.05 |
| 33 | FEPGPM- 33 | Grayish white | Black and brown with zonations | Regular | Uniform pluffy | Absent | Present | Coarse | 90.00 |
| 34 | FEPGPM- 34 | Green | Light green | Regular | Uniform granular | Present | Present | Coarse | 90.00 |
| 35 | FEPGPM- 35 | Black | White | Regular | Uniform granular | Absent | Present | Smooth | 70.23 |
| 36 | FEPGPM- 36 | Grayish black | Black | Regular | Uniform pluffy | Absent | Absent | Smooth | 75.03 |
| 37 | FEPGPM- 37 | Gray and white | Black | Irregular | Irregular pluffy | Absent | Absent | Smooth | 38.08 |
| 38 | FEPGPM- 38 | Light maroon | Cream | Irregular | Centered granular | Absent | Absent | Coarse | 39.02 |
| 39 | FEPGPM- 39 | Golden yellow and green | Brown | Irregular | Irregular pluffy | Absent | Absent | Coarse | 90.00 |
| 40 | FEPGPM- 40 | Brownish black | Black | Regular | Uniform flat | Absent | Absent | Smooth | 90.00 |

Table 2a: Grouping of fungal endophytic PGPMs based on colony diameter

| Sl. No. | Isolate code | Colony diameter | No. of isolates |
|---------|--|---------------------------|-----------------|
| 1 | FEPGPM-1, FEPGPM-3, FEPGPM-5, FEPGPM-7, FEPGPM-10, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-18, FEPGPM-23, EPGPM-24, FEPGPM-25, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-33, FEPGPM-34, FEPGPM-36, FEPGPM-39, FEPGPM-40 | Fast growth (75-90 mm) | 23 |
| 2 | FEPGPM-2, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-17, FEPGPM-20, FEPGPM-21, FEPGPM-26, FEPGPM-35 | Moderate (60-75 mm) | 9 |
| 3 | FEPGPM-4, FEPGPM-6, FEPGPM-12, FEPGPM-19, FEPGPM-22, FEPGPM-32, FEPGPM-37, FEPGPM-38 | Slow growth (30-60 mm) | 8 |

Table 2b: Grouping of fungal endophytic PGPMs based on colony color

| Sl. No. | Isolate code | Colony color | No. of isolates |
|---------|---|---------------------|-----------------|
| 1 | FEPGPM-2, FEPGPM-3, FEPGPM-5, FEPGPM-11, FEPGPM-15, FEPGPM-18, FEPGPM-32 | White | 7 |
| 2 | FEPGPM-1, FEPGPM-7, FEPGPM-10, FEPGPM-19, FEPGPM-20, FEPGPM-27, FEPGPM-35 | Black | 7 |
| 3 | FEPGPM-4, FEPGPM-13, FEPGPM-23, FEPGPM-17, FEPGPM-25, FEPGPM-26, FEPGPM-29, FEPGPM-36 | Grayish black | 8 |
| 4 | FEPGPM-12, FEPGPM-22, FEPGPM-30, FEPGPM-34 | Green | 4 |
| 5 | FEPGPM-6, FEPGPM-9, FEPGPM-14, FEPGPM-21, FEPGPM-33, FEPGPM-37 | Grey | 6 |
| 6 | FEPGPM-8, FEPGPM-40 | Brownish black | 2 |
| 7 | FEPGPM-38, FEPGPM-31 | Maroon | 2 |
| 8 | FEPGPM-24, FEPGPM-39 | Golden yellow white | 2 |
| 9 | FEPGPM-16 | Golden brown | 1 |
| 10 | FEPGPM-28 | Creamy | 1 |

Table 2c: Grouping of fungal endophytic PGPMs based on colony margin

| Sl. No. | Isolate code | Colony margin | No. of isolates |
|---------|--|---------------|-----------------|
| 1 | FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-20, FEPGPM-21, FEPGPM-23, FEPGPM-24, FEPGPM-26, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-33, FEPGPM-34, FEPGPM-35, FEPGPM-36, FEPGPM-40 | Regular | 30 |
| 2 | FEPGPM-1, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-19, FEPGPM-22, FEPGPM-25, FEPGPM-39, FEPGPM-37, FEPGPM-38 | Irregular | 10 |

Table 2d: Grouping of fungal endophytic PGPMs based on mycelial growth

| Sl. No. | Isolate code | Mycelial growth | No. of isolates |
|---------|---|-----------------------|-----------------|
| 1 | FEPGPM-3, FEPGPM-4, FEPGPM-8, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-21, FEPGPM-23, FEPGPM-25, FEPGPM-26, FEPGPM-29, FEPGPM-31, FEPGPM-33, FEPGPM-36 | Uniform pluffy | 16 |
| 2 | FEPGPM-1, FEPGPM-5, FEPGPM-7, FEPGPM-13, FEPGPM-27, FEPGPM-40 | Uniform flat | 6 |
| 3 | FEPGPM-12, FEPGPM-22, FEPGPM-34 | Uniform granular | 3 |
| 4 | FEPGPM-2, FEPGPM-9, FEPGPM-6 | Uniform light pluffy | 3 |
| 5 | FEPGPM-10, FEPGPM-28, FEPGPM-30 | Uniform flat granular | 3 |
| 6 | FEPGPM-11, FEPGPM-19, FEPGPM-20, FEPGPM-24, FEPGPM-32 | Centered pluffy | 5 |
| 7 | FEPGPM-37, FEPGPM-39 | Irregular pluffy | 2 |
| 8 | FEPGPM-38. | Centered granular | 1 |

Table 2e: Grouping of fungal endophytic PGPMs based on sectoring

| Sl. No. | Isolate code | Sectoring | No. of isolates |
|---------|--|-----------|-----------------|
| 1 | FEPGPM-2, FEPGPM-3, FEPGPM-7, FEPGPM-34 | Present | 4 |
| 2 | FEPGPM-1, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-8, FEPGPM-9, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-19, FEPGPM-20, FEPGPM-21, FEPGPM-22, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-26, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-33, FEPGPM-35, FEPGPM-36, FEPGPM-37, FEPGPM-38, FEPGPM-39, FEPGPM-40 | Absent | 36 |

Table 2f: Grouping of fungal endophytic PGPMs based on zonation

| Sl. No. | Isolate code | Zonation | No of isolates |
|---------|--|----------|----------------|
| 1 | FEPGPM-10, FEPGPM-19, FEPGPM-26, FEPGPM-28, FEPGPM-29, FEPGPM-31, FEPGPM-33, FEPGPM-34, FEPGPM-35 | Present | 9 |
| 2 | FEPGPM-1, FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-12, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-27, FEPGPM-20, FEPGPM-21, FEPGPM-22, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-30, FEPGPM-32, FEPGPM-36, FEPGPM-37, FEPGPM-38, FEPGPM-39, FEPGPM-40 | Absent | 31 |

Table 2g: Grouping of fungal endophytic PGPMs based on colony surface

| Sl. No. | Isolate code | Colony surface | No of isolates |
|---------|--|----------------|----------------|
| 1 | FEPGPM-1, FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-19, FEPGPM-20, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-26, FEPGPM-27, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-35, FEPGPM-36, FEPGPM-37, FEPGPM-40 | Smooth | 31 |
| 2 | FEPGPM-10, FEPGPM-12, FEPGPM-21, FEPGPM-22, FEPGPM-28, FEPGPM-33, FEPGPM-34, FEPGPM-38, FEPGPM-39 | Coarse | 9 |



FEPGPM-19, FEPGPM-20, FEPGPM-27, FEPGPM-35) black, eight (FEPGPM-4, FEPGPM-13, FEPGPM-23, FEPGPM-17, FEPGPM-25, FEPGPM-26, FEPGPM-29, FEPGPM-36) grayish black, four (FEPGPM-12, FEPGPM-22, FEPGPM-30, FEPGPM-34) green, six isolates (FEPGPM-6, FEPGPM-9, FEPGPM-14, FEPGPM-21, FEPGPM-33, FEPGPM-37) grey, two isolates (FEPGPM-8 and FEPGPM-40) brownish black, two isolates (FEPGPM-38 and FEPGPM-31) maroon color, two isolates (FEPGPM-24 and FEPGPM-39) golden yellow white, the color of FEPGPM-16 was golden brown and FEPGPM-28 creamy in color (Table 2b).

Colony margin

The colony margin of fungal endophytic PGPMs was categorized in to regular and irregular margin. The majority of PGPM isolates (FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-20, FEPGPM-21, FEPGPM-23, FEPGPM-24, FEPGPM-26, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-33, FEPGPM-34, FEPGPM-35, FEPGPM-36, FEPGPM-40) were regular in their margin. Whereas, FEPGPM-1, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-19, FEPGPM-22, FEPGPM-25, FEPGPM-39, FEPGPM-37, FEPGPM-38) were irregular at the margin (Table 2c).

Mycelial growth

The mycelial growth of PGPMs was categorized in to uniform pluffy, uniform flat, uniform granular, uniform light pluffy, uniform flat granular, centered pluffy, centered granular and irregular pluffy. Sixteen isolates (FEPGPM-3, FEPGPM-4, FEPGPM-8, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-21, FEPGPM-23, FEPGPM-25, FEPGPM-26, FEPGPM-29, FEPGPM-31, FEPGPM-33, FEPGPM-36) were found uniform pluffy, six isolates (FEPGPM-1, FEPGPM-5, FEPGPM-7, FEPGPM-13, FEPGPM-27, FEPGPM-40) uniform flat, three (FEPGPM-12, FEPGPM-22, FEPGPM-34) uniform granular, three (FEPGPM-2, FEPGPM-9 and FEPGPM-6) uniform light pluffy, three (FEPGPM-10, FEPGPM-28, FEPGPM-30) uniform flat granular, five (FEPGPM-11, FEPGPM-19, FEPGPM-20, FEPGPM-24, FEPGPM-32) centered pluffy, two (FEPGPM-37 and FEPGPM-39) irregular pluffy and only one isolate was centered granular (FEPGPM-38). (Table 2d).

Sectoring

The sectoring of PGPMs was shown by only three isolates *viz.*, FEPGPM-2, FEPGPM-3, FEPGPM-7, FEPGPM-34 while the remaining isolates (FEPGPM-1, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-8, FEPGPM-9, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-19, FEPGPM-20, FEPGPM-21, FEPGPM-22, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-26, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-33, FEPGPM-35, FEPGPM-36, FEPGPM-37, FEPGPM-38, FEPGPM-39, FEPGPM-40) does not exhibit any sectoring (Table 2e).

Zonation

Nine isolates (FEPGPM-20, FEPGPM-25, FEPGPM-28, FEPGPM-29, FEPGPM-31, FEPGPM-33, FEPGPM-34 and FEPGPM-35) exhibited zonation. Whereas the remaining thirty one isolates (FEPGPM-1, FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-19, FEPGPM-21, FEPGPM-22, FEPGPM-23, FEPGPM-24, FEPGPM-26, FEPGPM-27, FEPGPM-30, FEPGPM-32, FEPGPM-36, FEPGPM-37, FEPGPM-38, FEPGPM-39, FEPGPM-40) did not exhibited the zonation (Table 2f).

Colony surface

The colony surface of PGPMs was categorized in to smooth and coarse. The majority of the isolates (FEPGPM-1, FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-19, FEPGPM-20, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-26, FEPGPM-27, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-35, FEPGPM-36, FEPGPM-37, FEPGPM-40) showed smooth colony surface whereas only nine isolates (FEPGPM-10, FEPGPM-12, FEPGPM-21, FEPGPM-22, FEPGPM-28, FEPGPM-33, FEPGPM-34, FEPGPM-38, FEPGPM-39) have showed coarse colony surface (Table 2g).

The results on cultural characters of fungal endophytes in the present investigation indicated that the isolates varied with respect to colony diameter (30.05- 90.00 mm), colony pigmentation (white, black, gray, maroon, green, golden brown, golden yellow, grayish black, grayish white, light maroon, brownish black), colony margin (regular and irregular), mycelial growth (uniform pluffy, uniform flat, uniform granular, uniform light pluffy, uniform flat granular, centered pluffy, centered granular and irregular pluffy), sectoring, zonation and colony surface (smooth and coarse).

Similar kind of variation in cultural characters was reported by Shirasangi and Hegde (2018), Chen *et al.* (2012) and Hamzah *et al.* (2018). The studies conducted by Keerthi (2019) on cultural characters of eighteen fungal stem endophytes from sunflower showed that 6 were light grey, 5 white, 4 creamish white and remaining 3 dark grey were observed in present investigation. With respect to growth rate, 6 were slow (3.0-5.0 cm) and 5 were fast growing (7.1-9.0 cm). In the present investigation, the isolates showed fast, moderate and slow growth. The colony margin also varied in 40 isolates and similar results on margin observed by Keerthi (2019) where 8 were regular and 10 were irregular. However, with respect to texture, 7 were smooth and 11 were coarse. The elevation of colony indicated 12 were flat and 6 were raised.

4.2.2.1.2 Endophytic bacterial PGPMs

The bacterial isolates were isolated on nutrient agar medium and incubated for at 28 ± 2 °C for 48 h to obtain the colonies. After the incubation, the observations on the cultural characters were recorded and results are presented in Table 3 (Plate 7 and Plate 8).

Cultural characters

Colony color

The colony color of the bacterial colonies were distinct from each other as dark yellow, maroon, gray, cream, yellow, brown, white, creamy white, creamy yellow, light cream, light gray, light yellow. The isolates such as BEPGPM-1, BEPGPM-2, BEPGPM-8, BEPGPM-14, BEPGPM-15, BEPGPM-24, BEPGPM-26 and BEPGPM-30 were dark yellow, light cream, creamy, cream, maroon, yellow, white brown and light yellow in color, respectively. The twelve isolates (BEPGPM-3, BEPGPM-4, BEPGPM-5,

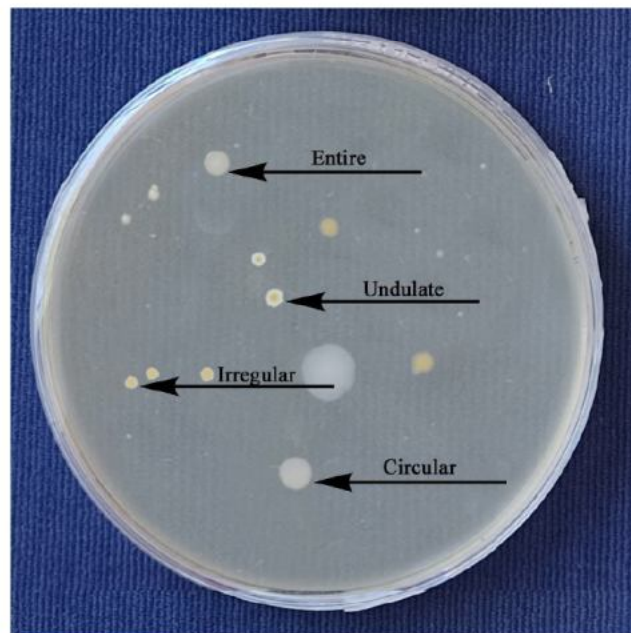
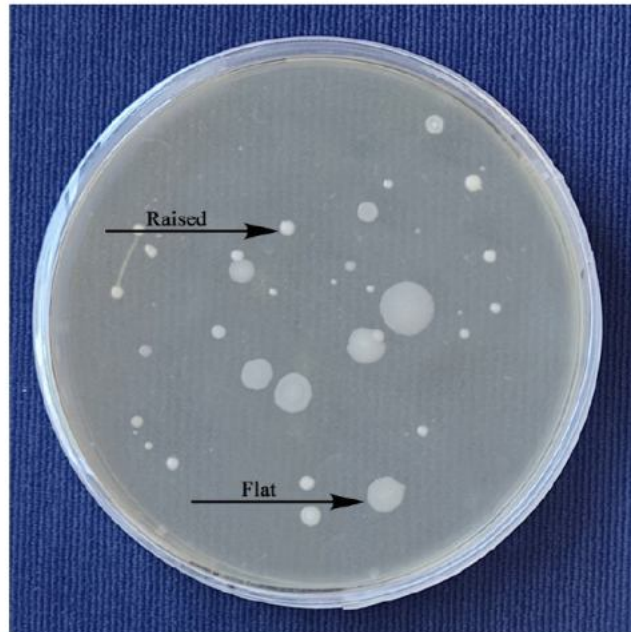


Plate 7: Representative colony characters of bacterial PGPMs

BEPGPM-9, BEPGPM-11, BEPGPM-13, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-22, BEPGPM-25 and BEPGPM-29) were gray, two (BEPGPM-10 and BEPGPM-28) white, three (BEPGPM-6, BEPGPM-12 and BEPGPM-27) creamy yellow and four (BEPGPM-7, BEPGPM-16, BEPGPM-21 and BEPGPM-23) were light gray (Table 3a).

Colony form

The colony form was categorized in to circular and irregular form. The nineteen isolates (BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30) were observed circular form. Whereas, eleven isolates such as BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26 and BEPGPM-27 were irregular in their form (Table 3b).

Colony elevation

The colony elevation of bacterial endophytic isolates was categorized in to flat and raised elevation. Twelve isolates BEPGPM- 1, BEPGPM- 2, BEPGPM- 3, BEPGPM- 4, BEPGPM- 6, BEPGPM- 11, BEPGPM- 12, BEPGPM- 14, BEPGPM- 17, BEPGPM- 18, BEPGPM-24, BEPGPM- 26 showed raised elevation and eighteen isolates BEPGPM-5, BEPGPM-7, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-15, BEPGPM-16, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM- 22, BEPGPM-23, BEPGPM-25, BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30 showed flat elevation (Table 3c).

Colony margin

The colony margin of bacterial endophytic isolates was categorized in to entire and undulated. Eighteen isolates (BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM- 10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30) were having entire margin. Twelve isolates (BEPGPM-4, BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17,

BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26, BEPGPM-27) were having undulated margin (Table 3d).

Staining reaction

Staining reaction was tested by using Gram's staining technique. Nineteen isolates (BEPGPM-1, BEPGPM-3, BEPGPM-4, BEPGPM-7, BEPGPM-8, BEPGPM-10, BEPGPM-11, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-19, BEPGPM-20, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-26 and BEPGPM-28) showed Gram negative in reaction. Whereas, eleven isolates (BEPGPM-5, BEPGPM-2, BEPGPM-6, BEPGPM-9, BEPGPM-12, BEPGPM-18, BEPGPM-21, BEPGPM-25, BEPGPM-27, BEPGPM-29 and BEPGPM-30) were Gram positive in their reaction (Table 3e,).

Cell shape

The twenty seven isolates (BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-10, BEPGPM-11, BEPGPM-12, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-25, BEPGPM-26 BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30) were rod shaped cells. On the other hand, only three isolates (BEPGPM-7, BEPGPM-8 and BEPGPM-20) were cocci in shape (Table 3f).

The cultural characters of bacterial endophytes in the present investigation indicated that the isolates varied with respect to colony color (dark yellow, maroon, gray, cream, yellow, brown, white, creamy white, creamy yellow, light cream, light gray, light yellow), colony form (circular and irregular), colony margin (entire and undulate), cell shape (rod and cocci) and staining reaction (Gram positive and Gram negative).

The similar variations in cultural characters like colony color, colony form and colony margin was observed by Hadimani (2018) who isolated bacterial endophytes from tomato plants. Out of eight effective bacterial endophytes, three isolates such as RBDNA-4, SBHKA-6 and SBBSA-11 showed yellowish color on nutrient agar. Whereas, RBDDE-14 and SBDVA-9 showed bright yellowish and dull creamish colonies on both nutrient agar and King's B media. In the present investigation also some isolates

Table 3: Cultural characters of endophytic bacterial PGPMs on nutrient agar

| Sl. No. | Isolate code | Colony characters | | | | Staining reaction | Cell shape |
|---------|--------------|-------------------|-------------|------------------|---------------|-------------------|------------|
| | | Colony color | Colony form | Colony elevation | Colony margin | | |
| 1 | BEPGPM- 1 | Dark yellow | Circular | Raised | Entire | - | Rod |
| 2 | BEPGPM- 2 | Light cream | Circular | Raised | Entire | + | Rod |
| 3 | BEPGPM- 3 | Gray | Circular | Raised | Entire | - | Rod |
| 4 | BEPGPM- 4 | Gray | Circular | Raised | Undulate | - | Rod |
| 5 | BEPGPM- 5 | Gray | Irregular | Flat | Undulate | + | Rod |
| 6 | BEPGPM- 6 | Creamy yellow | Circular | Raised | Entire | + | Rod |
| 7 | BEPGPM- 7 | Light gray | Irregular | Flat | Undulate | - | Cocci |
| 8 | BEPGPM- 8 | Creamy white | Circular | Flat | Entire | - | Cocci |
| 9 | BEPGPM- 9 | Gray | Circular | Flat | Entire | + | Rod |
| 10 | BEPGPM- 10 | White | Circular | Flat | Entire | - | Rod |
| 11 | BEPGPM- 11 | Gray | Irregular | Raised | Undulate | - | Rod |
| 12 | BEPGPM- 12 | Creamy yellow | Irregular | Raised | Undulate | + | Rod |
| 13 | BEPGPM- 13 | Gray | Circular | Flat | Entire | - | Rod |
| 14 | BEPGPM- 14 | Cream | Circular | Raised | Entire | - | Rod |
| 15 | BEPGPM- 15 | Maroon | Circular | Flat | Entire | - | Rod |
| 16 | BEPGPM- 16 | Light gray | Irregular | Flat | Undulate | - | Rod |

Contd.....

| Sl. No. | Isolate code | Colony characters | | | | Staining reaction | Cell shape |
|---------|--------------|-------------------|-------------|------------------|---------------|-------------------|------------|
| | | Color | Colony form | Colony elevation | Colony margin | | |
| 17 | BEPGPM- 17 | Gray | Irregular | Raised | Undulate | - | Rod |
| 18 | BEPGPM- 18 | Gray | Irregular | Raised | Undulate | + | Rod |
| 19 | BEPGPM- 19 | Gray | Circular | Flat | Entire | - | Rod |
| 20 | BEPGPM- 20 | Cream | Circular | Flat | Entire | - | Cocci |
| 21 | BEPGPM- 21 | Light gray | Circular | Flat | Entire | + | Rod |
| 22 | BEPGPM- 22 | Gray | Circular | Flat | Entire | - | Rod |
| 23 | BEPGPM- 23 | Light gray | Circular | Flat | Entire | - | Rod |
| 24 | BEPGPM-24 | Yellow | Irregular | Raised | Undulate | - | Rod |
| 25 | BEPGPM- 25 | Gray | Irregular | Flat | Undulate | + | Rod |
| 26 | BEPGPM- 26 | Brown | Irregular | Raised | Undulate | - | Rod |
| 27 | BEPGPM- 27 | Creamy yellow | Irregular | Flat | Undulate | + | Rod |
| 28 | BEPGPM- 28 | White | Circular | Flat | Entire | - | Rod |
| 29 | BEPGPM- 29 | Gray | Circular | Flat | Entire | + | Rod |
| 30 | BEPGPM- 30 | Light yellow | Circular | Flat | Entire | + | Rod |

Table 3a: Grouping of bacterial endophytic PGPMs based on colony color

| Sl. No. | Isolate code | Colony color | No. of isolates |
|---------|---|---------------|-----------------|
| 1 | BEPGPM-10, BEPGPM-28 | White | 2 |
| 2 | BEPGPM-6, BEPGPM-12, BEPGPM-27 | Creamy yellow | 3 |
| 3 | BEPGPM-7, BEPGPM-16, BEPGPM-21, BEPGPM-23 | Light grey | 4 |
| 4 | BEPGPM-1 | Dark yellow | 1 |
| 5 | BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-9, BEPGPM-11, BEPGPM-13, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-22, BEPGPM-25 and BEPGPM-29 | Grey | 12 |
| 6 | BEPGPM-2 | Light cream | 1 |
| 7 | BEPGPM-8 and BEPGPM-20 | Creamy | 2 |
| 8 | BEPGPM-14 | Cream | 1 |
| 9 | BEPGPM-15 | Maroon | 1 |
| 10 | BEPGPM-24 | Yellow | 1 |
| 11 | BEPGPM-26 | White brown | 1 |
| 12 | BEPGPM-30 | Light yellow | 1 |

Table 3b: Grouping of bacterial endophytic PGPMs based on colony form

| Sl. No. | Isolate code | Colony form | No. of isolates |
|---------|--|-------------|-----------------|
| 1 | BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30 | Circular | 19 |
| 2 | BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26 and BEPGPM-27 | Irregular | 11 |

Table 3c: Grouping of bacterial endophytic PGPMs based on colony elevation

| Sl. No. | Isolate code | Colony elevation | No of isolates |
|---------|--|------------------|----------------|
| 1 | BEPGPM- 1, BEPGPM- 2, BEPGPM- 3, BEPGPM- 4, BEPGPM- 6, BEPGPM- 11, BEPGPM- 12, BEPGPM- 14, BEPGPM- 17, BEPGPM- 18, BEPGPM-24, BEPGPM- 26 | Raised | 12 |
| 2 | BEPGPM-5, BEPGPM-7, BEPGPM-8, BEPGPM-9, BEPGPM-10, BEPGPM-13, BEPGPM-15, BEPGPM-16, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM- 22, BEPGPM-23, BEPGPM-25, BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30 | Flat | 18 |

Table 3d: Grouping of bacterial endophytic PGPMs based on colony margin

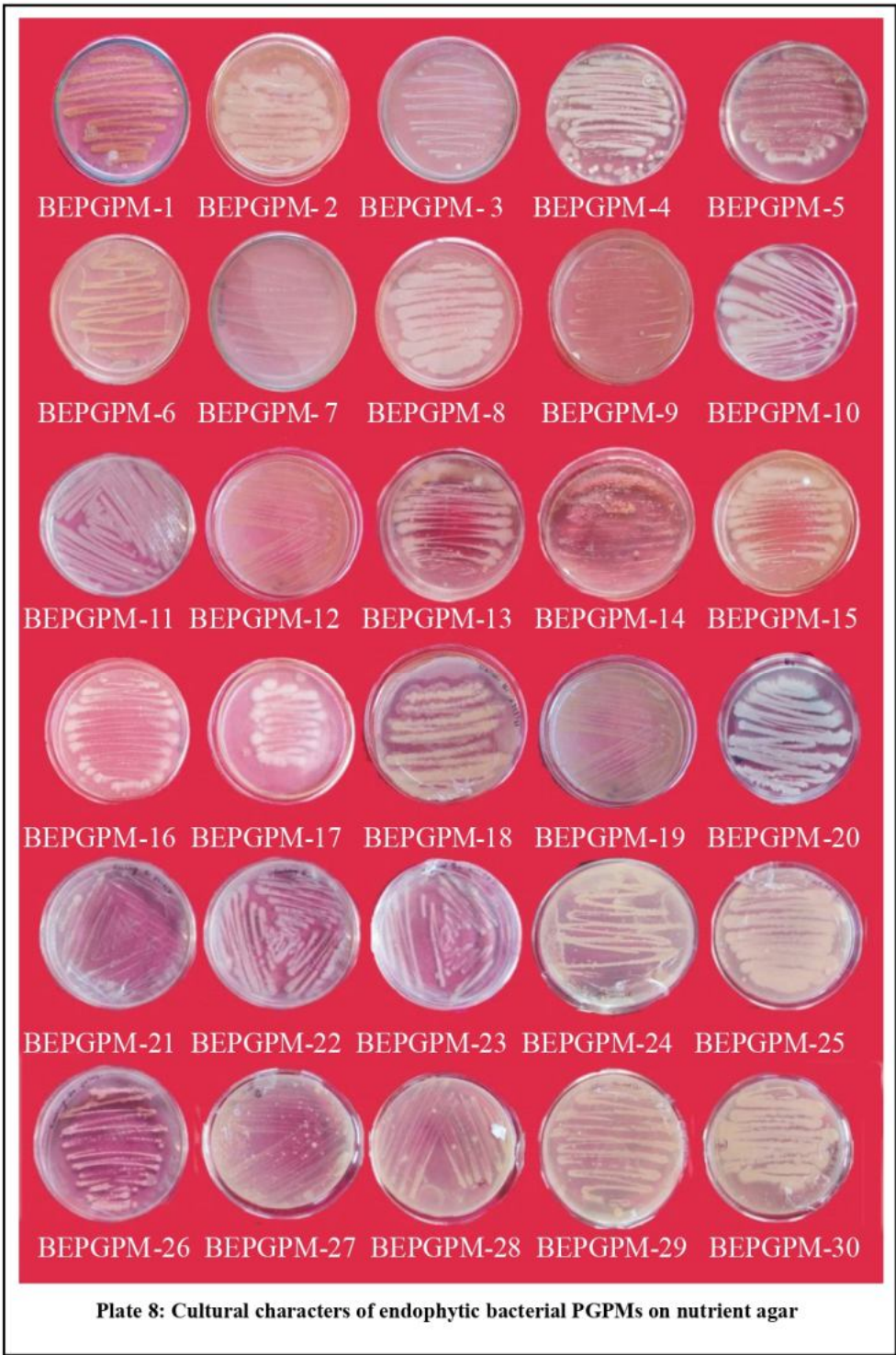
| Sl. No. | Isolate code | Colony margin | No of isolates |
|---------|---|---------------|----------------|
| 1 | BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-6, BEPGPM-8, BEPGPM-9, BEPGPM- 10, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-19, BEPGPM-20, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-28, BEPGPM-29, BEPGPM-30 | Entire | 18 |
| 2 | BEPGPM-4, BEPGPM-5, BEPGPM-7, BEPGPM-11, BEPGPM-12, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-24, BEPGPM-25, BEPGPM-26, BEPGPM-27 | Undulated | 12 |

Table 3e: Grouping of bacterial endophytic PGPMs based on staining reaction

| Sl. No. | Isolate code | Staining reaction | No. of isolates |
|---------|---|-------------------|-----------------|
| 1 | BEPGPM-1, BEPGPM-3, BEPGPM-4, BEPGPM-7, BEPGPM-8, BEPGPM-10, BEPGPM-11, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-19, BEPGPM-20, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-26 and BEPGPM-28 | Gram negative | 19 |
| 2 | BEPGPM-2, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-12, BEPGPM-18, BEPGPM-21, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30 | Gram positive | 11 |

Table 3f: Grouping of bacterial endophytic PGPMs based on cell shape

| Sl. No. | Isolate code | Cell shape | No. of isolates |
|----------------|--|-------------------|------------------------|
| 1 | BEPGPM-1, BEPGPM-2, BEPGPM-3, BEPGPM-4, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-10, BEPGPM-11, BEPGPM-12, BEPGPM-13, BEPGPM-14, BEPGPM-15, BEPGPM-16, BEPGPM-17, BEPGPM-18, BEPGPM-19, BEPGPM-21, BEPGPM-22, BEPGPM-23, BEPGPM-24, BEPGPM-25, BEPGPM-26 BEPGPM-27, BEPGPM-28, BEPGPM-29 and BEPGPM-30 | Rod shaped | 27 |
| 2 | BEPGPM-7, BEPGPM-8, BEPGPM-20 | Cocci | 3 |



produced creamish and yellow colored colonies. With regard to colony form, RBDNA-4 was irregular on both the media and remaining all isolates showed regular colony. Margin was undulating in RBDNA-4 on both the media and remaining isolates were of entire margin except SBKHA-2 and SBDOF-6 which showed undulated margin on nutrient agar. Similar variations were recorded with respect to colony form and colony margin in the present study.

The morphological characters such as staining reaction and cell shape also varied in the isolates. Likewise, Salo and Novero (2021) collected five bacterial samples from coconut plumule explants in tissue culture and plated on nutrient agar medium. Macroscopic features of the isolated bacterial colonies were assessed for the color of colony, elevation, margin, consistency and surface of the colony. The shape of isolates ranged from cocci to short rod. The isolate CEB1 was Gram-negative and coccobacillus in shape as it was intermediate between coccus and rod shape. Whereas, isolates CEB2, CEB3, CEB4 and CEB5 were Gram-positive and bacilli. In the present investigation also the PGPMs showed similar kind of variation with respect to cell shape and staining reaction. With respect to cell shape, some isolates were rod shaped and some were cocci. With respect to staining reaction, PGPMs showed Gram positive and Gram negative reaction.

4.2.2.2 Identification of rhizospheric PGPMs

Rhizosphere fungal and bacterial community can harbor beneficial organisms known as PGPMs. These organisms have the ability to colonize plant roots providing benefits to their hosts, by modulating the production of phytohormones, increasing the availability of soil nutrients and the resistance against pathogens. Hence, fifty six rhizospheric PGPMs (26 fungal and 30 bacterial) were isolated from chickpea rhizosphere and were studied for their cultural characters.

4.2.2.2.1 Rhizospheric fungal PGPMs

The twenty six fungal isolates were obtained on PDA medium and incubated for 7 days at 25 ± 2 °C to obtain the mycelial growth. After the incubation, the observations on the cultural characters were recorded. Based on the cultural characters the isolates were identified as *Trichoderma* isolates (Table 4, Plate 9) and results are presented below.

Colony diameter

The colony growth of the rhizospheric fungal PGPMs varied from 78.33 -90.00 mm. Ten isolates (SFPGPM-4, SFPGPM-5, SFPGPM-7, SFPGPM-10, SFPGPM-11, SFPGPM-12, SFPGPM-17, SFPGPM-20, SFPGPM-25, SFPGPM-26) showed 90.00 mm of colony growth and remaining sixteen isolates were in the range of 78.33-89.90 mm (Table 4a).

Colony color

The colony color of rhizospheric fungal PGPMs varied with dark green, whitish green and light green. Twelve isolates (SFPGPM-1, SFPGPM-2, SFPGPM-3, SFPGPM-4, SFPGPM-6, SFPGPM-10, SFPGPM-11, SFPGPM-14, SFPGPM-18, SFPGPM-19, SFPGPM-21, SFPGPM-22) were observed as dark green, thirteen (SFPGPM-5, SFPGPM-7, SFPGPM-8, SFPGPM-9, SFPGPM-12, SFPGPM-13, SFPGPM-23, SFPGPM-24, SFPGPM-25, SFPGPM-26, SFPGPM-15, SFPGPM-16, SFPGPM-17) showed whitish green and only one isolate that is SFPGPM- 20 was light green in color (Table 4b).

Colony shape

The shape of the colony was studied by categorizing in to irregular and circular colonies. Eighteen isolates (SFPGPM-1, SFPGPM-2, SFPGPM-5, SFPGPM-6, SFPGPM-7, SFPGPM-8, SFPGPM-9, SFPGPM-10, SFPGPM-11, SFPGPM-12, SFPGPM-13, SFPGPM-14, SFPGPM-15, SFPGPM-16, SFPGPM-18, SFPGPM-19, SFPGPM-24, SFPGPM-25) were showed irregular shape and eight (SFPGPM-3, SFPGPM-4, SFPGPM-17, SFPGPM-20, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-26) were circular (Table 4c).

Mycelial growth

Two types of growth were observed in the isolates (fluffy and raised as well as uniform pluffy). Twenty one isolates (SFPGPM-1, SFPGPM-2, SFPGPM-3, SFPGPM-4, SFPGPM-5, SFPGPM-6, SFPGP-7, SFPGPM-8, SFPGPM-9, SFPGPM-10, SFPGPM-11, SFPGPM-13, SFPGPM-14, SFPGPM-15, SFPGPM-16, SFPGPM-18, SFPGPM-19, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-24) were fluffy and raised. Whereas,

Table 4: Cultural characters of rhizospheric fungal PGPMs on potato dextrose agar

| Sl. No. | Isolate code | Colony color | Colony shape | Mycelial growth | Colony margin | Colony diameter (mm) |
|---------|--------------|---------------|--------------|-------------------|---------------|----------------------|
| 1 | SFPGPM-1 | Dark Green | Irregular | Fluffy and raised | Wavy | 81.67 |
| 2 | SFPGPM-2 | Dark Green | Irregular | Fluffy and raised | Smooth | 88.33 |
| 3 | SFPGPM-3 | Dark Green | Circular | Fluffy and raised | Smooth | 89.00 |
| 4 | SFPGPM-4 | Dark Green | Circular | Fluffy and raised | Smooth | 90.00 |
| 5 | SFPGPM-5 | Whitish green | Irregular | Fluffy and raised | Wavy | 90.00 |
| 6 | SFPGPM-6 | Dark Green | Irregular | Fluffy and raised | Wavy | 79.00 |
| 7 | SFPGPM-7 | Whitish green | Irregular | Fluffy and raised | Smooth | 90.00 |
| 8 | SFPGPM-8 | Whitish green | Irregular | Fluffy and raised | Wavy | 88.33 |
| 9 | SFPGPM-9 | Whitish green | Irregular | Fluffy and raised | Wavy | 89.78 |
| 10 | SFPGPM-10 | Dark Green | Irregular | Fluffy and raised | Smooth | 90.00 |
| 11 | SFPGPM-11 | Dark Green | Irregular | Fluffy and raised | Smooth | 90.00 |
| 12 | SFPGPM-12 | Whitish green | Irregular | Uniform fluffy | Smooth | 90.00 |
| 13 | SFPGPM-13 | Whitish green | Irregular | Fluffy and raised | Wavy | 86.23 |
| 14 | SFPGPM-14 | Dark Green | Irregular | Fluffy and raised | Smooth | 87.33 |
| 15 | SFPGPM-15 | Whitish green | Irregular | Fluffy and raised | Wavy | 89.80 |
| 16 | SFPGPM-16 | Whitish green | Irregular | Fluffy and raised | Wavy | 86.67 |
| 17 | SFPGPM-17 | Whitish green | Circular | Uniform fluffy | Smooth | 90.00 |
| 18 | SFPGPM-18 | Dark Green | Irregular | Fluffy and raised | Wavy | 89.00 |
| 19 | SFPGPM-19 | Dark Green | Irregular | Fluffy and raised | Smooth | 78.33 |
| 20 | SFPGPM-20 | Light green | Circular | Uniform fluffy | Smooth | 90.00 |
| 21 | SFPGPM-21 | Dark Green | Circular | Fluffy and raised | Smooth | 88.00 |
| 22 | SFPGPM-22 | Dark Green | Circular | Fluffy and raised | Smooth | 88.00 |
| 23 | SFPGPM-23 | Whitish green | Circular | Fluffy and raised | Smooth | 87.33 |
| 24 | SFPGPM-24 | Whitish green | Irregular | Fluffy and raised | Smooth | 88.00 |
| 25 | SFPGPM-25 | Whitish green | Irregular | Uniform fluffy | Smooth | 90.00 |
| 26 | SFPGPM-26 | Whitish green | Circular | Uniform fluffy | Smooth | 90.00 |

Table 4a: Grouping of rhizospheric fungal PGPMs based on colony diameter

| Sl. No. | Isolate code | Colony diameter | No. of isolates |
|---------|--|-----------------|-----------------|
| 1 | SFPGPM-4, SFPGPM-5, SFPGPM-7, SFPGPM-10, SFPGPM-11, SFPGPM-12, SFPGPM-17, SFPGPM-20, SFPGPM-25, SFPGPM-26 | 90.00 mm | 10 |
| 2 | SFPGPM-1, SFPGPM-2, SFPGPM-3, SFPGPM-6, SFPGPM-8, SFPGPM-9, SFPGPM-13, SFPGPM-14, SFPGPM-15, SFPGPM-16, SFPGPM-18, SFPGPM-19, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-24 | 78.33-89.90 mm | 16 |

Table 4b: Grouping of rhizospheric fungal PGPMs based on Colony color

| Sl. No. | Isolate code | Colony color | No. of isolates |
|---------|---|---------------|-----------------|
| 1 | SFPGPM-1, SFPGPM-2, SFPGPM-3, SFPGPM-4, SFPGPM-6, SFPGPM-10, SFPGPM-11, SFPGPM-14, SFPGPM-18, SFPGPM-19, SFPGPM-21, SFPGPM-22 | Dark green | 12 |
| 2 | SFPGPM-5, SFPGPM-7, SFPGPM-8, SFPGPM-9, SFPGPM-12, SFPGPM-13, SFPGPM-23, SFPGPM-24, SFPGPM-25, SFPGPM-26, SFPGPM-15, SFPGPM-16, SFPGPM-17 | Whitish green | 13 |
| 3 | SFPGPM-20 | Light green | 1 |

Table 4c: Grouping of rhizospheric fungal PGPMs based on colony shape

| Sl. No. | Isolate code | Colony shape | No. of isolates |
|---------|---|--------------|-----------------|
| 1 | SFPGPM-1, SFPGPM-2, SFPGPM-5, SFPGPM-6, SFPGPM-7, SFPGPM-8, SFPGPM-9, SFPGPM-10, SFPGPM-11, SFPGPM-12, SFPGPM-13, SFPGPM-14, SFPGPM-15, SFPGPM-16, SFPGPM-18, SFPGPM-19, SFPGPM-24, SFPGPM-25 | Irregular | 18 |
| 2 | SFPGPM-3, SFPGPM-4, SFPGPM-17, SFPGPM-20, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-26 | Circular | 8 |

Table 4d: Grouping of rhizospheric fungal PGPMs based on type of mycelial growth

| Sl. No. | Isolate code | Mycelial growth | No. of isolates |
|----------------|---|------------------------|------------------------|
| 1 | SFPGPM-1, SFPGPM-2, SFPGPM-3, SFPGPM-4, SFPGPM-5, SFPGPM-6, SFPGP-7, SFPGPM-8, SFPGPM-9, SFPGPM-10, SFPGPM-11, SFPGPM-13, SFPGPM-14, SFPGPM-15, SFPGPM-16, SFPGPM-18, SFPGPM-19, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-24 | Fluffy and raised | 21 |
| 2 | SFPGPM-12, SFPGPM-17, SFPGPM-20, SFPGPM-25, SFPGPM-26 | Uniform pluffy | 5 |

Table 4e: Grouping of rhizospheric fungal PGPMs based on type of colony margin

| Sl. No. | Isolate code | Colony margin | No. of isolates |
|----------------|---|----------------------|------------------------|
| 1 | SFPGPM-2, SFPGPM-3, SFPGPM-4, SFPGPM-7 SFPGPM-10 SFPGPM-11 SFPGPM-12, SFPGPM-14, SFPGPM-17, SFPGPM-19, SFPGPM-20, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-24, SFPGPM-25 and SFPGPM-26 | Smooth | 17 |
| 2 | SFPGPM-3, SFPGPM-4, SFPGPM-17, SFPGPM-20, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-26 | Wavy | 9 |



Plate 9: Cultural characters of rhizospheric fungal PGPMs on potato dextrose agar

five isolates (SFPGPM-12, SFPGPM-17, SFPGPM-20, SFPGPM-25, SFPGPM-26) were uniform pluffy in their mycelial growth (Table 4d).

Colony margin

The margin of the fungal isolates was categorized in to smooth and wavy. Seventeen isolates (SFPGPM-2, SFPGPM-3, SFPGPM-4, SFPGPM-7 SFPGPM-10 SFPGPM-11 SFPGPM-12, SFPGPM-14, SFPGPM-17, SFPGPM-19, SFPGPM-20, SFPGPM-21, SFPGPM-22, SFPGPM-23, SFPGPM-24, SFPGPM-25 and SFPGPM-26) showed smooth type of margin. Whereas, nine isolates (SFPGPM-1, SFPGPM-5, SFPGPM-6, SFPGPM-8, SFPGPM-9, SFPGPM-13, SFPGPM-15, SFPGPM-16, SFPGPM-18) were wavy in margin (Table 4e).

In the present investigation, the observations on cultural characters of fungal PGPMs varied with respect to colony color (dark green, whitish green and light green), colony shape (irregular and circular), mycelia growth (fluffy and raised as well as uniform pluffy) and type of margin (smooth and wavy). Likewise, Ranjana (2020) observed the cultural characteristics and growth rates of the twenty native isolates of *Trichoderma* spp. on PDA medium at 28 ± 1 °C. All the isolates showed difference in radial growth of mycelium at 72 h after incubation. Isolates TR-1, TR-13, TR-14 and TR-18 showed maximum growth of 90.00 mm compared to other isolates. All the isolates produced fluffy and raised type of colony growth and margin of colony varied from smooth to wavy as observed in the present investigation. The variation in cultural characters of eleven isolates of *Trichoderma* spp. also recorded varied rates of mycelia growth (Shruthi, 2017). The maximum radial growth of mycelium was 90 mm at 72 h of incubation. With respect to growth type, isolates showed fluffy and raised colony to appraised flat type. Margin of colony varied from uniform to irregular as observed in present study also.

4.2.2.2.2 Rhizospheric bacterial PGPMs

The bacterial isolates were obtained on nutrient agar medium and incubated for at 28 ± 2 °C for 48 h to obtain the colonies. After the incubation, the observations on the cultural and morphological characters were recorded and results are presented in Table 5 (Plate 10).

Cultural characters

Colony color

The colony color of rhizospheric bacterial PGPMs was very distinct and was categorized as grayish, grayish white, grayish white slimy, whitish and creamy white. Five isolates (SBPGPM-1, SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM- 15) were grayish white slimy in color, seventeen (SBPGPM-5, SBPGPM-6, SBPGPM-8, SBPGPM-10, SBPGPM-11, SBPGPM-12, SBPGPM-13, SBPGPM-14, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-21, SBPGPM-25, SBPGPM-26, SBPGPM-27, SBPGPM-29) were grayish white, five (SBPGPM-19, SBPGPM-22, SBPGPM-23, SBPGPM-24, SBPGPM-30) were creamy white, two (SBPGPM- 2 and SBPGPM-28) were whitish in color. Only one isolate (SBPGPM-3) showed grayish color (Table 5a).

Colony form

The colony form varied from regular to irregular. Seventeen isolates (SBPGPM-1, SBPGPM-3, SBPGPM-4, SBPGPM-6, SBPGPM-7, SBPGPM-8, SBPGPM-10, SBPGPM-11, SBPGPM-13, SBPGPM- 15, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-22, SBPGPM-24, SBPGPM-28) belong to regular form. Whereas thirteen isolates (SBPGPM-2, SBPGPM-5, SBPGPM-9, SBPGPM-12, SBPGPM-14, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-25, SBPGPM-26, SBPGPM-27, SBPGPM-29, SBPGPM-30) were irregular form (Table 5b).

Colony elevation

The results indicated that the bacterial cultures showed two types of elevation. Five isolates (SBPGPM-2, SBPGPM-5, SBPGPM-6, SBPGPM-13, SBPGPM-27) showed flat elevation and remaining twenty five isolates (SBPGPM-1, SBPGPM-3, SBPGPM-4, SBPGPM-7, SBPGPM- 8, SBPGPM-9, SBPGPM-10, SBPGPM-11, SBPGPM-12, SBPGPM-14, SBPGPM-15, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-19, SBPGPM-20, SBPGPM-21, SBPGPM-22, SBPGPM-23, SBPGPM-24, SBPGPM-25, SBPGPM-26, SBPGPM-28, SBPGPM-29, SBPGPM-30) belonged to raised elevation (Table 5c).

| Sl. No. | Isolate code | Colony characters | | | | Fluorescence under UV light | Cell shape | Staining reaction |
|---------|--------------|----------------------|-------------|------------------|---------------|-----------------------------|------------|-------------------|
| | | Colony color | Colony form | Colony elevation | Colony margin | | | |
| 1 | SBP GPM- 1 | Grayish white, slimy | Regular | Raised | Entire | + | Rod | - |
| 2 | SBP GPM- 2 | Whitish | Irregular | Flat | Undulate | - | Rod | - |
| 3 | SBP GPM- 3 | Grayish | Regular | Raised | Entire | - | Rod | + |
| 4 | SBP GPM- 4 | Grayish white, slimy | Regular | Raised | Entire | - | Rod | - |
| 5 | SBP GPM- 5 | Grayish white | Irregular | Flat | Undulate | - | Rod | + |
| 6 | SBP GPM- 6 | Grayish white | Regular | Flat | Entire | + | Rod | - |
| 7 | SBP GPM- 7 | Grayish white, slimy | Regular | Raised | Entire | - | Rod | - |
| 8 | SBP GPM- 8 | Grayish white | Regular | Raised | Entire | - | Rod | - |
| 9 | SBP GPM- 9 | Grayish white, slimy | Irregular | Raised | Undulate | - | Rod | - |
| 10 | SBP GPM- 10 | Grayish white | Regular | Raised | Entire | + | Rod | - |
| 11 | SBP GPM- 11 | Grayish white | Regular | Raised | Entire | - | Rod | - |
| 12 | SBP GPM- 12 | Grayish white | Irregular | Raised | Undulate | + | Rod | - |
| 13 | SBP GPM- 13 | Grayish white | Regular | Flat | Entire | - | Rod | - |
| 14 | SBP GPM- 14 | Grayish white | Irregular | Raised | Undulate | + | Rod | - |
| 15 | SBP GPM- 15 | Grayish white, slimy | Regular | Raised | Undulate | + | Rod | - |

Contd....

| Sl. No. | Isolate code | Colony characters | | | | Fluorescence under UV light | Cell shape | Staining reaction |
|---------|--------------|-------------------|-------------|------------------|---------------|-----------------------------|------------|-------------------|
| | | Colony color | Colony form | Colony elevation | Colony margin | | | |
| 16 | SBP GPM- 16 | Grayish white | Regular | Raised | Entire | - | Rod | - |
| 17 | SBP GPM- 17 | Grayish white | Regular | Raised | Entire | + | Rod | - |
| 18 | SBP GPM- 18 | Grayish white | Regular | Raised | Entire | + | Rod | - |
| 19 | SBP GPM- 19 | Creamy white | Irregular | Raised | Undulate | - | Rod | + |
| 20 | SBP GPM- 20 | Grayish white | Regular | Raised | Entire | - | Rod | - |
| 21 | SBP GPM- 21 | Grayish white | Irregular | Raised | Undulate | - | Rod | + |
| 22 | SBP GPM- 22 | Creamy white | Regular | Raised | Entire | + | Rod | - |
| 23 | SBP GPM- 23 | Creamy white | Irregular | Raised | Undulate | - | Rod | + |
| 24 | SBP GPM- 24 | Creamy white | Regular | Raised | Entire | + | Rod | - |
| 25 | SBP GPM- 25 | Grayish white | Irregular | Raised | Undulate | - | Rod | - |
| 26 | SBP GPM- 26 | Grayish white | Irregular | Raised | Undulate | - | Rod | - |
| 27 | SBP GPM- 27 | Grayish white | Irregular | Flat | Entire | + | Rod | - |
| 28 | SBP GPM- 28 | Whitish | Regular | Raised | Entire | - | Rod | + |
| 29 | SBP GPM- 29 | Grayish white | Irregular | Raised | Undulate | - | Rod | - |
| 30 | SBP GPM- 30 | Creamy white | Irregular | Raised | Undulate | - | Rod | + |

‘+’ - Indicates production of fluorescence under UV light

‘-’ - Indicates absence of fluorescence production under UV light

Table 5a: Grouping of rhizospheric bacterial PGPMs based on colony color

| Sl. No | Isolate code | Colony color | No. of isolates |
|--------|---|---------------------|-----------------|
| 1 | SBPGPM-1, SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM-15 | Grayish white slimy | 5 |
| 2 | SBPGPM-5, SBPGPM-6, SBPGPM-8, SBPGPM-10, SBPGPM-11, SBPGPM-12, SBPGPM-13, SBPGPM-14, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-21, SBPGPM-25, SBPGPM-26, SBPGPM-27, SBPGPM-29, | Grayish white | 17 |
| 3 | SBPGPM-19, SBPGPM-22, SBPGPM-23, SBPGPM-24, SBPGPM-30 | Creamy white | 5 |
| 4 | SBPGPM-2 and SBPGPM-28 | Whitish | 2 |
| 5 | SBPGPM-3 | Grayish | 1 |

Table 5b: Grouping of rhizospheric bacterial PGPMs based on colony form

| Sl. No | Isolate code | Colony form | No. of isolates |
|--------|---|-------------|-----------------|
| 1 | SBPGPM-1, SBPGPM-3, SBPGPM-4, SBPGPM-6, SBPGPM-7, SBPGPM-8, SBPGPM-10, SBPGPM-11, SBPGPM-13, SBPGPM-15, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-22, SBPGPM-24, SBPGPM-28 | Regular | 17 |
| 2 | SBPGPM-2, SBPGPM-5, SBPGPM-9, SBPGPM-12, SBPGPM-14, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-25, SBPGPM-26, SBPGPM-27, SBPGPM-29, SBPGPM-30 | Irregular | 13 |

Table 5c: Grouping of rhizospheric bacterial PGPMs based on elevation

| Sl. No | Isolate code | Elevation | No. of isolates |
|--------|---|-----------|-----------------|
| 1 | SBPGPM-2, SBPGPM-5, SBPGPM-6, SBPGPM-13, SBPGPM-27 | Flat | 5 |
| 2 | SBPGPM-1, SBPGPM-3, SBPGPM-4, SBPGPM-7, SBPGPM-8, SBPGPM-9, SBPGPM-10, SBPGPM-11, SBPGPM-12, SBPGPM-14, SBPGPM-15, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-19, SBPGPM-20, SBPGPM-21, SBPGPM-22, SBPGPM-23, SBPGPM-24, SBPGPM-25, SBPGPM-26, SBPGPM-28, SBPGPM-29, SBPGPM-30 | Raised | 25 |

Table 5d: Grouping of rhizospheric bacterial PGPMs based on colony margin

| Sl. No | Isolate code | Colony margin | No. of isolates |
|--------|---|---------------|-----------------|
| 1 | SBPGPM-1, SBPGPM-3, SBPGPM-4, SBPGPM-6, SBPGPM-7, SBPGPM-8, SBPGPM-10, SBPGPM-11, SBPGPM-13, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-22, SBPGPM-24, SBPGPM-27, SBPGPM-28 | Entire | 17 |
| 2 | SBPGPM-2, SBPGPM-5, SBPGPM-9, SBPGPM-12, SBPGPM-14, SBPGPM-15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-25, SBPGPM-26, SBPGPM-29, SBPGPM-30 | Undulate | 13 |

Table 5e: Grouping of rhizospheric bacterial PGPMs based on fluorescence under UV light

| Sl. No | Isolate code | Fluorescence under UV light | No. of isolates |
|--------|--|--|-----------------|
| 1 | SBPGPM-1, SBPGPM-6, SBPGPM-10, SBPGPM-12, SBPGPM-14, SBPGPM-15, SBPGPM-17, SBPGPM-18, SBPGPM-22, SBPGPM-24, SBPGPM-27 | Fluorescence under UV light | 11 |
| 2 | SBPGPM-2, SBPGPM-3, SBPGPM-4, SBPGPM-5, SBPGPM-7, SBPGPM-8, SBPGPM-9, SBPGPM-11, SBPGPM-13, SBPGPM-16, SBPGPM-19, SBPGPM-20, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-25, SBPGPM-26, SBPGPM-29, SBPGPM-30 | Absence of fluorescence under UV light | 19 |

Table 5f: Grouping of rhizospheric bacterial PGPMs based on staining reaction

| Sl. No | Isolate code | Staining reaction | No. of isolates |
|--------|--|-------------------|-----------------|
| 1 | SBPGPM-1, SBPGPM-2, SBPGPM-4, SBPGPM-6, SBPGPM-7, SBPGPM-8, SBPGPM-9, SBPGPM-10, SBPGPM-11, SBPGPM-12, SBPGPM-13, SBPGPM-14, SBPGPM-15, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-22, SBPGPM-24, SBPGPM-25, SBPGPM-26, SBPGPM-27, SBPGPM-29 | Gram negative | 23 |
| 2 | SBPGPM-3, SBPGPM-5, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-30 | Gram positive | 7 |



Plate 10: Cultural characters of rhizospheric bacterial PGPMs on nutrient agar

Colony margin

Two types of colony margins such as entire and undulated were recorded in the present studies. Out of thirty isolates, seventeen (SBPGPM-1, SBPGPM-3, SBPGPM-4, SBPGPM-6, SBPGPM- 7 SBPGPM- 8 SBPGPM-10, SBPGPM- 11 SBPGPM-13, SBPGPM- 16 SBPGPM- 17 SBPGPM-18, SBPGPM-20, SBPGPM-22, SBPGPM-24, SBPGPM-27, SBPGPM-28) recorded entire margin and rest of the thirteen isolates (SBPGPM-2, SBPGPM-5, SBPGPM-9, SBPGPM-12, SBPGPM-14, SBPGPM- 15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-25, SBPGPM-26, SBPGPM-29, SBPGPM-30) had undulated margin (Table 5d).

Fluorescence under UV light

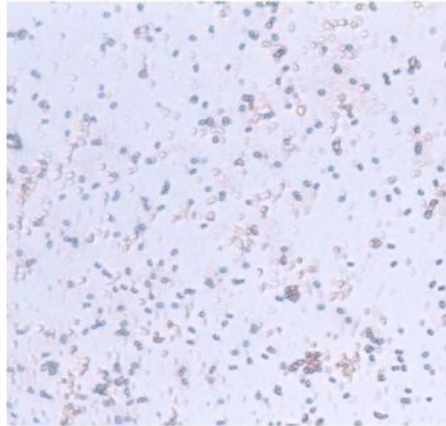
Eleven isolates (SBPGPM-1, SBPGPM-6, SBPGPM-10, SBPGPM-12, SBPGPM-14, SBPGPM- 15, SBPGPM-17, SBPGPM-18, SBPGPM-22, SBPGPM-24, SBPGPM-27) showed fluorescence under UV light and rest of the isolates (SBPGPM-2, SBPGPM-3, SBPGPM-4, SBPGPM-5, SBPGPM-7, SBPGPM-8, SBPGPM-9, SBPGPM-11, SBPGPM-13, SBPGPM-16, SBPGPM-19, SBPGPM-20, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-25, SBPGPM-26) did not produce fluorescence under UV light (Table 5e, Plate 11).

Cell shape

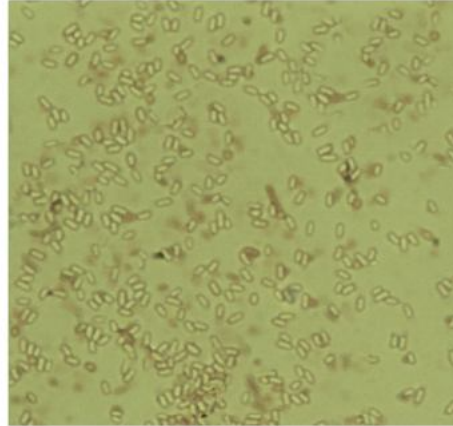
All the thirty isolates of rhizospheric bacterial PGPMs were rod shaped under microscopic observation during the study .

Staining reaction

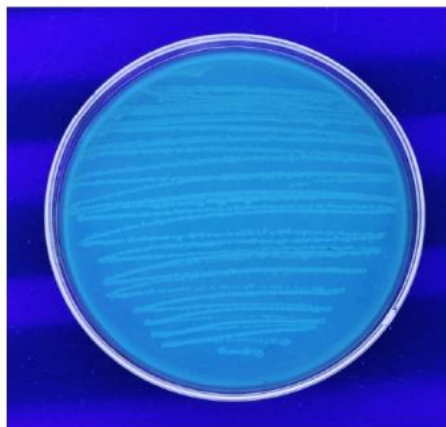
Results on staining reaction indicated that twenty three isolates (SBPGPM-1, SBPGPM-2, SBPGPM-4, SBPGPM-6, SBPGPM-7, SBPGPM-8, SBPGPM-9, SBPGPM-10, SBPGPM-11, SBPGPM-12, SBPGPM-13, SBPGPM-14, SBPGPM- 15, SBPGPM-16, SBPGPM-17, SBPGPM-18, SBPGPM-20, SBPGPM-22, SBPGPM-24, SBPGPM-25, SBPGPM-26, SBPGPM-27, SBPGPM-29) were Gram negative and remaining seven isolates (SBPGPM-3, SBPGPM-5, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-30) were Gram positive (Table 5f, Plate 11).



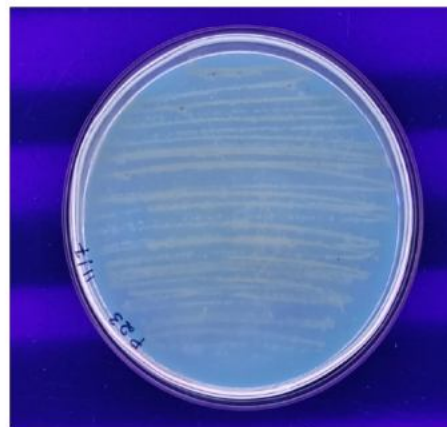
Gram positive and cocci shaped cells



Gram negative and rod shaped cells



Fluorescence production under UV spectroscopy



Absence of fluorescence production under UV spectroscopy

Plate 11. Staining reaction and fluorescence production by bacterial PGPMs

The cultural characters of bacterial entophytes in the present investigation varied with respect to colony color (grayish, grayish white, grayish white slimy, whitish and creamy white), colony form (regular to irregular), colony margin (entire and undulate) and elevation (raised and flat). The results are in trend with Ranjana (2020) who collected the native fluorescent *Pseudomonads* from soil and isolated by serial dilution technique on King's B medium. All the isolates produced slimy, irregular colonies with light yellowish green pigmentation and fluorescence under UV light. In the present investigation also PGPMs showed irregular colonies and exhibited fluorescence under UV spectroscopy. Under microscopic studies, the cells of all the isolates were recorded as rod shaped as like results obtained in the present study. In the gram's reaction all the isolates of fluorescent *Pseudomonads* showed Gram negative reaction. Whereas, in the present investigation, rhizospheric bacterial PGPMs showed both Gram positive and negative reaction.

The results of present investigation are also in accordance with another study conducted by Vinayarani and Prakash (2018) who collected 30 PGPR isolates from rhizosphere and 20 endophytic bacteria from different geographic regions of South India. Further, they reported that the isolates belonged to *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, *Alcaligenes*, *Acinetobacter*, *Ochrobactrum*, *Exiquebacterium*, *Rhizobium*, *Klebsiella*, *Citrobacter* and *Terribacillus* species. With respect to staining reaction and cell shape, the shape of all the isolates was motile rods. In case of Grams reaction, 43 were Gram negative while 7 were Gram-positive. In the present investigation also PGPMs showed Gram positive and Gram negative reaction with respect to staining reaction. Likewise, Kannan *et al.* (2018) collected five bacterial isolates (QAF 01, QAF 02, QAF 03, QAF 04 and QAF 05) from soil sample and were subjected to Gram's reactions and several biochemical characterizations. Among five isolates, three isolates were found to be gram positive and two were gram negative as well as all isolates were rod shaped as like the variation observed in case of staining reaction and cell shape in the present study.

4.2.3 Antagonistic potential of PGPMs against *R. bataticola*

Determination of antagonistic activity of PGPMs is a pre-requisite for selecting the potential isolate against the pathogen. Hence, all the 126 PGPMs were tested for their efficacy to inhibit the growth of pathogen in order to find the potential PGPMs.

4.2.3.1 Antagonistic potential of endophytic fungal PGPMs

All the forty isolates of fungal endophytic PGPMs were screened against *R. bataticola* for mycelial inhibition by dual culture technique. Results indicated that the per cent inhibition of mycelial growth varied greatly among the isolates. All the isolates of fungal endophytic PGPMs inhibited the mycelial growth of *R. bataticola*. The percent inhibition varied from 2.96-86.48 mm. However, per cent inhibition was highest (>50%) in eight isolates (FEPGPM-5, FEPGPM- 11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34 and FEPGPM-35) which varied from 52.96 to 86.48 per cent. It is also indicated that the per cent mycelial inhibition was highly significant between the isolates. All the isolates showed significant difference over the control. The maximum per cent inhibition of 86.48 was recorded in FEPGPM-34 followed by FEPGPM-35 (74.07). The efficacy of this particular isolate may be due to mycoparasitism where the overgrowth of the PGPM occurred by suppressing the growth of the pathogen which was seen in present studies. However, the minimum per cent inhibition of 2.96 was recorded in FEPGPM-6 (Table 6, Fig. 2, Plate 12). Hence from the results, eight fungal endophytes were considered as potential against *R. bataticola*.

As in the present investigation, Shirasangi and Hegde (2018) also reported that endophytes have antagonistic potential against *Rhizoctonia*. In their report, they collected total 66 fungal (26 from root, 15 from stem and 26 from leaf) endophytic isolates from different parts of healthy tomato samples, which were from 30 locations in three districts of northern Karnataka. A total of 66 fungal endophytes were evaluated against *R. solani* by dual culture technique to test antifungal activity of the endophytes. Among 26 fungal root endophytes, the isolate RFHKM-9 showed the maximum mycelial inhibition of 60.39 per cent against *R. solani* which was followed by RFBBA- 23 (54.51 %) and RFDHE-10 (51.76) as noticed in present investigation. In case of 15 fungal stem endophytes, isolate SFDDE-12 showed the maximum mycelial inhibition of 61.96 per cent followed by SFDDE-12 (54.90 %) against *R. solani*. Further, the maximum mycelial inhibition of *R. solani* was observed in LFDHO-3 (52.22 %), LFDLA-9 (51.48), LFHCH-13 (50.37), LFHCH-12 (49.63 %) and LFHMU16 (49.63 %) in case of leaf endophytes which were on par with each other. In the present investigation also fungal endophytic isolates (FEPGPM-5, FEPGPM- 11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34 and FEPGPM-35) inhibited the pathogen with per cent

Table 6: Antagonistic potential of endophytic fungal PGPMs against *R. bataticola* in dual culture assay

| Sl. No. | Isolate code | Colony growth* (mm) | Per cent mycelial inhibition* |
|---------|-----------------|---------------------|-------------------------------|
| 1 | FEPGPM- 1 | 63.00 | 30.00 (33.20) |
| 2 | FEPGPM- 2 | 66.67 | 25.93 (30.60) |
| 3 | FEPGPM- 3 | 75.33 | 16.30 (23.75) |
| 4 | FEPGPM- 4 | 63.33 | 29.63 (32.97) |
| 5 | FEPGPM- 5 | 38.00 | 57.78 (49.47) |
| 6 | FEPGPM- 6 | 87.33 | 2.96 (9.87) |
| 7 | FEPGPM- 7 | 62.67 | 30.37 (33.43) |
| 8 | FEPGPM- 8 | 70.33 | 21.85 (27.85) |
| 9 | FEPGPM- 9 | 71.33 | 20.74 (27.05) |
| 10 | FEPGPM- 10 | 59.50 | 33.89 (35.60) |
| 11 | FEPGPM- 11 | 31.33 | 65.19 (53.85) |
| 12 | FEPGPM- 12 | 52.33 | 42.96 (36.70) |
| 13 | FEPGPM- 13 | 64.33 | 28.52 (32.26) |
| 14 | FEPGPM- 14 | 59.50 | 33.89 (35.60) |
| 15 | FEPGPM- 15 | 57.00 | 36.67 (37.23) |
| 16 | FEPGPM- 16 | 34.67 | 61.48 (51.64) |
| 17 | FEPGPM- 17 | 65.33 | 27.41 (31.55) |
| 18 | FEPGPM- 18 | 82.33 | 8.52 (16.91) |
| 19 | FEPGPM- 19 | 73.00 | 18.89 (25.73) |
| 20 | FEPGPM- 20 | 71.00 | 21.11 (27.33) |
| 21 | FEPGPM- 21 | 62.17 | 30.93 (33.78) |
| 22 | FEPGPM- 22 | 66.33 | 26.30 (30.84) |
| 23 | FEPGPM- 23 | 82.67 | 8.15 (16.53) |
| 24 | FEPGPM- 24 | 33.33 | 62.96 (52.52) |
| 25 | FEPGPM- 25 | 71.00 | 21.11 (27.31) |
| 26 | FEPGPM- 26 | 61.33 | 31.85 (34.34) |
| 27 | FEPGPM- 27 | 23.33 | 69.63 (56.56) |
| 28 | FEPGPM- 28 | 35.00 | 61.11 (51.42) |
| 29 | FEPGPM- 29 | 55.33 | 38.52 (38.34) |
| 30 | FEPGPM- 30 | 52.00 | 42.22 (40.52) |
| 31 | FEPGPM- 31 | 66.67 | 25.93 (30.58) |
| 32 | FEPGPM- 32 | 83.33 | 7.41 (15.72) |
| 33 | FEPGPM- 33 | 71.33 | 20.74 (27.00) |
| 34 | FEPGPM- 34 | 12.17 | 86.48 (68.43) |
| 35 | FEPGPM- 35 | 27.33 | 74.07 (59.38) |
| 36 | FEPGPM- 36 | 65.67 | 27.04 (31.30) |
| 37 | FEPGPM- 37 | 70.33 | 21.85 (27.85) |
| 38 | FEPGPM- 38 | 70.00 | 22.22 (28.12) |
| 39 | FEPGPM- 39 | 66.33 | 26.30 (30.83) |
| 40 | FEPGPM- 40 | 77.00 | 14.44 (22.32) |
| | Control | 90.00 | 00.00 (00.00) |
| | S. Em ± | - | 1.03 |
| | CD at 1% | - | 3.83 |

*Mean of three replications, Figures in parentheses are arc sine transformed values



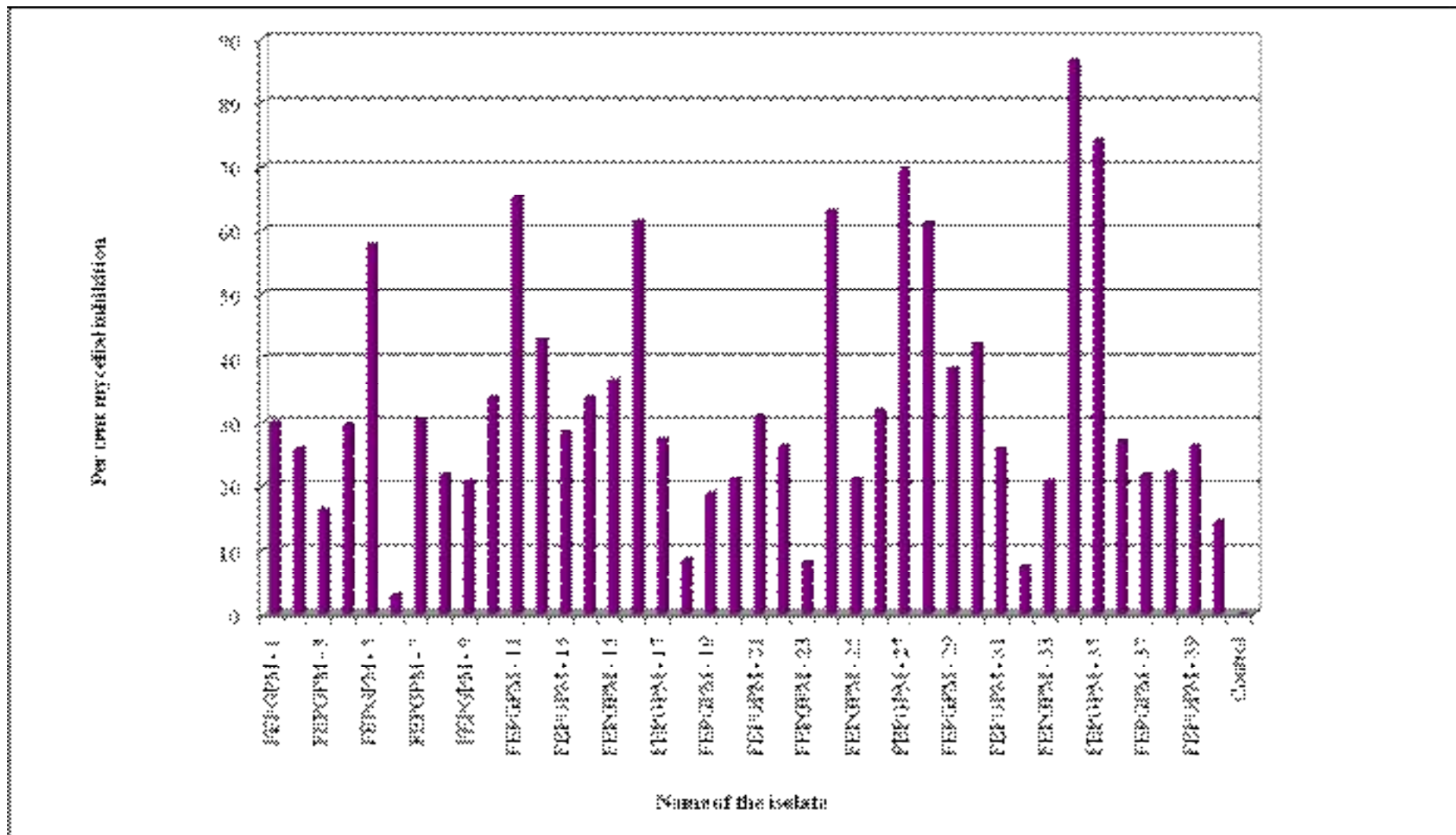


Fig. 2: Antagonistic potential of endophytic fungal PGPMs against *R. bataticola* in dual culture assay

mycelial inhibition varied from 52.96 to 86.48 per cent. indicating that the endophytes can inhibit the pathogen growth by certain mechanisms.

Eight isolates which proved to be efficient in the dual culture assay were selected for the further studies such as test on PGPR traits.

4.2.3.2 Antagonistic potential of endophytic bacterial PGPMs

Thirty isolates of bacterial endophytic PGPMs were screened against *R. bataticola* for mycelial inhibition by using dual culture technique. The results indicated that all the isolates inhibited the pathogen growth significantly. However, the inhibition percent varied from 4.26-83.15 mm. Among them, eight isolates (BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30) showed more than 50 per cent mycelial inhibition of pathogen which were on par with each other, highest being BEPGPM-30 (83.15). The reason behind the highest inhibition capacity may be due to the antibiosis or competition for the nutrients for their growth and production of HCN. The next best efficient antagonist isolate was BEPGPM-6 with the inhibition per cent of 62.96. The isolate BEPGPM-12 recorded minimum per cent mycelial inhibition with per cent inhibition of 4.26 (Table 7, Fig. 3, Plate 13).

Likewise, Chiranjeevi *et al.* (2020) assessed the antagonistic effect of endophytic bacterial isolates based on their ability to inhibit the pathogen growth in dual culture. A total of 40 endophytic bacterial isolates were evaluated for their antagonistic activity against chickpea dry root rot caused by the pathogen *R. bataticola*. From the data it is evident that, among the isolates tested CREB 37 showed significantly maximum inhibition (74.07 %) against *R. bataticola* followed by CREB 15 (71.11 %), CREB 21 (71.11 %), CREB 36 (64.44 %), CREB 16 (60.00 %). The least inhibition (0.00 %) was recorded in control. In the present study also eight isolates (BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30) showed more than 50 per cent mycelial inhibition of pathogen which were on par with each other, highest being BEPGPM-30 (83.15 %). Similarly, Bhavani *et al.* (2015) tested sixty three endophytic bacterial isolates for their antagonistic properties against *R. bataticola* causing dry root rot of chickpea by dual culture technique. Out of them, five isolates showed significant inhibition. The isolate B5 was shown the most significant

Table 7: Antagonistic potential of endophytic bacterial PGPMs against *R. bataticola* in dual culture assay

| Sl. No. | Isolate code | Colony growth* (mm) | Per cent mycelial inhibition* |
|-----------------|--------------|---------------------|-------------------------------|
| 1 | BEPGPM- 1 | 83.17 | 7.59 (15.99) |
| 2 | BEPGPM- 2 | 59.50 | 33.89 (35.60) |
| 3 | BEPGPM- 3 | 65.87 | 26.81 (31.18) |
| 4 | BEPGPM- 4 | 84.17 | 6.48 (14.75) |
| 5 | BEPGPM- 5 | 43.17 | 52.04 (46.16) |
| 6 | BEPGPM- 6 | 33.33 | 62.96 (52.51) |
| 7 | BEPGPM- 7 | 73.07 | 18.81 (25.70) |
| 8 | BEPGPM- 8 | 82.27 | 8.59 (17.04) |
| 9 | BEPGPM- 9 | 42.47 | 52.81 (46.61) |
| 10 | BEPGPM- 10 | 83.17 | 7.59 (15.99) |
| 11 | BEPGPM- 11 | 83.90 | 6.78 (15.09) |
| 12 | BEPGPM- 12 | 86.17 | 4.26 (11.91) |
| 13 | BEPGPM- 13 | 83.00 | 7.78 (16.19) |
| 14 | BEPGPM- 14 | 64.00 | 28.89 (32.51) |
| 15 | BEPGPM- 15 | 41.50 | 53.89 (47.32) |
| 16 | BEPGPM- 16 | 56.50 | 37.22 (37.59) |
| 17 | BEPGPM- 17 | 53.67 | 40.37 (39.44) |
| 18 | BEPGPM- 18 | 75.40 | 16.22 (23.75) |
| 19 | BEPGPM- 19 | 57.33 | 36.30 (37.04) |
| 20 | BEPGPM- 20 | 58.00 | 35.56 (36.60) |
| 21 | BEPGPM- 21 | 52.93 | 41.19 (39.92) |
| 22 | BEPGPM- 22 | 71.83 | 20.19 (26.69) |
| 23 | BEPGPM- 23 | 64.10 | 28.78 (32.44) |
| 24 | BEPGPM- 24 | 55.87 | 37.93 (38.01) |
| 25 | BEPGPM- 25 | 44.00 | 51.11 (45.63) |
| 26 | BEPGPM- 26 | 57.47 | 36.15 (36.95) |
| 27 | BEPGPM- 27 | 42.03 | 53.30 (46.88) |
| 28 | BEPGPM- 28 | 62.33 | 30.74 (33.67) |
| 29 | BEPGPM- 29 | 42.77 | 52.48 (46.42) |
| 30 | BEPGPM- 30 | 15.17 | 83.15 (65.75) |
| | Control | 90.00 | 00.00 (00.00) |
| S. Em ± | | - | 0.79 |
| CD at 1% | | - | 2.98 |

*Mean of three replications, Figures in parentheses are arc sine transformed values



Plate 13: Inhibition of *R. bataticola* by endophytic bacterial PGPMs in dual culture assay

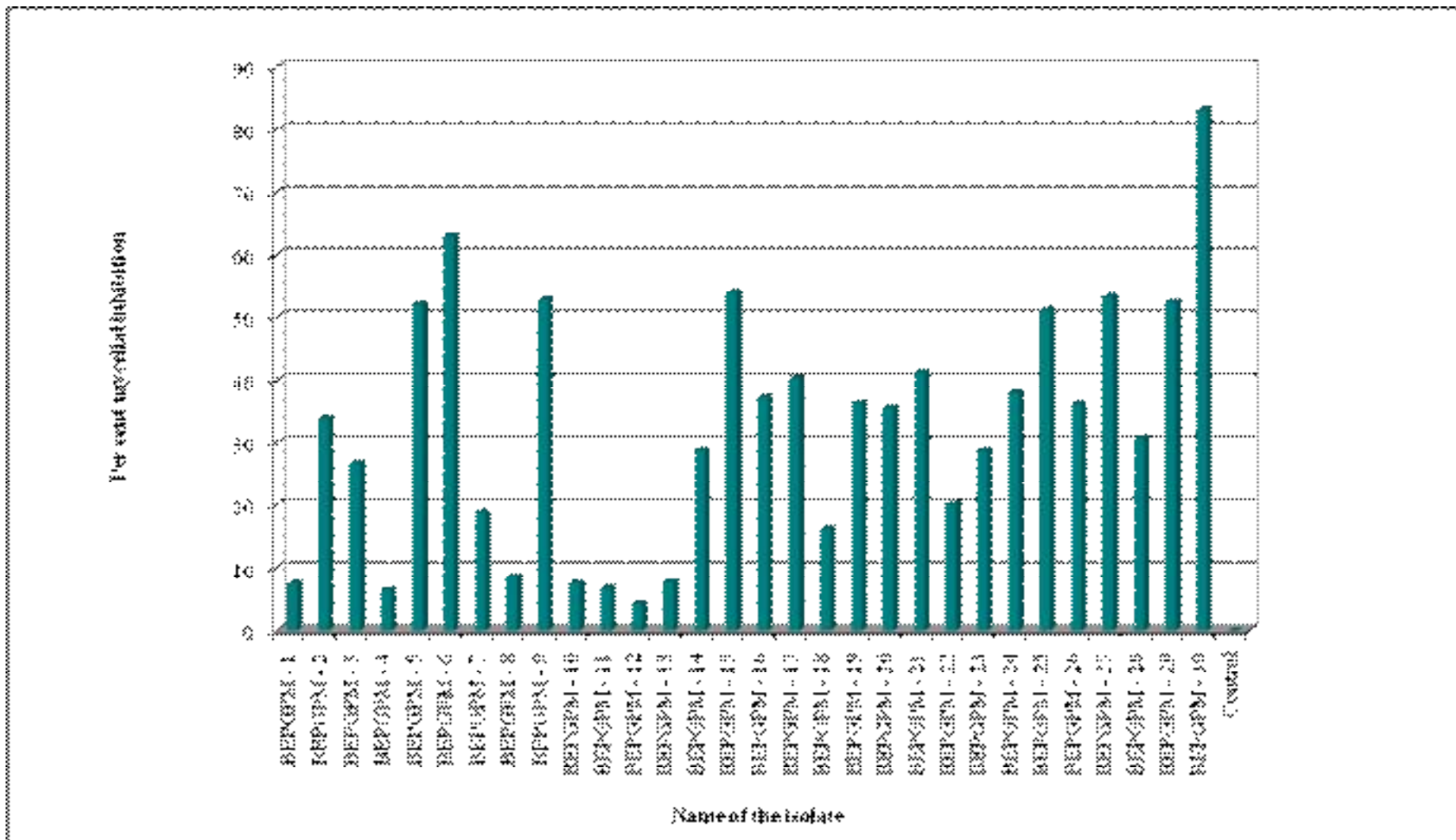


Fig. 3: Antagonistic potential of endophytic bacterial PGPMs against *R. bataticola* in dual culture assay

inhibition with 81 per cent, K1 with 77, K2 with 75, C2 and A8 with 74 per cent respectively.

All the eight isolates which were proved to be efficient in inhibiting the pathogen in the dual culture assay were selected for further studies on PGPR traits.

4.2.3.3 Antagonistic potential of rhizospheric fungal PGPMs

The antagonistic potential of twenty six fungal rhizospheric PGPMs against *R. bataticola* was evaluated by using dual culture technique. The result indicated that per cent mycelial inhibition by PGPMs ranged from 49.39 - 88.44 mm. Thirteen isolates (SFPGPM-1, SFPGPM-2, SFPGPM-3, SFPGPM-9, SFPGPM-10, SFPGPM-13, SFPGPM-14, SFPGPM-15, SFPGPM-19, SFPGPM-21, SFPGPM-22, SFPGPM-23 and SFPGPM-26) showed highest efficacy with more than 50 per cent of inhibition, out of which, SFPGPM-13 (88.44) was the most effective one. This isolate was followed by SFPGPM-3 with per cent inhibition of 80.67 when compared to SFPGPM-12 which showed the least per cent mycelial inhibition of 49.39 (Table 8, Fig. 4, Plate 14). The efficacy of inhibiting the pathogen could be due to mycoparasitism, competition for space or antibiosis or a volatile organic compound. The results of present investigation are also in trend with Nagamani *et al.* (2015) who tested 20 *Trichoderma* isolates against *R. bataticola*. Among them, *T. harzianum* (KNN 4) showed maximum inhibition of mycelia growth of 81.1 per cent followed by *T. harzianum* (ATPP 6) with inhibition of 79.3 per cent. The least inhibition (66.3%) was observed in *T. viride* (KJ 12) isolate. In the present study, similar results were obtained with respect to inhibition per cent with SFPGPM-13 (88.44 %) being most effective. In another report, four bioagents tested for their efficacy in inhibiting mycelial growth of *R. bataticola*. The results indicated that *T. harzianum* is highly antagonistic to *R. bataticola* by suppressing its growth by 78.67 per cent (Veenashri *et al.*, 2019). Similarly, in the present investigation almost all the *Trichoderma* isolates inhibited the pathogen with 49-55 per cent mycelial inhibition. However, only three isolates (SFPGPM-3, SPGPM-9, SFPGPM-13) showed considerable per cent mycelial inhibition (>70 per cent) (Fig.4). Hence, only three isolates were selected for further studies on PGP traits.

Table 8: Antagonistic potential of rhizospheric fungal PGPMs against *R. bataticola* in dual culture assay

| Sl. No | Isolate code | Colony growth* (mm) | Per cent mycelial inhibition* |
|-----------------|--------------|---------------------|-------------------------------|
| 1 | SFPGPM-1 | 35.75 | 60.28 (50.92) |
| 2 | SFPGPM-2 | 37.15 | 58.72 (50.02) |
| 3 | SFPGPM-3 | 17.40 | 80.67 (64.38) |
| 4 | SFPGPM-4 | 45.45 | 49.50 (44.71) |
| 5 | SFPGPM-5 | 45.50 | 49.44 (44.68) |
| 6 | SFPGPM-6 | 45.40 | 49.56 (44.74) |
| 7 | SFPGPM-7 | 45.50 | 49.44 (44.68) |
| 8 | SFPGPM-8 | 45.35 | 49.61 (44.77) |
| 9 | SFPGPM-9 | 23.75 | 73.61 (59.08) |
| 10 | SFPGPM-10 | 43.50 | 51.67 (45.95) |
| 11 | SFPGPM-11 | 45.50 | 49.44 (44.68) |
| 12 | SFPGPM-12 | 45.55 | 49.39 (44.64) |
| 13 | SFPGPM-13 | 10.40 | 88.44 (70.12) |
| 14 | SFPGPM-14 | 44.90 | 50.11 (45.06) |
| 15 | SFPGPM-15 | 42.40 | 52.89 (46.65) |
| 16 | SFPGPM-16 | 45.15 | 49.83 (44.90) |
| 17 | SFPGPM-17 | 45.40 | 49.56 (44.74) |
| 18 | SFPGPM-18 | 45.45 | 49.50 (44.71) |
| 19 | SFPGPM-19 | 42.60 | 52.67 (46.52) |
| 20 | SFPGPM-20 | 45.50 | 49.44 (44.68) |
| 21 | SFPGPM-21 | 42.60 | 52.67 (46.52) |
| 22 | SFPGPM-22 | 40.65 | 54.83 (47.77) |
| 23 | SFPGPM-23 | 40.75 | 54.72 (47.70) |
| 24 | SFPGPM-24 | 45.30 | 49.67 (44.80) |
| 25 | SFPGPM-25 | 45.40 | 49.56 (44.74) |
| 26 | SFPGPM-26 | 42.50 | 52.78 (46.59) |
| | Control | 90.00 | 0.00 (0.00) |
| S. Em ± | | - | 0.95 |
| CD at 1% | | - | 3.74 |

*Mean of three replications, Figures in parentheses are arc sine transformed values



Plate 14: Inhibition of *R. bataticola* by rhizospheric fungal PGPMs in dual culture assay

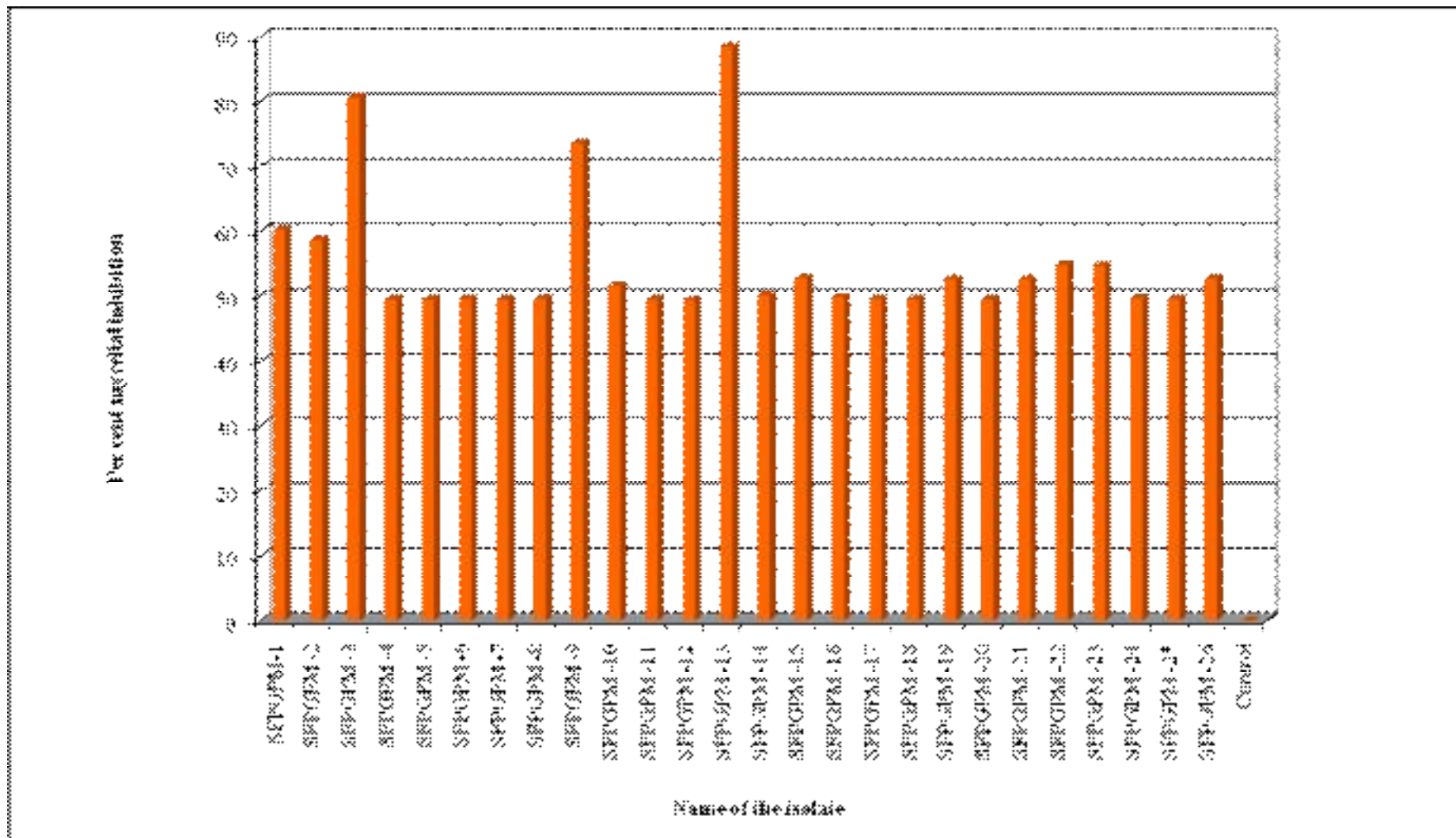


Fig. 4: Antagonistic potential of rhizospheric fungal PGPMs against *R. bataticolain* in dual culture assay

4.2.3.4 Antagonistic potential of rhizospheric bacterial PGPMs

In the present investigation, thirty bacterial rhizospheric PGPMs were tested for their antagonistic potential against *R. bataticola*. The mycelial per cent inhibition of pathogen varied from 2.59 - 83.89 mm. The mycelial inhibition of more than 50 per cent was observed in nine isolates viz., SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM-15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28 and SBPGPM-30. Among them, the highest inhibition was found in the SBPGPM-21 (83.89 %) followed by SFPGPM-30 (76.30 %). The maximum inhibition by the PGPM entails the involvement of mechanisms such as competition for nutrients and space, production of antibiotics, siderophores, HCN, inducing systemic resistance and/or production of hydrolytic enzymes as cell wall components of fungi are made up of chitin and beta-glucan. However, the minimum mycelial inhibition was observed in SBPGPM-22 with per cent inhibition of 2.59 (Table 9, Fig. 5, Plate 15). Similarly, Pandey *et al.* (2016) studied the antagonistic action of fluorescent *Pseudomonas* which was isolated from native soil (Raipur), tested against three different isolates of *F. oxysporum* f. sp. *ciceris* by dual culture technique. The fluorescent *Pseudomonas* inhibited the growth of pathogen isolates such as *F. oxysporum* f. sp. *ciceris* 1, *F. oxysporum* f. sp. *ciceris* 2 and *F. oxysporum* f. sp. *ciceris* 3 to the extent of 46.70, 46.83 and 46.03 per cent, respectively. Likewise, *B. subtilis* strain PRBS-1 and AP-3 inhibited five soybean seed pathogenic fungi, viz., *Rhizoctonia solani*, *C. truncatum*, *S. sclerotium*, *M. phaseolina*, and *Phomopsis* spp. under *in vitro* conditions (Araujo *et al.*, 2005). In the current study also the rhizospheric bacterial PGPMs could able to inhibit the *R. bataticola* with considerable per cent mycelial inhibition which indicated that rhizospheric bacteria can inhibit the fungal pathogen.

Nine isolates which were proven to potential in inhibiting the pathogen in the dual culture assay were selected for further studies on PGP traits.

4.3 Studies on plant growth promoting traits of potential PGPMs

Plant growth promoting microorganisms significantly ameliorate plant growth by a number of mechanisms including increase of uptake of essential nutrients such as phosphate, ammonia, and nitrogen, producing phytohormones such as indole-3-acetic acid and gibberellins. Hence, twenty eight potential PGPMs (fungal as well as bacterial endophytic and rhizospheric) which were efficient in inhibition of pathogen in the dual

Table 9: Antagonistic potential of rhizospheric bacterial PGPMs against *R. bataticola* in dual culture assay

| Sl. No. | Isolate code | Colony growth* (mm) | Per cent mycelial inhibition* |
|---------|-----------------|---------------------|-------------------------------|
| 1 | SBP GPM- 1 | 59.67 | 33.70 (35.48) |
| 2 | SBP GPM- 2 | 73.17 | 18.70 (25.62) |
| 3 | SBP GPM- 3 | 64.00 | 28.89 (32.51) |
| 4 | SBP GPM- 4 | 36.67 | 59.26 (50.33) |
| 5 | SBP GPM- 5 | 70.00 | 22.22 (28.12) |
| 6 | SBP GPM- 6 | 52.67 | 41.48 (40.09) |
| 7 | SBP GPM- 7 | 37.17 | 58.70 (50.01) |
| 8 | SBP GPM- 8 | 64.83 | 27.96 (31.92) |
| 9 | SBP GPM- 9 | 36.50 | 59.44 (50.44) |
| 10 | SBP GPM- 10 | 84.50 | 6.11 (14.31) |
| 11 | SBP GPM- 11 | 84.33 | 6.30 (14.53) |
| 12 | SBP GPM- 12 | 79.00 | 12.22 (20.46) |
| 13 | SBP GPM- 13 | 79.83 | 11.30 (19.64) |
| 14 | SBP GPM- 14 | 54.83 | 39.07 (38.68) |
| 15 | SBP GPM- 15 | 38.83 | 56.85 (48.93) |
| 16 | SBP GPM- 16 | 70.67 | 21.48 (27.61) |
| 17 | SBP GPM- 17 | 63.50 | 29.44 (32.86) |
| 18 | SBP GPM- 18 | 65.33 | 27.41 (31.56) |
| 19 | SBP GPM- 19 | 42.67 | 52.59 (46.48) |
| 20 | SBP GPM- 20 | 71.27 | 21.26 (27.45) |
| 21 | SBP GPM- 21 | 14.97 | 83.89 (66.33) |
| 22 | SBP GPM- 22 | 88.00 | 2.59 (9.26) |
| 23 | SBP GPM- 23 | 31.33 | 67.41 (55.18) |
| 24 | SBP GPM- 24 | 65.33 | 28.33 (32.16) |
| 25 | SBP GPM- 25 | 68.00 | 26.30 (30.85) |
| 26 | SBP GPM- 26 | 65.23 | 29.44 (32.86) |
| 27 | SBP GPM- 27 | 70.00 | 22.59 (28.38) |
| 28 | SBP GPM- 28 | 44.97 | 50.93 (45.52) |
| 29 | SBP GPM- 29 | 84.50 | 9.26 (17.71) |
| 30 | SBP GPM- 30 | 22.67 | 76.30 (60.86) |
| | Control | 90.00 | 0.00 (0.00) |
| | S. Em ± | - | 1.06 |
| | CD at 1% | - | 3.97 |

*Mean of three replications, Figures in parentheses are arc sine transformed values

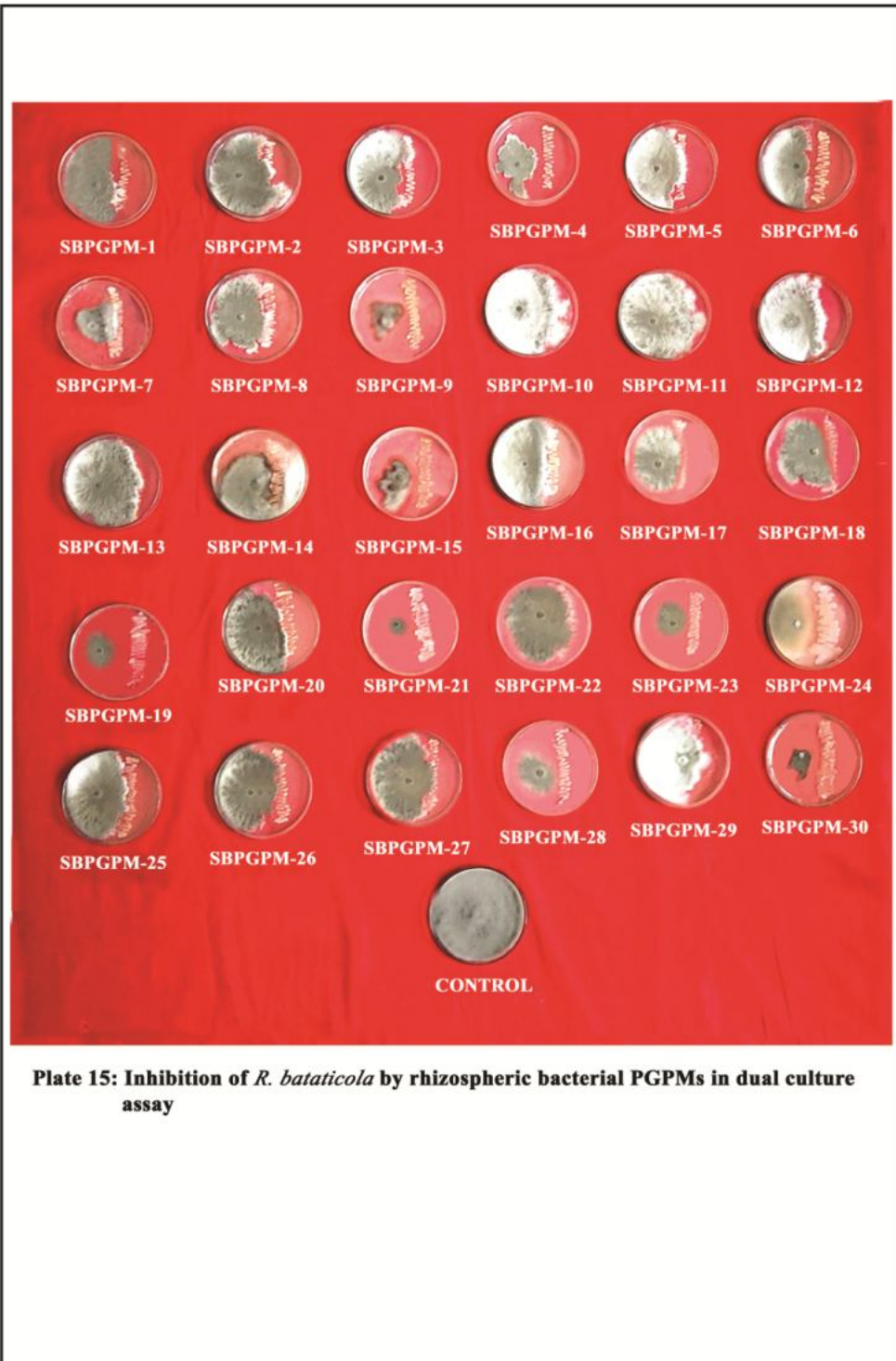


Plate 15: Inhibition of *R. bataticola* by rhizospheric bacterial PGPMs in dual culture assay

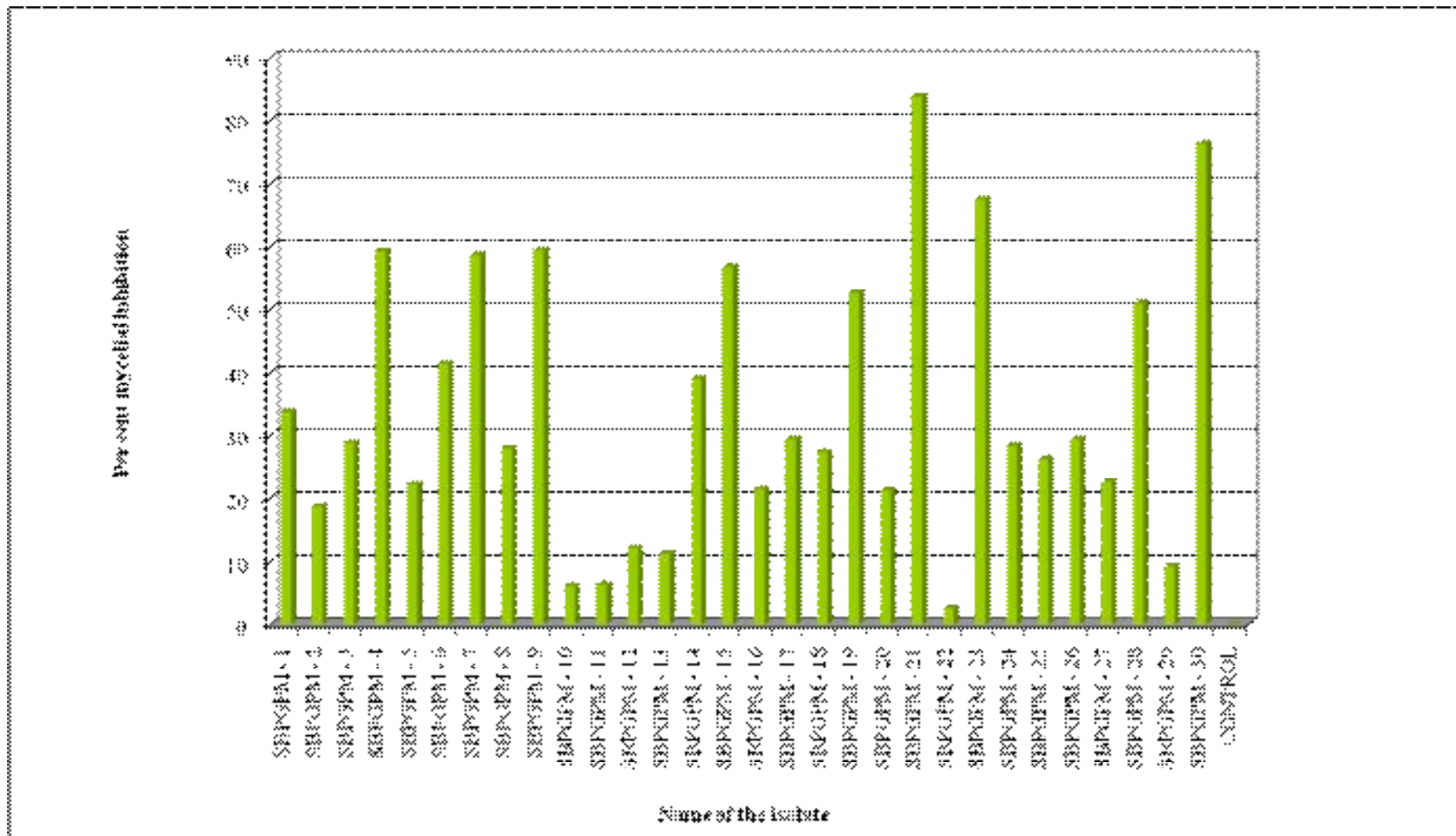


Fig. 5: Antagonistic potential of rhizospheric bacterial PGPMs against *R. bataticola* in dual culture assay

culture assay were further screened for the production of plant growth promoting traits like IAA production, siderophore production, ammonia production, phosphate solubilization and HCN production (Table 10).

4.3.1 IAA production

Indole-3-acetic acid is the most common, naturally occurring plant hormone. IAA influences the process of forming plant tissues, namely growth, division and cell differentiation and protein synthesis. A diverse genus of microbes can augment significant amount of IAA. That being the case, an effort to test the ability of twenty eight potential PGPMs for IAA production. In the present study, all twenty eight potential PGPMs (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34, FEPGPM-35, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30, SFPGPM-3, SFPGPM-9, SFPGPM-13, SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM-15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-30) exhibited pink color which indicated the production of indole-3-acetic acid as detected by the Salkowaski's reagent (Plate 16a). The results indicated that PGPMs have the ability to induce plant growth promotion of chickpea through the synthesis of IAA which helped in plant cell elongation, proliferation and also indirectly supported the growth of root and shoot. These results were in accordance with Restu and Payangan (2019) who aimed to identify rhizosphere fungi and evaluate the ability of IAA production. They obtained five fungi (*Aspergillus*, *Trichoderma*, *Rhizopus*, *Penicillium* and *Fusarium*). In the present investigation also the rhizospheric and endophytic fungi were able to produce the indole acetic acid. Other workers undertook a study for isolation of *P. fluorescens* and *B. subtilis* from rhizosphere of onion and analysis of these bacteria for *in vitro* indole acetic acid production. The results indicated that the two tested microorganisms exhibited a pink to red color with a little variation in intensity (Reetha *et al.*, 2014). Similarly, in the current study also the rhizospheric and endophytic bacterial isolates produced the indole acetic acid.

4.3.2 Siderophore production

Siderophores are low molecular weight iron chelating secondary metabolites synthesized by various groups of microorganisms which help in scavenging iron-limited

Table 10: Screening of potential PGPMs for plant growth promoting traits

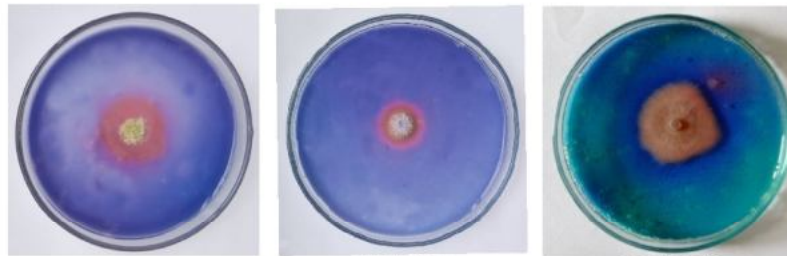
| Sl. No. | Isolate code | IAA production | Siderophore production | Ammonium production | Phosphate solubilization | HCN production |
|---------|--------------|----------------|------------------------|---------------------|--------------------------|----------------|
| 1 | FEPGPM- 5 | + | + | + | + | + |
| 2 | FEPGPM- 11 | + | + | + | + | + |
| 3 | FEPGPM- 16 | + | + | + | + | + |
| 4 | FEPGPM- 24 | + | + | + | + | + |
| 5 | FEPGPM- 27 | + | + | + | + | + |
| 6 | FEPGPM- 28 | + | + | + | + | + |
| 7 | FEPGPM- 34 | + | + | + | + | + |
| 8 | FEPGPM- 35 | + | + | + | - | - |
| 9 | BEPGPM- 5 | + | - | + | + | + |
| 10 | BEPGPM- 6 | + | - | + | - | + |
| 11 | BEPGPM- 9 | + | - | + | + | + |
| 12 | BEPGPM- 15 | + | - | + | + | - |
| 13 | BEPGPM- 25 | + | - | + | - | + |
| 14 | BEPGPM- 27 | + | - | + | + | + |
| 15 | BEPGPM- 29 | + | - | + | + | + |
| 16 | BEPGPM- 30 | + | - | + | + | + |
| 17 | SFPGPM- 3 | + | + | + | + | + |
| 18 | SFPGPM- 9 | + | + | + | + | + |
| 19 | SFPGPM- 13 | + | + | + | + | + |
| 20 | SBPGPM- 4 | + | - | + | - | - |
| 21 | SBPGPM- 7 | + | - | + | - | - |
| 22 | SBPGPM- 9 | + | - | + | + | - |
| 23 | SBPGPM- 15 | + | - | + | - | - |
| 24 | SBPGPM- 19 | + | - | + | + | + |
| 25 | SBPGPM- 21 | + | - | + | + | + |
| 26 | SBPGPM- 23 | + | - | + | + | + |
| 27 | SBPGPM- 28 | + | - | + | + | + |
| 28 | SBPGPM- 30 | + | - | + | - | + |

‘+’ - Indicates positive reaction for the test

‘-’ - Indicates negative reaction for the test



IAA production



SFPGPM-3

SFPGPM-9

SFPGPM-13

Siderophore production



Ammonium production

Plate 16a: Plant growth promoting traits exhibited by representative potential PGPMs

conditions. Siderophore produced by microbes facilitate the plant growth by providing iron to plants and creates scarcity of nutrients for the pathogen, there by suppresses the pathogen population. On that account, all the twenty eight potential PGPMs were tested for their ability to siderophore production. The results revealed that eleven endophytic as well as rhizospheric potential fungal PGPMs (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34, FEPGPM-35, SFPGPM-3, SFPGPM-9 and SFPGPM-13) produced siderophores, which was indicated by production of yellow/orange colored zone on blue agar medium. Rest of the isolates did not produce siderophores (Plate 16a). The result implied that the PGPMs which showed positive reaction for siderophore production, developed specific mechanism for uptake of iron by releasing siderophores to scavenge iron. The results of present investigation were supported by Lacava *et al.* (2008) who reported that siderophores arrest insoluble ferric iron from different environments. The fungal and bacterial species in soil and plant endophytes synthesize hydroxamate and carboxylate type siderophores of several classes - coprogens, fusarinines and ferrichromes (Khan *et al.*, 2018).

In another study, eighty four fungal endophytes were isolated from nodules and roots of *C. arietinum* and *P. sativum* plants and screened for production of siderophores. Among eighty four isolates, fourteen endophytes produced siderophores. The quantitative analysis was also done and ten best siderophore producers (above 65 per cent siderophore units) were characterized for the type of siderophore produced. Most of them produced hydroxamate and carboxylate type of siderophores (Maheshwari *et al.*, 2019). In the current study also the fungal endophytes tested positive for siderophore production. These results indicate that the fungal endophytes can produce the siderophores for the plant growth promotion and also for the suppression of the pathogen.

4.3.3 Ammonium production

Ammonium plays a key role in the plant growth by providing the nitrogen to plants. Thus, twenty eight potential PGPMs were subjected for ammonium production. The results showed that all of them (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34, FEPGPM-35, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30, SFPGPM-3, SFPGPM-9, SFPGPM-13, SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM-15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-30)

were found positive for production of ammonium by exhibiting yellow color (Plate 16a). The results also indicated that the PGPMs which could produce the ammonia can supply the crops with a sufficient amount of ammonium required for root and shoot elongation and consequently promote plant growth. There is a report given by Hassan (2017) that endophytes produced plant growth-promoting substances in *Teucrium polium*. The bacterial endophytes viz., *B. cereus* and *B. subtilis* and fungal endophytes, *P. chrysogenum* and *P. crustosum* also produced indole acetic acid, ammonium and phosphate. In the present investigation, the ammonium production was found in both fungal PGPMs and bacterial PGPMs.

4.3.4 Phosphate solubilization

Phosphorus is one of the macronutrients required for plant growth promotion. In most cases, phosphorus is present in the soil as insoluble inorganic forms. But, different rhizospheric and endophytic PGPM strains have the efficacy to convert it from an unavailable to available source for plant uptake. In the present study, the phosphate solubilizing activity for all PGPM isolates was assessed on Pikoviskaya medium supplemented with tri-calcium phosphate as an inorganic phosphate source. Out of twenty eight potential PGPMs assessed, twenty two isolates (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34, BEPGPM-5, BEPGPM-9, BEPGPM-15, BEPGPM-27, BEPGPM-29, BEPGPM-30, SFPGPM-3, SFPGPM-9, SFPGPM-13, SBPGPM-9, SBPGPM-19, SBPGPM-21, SBPGPM- 23, SBPGPM-28) were positive for the reaction (Plate 16b). The result depicts that the phosphate solubilizing PGPMs can be used for increasing crop production and promote plant growth by availability of phosphate. Similarly, Khalil *et al.* (2021) reported that three endophytic fungi isolated from matured leaves of *Ephedra pachyclada* such as *P. crustosum* EP-2, *P. chrysogenum* EP-3 and *A. flavus* EP-14 were able to form clear zones, suggesting a phosphate solubilizing ability. In the current study also the twenty two isolates which showed positive reaction for phosphate solubilization, exhibited the clear zone formation.

4.3.5 HCN Production

HCN is recognized as a biocontrol agent, based on its ascribed toxicity against plant pathogens. In case of HCN production by twenty eight potential PGPMs, twenty

two isolates (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34, BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30, SFPGPM-3, SFPGPM-9, SFPGPM-13, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28, SBPGPM-30) produced light reddish brown color on the filter paper which indicated the production of HCN (Plate 16b). There is a previous report similar to present investigation in which one hundred and fifty strains (endophytic and rhizospheric isolates) isolated from *Canola* were characterized for plant growth promoting traits. Among them, hundred isolates produced indole-3-acetic acid, seventeen isolates solubilized phosphate, forty four isolates produced siderophores, thirty four produced 1-aminocyclopropane-1-carboxylate deaminase and five produced hydrocyanic acid (Etesami *et al.*, 2014). In the present investigation also both the endophytic and rhizospheric PGPMs could produce similar PGP traits.

It is clear from the present investigation that nineteen PGPMs *viz.*, seven fungal endophytic PGPMs (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34), five bacterial endophytes (BEPGPM-5, BEPGPM-9, BEPGPM-27, BEPGPM-29, BEPGPM-30), three rhizospheric fungi (SFPGPM-3, SFPGPM-9, SFPGPM-13) and four rhizospheric bacteria (SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28) showed positive reaction for maximum PGP traits tested. Hence all the nineteen PGPMs were further carried for molecular characterization.

4.4 Molecular characterization and diversity analysis of potential PGPMs and their screening for bio-active compound production against *R. bataticola*

Nineteen potential PGPMs (7 fungal endophytes, 5 bacterial endophytes, 3 rhizospheric fungi and 4 rhizospheric bacteria) were selected for molecular characterization and diversity analysis studies.

4.4.1 Molecular characterization and diversity analysis of potential PGPMs

For molecular characterization, nineteen PGPMs as listed above were subjected for analysis using ITS and 16S rRNA sequences for fungal and bacterial PGPMs, respectively. The ITS gene regions were successfully amplified and were 500-600 bp long and amplified 16S rRNA gene regions were of 1500 bp long (Plate 17 and Plate 18). The BLAST results revealed that nineteen PGPM isolates showed 99-100 per cent

similarity with other strains from GenBank. The accession numbers of both endophytic and rhizospheric PGPMs are summarized in Tables and sequences of all the nineteen isolates are presented in Appendices I.

4.4.1.1 Endophytic fungal PGPMs

Seven endophytic fungal isolates were identified based on ITS gene sequencing using ITS-1 and ITS-4 primers for PCR amplification. Both the primers produced amplified product size of 500-650 bp in all the isolates. Further, the sequences were BLAST searched and results showed that isolates such as FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34 showed 100 per cent similarity with *Fusarium sulawesiense*, *Fusarium incarnatum*, *Fusarium nygamai*, *Fusarium equeseti*, *Fusarium proliferatum*, *Aspergillus nidulans*, *Trichoderma asperellum* with accession numbers ON514084, ON595690, ON564638, ON514139, ON514349, ON564645 and ON514144, respectively (Table 11). Thus, the endophytic fungal isolates such as FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28 and FEPGPM-34 identified as *F. sulawesiense*, *F. incarnatum*, *F. nygamai*, *F. equeseti*, *F. proliferatum*, *A. nidulans* and *T. asperellum*, respectively.

Fusarium sp. belongs to the group of soil borne microflora which is known to cause wilts and root rots in plants, however, some species are non-pathogenic and are good bio-control agents (Kaur *et al.*, 2010). In the present investigation, the fungal endophytes such as *F. sulawesiense*, *F. incarnatum*, *F. nygamai*, *F. equeseti* and *F. proliferatum* are new reports for pathogen inhibition and for growth promoting activity.

Diversity analysis (Phylogenetic tree)

The dendrogram was constructed using the neighbor-joining tree method using sequence of reference organisms obtained from the Genbank to understand the relationship among seven endophytic fungal PGPMs (Fig. 6). Phylogenetic tree showed closely related nucleotide sequences. The phylogenetic tree divided the isolates in to two clusters. In the cluster-I contained two sub clusters. Sub cluster I had four branches. In branch I, *F. incarnatum* (FEPGPM-11) shared 62 per cent similarity with reference isolate *F. equeseti*. In branch II, *T. asperellum* (FEPGPM-34) shared 100 per cent similarity with *T. asperellum* strain IIPR-81. In branch III, *A. nidulans* (FEPGPM-28) shared 24 per cent similarity with *F. equeseti* (FEPGPM-24) and in branch IV, *F.*

proliferatum shared 40 per cent similarity with *F. sulawesiense* reference strain. In sub cluster-II, *F. nygamai* shared 99 per cent similarity with *F. nygamai* reference strain. In the cluster II, *F. sulawesiense* was outgrouped. *A. nidulans* and *F. equeseti* both belongs to Ascomycota. Hence, they showed 24 per cent similarity in the evolutionary tree. Further, sequence identity matrix was also constructed in order to know the similarity per cent and diversity between the isolates (Table 12). The highest percent identity (86 %) showed by *F. equeseti* with *F. proliferatum*. Whereas, least per cent identity was 38 per cent between *T. asperellum* and *F. proliferatum*.

Similarly, Mbilu *et al.* (2018) carried out the characterization of 60 fungal endophytes isolated from *Warburgia ugandensis* using molecular tools to identify the isolates up to species level as carried out in the present investigation. The results revealed that the isolates were placed in fourteen different genera namely *Nigrospora*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Phomopsis*, *Colletotrichum*, *Alternaria*, *Cochliobolus*, *Bionectria*, *Phyllosticta*, *Guignardia*, *Tricharina*, *Diaporthe* and *Trichoderma* through diversity analysis by constructing phylogenetic tree. In the present investigation also the identified isolates were belonging to different genera like *Fusarium*, *Aspergillus* and *Trichoderma*. In another molecular diversity analysis study, fifteen endophytic fungi were isolated from matured leaves of *Ephedra pachyclada* and were subjected to ITS sequence analysis. They reported that the endophytes were identified *A. flavus* (EP-14) and *A. niger* (EP-15) (Khalil *et al.*, 2021). The BLAST analysis also revealed 98-99 per cent identity with ITS sequences of the related species. In the present investigation also FEPGPM-28 was identified as *A. nidulans* and the phylogenetic tree was constructed based on ITS gene sequences.

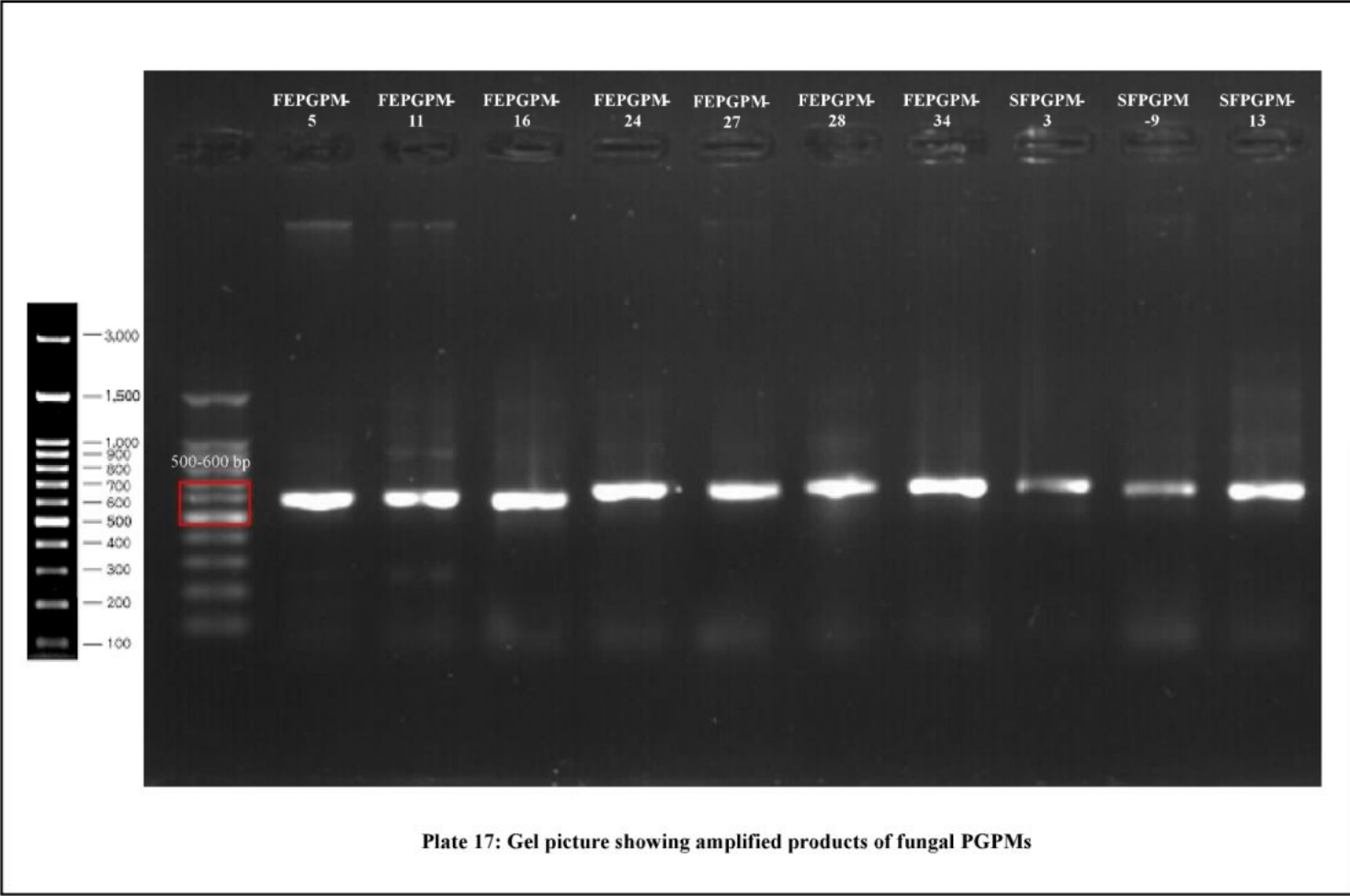
Noveriza *et al.* (2022) isolated endophytic fungi by direct planting method, purification and characterization of fifty isolates were conducted on potato dextrose agar media. Based on ITS sequence analysis, the DNA sequences of isolate A-TKR1 were identical to *A. flavus*, isolate ETKR3 to *Penicillium citrinum* and isolate ECT- TKR7 to *Neofusicoccum parvum*. Phylogenetic analysis was constructed using the UPGMA method based on ITS DNA sequences of three endophytic fungi from *Eucalyptus* leaves and their similarity with fungal isolates from the Gene Bank. The similarity of those isolates was 100, 99.81, 99.82 per cent, respectively. Similarly, in the present study also the diversity analysis was studied by constructing phylogenetic tree. Jeewon *et al.* (2013)

Table 11: Molecular characterization of endophytic fungal PGPMs by ITS sequencing

| SL. No | Isolate code | Strain | Accession Number | Percent identity | Reference organism |
|--------|--------------|--------|------------------|------------------|------------------------------|
| 1 | FEPGPM- 5 | FE -5 | ON514084 | 100% | <i>Fusarium sulawesiense</i> |
| 2 | FEPGPM- 11 | FE-11 | ON595690 | 100% | <i>Fusarium incarnatum</i> |
| 3 | FEPGPM- 16 | FE-16 | ON564638 | 100% | <i>Fusarium nygamai</i> |
| 4 | FEPGPM- 24 | FE-24 | ON514139 | 100% | <i>Fusarium equeseti</i> |
| 5 | FEPGPM- 27 | FE-27 | ON514349 | 100% | <i>Fusarium proliferatum</i> |
| 6 | FEPGPM- 28 | FE -28 | ON564645 | 100% | <i>Aspergillus nidulans</i> |
| 7 | FEPGPM- 34 | FE- 34 | ON514144 | 100% | <i>Trichodema asperillum</i> |

Table 12: Sequence identity matrix of endophytic fungal PGPMs

| Sequence name | <i>Fusarium incarnatum</i> (FEPGPM-11) | <i>Fusarium nygamai</i> (FEPGPM-16) | <i>Trichodema asperillum</i> (FEPGPM-34) | <i>Aspergillus nidulans</i> (FEPGPM-28) | <i>Fusarium sulawesiense</i> (FEPGPM-5) | <i>Fusarium equeseti</i> (FEPGPM-24) | <i>Fusarium proliferatum</i> (FEPGPM-27) |
|---|---|--|---|--|--|---|---|
| <i>Fusarium incarnatum</i> (FEPGPM-11) | ID | | | | | | |
| <i>Fusarium nygamai</i> (FEPGPM-16) | 65% | ID | | | | | |
| <i>Trichodema asperillum</i> (FEPGPM-34) | 67% | 59% | ID | | | | |
| <i>Aspergillus nidulans</i> (FEPGPM-28) | 57% | 60% | 54% | ID | | | |
| <i>Fusarium sulawesiense</i> (FEPGPM-5) | 71% | 71% | 54% | 56% | ID | | |
| <i>Fusarium equeseti</i> (FEPGPM-24) | 53% | 47% | 38% | 41% | 39% | ID | |
| <i>Fusarium proliferatum</i> (FEPGPM-27) | 49% | 54% | 38% | 42% | 42% | 86% | ID |



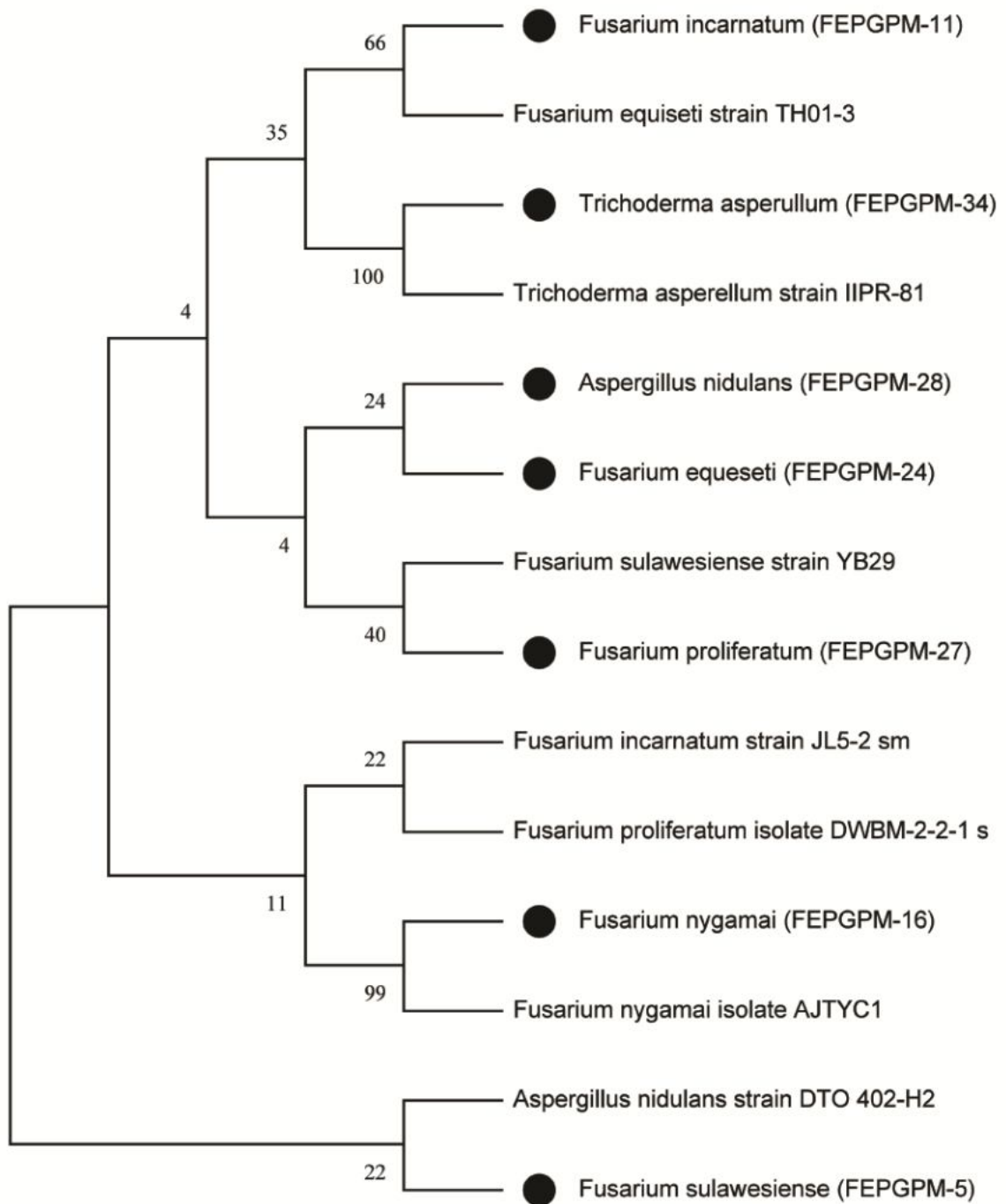


Fig. 6: Phylogenetic tree depicting diversity among the endophytic fungal PGPM isolates

reported that fungi (both endophytes and saprobes) were isolated from *Antidesma madagascariense*, a medicinal plant in Mauritius, in view of identifying potential isolates for screening of fungi for pharmaceutical importance. The diversity analysis of fungi was investigated by constructing the phylogenetic tree based on rDNA sequence analysis. Fungi were related to *Aspergillus*, *Guignardia*, *Fusarium*, *Penicillium*, *Pestalotiopsis* and *Trichoderma*. Phylogenetic analysis revealed that fungi recovered were belong to 5 different fungal lineages (Hypocreaceae, Trichocomaceae, Nectriaceae, Xylariaceae, and Botryosphaeriaceae). DNA data from the ITS regions were reliable in classification of all recovered isolates up to genus level, but identification to an exact species name was not possible at this stage. Hence these reports supports the present investigation with respect to diversity analysis in which endophytic fungal PGPMs belong to three different genus *Fusarium*, *Trichoderma* and *Aspergillus*.

4.4.1.2 Rhizospheric fungal PGPMs

The molecular identification of rhizospheric fungal PGPMs was done by nucleotide sequencing of ITS region. The BLAST data results of three rhizospheric fungal isolates (SFPGPM-3, SFPGPM-9 and SFPGPM-13) revealed 100 per cent homology with *Trichoderma asperelloides*, *Trichoderma asperellum*, *Trichoderma harzianum* and assigned with accession numbers. ON513885, ON514143 ON514140, respectively by GenBank (Table 13). Hence, SFPGPM-3, SFPGPM-9 and SFPGPM-13 are identified as *T. asperelloides*, *T. asperellum* and *T. harzianum*.

Diversity analysis (Phylogenetic tree)

To know the diversity among three rhizospheric fungal PGPMs, Phylogenetic tree was constructed using neighbor-joining tree method in Mega 11 software. The phylogenetic relationships between rhizospheric fungal PGPMs and among top most hits against GenBank showed two clusters, keeping *T. harzianum* isolate *UOM PGPF 03* as an out group in cluster-II. In the cluster-I, there were two sub groups. Sub group I comprises of *T. asperelloides* (SFPGPM-3) which showed 67 per cent similarity with *T. asperelloides* isolate NT43. Sub group-II comprises *T. asperellum* (SFPGPM- 9), *T. harzianum* (SFPGPM- 13) with 99 per cent similarity and 75 per cent with *T. asperellum* isolate MICO1211 (Fig. 7). With respect to sequence identity matrix, the highest per cent

Table 13: Molecular characterization of rhizospheric fungal PGPMs by ITS sequencing

| SL. No | Isolate code | Strain | Accession Number | Percent identity | Reference organism |
|--------|--------------|--------|------------------|------------------|----------------------------------|
| 1 | SFPGPM-3 | T3 | ON513885 | 100% | <i>Trichoderma asperelloides</i> |
| 2 | SFPGPM-9 | T9 | ON514143 | 100% | <i>Trichoderma asperellum</i> |
| 3 | SFPGPM-13 | T13 | ON514140 | 100% | <i>Trichoderma harzianum</i> |

Table 14: Sequence identity matrix of rhizospheric fungal PGPMs

| Sequence name | <i>Trichoderma asperelloides</i> (SFPGPM-3) | <i>Trichoderma asperellum</i> (SFPGPM-9) | <i>Trichoderma harzianum</i> (SFPGPM-13) |
|---|---|--|--|
| <i>Trichoderma asperelloides</i> (SFPGPM-3) | ID | | |
| <i>Trichoderma asperellum</i> (SFPGPM-9) | 93% | ID | |
| <i>Trichoderma harzianum</i> (SFPGPM-13) | 60% | 65% | ID |

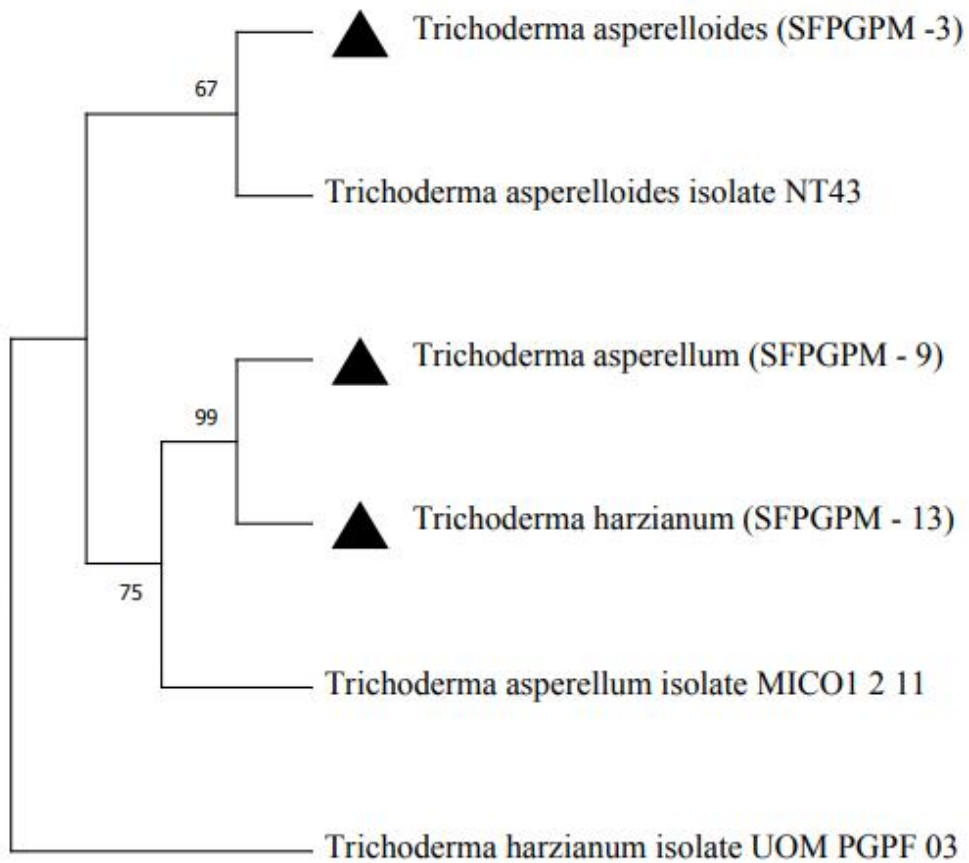


Fig. 7: Phylogenetic tree depicting diversity among the rhizospheric fungal PGPM isolates

of identity 93 per cent shared with *T. asperellum* and *T. asperelloides*. Whereas, least per cent identity was observed *T. harzianum* and *T. asperelloides* (Table 14).

Similar results were obtained by the molecular study conducted by Nagamani *et al.* (2017) who studied sixteen *Trichoderma* isolates from different chickpea growing areas of Andhra Pradesh region by ITS sequencing. Phylogenetic tree was constructed containing isolates belonging to two groups in order to study the diversity between them. Group I included *T. asperellum*, *T. longibrachiatum*, *Trichoderma* spp. and Group II was outgroup included *T. viride*, *T. asperellum* and *T. asperelloides* complex. In the present investigation also *T. asperellum*, *T. asperelloides* and *T. harzianum* were identified and in the phylogenetic tree *T. asperelloides* was outgrouped. Another report on molecular characterization of the *Trichoderma* isolates collected from rhizosphere of chickpea, pigeonpea and lentil crop was reported by Shahid *et al.* (2014) using microsatellite-primed polymerase chain reaction (MP-PCR) and ribosomal DNA (rDNA) sequence analysis. The genetic relatedness among fifteen isolates of *Trichoderma* sp. was analyzed with six micro-satellite primers. ITS-PCR of rDNA region with ITS1 and ITS4 primers produced 600 bp products in all isolates as obtained in the present study with the range of 500-600 bp. The results of phylogenetic analysis showed 83.7 per cent genetic diversity among the isolates with the formation of four clusters. Thus, *Trichoderma* isolates were identified.

4.4.1.3 Endophytic bacterial PGPMs

16S rRNA gene sequencing was done for the identification of five endophytic bacterial isolates. The universal primers (16Sr DNA F and 16Sr DNA R) were used for PCR amplification, which produced amplified products of size 1500 bp. BLAST results of 16S rRNA gene sequence revealed that all the endophytic bacterial PGPMs showed homology with genus *Bacillus* but with different species. The four isolates (BEPGPM-5, BEPGPM-27, BEPGPM-28, BEPGPM-30) showed 100 per cent homology with *Bacillus tropicus* (ON564730), *Bacillus pacificus* (ON564773), *Bacillus cereus* (ON564610) and *Bacillus subtilis* (ON564689), respectively with assigned accession numbers. While, isolate BEPGPM-9 showed 99.69 per cent homology with *Bacillus tropicus* (ON564907) (Table 15). As result, the isolates namely, BEPGPM-5, BEPGPM-9, BEPGPM-27, BEPGPM-28, BEPGPM-30 were confirmed as *B. tropicus*, *B. tropicus*, *B. pacificus*, *B. cereus*, *B. subtilis*, respectively.

Table 15: Molecular characterization of endophytic bacterial PGPMs by 16S rDNA sequencing

| SL. No | Isolate code | Strain | Accession Number | Percent identity | Reference organism |
|--------|--------------|---------|------------------|------------------|---------------------------|
| 1 | BEPGPM- 5 | Gada B2 | ON564730 | 100% | <i>Bacillus tropicus</i> |
| 2 | BEPGPM - 9 | ZARS | ON564907 | 99.69% | <i>Bacillus tropicus</i> |
| 3 | BEPGPM- 27 | HRS | ON564773 | 100% | <i>Bacillus pacificus</i> |
| 4 | BEPGPM- 28 | B1 | ON564610 | 100% | <i>Bacillus cereus</i> |
| 5 | BEPGPM- 30 | B4 | ON564689 | 100% | <i>Bacillus subtilis</i> |

Table 16: Sequence identity matrix of endophytic bacterial PGPMs

| Sequence name | <i>Bacillus cereus</i> (BEPGPM-27) | <i>Bacillus subtilis</i> (BEPGPM-30) | <i>Bacillus tropicus</i> (BEPGPM-5) | <i>Bacillus pacificus</i> (BEPGPM- 25) | <i>Bacillus tropicus</i> (BEPGPM -9) |
|---------------------------------------|---------------------------------------|---|--|---|---|
| <i>Bacillus cereus</i> (BEPGPM-27) | ID | | | | |
| <i>Bacillus subtilis</i> (BEPGPM-30) | 76% | ID | | | |
| <i>Bacillus tropicus</i> (BEPGPM-5) | 99% | 76% | ID | | |
| <i>Bacillus pacificus</i> (BEPGPM-25) | 61% | 71% | 61% | ID | |
| <i>Bacillus tropicus</i> (BEPGPM-9) | 79% | 61% | 80% | 49% | ID |

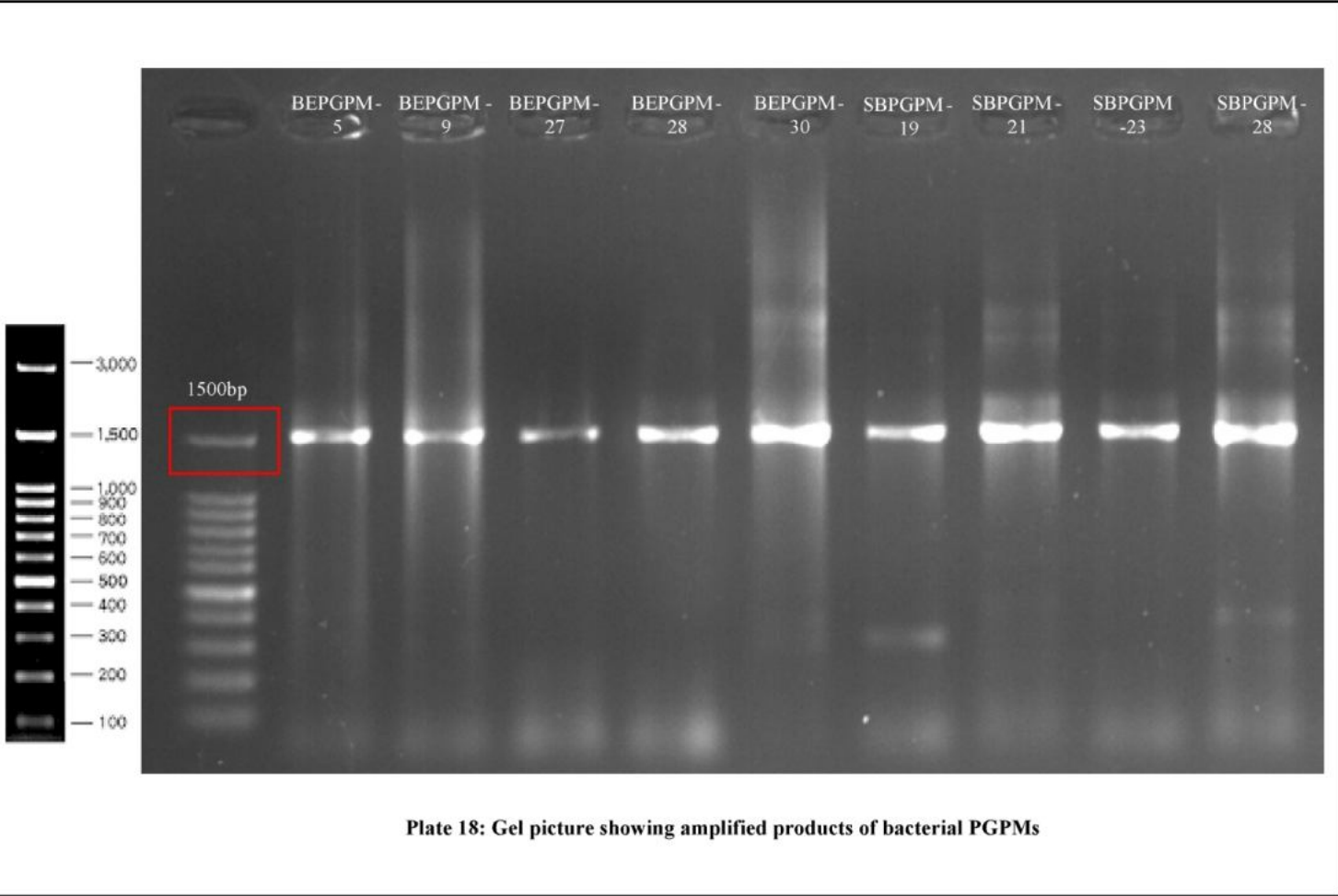


Plate 18: Gel picture showing amplified products of bacterial PGPMs

Likewise, Hernández *et. al.* (2014) reported that many species of *Bacillus* including *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. cereus*, *B. mycoides* and *B. thuringiensis*, are known to suppress growth of several fungal pathogens such as *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Sclerotium*, *Gaeummanomyces*, *Nectria*, *Pythium*, *Phytophthora* and *Verticillium*. The main property of antagonist bacterial strains is production of antifungal antibiotics, which seem to play a major role in biological control of plant pathogens. In the present investigation also BEPGPM-28 and BEPGPM-30 were identified as *B. cereus* and *B. subtilis* respectively and were effective in mycelial inhibition *R. bataticola* *in vitro*. Another similar investigation was carried out by Dong *et al.* (2021) who isolated total of 53 bacterial strains from the rhizosphere soil, nine of which exhibited good bio-control effect against the pathogenic bacteria of soft rot disease caused by *Pectobacterium carotovorum* sub sp. *carotovorum*. Four strains such as CAB-L005, CAB-L012, CAB-L014 and CAB-L022 exhibited strong antagonistic effects. On the basis of the sequence homology of 16S rRNA genes, the similarity between strain CAB-L005 and *B. tropicus* was 100 per cent, that between strain CAB-L012 and *B. subtilis* was 99 per cent, and that between strain CAB-L014 and *B. tequilensis* was 100 per cent and similarity between strain CAB-L022 and *B. cereus* was 100 per cent. The isolated bacteria demonstrated good biocontrol effects in field experiments. In the present investigation also *B. tropicus*, *B. cereus* and *B. subtilis* could inhibit the *R. bataticola* *in vitro*, among them *B. tropicus* is a new outcome from the present study, which exhibited the antagonistic potential against *R. bataticola* *in vitro* with plant growth promoting traits.

Diversity analysis

Phylogram of five endophytic bacterial PGPMs was constructed to analyze the diversity among them (Fig. 8). The results in the dendrogram showed two major clusters. Cluster-I comprised two sub groups. In sub-group I, *B. pacificus* (BEPGPM-25) shared 36 per cent similarity with reference organism *B. cereus* strain Pm-L3. Whereas, *B. cereus* (BEPGPM-27) shared 16 per cent similarity between *B. pacificus* (BEPGPM-25) and *B. cereus* strain Pm-L3. *B. tropicus* (BEPGPM-9) was outgrouped from *B. pacificus* (BEPGPM-25), *B. cereus* (BEPGPM-27) and two reference *B. cereus* strains. Sub-group II consists of *B. tropicus* (BEPGPM 5) which was outgrouped from *B. pacificus* and *B. tropicus* reference strains. Cluster-II consists of *B. subtilis* (BEPGPM 30) shared 99 per cent similarity with *B. subtilis* strain DE-7 (Fig. 8). With respect to sequence identity

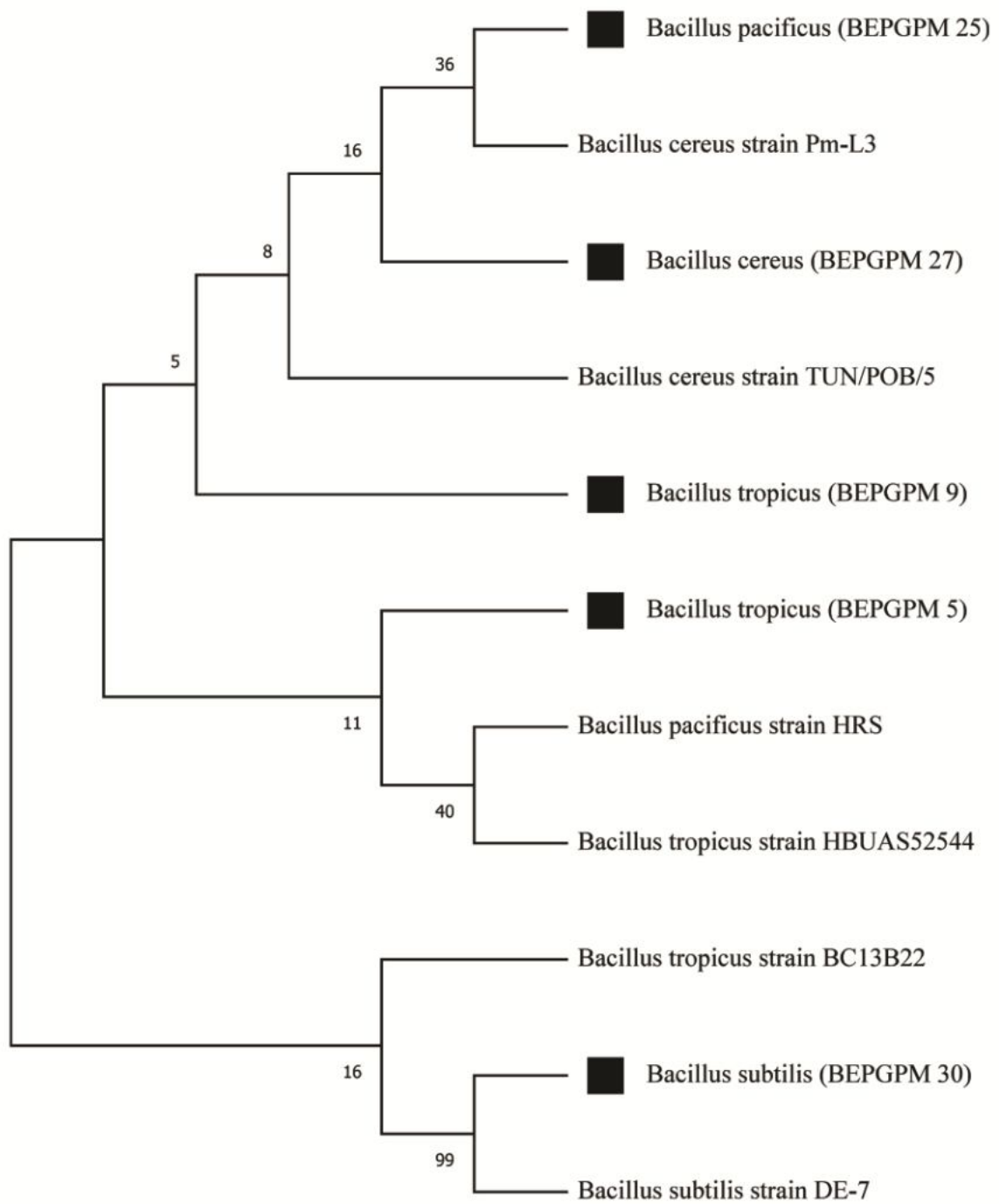


Fig. 8: Phylogenetic tree depicting diversity among the endophytic bacterial PGPM isolates

matrix, the highest per cent of identity 99 per cent shared with *B. cereus* and *B. tropicus*. Whereas, least per cent identity was observed with *B. pacificus* and *B. tropicus* (Table 16).

The results of present investigation were supported by previous reports that *B. subtilis* is commonly found in the soil or on plant surfaces (Abd Allah *et al.* 2017). They are also considered as common endophytes found in various plants. The BLAST analysis of the bacterial DNA sequences aligned isolate CEB 1 with the 16S rDNA sequence of *Pantoea dispersa* with 96 per cent identity while CEB 2, CEB 3, CEB 4 and CEB 5 aligned with the 16S rDNA sequences of *B. subtilis* strains with 99 per cent identity as observed in the study. The assembled sequences ranged from 1255 to 1459 bp which fell within the expected nucleotide length of 16S rDNA fragments. In the present investigation also the nucleotide length was 1500 bp which indicated the 16S rDNA and based on BLAST results BEPGPM-5, BEPGPM-9, BEPGPM-27, BEPGPM-28 and BEPGPM-30 were confirmed as *B. tropicus*, *B. tropicus*, *B. pacificus*, *B. cereus*, *B. subtilis*. Phylogenetic analysis revealed that CEB 2, CEB 3, CEB 4 and CEB 5 were grouped together with *B. subtilis* with a bootstrap value of 91 per cent while CEB 1 was clustered with *P. dispersa* with a bootstrap value of 99 per cent. CEB 2 branched off from the other *Bacillus* isolates (Salo and Novero, 2021). In the current study also the diversity analysis among the *Bacillus* sp. was studied by constructing phylogenetic tree.

4.4.1.4 Rhizospheric bacterial PGPMs

Four highly potential rhizospheric bacterial isolates subjected to molecular identification using 16S rRNA gene sequencing and the PCR amplified products of all four isolates were of 1500 bp. Further diversity analysis among them was studied by phylogenetic analysis. The results of phylogeny showed homology of SBPGPM- 19, SBPGPM- 21 SBPGPM- 23, SBPGPM- 28 with *B. cereus* (100 %), *B. velezensis* (100 %), *B. subtilis* (100 %), *B. subtilis* sub sp. *subtilis* (99.34 %). The allocated accession numbers for all four isolates were ON567448, ON568504, ON566236 and ON566124 respectively (Table 17).

Similar investigation was carried out by Osman and Yin (2018) who isolated the PGPRs from pea plant (SE-7, LE-26, SQ-7 and SQ-9), which could promote growth through genes that encode for the synthesis of specific growth stimuli or other growth-

promoting traits such as vitamins, antibiotics, and secondary metabolites. Sequencing of 16S ribosomal RNA showed that these strains were mostly similar to *Bacillus* sp. (99 % similarity). Using the EzBioCloud 16S rRNA database, it was found that one strain was likely to be *B. paramycooides* based on 100 per cent similarity, two strains were *B. wiedmannii* based on 99.05 and 100 per cent similarity, and the remaining strain was *B. amyloliquefaciens* based on 99.64 per cent similarity. In the present investigation, the rhizospheric bacterial PGPMs such as SBPGPM- 19, SBPGPM- 21 and SBPGPM- 23 showed 100 per cent homology with genus *Bacillus* but with different species based on BLAST results of 16S rDNA. Moon *et al.* (2021) reported the potentiality of *B. velezensis* CE 100 in controlling Phytophthora root rot diseases and promoting the growth of *C. obtusa* seedlings. *B. velezensis* CE 100 produced β -1,3-glucanase and protease enzymes, which degrade the β -glucan and protein components of phytopathogenic oomycetes cell-wall, causing mycelial growth inhibition of *P. boehmeriae*, *P. cinnamomi*, *P. drechsleri* and *P. erythroseptica* by 54.6, 62.6, 74.3 and 73.7 per cent, respectively. *B. velezensis* CE 100 also increased the survival rate of *C. obtusa* seedlings 2.0-fold and 1.7-fold compared to control. Juan *et al.* (2016) reported *B. velezensis* RC 218 as a biocontrol agent to reduce Fusarium head blight and deoxynivalenol accumulation. It was demonstrated to have antagonist activity against the plant pathogen under *in vitro* and greenhouse assays. But in case of chickpea it is a new outcome that *B. velezensis* could inhibit the *R. bataticola* and exhibited plant growth promoting activity.

Diversity analysis

The diversity studies by constructing phylogenetic tree indicated two major clusters. Cluster-I consists two branches. In branch I, *B. subtilis* (SBPGPM 23) shared 33 per cent similarity with *B. velezensis* strain DRC-18-2D. In branch II *B. subtilis* sub sp. *subtilis* (SBPGPM-28) shared 23 per cent similarity with *B. subtilis* strain LK-3. In cluster II, *B. cereus* (SBPGPM-19) shared 41 per cent similarity with *B. subtilis* sub sp. *subtilis* strain 3619. Whereas, *B. velezensis* (SBPGPM-21) was out grouped (Fig. 9). In the sequence identity matrix, *Bacillus subtilis* sub sp. *subtilis* shared highest percent identity of 97 per cent with *B. velezensis*. Whereas, *B. cereus* shared least percent identity of 60 per cent with *B. subtilis* (Table 18).

Similarly, the Phylogenetic tree of rhizospheric and endophytic bacterial isolates constructed from 16s rDNA sequences along with the related reference species retrieved

Table 17: Molecular characterization of rhizospheric bacterial PGPMs by 16S rDNA sequencing

| SL. No | Isolate code | Strain | Accession Number | Percent identity | Reference organism |
|--------|--------------|--------|------------------|------------------|--|
| 1 | SBP GPM- 19 | P 19 | ON567448 | 100% | <i>Bacillus cereus</i> |
| 2 | SBP GPM- 21 | P 21 | ON568504 | 100% | <i>Bacillus velezensis</i> |
| 3 | SBP GPM -23 | P 23 | ON566236 | 100% | <i>Bacillus.subtilis</i> |
| 4 | SBP GPM- 28 | P 28 | ON566124 | 99.34% | <i>Bacillus subtilis</i> sub sp. <i>subtilis</i> |

Table 18: Sequence identity matrix of rhizospheric bacterial PGPMs

| Sequence name | <i>Bacillus subtilis</i> (SBPGPM-23) | <i>Bacillus subtilis</i> sub sp. <i>subtilis</i> (SBPGPM-28) | <i>Bacillus cereus</i> (SBPGPM -19) | <i>Bacillus velezensis</i> (SBPGPM -21) |
|--|--------------------------------------|--|-------------------------------------|---|
| <i>Bacillus subtilis</i> (SBPGPM-23) | ID | | | |
| <i>Bacillus subtilis</i> sub sp. <i>subtilis</i> (SBPGPM-28) | 63% | ID | | |
| <i>Bacillus cereus</i> (SBPGPM -19) | 60% | 92% | ID | |
| <i>Bacillus velezensis</i> (SBPGPM -21) | 63% | 97% | 94% | ID |

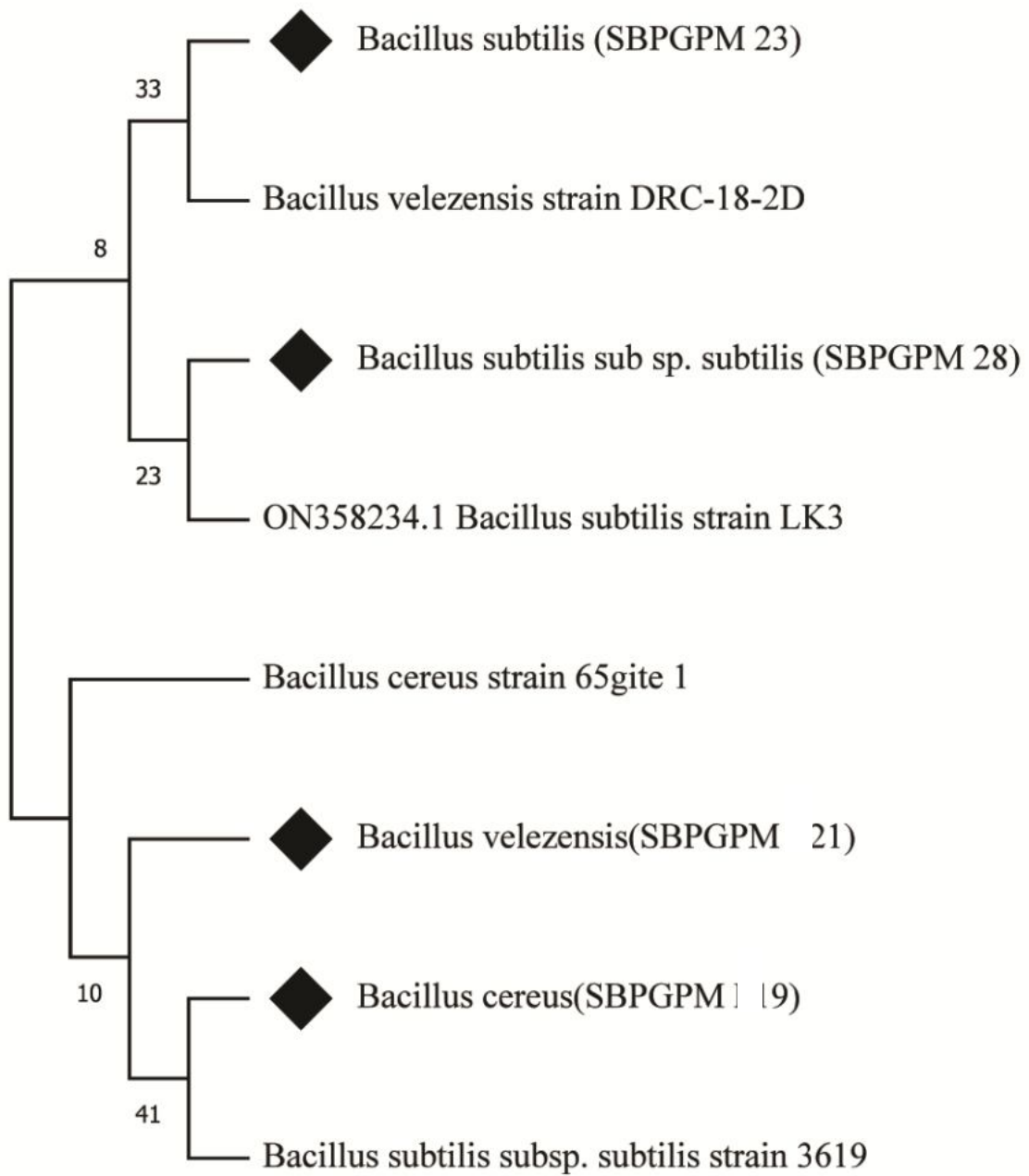


Fig. 9: Phylogenetic tree depicting diversity among the rhizospheric bacterial PGPM isolates

from Genbank of NCBI confirmed these isolates belong to *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, *Alcaligenes*, *Acinetobacter*, *Ochrobactrum*, *Exiquebacterium*, *Rhizobium*, *Klebsiella*, *Citrobacter* and *Terribacillus* species by clustering of each of the isolate to its corresponding group (Vinayarani and Prakash, 2018). In the present study also *Bacillus* sp. were confirmed with Genbank assigned accession numbers. With the same background, Yang *et al.* (2020) isolated seven endophytic bacterial strains, 665L2, 725L2, 725R2, 92R2, 728R3, 728R4 and 2416T3 from seeds of five healthy maize varieties and all the five isolates were identified as *B. velezensis* by polyphasic taxonomy based on 16S rRNA and *gyrA* gene phylogenetic analysis. Similarly, Sayed *et al.* (2014) grouped 66 isolates in accordance with the dendrogram of genetic similarity using Dice similarity coefficient index, into twenty three different groups namely; *Bacillus*, *Enterobacter* and *Pseudomonas*. According to BLAST similarity matches, the *Bacillus* group was found to include five different strains, CSS01 (99.58 % similarity to *Bacillus* sp. strain ZB2), GLS01 (99.6 % similarity to *B. subtilis* strain KTH-61), TAS04 (99.2 % similarity to *B. subtilis* strain M61), TTP02 (99.4 % similarity to *B. subtilis* strain LLS- M3-11), and TTP05 (97.95 % similarity to *B. subtilis* strain CD-6). In the present investigation also *B. subtilis*, *B. velezensis* and *B. subtilis* sub sp. *subtilis* were identified.

4.4.2 Screening for bio-active compounds production by potential PGPMs against *R. bataticola*

Nineteen PGPMs (10 fungal PGPMs and 9 bacterial PGPMs) which were subjected for molecular identification were also screened for the production of bio-active compounds for the inhibition of pathogen by using inverted plate technique.

4.4.2.1 Fungal PGPMs

Endophytic fungal PGPMs

All the seven highly potential fungal endophytic PGPMs produced the bio-active compounds and the per cent mycelial inhibition of pathogen ranged from 25.47 to 74.61. Among seven fungal PGPM isolates, the maximum inhibition of pathogen mycelial growth was recorded in *T. asperellum* with per cent inhibition of 74.61. The next best isolates were *F. incarnatum* and *F. sulawesiense* with inhibition per cent of 57.14 and 51.11, respectively. However, *F. proliferatum* recorded minimum per cent inhibition of

about 25.47 (Table 19, Plate 19). The maximum inhibition of pathogen mycelial growth by *T. asperellum* may be due to the production of bio-active compounds which are antimicrobial or antifungal. This indicated that the endophytic fungal PGPMs indirectly inhibit *R. bataticola* by inducing the expression of defense genes encoding the production of salicylic and jasmonic acids and by interacting with hydrolytic enzymes.

Rhizospheric fungal PGPMs

Three potential rhizospheric fungal PGPMs were screened for the production of bio-active compounds and as result, all the three isolates showed more than 50 per cent inhibition of pathogen mycelial growth and were on par with each other. The maximum mycelial inhibition of pathogen was shown by *T. harzianum* with per cent inhibition of 67.22. The least mycelial inhibition showed by *T. asperelloides* with inhibition per cent of 30.33 (Table 20, Plate 20). The results depicts that rhizospheric fungal PGPM *T. harzianum* was effective in production of bio-active compounds which were antifungal in nature.

The results are in trend with Ranjana (2020) who tested twenty native *Trichoderma* isolates were tested for volatile compounds production in inverted plate technique. The results showed that all the isolates produced considerable amount of volatile compounds which varied among isolates. Higher concentration of volatile compounds was produced by isolate TR-14 (72.22 %) followed by TR-19 (66.67 %), TR-9 (65.93 %) and lower concentration of volatile compounds was produced by TR-18 (2.59 %). All the isolates have shown significant difference in mycelial growth inhibition when compared to the control as the results obtained in the present investigation in which the maximum mycelial inhibition of pathogen exhibited by *T. harzianum* with per cent inhibition of 67.22. Another work on similar study depicts that bio-active compounds produced by *Trichoderma* isolates (IPT 2, 6, 9, 11, 16, 20, 24, 28 and 31) reduced the growth of *F. oxysporum* f. sp. *ciceris* by 25.00 - 65.30 per cent and conidia production by 25.00-87.50 per cent and were highly effective (Shubha *et al.*, 2013). Similarly, Nagamani *et al.* (2017) collected and tested twenty *Trichoderma* spp. for their antagonism against *F. oxysporum* f. sp. *ciceris*. All the *Trichoderma* isolates produced toxic volatile metabolites having significant effect in reducing the radial growth of test pathogen. Volatile metabolites produced by *T. asperellum* (ATPU 1) was found most efficacious in reducing the mycelial growth of *F. oxysporum* f. sp. *ciceris* by 86.70 per cent followed by

Table 19: Screening of endophytic fungal PGPMs for the production of bio active compounds against mycelial inhibition of *R. bataticola* by invert plate technique

| Sl. No | Isolate name | Colony growth* (mm) | Per cent mycelial inhibition* |
|--------------|-------------------------------|---------------------|-------------------------------|
| 1 | <i>Fusarium sulawesiense</i> | 44.00 | 51.11 (45.63) |
| 2 | <i>Fusarium incarnatum</i> | 38.58 | 57.14 (49.10) |
| 3 | <i>Fusarium nygamai</i> | 55.28 | 38.58 (38.39) |
| 4 | <i>Fusarium equeseti</i> | 62.10 | 31.00 (33.83) |
| 5 | <i>Fusarium proliferatum</i> | 67.08 | 25.47 (30.30) |
| 6 | <i>Aspergillus nidulans</i> | 53.51 | 40.54 (39.54) |
| 7 | <i>Trichoderma asperellum</i> | 22.85 | 74.61 (59.74) |
| Control | | 90.00 | 0.00 (0.00) |
| S. Em. \pm | | - | 0.34 |
| CD at 1% | | - | 1.40 |

*Mean of three replications, Figures in parentheses are arc sine transformed values

Table 20: Screening of rhizospheric fungal PGPMs for the production of bio active compounds against mycelial inhibition of *R. bataticola* by invert plate technique

| Sl. No. | Isolate name | Colony growth* (mm) | Per cent mycelial inhibition* |
|-------------|----------------------------------|---------------------|-------------------------------|
| 1 | <i>Trichoderma asperelloides</i> | 30.33 | 66.30 (54.50) |
| 2 | <i>Trichoderma asperellum</i> | 32.40 | 64.00 (53.12) |
| 3 | <i>Trichoderma harzianum</i> | 28.50 | 67.22 (55.07) |
| Control | | 90.00 | 0.00 (0.00) |
| S. Em \pm | | - | 0.42 |
| CD at 1% | | - | 1.59 |

*Mean of three replications, Figures in parentheses are arc sine transformed values

T. asperellum (KNPG 3) and *T. harzianum* (ATPP 6) recording 83.90 and 83.50 per cent inhibition over control, respectively. Similarly, in the present investigation, *T. harzianum* showed considerable per cent of mycelial inhibition, which indicates the production of bio-active compounds.

4.4.2.2 Bacterial PGPMs

Endophytic bacterial PGPMs

Among the five potential endophytic bacterial isolates screened for the production of bio-active compounds, *B. subtilis* has showed maximum per cent inhibition of 60.61, which was followed by *B. tropicus* (53.28). However, the minimum per cent mycelial inhibition of 29.50 was shown by *B. pacificus* (Table 21, Plate 21).

Rhizospheric bacterial PGPMs

The results of screening of four rhizospheric bacterial PGPMs for the production of bio-active compounds showed significant difference among them. The maximum mycelial inhibition of pathogen was recorded in *B. velezensis* with per cent inhibition of 59.06 followed by *B. cereus* (50.78 %). While, minimum mycelial inhibition of pathogen was noticed in *B. subtilis sub sp. subtilis* with per cent inhibition of 45.53 (Table 22, Plate 22). The results are in accordance with Ranjana (2020) who tested twenty native isolates of fluorescent *Pseudomonas* isolated from chickpea rhizosphere for volatile compounds production in inverted plate technique. The results showed that the isolates produced considerable amount of volatile compounds which varied within the isolates. Higher concentration of volatile compounds was produced by isolate PF-19 (66.67 %) followed by PF-14 (64.44 %), PF-6 (56.67 %) and lower concentration of volatile compounds was produced by PF-9 (4.44 %). All the isolates have shown significant difference in mycelial growth inhibition when compared to the control. In the present investigation also the rhizospheric bacterial isolates significantly inhibited the pathogen by producing the bio-active compounds.

Hence, from the present investigation, it is clear that both endophytic and rhizospheric PGPMs can produce the bio-active compounds which have antifungal activity against *R. bataticola*. In the study, four isolates *T. asperellum*, *T.harzianum*, *B.subtilis* and *B. velezensis* showed maximum inhibition against the pathogen which

Table 21: Screening of bacterial endophytic PGPMs for the production of bio active compounds against mycelial inhibition of *R. bataticola* by invert plate technique

| Sl. No | Isolate name | Colony growth* (mm) | Per cent mycelial inhibition* |
|-----------------|---------------------------|---------------------|-------------------------------|
| 1 | <i>Bacillus tropicus</i> | 47.80 | 46.89 (43.21) |
| 2 | <i>Bacillus tropicus</i> | 42.05 | 53.28 (46.87) |
| 3 | <i>Bacillus pacificus</i> | 63.45 | 29.50 (32.88) |
| 4 | <i>Bacillus cereus</i> | 57.45 | 36.17 (36.96) |
| 5 | <i>Bacillus subtilis</i> | 35.45 | 60.61 (51.12) |
| Control | | 90.00 | 0.00 (0.00) |
| S. Em. ± | | - | 0.44 |
| CD at 1% | | - | 1.85 |

*Mean of three replications, Figures in parentheses are arc sine transformed values

Table 22: Screening of rhizospheric bacterial PGPMs for the production of bio active compounds against mycelial inhibition of *R. bataticola* by invert plate technique

| Sl. No | Isolate name | Colony growth* (mm) | Per cent mycelial inhibition* |
|-----------------|--|---------------------|-------------------------------|
| 1 | <i>Bacillus cereus</i> | 44.30 | 50.78 (45.44) |
| 2 | <i>Bacillus velezensis</i> | 36.85 | 59.06 (50.21) |
| 3 | <i>Bacillus subtilis</i> | 44.68 | 50.36 (45.20) |
| 4 | <i>Bacillus subtilis</i> sub sp. <i>subtilis</i> | 49.03 | 45.53 (42.43) |
| Control | | 90.00 | 0.00 (0.00) |
| S. Em. ± | | - | 0.88 |
| CD at 1% | | - | 3.68 |

*Mean of three replications, Figures in parentheses are arc sine transformed values

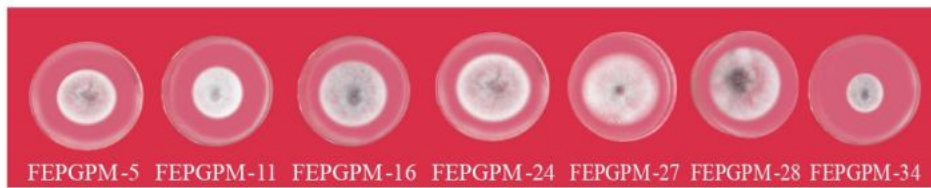


Plate 19: Mycelial inhibition of *R. bataticola* by endophytic fungal PGPMs indicating production of bio-active compounds

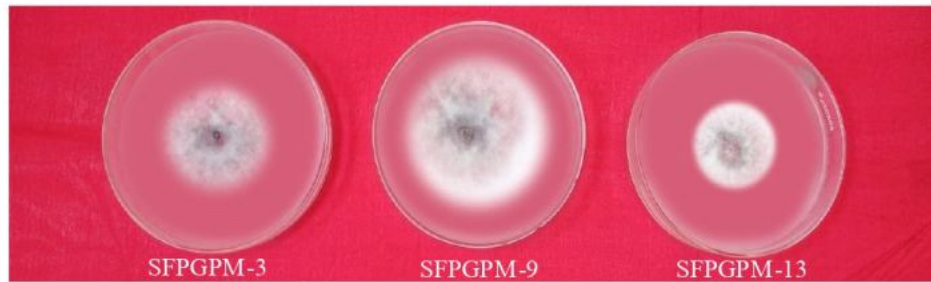


Plate 20: Mycelial inhibition of *R. bataticola* by rhizospheric fungal PGPMs indicating production of bio-active compounds

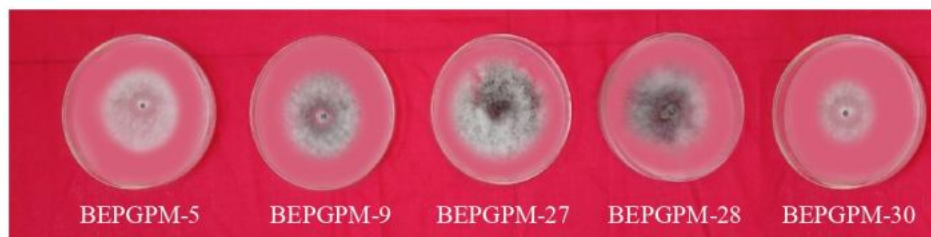


Plate 21: Mycelial inhibition of *R. bataticola* by endophytic bacterial PGPMs indicating production of bio-active compounds

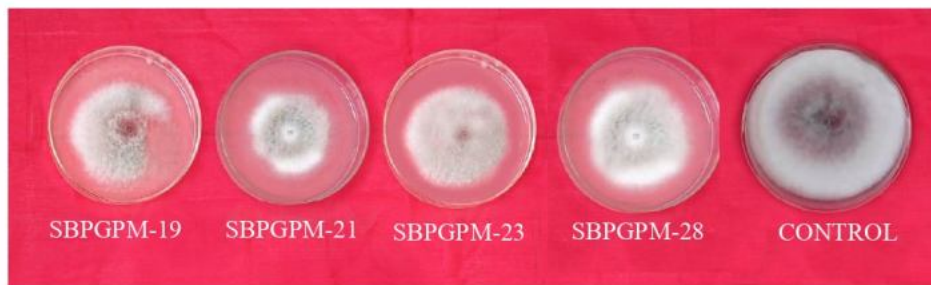


Plate 22: Mycelial inhibition of *R. bataticola* by rhizospheric bacterial PGPMs indicating production of bio-active compounds

indicated the highest capacity of isolates for the production of bio active compounds. Further, these four isolates were used for extraction and identification of bio-active compounds.

4.5 Extraction, identification of bio-active compounds and screening of crude extract from potential PGPMs responsible for inhibition of *Rhizoctonia bataticola*

The four isolates, *T. asperellum*, *T. harzianum*, *B. subtilis* and *B. velezensis* which showed maximum inhibition in the inverted plate technique were used for the extraction and identification of bio-active compounds responsible for inhibition of *R. bataticola*.

4.5.1. Extraction of bio-active compounds from fungal PGPMs

Fungal PGPMs have been reported to potential for production of an array of bio-active metabolites with antifungal properties which enables the host plants to meet possible challenges generated by phytopathogenic fungi. Hence, an effort was made to identify the bio-active compounds from one endophytic *T. asperellum* and one rhizospheric fungal PGPM *T. harzianum* each of which showed highest per cent inhibition in inverted plate technique into ethyl acetate (EtOAc) at the ratio of 1:1 (v/v) by solvent extraction method and putative identification was done with the aid of GC-MS/MS analysis.

4.5.1.1. Endophytic fungal PGPMs

The GC-MS/ MS results of ethyl acetate extract of *T. asperellum* showed the presence of 65 compounds at different retention time ranging from 3.166 to 30.91 min. The mass to charge (m/z) ratio of compounds ranging from 42.7 to 281.8 (Table 23). The chromatogram of compounds showed different peaks (Fig. 10). Among 65 compounds identified, thirteen compounds namely, D- Alanine, 2-Decanol, Butanal 3-methyl, Butanol 3- hydroxy, 1,2-benzenediol,4-Methyl-, Propanamide 2-hydroxy, Boronic acid, Phthalic acid, 1 iso-Propyl-3,6-diazahomoadamantan-9-ol,1-Adamantylamine and 2-Adamantylamine have antimicrobial properties (Table 24). Hence these compounds were responsible for the inhibition of the pathogen and might process antifungal activity against the *R. bataticola*. Among thirteen bio-active compounds Butanal, 3-hydroxy- was

**Table 23: List of compounds identified in acetone extract of fungal endophyte
asperellum in GC-MS/MS analysis**

T.

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|--|--|--------|-----------|--------|
| 1 | C ₄ H ₈ O ₂ | Butanal, 3-hydroxy- | 3.166 | 42.7 | 106059 |
| 2 | C ₃ H ₇ NO ₂ | Propanamide, 2-hydroxy- | 3.259 | 44.6 | 5792 |
| 3 | C ₄ H ₈ O ₂ | Ethyl Acetate | 3.323 | 43.6 | 16507 |
| 4 | C ₁₁ H ₂₁ ClO ₂ | 6-Chlorohexanoic acid, 3-methylbut-2-yl ester | 3.557 | 43.6 | 8179 |
| 5 | C ₃ H ₇ NO ₂ | D-Alanine | 3.878 | 43.6 | 3047 |
| 6 | C ₄ H ₁₀ O ₃ | 1,2,3-Butanetriol | 4.739 | 43.6 | 3734 |
| 7 | C ₂ H ₇ N | Dimethylamine | 5.657 | 43.6 | 2577 |
| 8 | C ₁₀ H ₂₂ O | 2-Decanol | 6.154 | 43.6 | 19531 |
| 9 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 6.378 | 43.7 | 2542 |
| 10 | C ₅ H ₁₀ O | Butanal, 3-methyl- | 6.398 | 44.6 | 3662 |
| 11 | C ₂₄ H ₄₅ NS | Ethylamine, N,N-dinonyl-2-(2thiophenyl)- | 6.465 | 281.6 | 2326 |
| 12 | C ₆ H ₇ NO | Piconol | 7.252 | 108.6 | 31999 |
| 13 | C ₇ H ₈ O ₂ | 1,2-Benzenediol, 4-methyl- | 8.422 | 77.6 | 15548 |
| 14 | C ₃ H ₇ NO ₂ | Propanamide, 2-hydroxy- | 9.517 | 44.6 | 2688 |
| 15 | C ₇ H ₁₅ NO | 5-Aziridinopentanol | 10.443 | 43.6 | 49562 |
| 16 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 10.586 | 44.6 | 8071 |
| 17 | C ₂₄ H ₄₅ NS | Ethylamine, N,N-dinonyl-2-(2thiophenyl)- | 11.143 | 281.8 | 6738 |
| 18 | C ₉ H ₁₃ NO | Benzyl alcohol, 3-ethylamino- | 12.051 | 135.8 | 56454 |
| 19 | C ₁₁ H ₁₂ O ₄ | 1-(3,4-methylenedioxyphenyl)-1-methoxypropan-2-one | 13.009 | 164.7 | 63829 |
| 20 | C ₁₉ H ₄₁ N | 2-Aminononadecane | 13.358 | 44.5 | 3951 |
| 21 | C ₆ H ₁₄ N ₂ | N-[4-Aminobutyl]aziridine | 13.458 | 69.6 | 55210 |
| 22 | C ₁₉ H ₄₁ N | 2-Aminononadecane | 13.559 | 44.5 | 5721 |
| 23 | C ₅ H ₁₂ O ₄ | 1-Deoxy-d-arabitol | 13.853 | 44.5 | 2245 |
| 24 | C ₅ H ₁₂ O | 2-Butanol, 3-methyl- | 14.198 | 44.6 | 2514 |
| 25 | C ₇ H ₁₆ O | 2-Hexanol, 3-methyl- | 14.546 | 44.6 | 3967 |
| 26 | C ₁₀ H ₁₄ BeO ₄ | Beryllium, bis(2,4-pentanedionatoO,O)-, (T-4)- | 15.097 | 191.9 | 83715 |
| 27 | C ₁₄ H ₁₉ NO ₅ | 2-(2,6-Dimethoxybenzoylamino)propionic acid, ethyl ester | 15.318 | 164.6 | 4863 |
| 28 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 15.687 | 44.6 | 2150 |

Contd.....

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|--|--|--------|-----------|-------|
| 29 | C ₇ H ₁₅ N | Nanofin | 16.256 | 69.6 | 43842 |
| 30 | C ₂ H ₇ BO ₂ | Boronic acid, ethyl- | 16.362 | 44.6 | 4536 |
| 31 | C ₁₁ H ₂₃ N ₅ OSi | Desethyl terbumeton, TMS derivative | 17.722 | 44.5 | 4177 |
| 32 | C ₁₁ H ₂₃ NOSi | 4-Isobutyl-2-Pyrrolidione 1-TMS Deriv | 18.44 | 44.5 | 3790 |
| 33 | C ₃ H ₇ NO ₂ | Alanine | 18.825 | 44.6 | 2298 |
| 34 | C ₂ H ₇ BO ₂ | Boronic acid, ethyl- | 18.925 | 44.6 | 2921 |
| 35 | C ₁₄ H ₁₆ N ₂ | Benzenamine, 4,4'-(1,2-ethanediy)bis- | 19.103 | 105.6 | 16958 |
| 36 | C ₁₀ H ₂₂ O | Methyl nonyl ether | 19.531 | 44.6 | 25523 |
| 37 | C ₂₀ H ₃₀ O ₅ | Phthalic acid, 2-(2-methoxyethyl)hexyl propyl ester | 20.87 | 44.5 | 6987 |
| 38 | C ₉ H ₂₀ O | 2-Octanol, 3-methyl- | 21.975 | 44.6 | 4110 |
| 39 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 22.503 | 149.7 | 3193 |
| 40 | C ₁₀ H ₂₂ O | Methyl nonyl ether | 23.005 | 44.7 | 2163 |
| 41 | C ₁₀ H ₂₂ O | Methyl nonyl ether | 23.02 | 44.7 | 8592 |
| 42 | C ₁₁ H ₁₂ O ₄ | 3,4-Dimethoxycinnamic acid | 23.42 | 44.5 | 3640 |
| 43 | C ₂₀ H ₄₃ N | 2-Butylamine, N-hexadecyl- | 23.936 | 44.5 | 3077 |
| 44 | C ₁₀ H ₁₇ N ₅ OSi | 6-(2-Trimethylsilylethoxy)-9H-purin-2ylamine | 24.228 | 44.6 | 2347 |
| 45 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 24.585 | 44.6 | 2347 |
| 46 | C ₂₀ H ₃₂ N ₂ Si ₂ | o-Tolidine, N,N'-bis(trimethylsilyl)- | 25.564 | 207.7 | 2538 |
| 47 | C ₁₂ H ₂₀ N ₂ O | 1-iso-Propyl-3,6-diazahomoadamantan-9-one | 25.838 | 57.6 | 14258 |
| 48 | C ₅ H ₁₀ O ₄ | 1,2,3,4-Cyclopent anet etrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)- | 25.863 | 44.6 | 8011 |
| 49 | C ₆ H ₁₂ N ₂ O ₆ | Hexamethylenetriperoxidediamine | 25.881 | 73.5 | 7928 |
| 50 | C ₁₃ H ₂₈ O | 2-Tridecanol | 26.489 | 44.6 | 2638 |
| 51 | C ₁₉ H ₄₁ N | 1-Octadecanamine, N-methyl- | 26.529 | 44.6 | 2228 |
| 52 | C ₈ H ₁₈ O | 2-Heptanol, 6-methyl- | 26.706 | 44.6 | 2186 |
| 53 | C ₁₀ H ₂₈ O ₄ Si ₃ | Silicic acid, diethyl bis(trimethylsilyl) ester | 27.758 | 44.6 | 3084 |
| 54 | C ₁₉ H ₄₁ N | Ethylamine, N-methyl-N-hexadecyl- | 28.05 | 44.6 | 4336 |

Contd.....

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|---|---|--------|-----------|-------|
| 55 | C ₁₉ H ₂₅ N ₃ OSi | 2-(2-tert-Butyldimethylsilyloxy-5methylphenyl)benzotriazole | 28.077 | 44.5 | 2451 |
| 56 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 28.195 | 57.6 | 11029 |
| 57 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 28.384 | 207.7 | 4325 |
| 58 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 28.469 | 44.6 | 2131 |
| 59 | C ₁₃ H ₂₂ N ₂ O | Propanenitrile, 3-(5-diethylamino-1methyl-3-pentyloxy)- | 28.614 | 44.6 | 2219 |
| 60 | C ₁₂ H ₁₆ O ₃ | Ethanol, 2-[4-vinyl-2-methoxy-6methyl]phenoxy- | 29.24 | 44.6 | 2547 |
| 61 | C ₁₂ H ₂₅ B ₂ NO ₂ | Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]- | 29.42 | 44.5 | 3048 |
| 62 | C ₁₁ H ₂₃ N ₅ OSi ₂ | 4,6-Diamino-5-formamidopyrimidine, N,N'-bis(trimethylsilyl)- | 30.41 | 207.6 | 2500 |
| 63 | C ₁₆ H ₃₁ NSi | 1-Adamantylamine, N-tert butyldimethylsilyl- | 30.69 | 207.6 | 2260 |
| 64 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 30.86 | 207.7 | 3271 |
| 65 | C ₁₆ H ₂₉ NO ₃ | Perhydrohistrionicotoxin, 2-depentyloxy-, methoxyformate(ester) | 30.91 | 207.7 | 2443 |

Table 24: Bio-active compounds with anti-microbial property identified in acetone extract of fungal endophyte *T. asperellum* in GC-MS/MS

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|--|---|--------|-----------|--------|
| 1 | C ₄ H ₈ O ₂ | Butanal, 3-hydroxy- | 3.166 | 42.7 | 106059 |
| 2 | C ₃ H ₇ NO ₂ | D-Alanine | 3.878 | 43.6 | 3047 |
| 3 | C ₁₀ H ₂₂ O | 2-Decanol | 6.154 | 43.6 | 19531 |
| 4 | C ₅ H ₁₀ O | Butanal, 3-methyl- | 6.398 | 44.6 | 3662 |
| 5 | C ₇ H ₈ O ₂ | 1,2-Benzenediol, 4-methyl- | 8.422 | 77.6 | 15548 |
| 6 | C ₃ H ₇ NO ₂ | Propanamide, 2-hydroxy- | 9.517 | 44.6 | 2688 |
| 7 | C ₂ H ₇ BO ₂ | Boronic acid, ethyl- | 16.362 | 44.6 | 4536 |
| 8 | C ₂₀ H ₃₀ O ₅ | Phthalic acid, 2-(2-methoxyethyl)hexyl propyl ester | 20.87 | 44.5 | 6987 |
| 9 | C ₁₂ H ₂₀ N ₂ O | 1-iso-Propyl-3,6-diazahomoadamantan- 9-one | 25.838 | 57.6 | 14258 |
| 10 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 28.384 | 207.7 | 4325 |
| 11 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 28.469 | 44.6 | 2131 |
| 12 | C ₁₆ H ₃₁ NSi | 1-Adamantylamine, N-tertbutyldimethylsilyl- | 30.69 | 207.6 | 2260 |
| 13 | C ₁₆ H ₃₁ NSi | 2-Adamantylamine, TBDMS derivative | 30.86 | 207.7 | 3271 |

found with maximum area of 106059 and retention time of 3.166 as compared to other compounds found (Fig. 11). The structure of compound is shown in Fig. 12.

The thirteen compounds which showed anti microbial property were reported by the previous studies conducted by Marcone *et al.* (2019) who proved the antibacterial activity of D-amino acid oxidase against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli*) bacteria. D-Alanine is a D-amino acid oxidase substrate (DAAO). The DAAO inhibited the growth of all the strains tested only when D-alanine was present in the medium. They reported that antibacterial activity was due to hydrogen peroxide production. 2-Decanol is a long-chain fatty alcohol. Togashi *et al.* (2007) investigated the antibacterial activity against *Staphylococcus aureus* of long-chain fatty alcohols with a focus on normal alcohols. The results declared that 1-Nonanol, 1-decanol and 1-undecanol had bactericidal activity and membrane-damaging activity. Al-fatimi (2018) reported that *O. majorana* extract containing five volatile non terpenoid compounds: 2-methylpropanal, 2-methyl, butanal, 3-methyl, butanal 3-hydroxy, pentanal and methyl 2-methyl butanoate showed potent antimicrobial and antioxidant activities. Further compound 4-Methyl-1,2-benzenediol also used in the synthesis of antibacterial agents and antioxidants.

Guy *et al.* (2019) reported that boronic acids have antimicrobial property for pathogen-selective killing of *Mycobacterium tuberculosis* by targeting extracellular glycans. Osuntokun and Cristina (2019) have conducted study on chemical identification, antimicrobial and synergistic efficacy of extracted essential oils from ethyl acetate extract of *Spondias mombin*. The structures of the isolated compounds were determined by using data obtained from GC-MS spectrum. The compound isolated include Aspidofractinine-3-methanol, Phthalic acid, 2-ethylhexyl tetradecyl ester, Phthalic acid, di (2-propylpentyl ester), (9-(2', 2'-Dimethylpropanoilhydrazono)-3, 6-dichloro-2,7- bis-[2-(diethylamino)-ethoxy]fluorine) and Tere phthalic acid, dodecyl 2-ethylhexyl ester. These compounds have individual and synergistic activity against Gram negative (*E. coli*), Gram-positive (*B. subtilis*) and (*A. flavus*) at 10, 5, 2.5 and 1.25 g/mL. Al-Garaawi (2019) identified thirty four bioactive phytochemical compounds of *Cyperus alternifolius* L in GCMS analysis. The identification of phytochemical compounds was based on the retention time, peak area, MS Fragmentation and molecular weight. As in the present investigation, *Cyperus alternifolius* also showed the presence of the Phthalic acid and 1-Propyl-3,6-

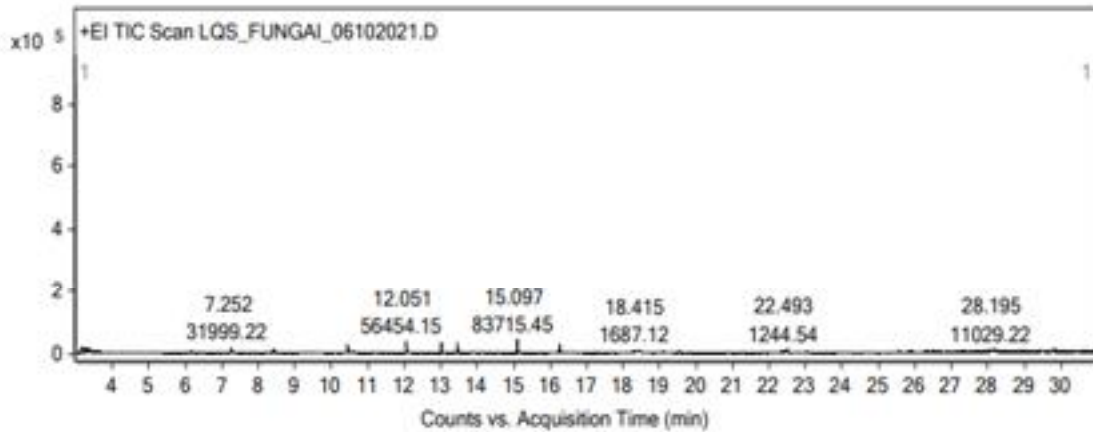


Fig. 10: Chromatogram of compounds of *T. asperellum* extract obtained from GC-MS/MS analysis

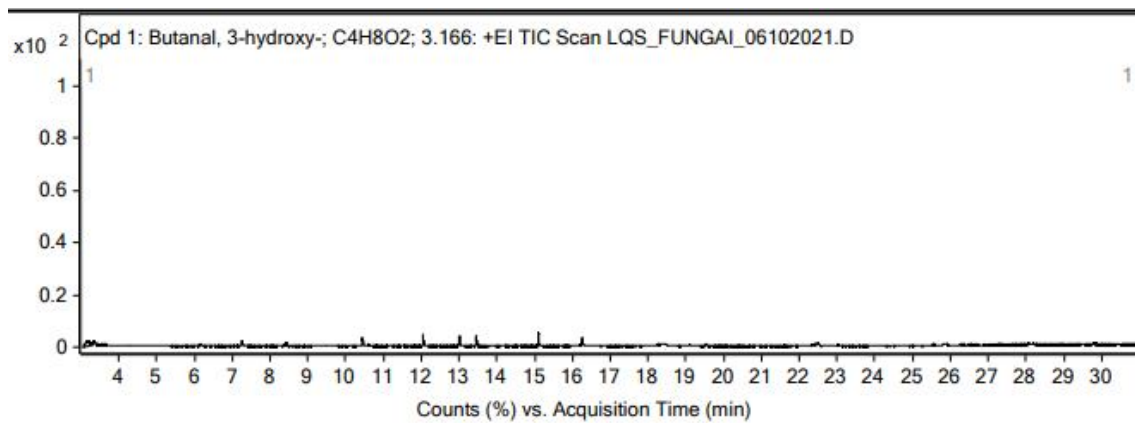


Fig. 11: Mass spectrum of Butanal, 3-hydroxy- obtained from *T. asperellum* in GC-MS/MS analysis

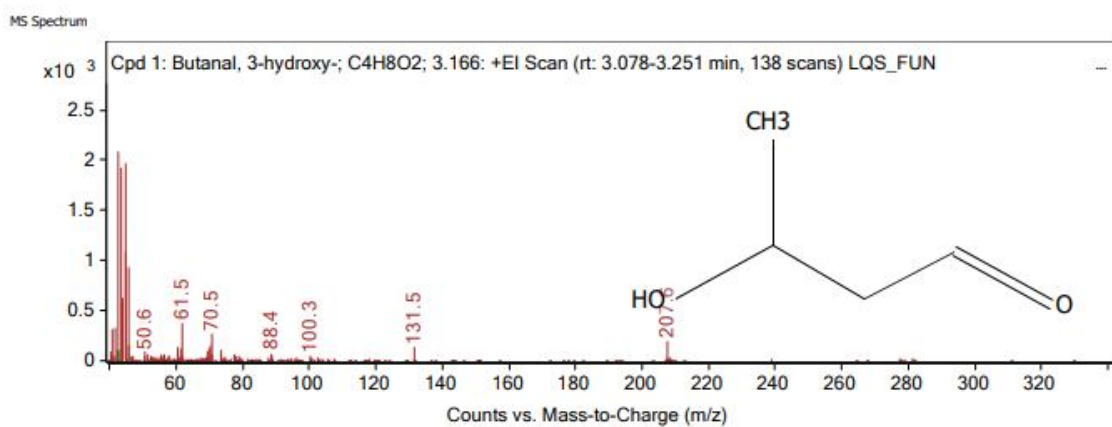


Fig. 12: Mass fragmentation and structure of Butanal, 3-hydroxy- obtained from *T. asperellum* in GC-MS/MS analysis

diazahomoadamantan-9-ol among thirty four compounds. The chemical constitutions were useful as antimicrobial and antifungal activity. Adamantanamine derivatives (1-Adamantylamine and 2-Adamantylamine) also recorded to have antimicrobial activities (Magarian and Sorenson, 1976).

Similar work was carried out by Ranjana (2020) who subjected the ethyl acetate extract of *T.asperellum* dissolved in acetone to gas chromatography-tandem mass spectrometry (GC-MS) analysis. The GC-MS analysis revealed that the extract showed the presence of 29 compounds at different retention time ranging from 4.910 to 21.868 min. The mass to charge (m/z) ratio of compounds ranged from 43 to 190. The chromatogram of compounds showed different peaks. The compound 2-Imidazol-1-ylmethyl-pyridine 1-oxide (C₉H₉N₃O) was found with maximum area of 14375440 and retention time of 19.842 as compared to other compounds found. Likewise, in the present investigation, Butanal, 3-hydroxy- was found with maximum area of 106059 and retention time of 3.166 as compared to other compounds found. Nitish and Kumar (2017) also characterized volatile secondary metabolites from the culture filtrate of *Trichoderma asperellum* strain using Gas chromatography-Mass spectrometry (GC-MS). Results of GC-MS detected 43 secondary metabolites in the *T. asperellum* strain including many important volatile secondary metabolites such as 1,2-Benzenedicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester (peak area - 3.59 %), 1,2-Benzenedicarboxylic acid dibutyl ester (peak area - 2.02 %), 2H-Pyran-2-one (peak area - 66.63 %), palmitic acid (peak area - 2.86 %), several phenolic isomers, methyl cyclohexane *etc.*, all reportedly having effective pesticidal activity. These reports confirmed the findings of present investigation.

4.5.1.2 Rhizospheric fungal PGPMs

After solvent extraction method, the ethyl acetate extract of *T. harzianum* dissolved in acetone was subjected to GC-MS analysis. The GC-MS analysis revealed that the extract showed the presence of 62 compounds at different retention time ranging from 3.464 to 29.818 min. The mass to charge (m/z) ratio of the compounds were ranging from 43.6 to 191.9 (Table 25). The chromatogram of compounds showed different peaks (Fig. 13). However, among 62 compounds, 20 compounds have antimicrobial property based the previous reports such as Pyridine, 3-ethyl (Marinescu and Popa, 2022), Butane-2-one, 3-methyl-3-(2-oxopropylamino)- (Al-Wathnani *et al.*, 2012), 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- and 1,3-Propanediamine, N,N-diethyl-N'-methyl- (Culler *et al.*,

Table 25: List of compounds identified in acetone extract of rhizospheric fungi *T. harzianum* in GC-MS/MS analysis

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|---|---|--------|-----------|---------|
| 1 | C ₇ H ₉ N | Pyridine, 3-ethyl- | 3.464 | 91.7 | 747799 |
| 2 | C ₆ H ₁₆ N ₄ | Diazene, [1-(2,2-dimethylhydrazino)ethyl]ethyl- | 4.485 | 43.7 | 119646 |
| 3 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 5.269 | 57.6 | 35732 |
| 4 | C ₈ H ₁₅ NO ₂ | Butane-2-one, 3-methyl-3-(2oxopropylamino)- | 5.453 | 43.6 | 57446 |
| 5 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 6.392 | 57.7 | 118934 |
| 6 | C ₆ H ₁₄ Si | 1-Methylsilacyclohexane | 6.64 | 71.8 | 344970 |
| 7 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 6.746 | 57.7 | 405863 |
| 8 | C ₆ H ₁₄ O ₃ | 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- | 6.952 | 56.6 | 98983 |
| 9 | C ₇ H ₁₄ N ₂ O | Azetidin-2-one 3,3-dimethyl-4-(1aminoethyl)- | 7.283 | 57.6 | 55232 |
| 10 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 8.307 | 69.7 | 557294 |
| 11 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 8.394 | 69.8 | 590238 |
| 12 | C ₈ H ₂₀ N ₂ O | Octan-8-ol, 2,5-diaza-2,5-dimethyl- | 8.606 | 57.8 | 485993 |
| 13 | C ₁₉ H ₄₀ O | Dodecyl heptyl ether | 10.442 | 43.7 | 47552 |
| 14 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 10.586 | 57.8 | 586563 |
| 15 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 10.825 | 43.6 | 42940 |
| 16 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 11.179 | 71.7 | 134025 |
| 17 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 11.315 | 71.8 | 251053 |
| 18 | C ₁₈ H ₃₆ O ₃ | Carbonic acid, 2-ethylhexyl nonyl ester | 11.383 | 70.6 | 70937 |
| 19 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 11.423 | 71.7 | 154749 |
| 20 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 11.961 | 83.7 | 131890 |
| 21 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 12.255 | 69.8 | 683367 |
| 22 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 12.38 | 69.8 | 1092957 |
| 23 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 12.5 | 69.8 | 828295 |
| 24 | C ₁₂ H ₂₅ N | 1-(1-Azacyclopropyl)decane | 13.455 | 83.6 | 61798 |
| 25 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 13.561 | 57.8 | 199803 |

Contd.....

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|--|--|--------|-----------|---------|
| 26 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.132 | 71.6 | 46345 |
| 27 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.27 | 71.7 | 104595 |
| 28 | C ₂₁ H ₂₅ NO ₂ | Piperidolate | 14.394 | 95.7 | 857400 |
| 29 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 14.513 | 71.7 | 201921 |
| 30 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 14.648 | 57.7 | 251676 |
| 31 | C ₇ H ₁₈ N ₂ | 1,3-Propanediamine, N,N-diethyl- | 14.779 | 71.7 | 86859 |
| 32 | C ₁₀ H ₁₄ BeO ₄ | Beryllium, bis(2,4-pentanedionatoO,O')-, (T-4)- | 15.1 | 191.9 | 1105991 |
| 33 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.26 | 69.6 | 184031 |
| 34 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.396 | 69.7 | 271943 |
| 35 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.512 | 69.6 | 243345 |
| 36 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.659 | 69.7 | 304850 |
| 37 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.817 | 69.7 | 161868 |
| 38 | C ₁₂ H ₂₅ N | 1-(1-Azacyclopropyl)decane | 16.257 | 55.6 | 61652 |
| 39 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 16.368 | 57.6 | 45906 |
| 40 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.581 | 57.6 | 38609 |
| 41 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.733 | 71.6 | 74039 |
| 42 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 17.857 | 57.6 | 42770 |
| 43 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.9 | 71.6 | 38481 |
| 44 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 18.265 | 71.7 | 37961 |
| 45 | C ₁₄ H ₂₇ NO | Acrylamide, N-undecyl- | 18.627 | 69.6 | 55361 |
| 46 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 18.784 | 69.6 | 157388 |
| 47 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 18.927 | 69.6 | 166704 |
| 48 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 19.11 | 69.7 | 295721 |
| 49 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.256 | 69.6 | 217322 |

Contd.....

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|--|---|--------|-----------|--------|
| 50 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.479 | 69.6 | 197288 |
| 51 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.68 | 69.7 | 170545 |
| 52 | C ₁₂ H ₁₅ NO ₄ | 4-Nitrobenzoic acid, 3-pentyl ester | 20.864 | 149.7 | 38786 |
| 53 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 21.676 | 149.8 | 318883 |
| 54 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 22.049 | 149.6 | 81814 |
| 55 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 22.504 | 149.8 | 362356 |
| 56 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 22.876 | 149.8 | 265714 |
| 57 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 23.365 | 149.6 | 40472 |
| 58 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 24.165 | 149.7 | 89684 |
| 59 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 25.891 | 57.6 | 33436 |
| 60 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 26.45 | 69.6 | 49962 |
| 61 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 27.272 | 57.6 | 51901 |
| 62 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 29.818 | 57.6 | 46015 |

Table 26: Bio-active compounds with anti microbial property identified in acetone extract of rhizospheric fungi *T. harzianum* in GC-MS/MS analysis

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|--|--|--------|-----------|--------|
| 1 | C ₇ H ₉ N | Pyridine, 3-ethyl- | 3.464 | 91.7 | 747799 |
| 2 | C ₈ H ₁₅ NO ₂ | Butane-2-one, 3-methyl-3-(2oxopropylamino)- | 5.453 | 43.6 | 57446 |
| 3 | C ₆ H ₁₄ O ₃ | 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- | 6.952 | 56.6 | 98983 |
| 4 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 10.586 | 57.8 | 586563 |
| 5 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 10.825 | 43.6 | 42940 |
| 6 | C ₁₈ H ₃₆ O ₃ | Carbonic acid, 2-ethylhexyl nonyl ester | 11.383 | 70.6 | 70937 |
| 7 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.132 | 71.6 | 46345 |
| 8 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.27 | 71.7 | 104595 |
| 9 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 16.368 | 57.6 | 45906 |
| 10 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.581 | 57.6 | 38609 |
| 11 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.733 | 71.6 | 74039 |
| 12 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.9 | 71.6 | 38481 |
| 13 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 18.265 | 71.7 | 37961 |
| 14 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 18.927 | 69.6 | 166704 |
| 15 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.256 | 69.6 | 217322 |
| 16 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.479 | 69.6 | 197288 |
| 17 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.68 | 69.7 | 170545 |
| 18 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 26.45 | 69.6 | 49962 |
| 19 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 27.272 | 57.6 | 51901 |
| 20 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 29.818 | 57.6 | 46015 |

1979), Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)- (Bhalla *et al.*, 2021), Carbonic acid, 2-ethylhexyl nonyl ester (Liu *et al.*, 2018), 4-Nitrobenzoic acid, 3-pentyl ester (Chowdhury *et al.* 2017), N- [Dimethylaminomethyl] aziridine and N-[3-[N-Aziridyl]propyl]-3-dimethylaminopropylamine (Kowalczyk *et al.*, 2017) (Table 26). Hence, these compounds are involved in the defence mechanism against *R. bataticola*. Among 20 compounds, Pyridine, 3-ethyl- was found with maximum area of 747799 and retention time of 3.464 as compared to other compounds found (Fig. 14). The structure of compound is shown in Fig. 15.

The compound N- [Dimethylaminomethyl] aziridine and N-[3-[N-Aziridyl]propyl]-3-dimethylaminopropylamine identified in the present investigation, belongs to azirididine group of chemical compounds. Aziridine-containing compounds possess many biological activities especially antitumor and antibacterial ones, due to the presence of the aziridine ring. These compounds were screened for their *in vitro* antimicrobial activity against a panel of Gram-positive and Gram-negative strains of bacteria and were appeared to be promising agents against reference strains of *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermid* (Kowalczyk *et al.*, 2017). The results are also in accordance with Avinash (2017) who screened thirty five isolates of *Trichoderma* species for production of volatile compounds, two isolates *viz.*, Tri-4 (*T. viridae*) and Tri-12 (*T. hamatum*) performed better under *in vitro* against major fungal pathogens *viz.*, *F. oxysporum* f. sp. *udum*, *R. bataticola*, *S. rolfsii* and *A. alternata* and extracted and characterized secondary metabolites from isolate Tri-4 (*T. viridae*), LCMS result confirmed the presence of antifungal metabolites *viz.*, ferulic acid (C₁₀H₁₀O₄) at 195.11m/z, harzianic acid (C₁₉H₂₇NO₆) at 366.31 and viridofungin A (C₃₁H₄₆O₁₀N) at 592.31 with retention time of 1.94, 3.46 and 3.15 min respectively. Similarly, the secondary metabolites from culture filtrate and mycelial mass of potential isolates of *T. viride* (IARI P1 and IARI P2), *T. virens* (IARI P3) and *T. harzianum* (IARI P4) were extracted by solvent extraction and soxhlet water bath distillation methods and were chemically characterized by GC-MS/MS. The compounds observed were, 6-nonylene alcohol, massoilactone, methyl-cyclopentane, methyl cyclohexane, N-methyl pyrrolidine, dermadin, ketotriol, koningin-A, 3-methyl-heptadecanol, 2-methyl heptadecanol, palmitic acid, 3-(2'-hydroxypropyl)-4-(hexa-2'- 4-dineyl) -2- (5H)-furanone and 3-(propenone)- 4-(hexa-2'-4'-dineyl)- 2-(5H)-furanone (Dubey *et al.*, 2011).

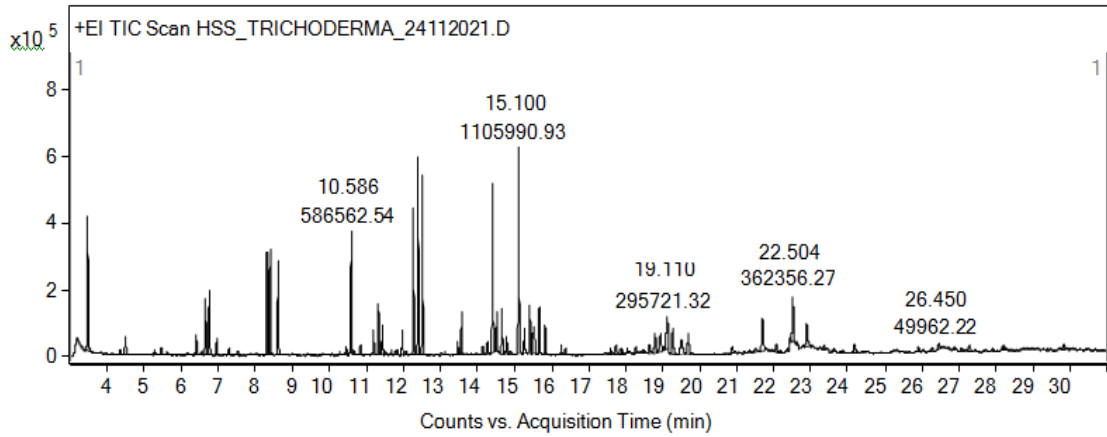


Fig 13: Chromatogram of compounds of *T. harzianum* extract obtained from GC-MS/MS analysis

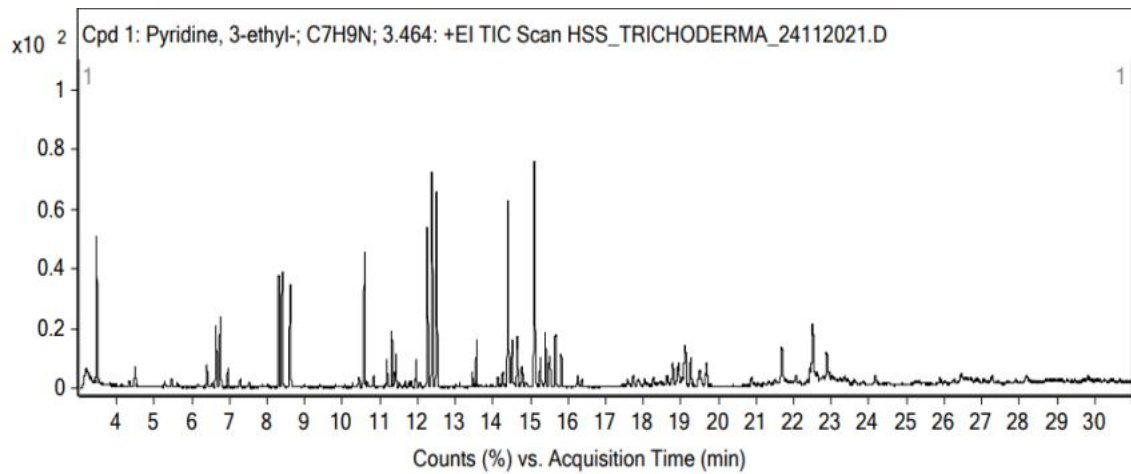


Fig. 14: Mass spectrum of Pyridine, 3-ethyl- obtained from *T. harzianum* in GC-MS/MS analysis

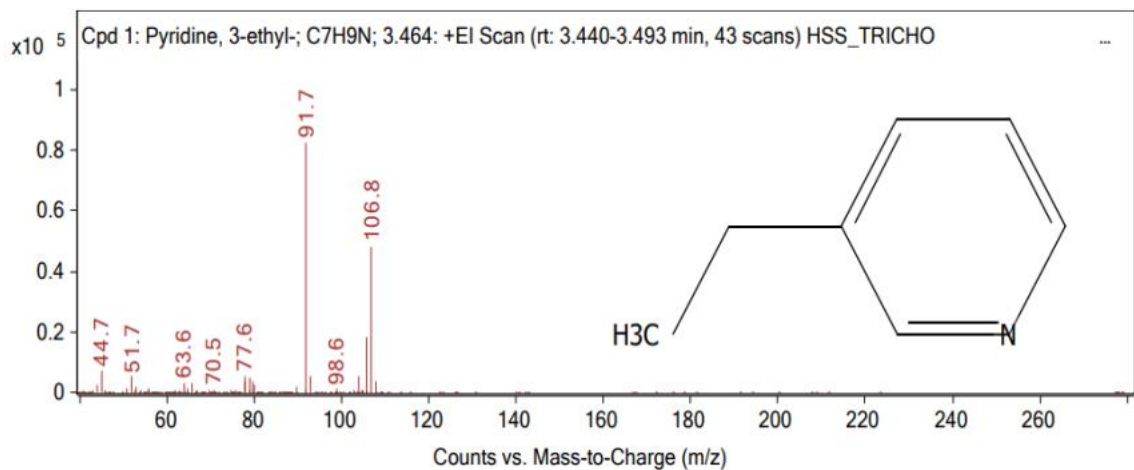


Fig. 15: Mass fragmentation and structure of Pyridine, 3-ethyl- obtained from *T. harzianum* in GC-MS/MS analysis

4.5.2 Extraction of bio-active compounds from bacterial PGPMs

An attempt was made to identify the bio-active compounds produced from one endophytic bacteria *B. subtilis* and one rhizospheric bacterial PGPM *B. velezensis* by using solvent extraction method into ethyl acetate. The ethyl acetate extract of dissolved in methanol: chloroform (2:2) was subjected to gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis.

4.5.2.1 Endophytic bacterial PGPMs

Endophytic bacteria belong to various genera and many of the biological active substances extracted from endophytic bacteria are reported to be novel with various bioactivities (Golinska *et al.*, 2015). The GC-MS/MS analysis revealed that the extract of *Bacillus subtilis* showed the presence of 60 compounds at different retention time ranging from 3.139 to 29.737. The mass to charge (m/z) ratio of the compounds were ranging from 45.8 and 205.9 (Table 27). The chromatogram of compounds showed different peaks (Fig. 16). Among them 9 compounds such as 2,3-butanediol, 2-pyridinecarboxylic acid, Benzamide, N,N-dimethyl-, Benzenamine, 4-methoxy-3,5- dimethyl-, 1,2-Benzenedicarboxylic acid, Pyridine, 3-butyl-, Acetamide, N-butyl-N-propyl-, Octanoic acid and Succinic acid have antimicrobial properties as recorded in the previous reports (Table 28). Hence they may exhibited the antifungal activity against pathogen. Among 9 bio-active compounds, 2, 3-Butanediol, dinitrate was found with maximum area of 1581999 and retention time of 3.33 as compared to other compounds found (Fig. 17). The structure of compound is shown in Fig. 18.

The compound 2, 3-butanediol obtained in the present investigation, is well-characterized bacterial volatile, produced by the rhizobacterium *B. subtilis*. Pepper roots treated with 2,3-butanediol suppressed growth of the soil-borne pathogen *R. solanacearum*. Hence it was proved that 2,3-butanediol has antimicrobial property (Yi *et al.*, 2016). The compound 2-pyridinecarboxylic acid also referred as picolinic acid. Oladipo *et al.* (2013) tested the antibacterial properties of picolinic acid against selected test organisms. The results obtained established that picolinic acid has antibacterial activities against *S. marcescens*, *K. pneumoniae*, *E. coli*, *S. flexneri*, *B. cereus*, *P. vulgaris*, *M. luteus* with minimum inhibitory concentration of 0.5 mg/mL. It also had great antibacterial activity against *P. mirabilis* at minimum inhibitory concentration of 1.5

Table 27: List of compounds identified in methanol chloroform extract of bacterial endophyte *Bacillus subtilis* in GC-MS/MS analysis

| Sl. No. | Formula | Name | RT | m/z ratio | Area |
|---------|---|---|--------|-----------|---------|
| 1 | C ₅ H ₁₃ NO ₂ | Ethanamine, 2,2-dimethoxy-N-methyl- | 3.139 | 45.8 | 367976 |
| 2 | C ₄ H ₈ N ₂ O ₆ | 2,3-Butanediol, dinitrate | 3.33 | 45.8 | 1581999 |
| 3 | C ₆ H ₇ NO | Piconol | 7.274 | 108.7 | 263657 |
| 4 | C ₆ H ₇ NO | 1H-Pyrrole-2-carboxaldehyde, 1-methyl- | 8.234 | 107.7 | 79433 |
| 5 | C ₇ H ₇ NO ₂ | 2-Pyridinecarboxylic acid, 6-methyl- | 9.004 | 91.7 | 78078 |
| 6 | C ₅ H ₁₄ N ₂ O | 1-(2-Aminoethylamino)-2-propanol | 9.155 | 69.7 | 220857 |
| 7 | C ₆ H ₅ NO ₂ | 4-Pyridinecarboxylic acid | 9.947 | 105.7 | 221707 |
| 8 | C ₉ H ₇ N | Benzonitrile, 4-ethenyl- | 10.277 | 128.8 | 546635 |
| 9 | C ₇ H ₁₅ NO | 5-Aziridinopentanol | 10.454 | 55.7 | 364148 |
| 10 | C ₇ H ₇ NO ₂ | 3-Pyridineacetic acid | 11.439 | 91.8 | 2066216 |
| 11 | C ₉ H ₁₈ O ₂ | 2,2-Diisopropyl-1,3-dioxolane | 11.532 | 114.8 | 230486 |
| 12 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 11.872 | 71.8 | 353907 |
| 13 | C ₉ H ₁₉ NO | Acetamide, N-butyl-N-propyl- | 12.001 | 71.6 | 70965 |
| 14 | C ₉ H ₁₃ NO | Benzenamine, 4-methoxy-3,5-dimethyl- | 12.08 | 135.7 | 149101 |
| 15 | C ₉ H ₁₁ NO | Benzamide, N,N-dimethyl- | 12.389 | 104.7 | 687695 |
| 16 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 12.551 | 57.7 | 209722 |
| 17 | C ₈ H ₉ NO ₂ | Pyridine-2-propionic acid | 12.691 | 91.8 | 1481864 |
| 18 | C ₉ H ₁₁ NO ₂ | Benzene, 1-amino-2-acetyl-4-methoxy- | 13.014 | 164.7 | 69544 |
| 19 | C ₆ H ₁₄ N ₂ | N-[4-Aminobutyl]aziridine | 13.46 | 83.8 | 782264 |
| 20 | C ₉ H ₁₃ N | Pyridine, 3-butyl- | 13.561 | 91.6 | 92316 |
| 21 | C ₈ H ₇ NO ₄ | 1,2-Benzenedicarboxylic acid, 3-amino- | 14.116 | 118.7 | 182585 |
| 22 | C ₉ H ₂₀ N ₄ | 2,4-Diazapentane, N,N'-dimethyl-3,3bis[cycloazapropyl]- | 14.796 | 57.6 | 80771 |
| 23 | C ₈ H ₁₉ N | (1-Ethyl-2-methylpropyl)dimethylamine | 14.863 | 85.8 | 397032 |

Contd.....

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|---|--|--------|-----------|---------|
| 24 | C ₁₀ H ₁₄ BeO ₄ | Beryllium, bis(2,4-pentanedionatoO,O)-, (T-4)- | 15.099 | 191.9 | 5856802 |
| 25 | C ₁₃ H ₁₉ NO ₂ | Benzoic acid, 4-diethylamino-, ethyl ester | 15.143 | 205.9 | 267685 |
| 26 | C ₉ H ₁₉ NO | Acetamide, N-butyl-N-propyl- | 15.5 | 71.7 | 155374 |
| 27 | C ₆ H ₁₂ N ₂ O | 1-Isopropylimidazolidin-2-one | 15.66 | 112.7 | 134767 |
| 28 | C ₉ H ₂₁ N ₃ | 1-Piperazineethanamine, N,N,4-trimethyl- | 15.78 | 112.7 | 63937 |
| 29 | C ₉ H ₁₉ N ₅ | Tris(aziridinomethyl)hydrazine | 16.257 | 83.7 | 1121189 |
| 30 | C ₁₄ H ₂₅ N ₃ | 3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino]propionitrile | 16.364 | 57.6 | 75868 |
| 31 | C ₁₁ H ₂₂ N ₂ O | 2-[3-[N-Aziridyl]propyl]aminomethyltetrahydro pyran | 17.747 | 57.6 | 95625 |
| 32 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 17.957 | 71.6 | 101591 |
| 33 | C ₈ H ₁₅ N ₃ O | 4-Imidazolidinone, 2,2-dimethyl-3-[(1-methylethylidene)amino]- | 18.146 | 71.7 | 616722 |
| 34 | C ₁₂ H ₂₃ NO ₂ | Octanoic acid, morpholide | 18.393 | 128.7 | 133580 |
| 35 | C ₇ H ₁₂ N ₄ O ₂ | 6-t-Butylamino-[1,3,5]triazine-2,4-diol | 18.52 | 128.6 | 145177 |
| 36 | C ₈ H ₁₃ NO ₂ | 3-Piperidinone, 1-acetyl-6-methyl- | 18.814 | 154.8 | 390372 |
| 37 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 18.864 | 71.7 | 103433 |
| 38 | C ₁₄ H ₂₇ NO ₂ S | S-Allyl-L-cysteine, N-(n-butyl)-, n-butyl ester | 19.004 | 73.7 | 4118008 |
| 39 | C ₆ H ₁₄ N ₂ | N-[4-Aminobutyl]aziridine | 19.532 | 83.8 | 1077345 |
| 40 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.651 | 57.6 | 59072 |
| 41 | C ₆ H ₉ N ₃ O ₂ | 6-Azathymine, Bis(methyl) ether | 19.882 | 154.8 | 469934 |
| 42 | C ₁₂ H ₁₉ NO ₂ Si | 4'-Methoxyacetophenone oxime, TMS derivative | 20.085 | 147.6 | 78968 |
| 43 | C ₁₃ H ₁₇ N ₃ O ₄ | Ethanol, 2-[4-(4-nitrobenzoyl-1-piperazinyl)- | 20.192 | 247.9 | 77964 |
| 44 | C ₄ H ₃ N ₃ O ₄ | 5-Nitro-4,6-pyrimidinediol | 20.306 | 156.7 | 84618 |
| 45 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 20.865 | 149.8 | 528520 |

Contd.....

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|--|---|--------|-----------|---------|
| 46 | C ₁₃ H ₁₆ F ₂ O ₂ | 2,6-Difluoro-3-methylbenzoic acid, 3methylbutyl-2 ester | 21.526 | 154.8 | 1046708 |
| 47 | C ₁₃ H ₁₉ NO ₂ | Carnegine | 21.758 | 57.7 | 135331 |
| 48 | C ₁₃ H ₁₆ F ₂ O ₂ | 2,6-Difluoro-3-methylbenzoic acid, 3methylbutyl-2 ester | 21.829 | 154.8 | 2215062 |
| 49 | C ₁₅ H ₁₉ FO ₄ | Succinic acid, 2-fluorophenyl neopentyl ester | 21.98 | 70.7 | 150954 |
| 50 | C ₁₈ H ₁₅ NOS | 2-(p-Methoxyphenyl)-8-methyl-8Hthieno(2,3-b)indole | 22.163 | 278 | 365538 |
| 51 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 22.494 | 149.8 | 2437173 |
| 52 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 23.024 | 83.7 | 818988 |
| 53 | C ₁₁ H ₂₃ NO | Acetamide, N-butyl-N-pentyl- | 25.5 | 71.6 | 183105 |
| 54 | C ₆ H ₉ N ₃ O ₃ | 1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trimethyl- | 25.836 | 170.8 | 397977 |
| 55 | C ₁₁ H ₂₄ OSi | 1-Butoxy-1-ethyl-1-silacyclohexane | 25.908 | 170.8 | 564086 |
| 56 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 26.085 | 57.6 | 110594 |
| 57 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 26.465 | 83.7 | 482260 |
| 58 | C ₁₃ H ₂₄ F ₃ NO ₂ | 3-(2-Ethylhexoxy)propan-1-amine, Ntrifluoroacetyl | 28.519 | 125.6 | 61098 |
| 59 | C ₁₆ H ₂₃ NO | Butan-1-one, 1-(3-methylpiperidin-1-yl)2-phenyl- | 29.256 | 125.8 | 785298 |
| 60 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 29.737 | 57.7 | 307746 |

Table 28: Bio-active compounds with anti-microbial property identified in acetone extract of bacterial endophyte *Bacillus subtilis* in GC-MS/MS analysis

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|---|---|--------|-----------|---------|
| 1 | C ₄ H ₈ N ₂ O ₆ | 2,3-Butanediol, dinitrate | 3.33 | 45.8 | 1581999 |
| 2 | C ₇ H ₇ NO ₂ | 2-Pyridinecarboxylic acid, 6-methyl- | 9.004 | 91.7 | 78078 |
| 3 | C ₉ H ₁₃ NO | Benzenamine, 4-methoxy-3,5-dimethyl- | 12.08 | 135.7 | 149101 |
| 4 | C ₉ H ₁₁ NO | Benzamide, N,N-dimethyl- | 12.389 | 104.7 | 687695 |
| 5 | C ₉ H ₁₃ N | Pyridine, 3-butyl- | 13.561 | 91.6 | 92316 |
| 6 | C ₈ H ₇ NO ₄ | 1,2-Benzenedicarboxylic acid, 3-amino- | 14.116 | 118.7 | 182585 |
| 7 | C ₉ H ₁₉ NO | Acetamide, N-butyl-N-propyl- | 15.5 | 71.7 | 155374 |
| 8 | C ₁₂ H ₂₃ NO ₂ | Octanoic acid, morpholide | 18.393 | 128.7 | 133580 |
| 9 | C ₁₅ H ₁₉ FO ₄ | Succinic acid, 2-fluorophenyl neopentyl ester | 21.98 | 70.7 | 150954 |

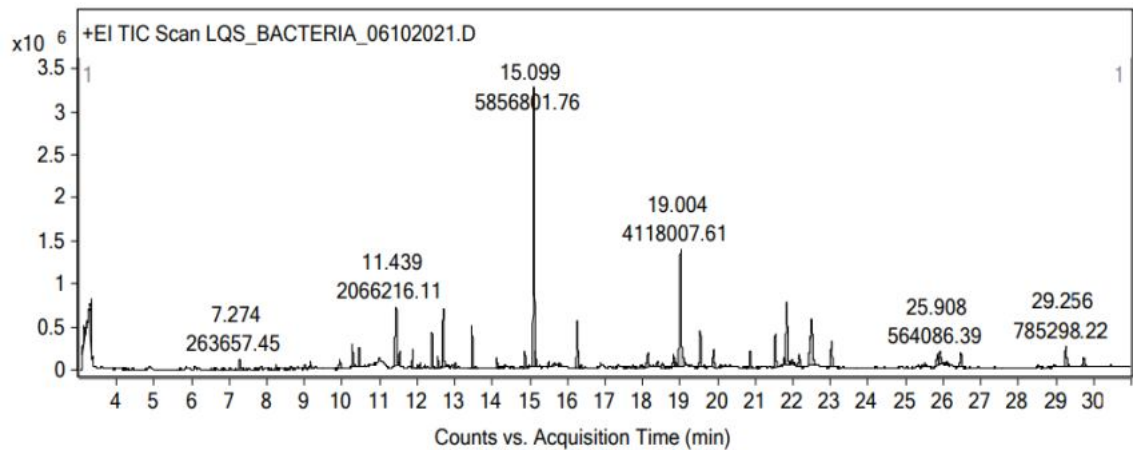


Fig. 16: Chromatogram of compounds of *Bacillus subtilis* extract obtained from GC-MS/MS analysis

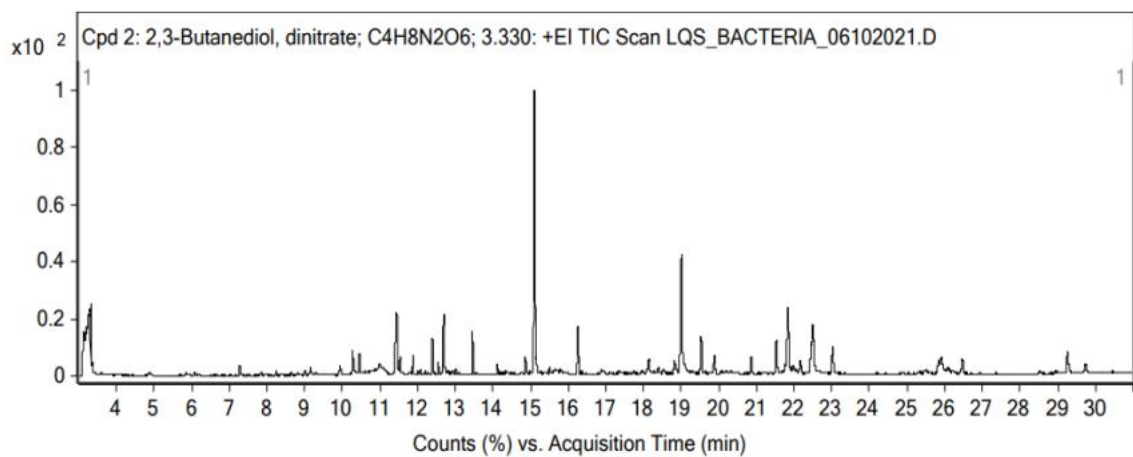


Fig. 17: Mass spectrum of 2, 3-Butanediol, dinitrate obtained from *Bacillus subtilis* in GC-MS/MS analysis

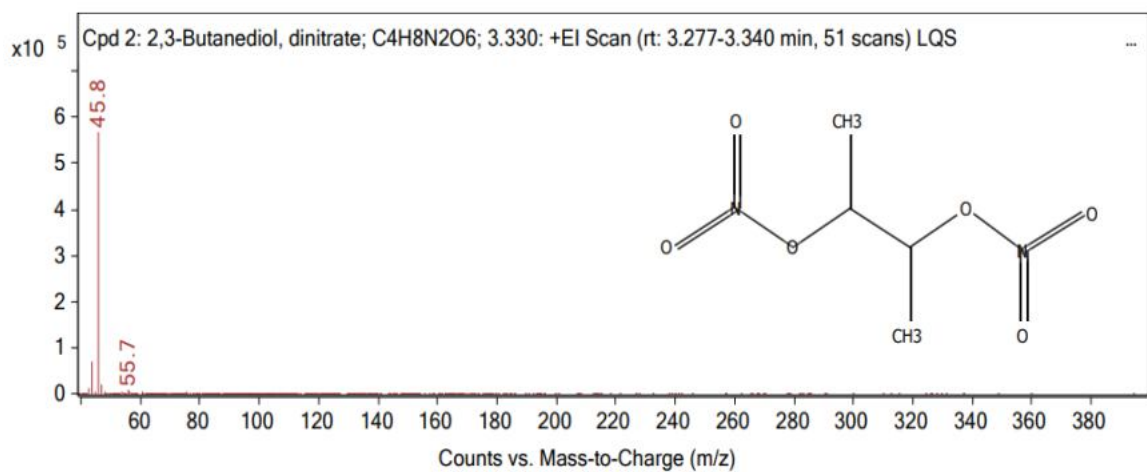


Fig. 18: Mass fragmentation and structure of 2, 3-Butanediol, dinitrate obtained from *Bacillus subtilis* in GC-MS/MS analysis

mg/L. Picolinic acid evoked strong antibacterial activities against *B. subtilis* and *S. aureus* at minimum inhibitory concentration of 2.0 mg/mL. The compounds, 1,2-Benzenedicarboxylic acid, 3-amino (Beulah *et al.*, 2018), Pyridine, 3-butyl-, (Shaikh *et al.*, 2011) Acetamide, N-butyl-N-propyl- (Kaplancikli *et al.*, 2012), Octanoic acid (Rosenblatt *et al.*, 2017) and Succinic acid (Iqbal *et al.*, 2017) have reported to have antimicrobial activity.

However, Benzenamine, 4-methoxy-3, 5- dimethyl and Benzamide, N,N-dimethyl-, belong to benzimidazole group of compounds. Choudari *et al.* (2016) screened benzimidazoles compounds for antibacterial and antifungal activity by disc diffusion methods on nutrient agar medium. The results revealed that compounds were found to be potent against *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. niger*. Hence they concluded that benzimidazole moiety displayed valuable biological activities and exhibited significant antibacterial and antifungal activities. Hence in the present investigation also this compound showed the antifungal activity for the inhibition of *R. bataticola*.

Likely, Kai (2020) reported that using *in vitro* volatile-collection systems, 26 strains of *B. subtilis* isolated from different habitats were found to produce in total 231 volatile secondary metabolites. These volatile secondary metabolites comprised mainly hydrocarbons, ketones, alcohols, aldehydes, ester, acids, aromatics, sulfur- and nitrogen-containing compounds. The production and release of several volatile bioactive compounds was retained in isolates of the species *B. subtilis*, while volatiles without a described function were seemed to be isolate-specifically produced. Hence these reports proved the present investigation.

4.5.2.2 Rhizospheric bacterial PGPMs

The GC-MS/MS analysis revealed that the extract of *B. velezensis* showed the presence of 61 compounds at different retention time ranging from 3.484 to 27.273. The mass to charge (m/z) ratio of the compounds were ranging from 43.6 and 191.6 (Table 29). The chromatogram of compounds showed different peaks (Fig. 19). Among 61 compounds, 11 compounds such as N- [Dimethylaminomethyl]aziridine, N-[3-Methylaminopropyl] aziridine, 1,2-Propanediol, 3-methoxy, 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, (2,2- Dimethylcyclobutyl)methylamine, 1,3-Propanediamine,

N,N-diethyl-N'-methyl-, 4-Nitrobenzoic acid, 3-pentyl ester recorded to have antimicrobial properties in the previous reports which are responsible for the inhibition of the pathogen (Table 30). Among 11 compounds, N-[Dimethylaminomethyl] aziridine was found with maximum area of 612698 and retention time of 10.597 as compared to other compounds found (Fig. 20). The structure of compound is shown in Fig. 21.

The present investigation was also supported by the previous reports which suggest that *B. licheniformis* and *B. subtilis* are two of the most commercially important bacteria, used for the production of a range of metabolites (vitamins, amino acids and antibiotics) and industrial enzymes (Harwood, 1992). They are closely related to other members of the *B. subtilis* species complex (*B. subtilis* group) that also includes *B. amyloliquefaciens*, *B. atrophaeus*, *B. mojavensis*, *B. paralicheniformis*, *B. pumilus*, *B. tequilensis*, *B. vallismortis* and *B. velezensis*. Members of the *B. subtilis* group have long been known to produce a range of secondary metabolites, including polyketides (PKs), terpenes and siderophores, as well as ribosomally and non-ribosomally synthesised peptides. For decades the identification of secondary metabolites and antimicrobial peptides (AMPs) was based primarily on their extraction from the culture medium, often because of their inhibitory effect on other bacteria and fungi. This was followed by analysis of their chemical composition and structure, and subsequent identification of the genes involved in their synthesis (Harwood *et al.*, 2018). In the present investigation also the extraction of bio active compounds was carried out from *B. velezensis*.

The compound N-[Dimethylaminomethyl] aziridine and N-[3-Methylaminopropyl] aziridine identified in the present study belongs to aziridine group of chemical compounds. Aziridine-containing compounds possess many biological activities especially antibacterial ones, due to the presence of the aziridine ring. These compounds were screened for their *in vitro* antimicrobial activity against a panel of Gram-positive and Gram-negative strains of bacteria (Kowalczyk *et al.*, 2017). Aldakheel *et al.* (2020) assessed the antibacterial and *in vitro* cytotoxicity of the bioactive components present in *Moringa oleifera* seed (MOS) extract. The outcome was validated using gas chromatography–mass spectrometry. They reported that bactericidal efficacy of the alcoholic MOS extract was examined against *E.coli* and *Staphylococcus aureus* by agar well diffusion assays which depicted greater inhibition against Gram-positive bacteria. Among them 1,2-Propanediol, 3-methoxy, 1,3-Propanediol, 2-ethyl-2-

Table 29: List of compounds identified in methanol chloroform extract of rhizospheric bacterial *Bacillus velezensis* in GC-MS/MS analysis

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|--|---|--------|-----------|---------|
| 1 | C ₇ H ₉ N | Pyridine, 3-ethyl- | 3.484 | 91.7 | 672967 |
| 2 | C ₆ H ₁₆ N ₄ | Diazene, [1-(2,2-dimethylhydrazino)ethyl]ethyl- | 4.516 | 43.7 | 130612 |
| 3 | C ₈ H ₁₅ NO ₂ | Butane-2-one, 3-methyl-3-(2oxopropylamino)- | 5.491 | 43.6 | 50712 |
| 4 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 6.422 | 57.7 | 124073 |
| 5 | C ₆ H ₁₄ N ₂ | Butanal, dimethylhydrazone | 6.666 | 71.8 | 321857 |
| 6 | C ₆ H ₁₄ Si | 1,1,2-Trimethyl-1-silacyclobutane | 6.771 | 71.8 | 379922 |
| 7 | C ₆ H ₁₄ O ₃ | 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- | 6.974 | 43.7 | 101700 |
| 8 | C ₇ H ₁₅ N | (2,2-Dimethylcyclobutyl)methylamine | 7.303 | 43.6 | 57689 |
| 9 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 8.325 | 69.8 | 553753 |
| 10 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 8.414 | 69.7 | 552994 |
| 11 | C ₅ H ₁₃ NO ₂ | 1,2-Propanediol, 3-(dimethylamino)- | 8.624 | 57.8 | 502972 |
| 12 | C ₇ H ₇ NO ₂ | 2-Pyridinecarboxylic acid, 6-methyl- | 9.007 | 91.7 | 83937 |
| 13 | C ₁₁ H ₂₃ N | n-Nonane, 1-[1-cycloazapropyl]- | 10.456 | 83.6 | 43777 |
| 14 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 10.597 | 57.8 | 612698 |
| 15 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 10.84 | 85.6 | 59067 |
| 16 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 11.19 | 57.7 | 133701 |
| 17 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 11.324 | 71.8 | 257080 |
| 18 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 11.395 | 70.7 | 72384 |
| 19 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 11.434 | 71.7 | 160306 |
| 20 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 11.969 | 84.7 | 146915 |
| 21 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 12.263 | 69.8 | 740061 |
| 22 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 12.387 | 69.8 | 1200002 |
| 23 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 12.509 | 69.8 | 885618 |
| 24 | C ₇ H ₁₅ NO | 5-Aziridinopentanol | 13.462 | 83.7 | 59508 |

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|--|---|--------|-----------|---------|
| 25 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 13.567 | 71.7 | 198281 |
| 26 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 14.138 | 71.6 | 44684 |
| 27 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.271 | 57.7 | 103371 |
| 28 | C ₁₁ H ₂₃ NO | Propionamide, N-propyl-N-pentyl- | 14.405 | 71.7 | 154565 |
| 29 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 14.518 | 57.7 | 259386 |
| 30 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.653 | 71.7 | 257135 |
| 31 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.782 | 71.7 | 79714 |
| 32 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 14.868 | 69.6 | 46542 |
| 33 | C ₁₀ H ₁₄ BeO ₄ | Beryllium, bis(2,4-pentanedionatoO,O)-, (T-4)- | 15.102 | 191.9 | 1336762 |
| 34 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 15.264 | 83.6 | 199892 |
| 35 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.403 | 69.7 | 296388 |
| 36 | C ₆ H ₁₄ N ₂ | N-[3-Methylaminopropyl]aziridine | 15.509 | 69.7 | 271287 |
| 37 | C ₁₄ H ₃₂ N ₄ | 1,4,8,11-Tetraazacyclotetradecane, 1,4,8,11-tetramethyl- | 15.662 | 69.7 | 332651 |
| 38 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 15.821 | 69.7 | 173721 |
| 39 | C ₁₂ H ₂₅ N | 1-(1-Azacyclopropyl)decane | 16.26 | 83.6 | 65843 |
| 40 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 16.371 | 85.6 | 40237 |
| 41 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.736 | 71.6 | 85271 |
| 42 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 17.902 | 71.6 | 45900 |
| 43 | C ₈ H ₁₇ N ₃ | 1,3-Propanediamine, N'-(ethylcarbonimidoyl)-N,N-dimethyl- | 18.634 | 69.6 | 82392 |
| 44 | C ₁₂ H ₂₆ N ₂ | cis-1,4-Cyclohexanediamine, N1,N1,N4triethyl | 18.785 | 83.6 | 185641 |
| 45 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 18.926 | 69.6 | 174573 |
| 46 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 19.115 | 69.7 | 394913 |
| 47 | C ₁₀ H ₂₁ N | Cyclohexanamine, N,N-diethyl- | 19.265 | 69.7 | 239888 |

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|--|---|--------|-----------|--------|
| 48 | C ₁₁ H ₂₃ N | 1-Butanamine, N-(1-propylbutylidene)- | 19.495 | 69.6 | 216175 |
| 49 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 19.686 | 69.6 | 180877 |
| 50 | C ₁₂ H ₁₅ NO ₄ | 4-Nitrobenzoic acid, 3-pentyl ester | 20.872 | 149.6 | 49959 |
| 51 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 21.508 | 57.6 | 49594 |
| 52 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 21.68 | 149.8 | 303516 |
| 53 | C ₇ H ₁₃ N ₃ O | 1,3,5-Triazaadamantan-7-ol | 21.817 | 154.7 | 53121 |
| 54 | C ₈ H ₁₉ NO ₂ S | Methanesulphonamide, N-(2-pentyl)-Nethyl- | 22.057 | 149.7 | 89851 |
| 55 | C ₇ H ₁₈ N ₂ | 1,3-Propanediamine, N,N-diethyl- | 22.442 | 73.6 | 89387 |
| 56 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 22.501 | 149.8 | 480811 |
| 57 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 22.874 | 149.7 | 76622 |
| 58 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 22.883 | 149.8 | 45293 |
| 59 | C ₁₃ H ₂₁ NO | 1-(Hexahydropyrrolizin-3-ylidene)-3,3dimethyl-butan-2-one | 24.166 | 149.7 | 90989 |
| 60 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 26.446 | 69.6 | 42805 |
| 61 | C ₁₀ H ₂₃ N ₃ | N-[3-[N-Aziridyl]propyl]-3dimethylaminopropylamine | 27.273 | 57.6 | 54785 |

Table 30: Bio-active compounds with antimicrobial property identified in acetone extract of rhizospheric bacteria *Bacillus velezensis* in GC-MS/MS analysis

| Sl. No. | DB Formula | Name | RT | m/z ratio | Area |
|---------|---|---|--------|-----------|--------|
| 1 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 6.422 | 57.7 | 124073 |
| 2 | C ₇ H ₁₅ N | (2,2-Dimethylcyclobutyl)methylamine | 7.303 | 43.6 | 57689 |
| 3 | C ₅ H ₁₃ NO ₂ | 1,2-Propanediol, 3-(dimethylamino)- | 8.624 | 57.8 | 502972 |
| 4 | C ₅ H ₁₂ N ₂ | N-[Dimethylaminomethyl]aziridine | 10.597 | 57.8 | 612698 |
| 5 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 11.434 | 71.7 | 160306 |
| 6 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 11.324 | 71.8 | 257080 |
| 7 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl- | 14.271 | 57.7 | 103371 |
| 8 | C ₈ H ₁₇ N ₃ | 1,3-Propanediamine, N'-(-ethylcarbonimidoyl)-N,N-dimethyl | 18.634 | 69.6 | 82392 |
| 9 | C ₈ H ₂₀ N ₂ | 1,3-Propanediamine, N,N-diethyl-N'methyl | 16.371 | 85.6 | 40237 |
| 10 | C ₈ H ₁₇ N ₃ | 1,3-Propanediamine, N'-(ethylcarbonimidoyl)-N,N-dimethyl- | 18.634 | 69.6 | 82392 |
| 11 | C ₁₂ H ₁₅ NO ₄ | 4-Nitrobenzoic acid, 3-pentyl ester | 20.872 | 149.6 | 49959 |

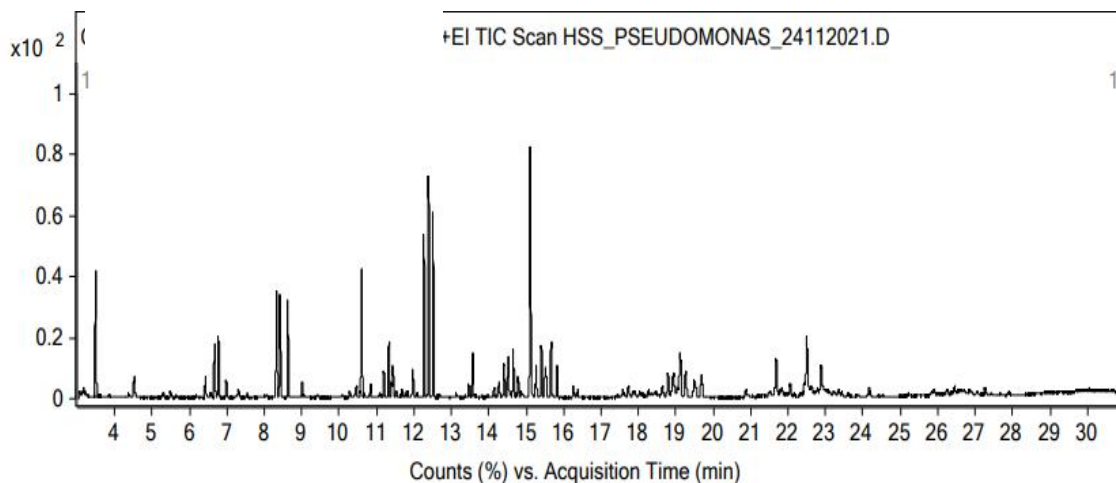


Fig. 19: Chromatogram of compounds of *Bacillus velezensis* extract obtained from GC-MS/MS analysis

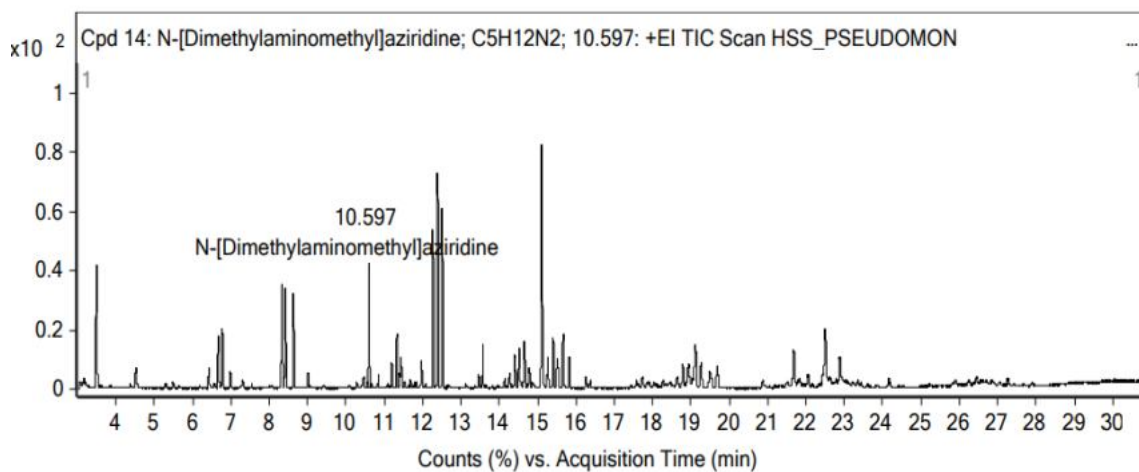


Fig. 20: Compound chromatogram of N-[Dimethylaminomethyl] aziridine obtained from *Bacillus velezensis* in GC-MS/MS analysis

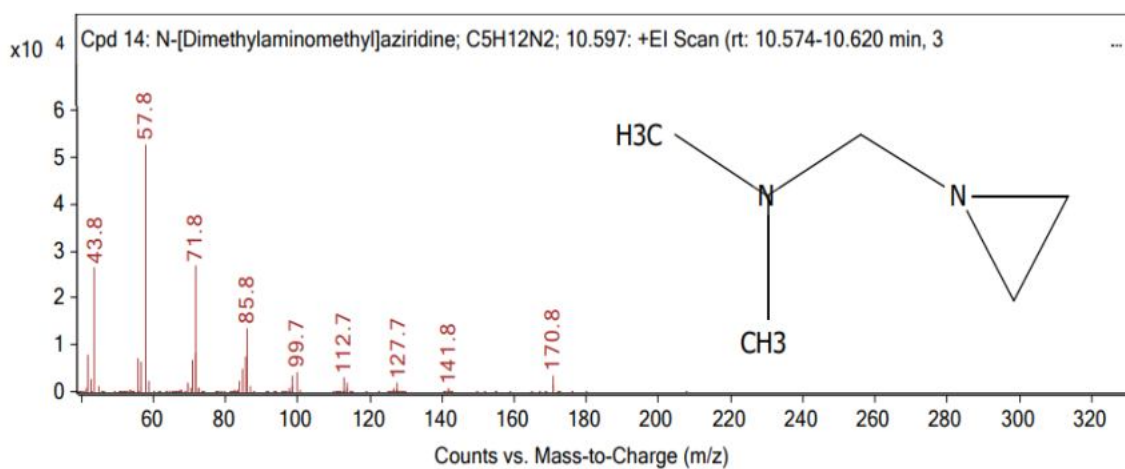


Fig. 21: Mass fragmentation and structure of N-[Dimethylaminomethyl] aziridine obtained from *Bacillus velezensis* in GC-MS/MS analysis

(hydroxymethyl)- are alcoholic compounds which are also found in present investigation, showed the antimicrobial properties. Sharara (2017) reported (2,2-Dimethylcyclobutyl)methylamine as a volatile compound in the fenugreek seeds. 1, 3-Propanediamine, N,Ndiethyl-N'-methyl- (Culler *et al.*, 1979), 4-Nitrobenzoic acid, 3-pentyl ester (Chowdhury *et al.* 2017) also recorded for their anti microbial activity. Hence, in the present investigation these compounds influenced the inhibition of the pathogen.

4.5.3 Screening for anti-microbial activity of crude extract of potential PGPMs

The crude extract of four potential PGPMs *viz.*, *T. asperellum*, *T. harzianum*, *B. subtilis* and *B. vazezensis* which produced bio-active compounds in the above study was further subjected for anti-microbial activity against *R. bataticola* using poisoned food technique at different concentrations *viz.*, 500, 1000 and 2000 ppm. Among endophytic PGPMs, *T. asperellum* and *B. subtilis*, the maximum inhibition was observed at 2000 ppm with per cent inhibition of 71.22 and 50.59 respectively. In case of rhizospheric isolates also *T. harzianum* and *B. vazezensis*, showed maximum inhibition of pathogen at 2000 ppm concentration with per cent inhibition of 78.85 and 68.52 respectively (Table 31, Plate 23). Hence in the test of antimicrobial activity of crude extract of potential PGPMs, the results revealed that *T. asperellum*, *B. subtilis*, *T. harzianum*, *B. vazezensis* could inhibit the pathogen effectively at 2000 ppm concentration. Among them, *T. harzianum* showed maximum inhibition followed by *Trichoderma asperellum*. Whereas, *B. subtilis* showed least inhibition. Hence it is clear from the study that the crude extract of bio-active compounds was highly effective to suppress the growth of fungal pathogens.

The similar inhibition per cent results were observed by Ranjana (2020) who reported that the volatile constituents of *T. asperellum* significantly inhibited the growth of pathogen at all the concentrations tested. Maximum inhibition of pathogen was observed at 15 per cent concentration (75.93 %) followed by 64.07 per cent and 44.44 per cent at 10 and 5 per cent concentrations, respectively. Maximum inhibition of pathogen observed at 15 per cent concentration (73.33 %) followed by 62.96 per cent and 31.48 per cent at 10 and 5 per cent concentrations, respectively in case of volatile constituents of *P. fluorescens* (PF-19) .

Table 31: Screening for antimicrobial activity of crude extract of PGPMs against *R. bataticola* using poisoned food technique

| Name of the isolate | Concentration of crude extract (%) | | | | | |
|-------------------------------|------------------------------------|----------|----------|-------------------------------|---------------|---------------|
| | Colony diameter (mm)* | | | Per cent mycelial inhibition* | | |
| | 500 ppm | 1000 ppm | 2000 ppm | 500 ppm | 1000 ppm | 2000 ppm |
| Endophytic PGPMs | | | | | | |
| <i>Trichoderma asperellum</i> | 88.50 | 85.57 | 44.43 | 1.67 (7.42) | 4.93 (12.82) | 71.22 (57.55) |
| <i>Bacillus subtilis</i> | 88.33 | 76.67 | 44.40 | 1.85 (7.82) | 14.81 (22.63) | 50.59 (45.33) |
| Rhizospheric PGPMs | | | | | | |
| <i>Trichoderma harzianum</i> | 88.83 | 77.17 | 30.53 | 1.30 (6.54) | 14.26 (22.18) | 78.85 (62.61) |
| <i>Bacillus velezensis</i> | 87.97 | 81.33 | 37.33 | 2.26 (8.64) | 9.63 (18.08) | 68.52 (55.86) |
| Control | 90.00 | 90.00 | 90.00 | 00.00 | 00.00 | 00.00 |
| SEM ± | | | | 0.99 | 0.40 | 0.71 |
| CD AT 1% | | | | 4.45 | 1.77 | 3.17 |

*Mean of three replications

Figures in parentheses are arc sine transformed values

Mycelial growth of *R. bataticola* is 90.00 mm (Control)

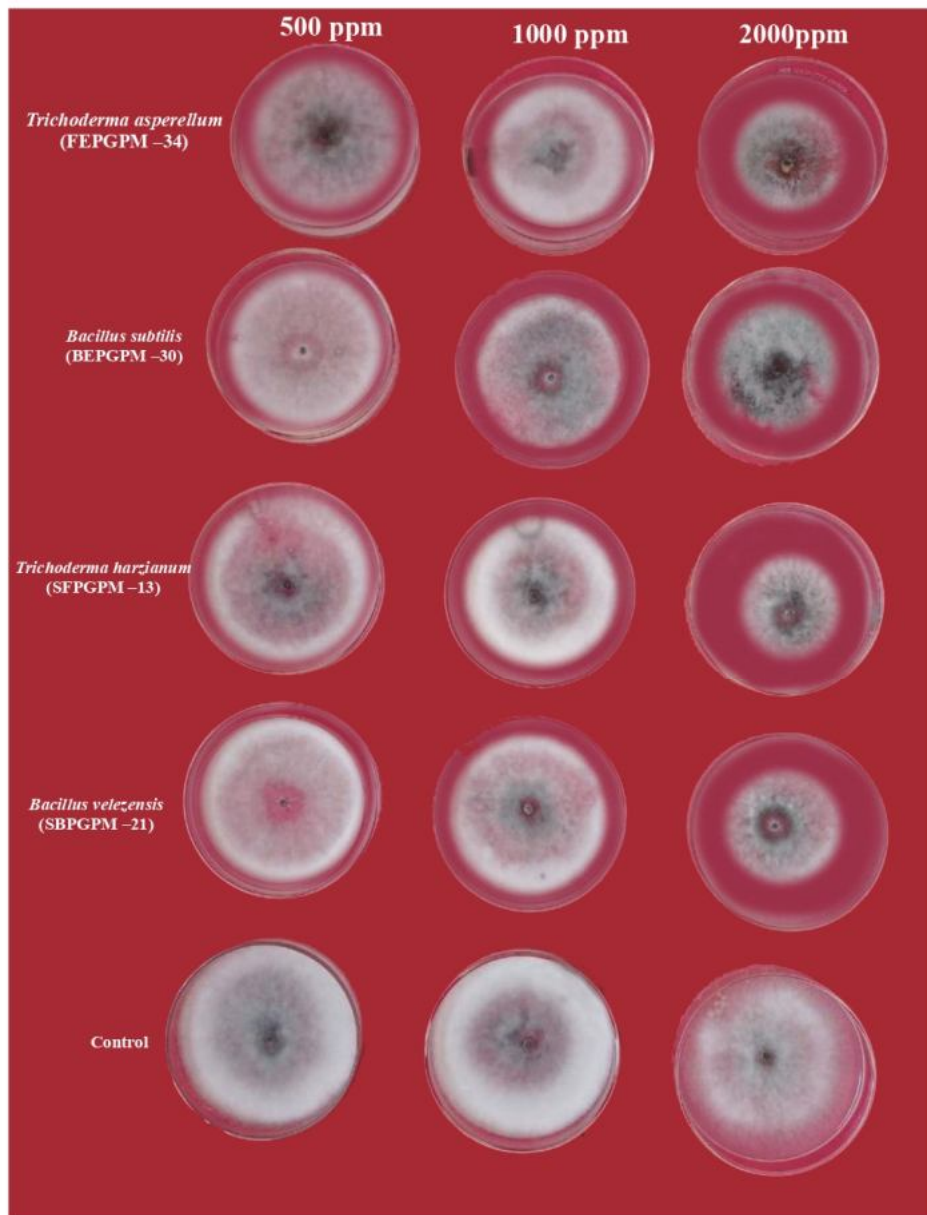


Plate 23: Mycelial inhibition of *R. bataticola* by crude extract of potential PGPMs at different concentration

In another study conducted by Makuwa and Dlamini (2021) determined the antibacterial activity of endophytic bacteria's crude extracts against five pathogenic test strains: Gram-negative bacteria *E. coli*, *P. aeruginosa* and *Klebsiella oxytoca* Gram-positive bacteria *Staphylococcus aureus* and *B. cereus*. The inhibition activity varied depending on the test strain used, most extracts showed interesting results, and the positive control *Streptomycin* at 1 at mg/mL was effective against all the test strains. Minimum inhibition concentration of the extracted crude extracts from bacterial endophyte ranged from 0.312 at mg/mL to 10 at mg/mL. The lowest MIC value was 0.312 at mg/mL and other crude extracts indicated MIC values higher than 1 at mg/mL.

4.6 Management of dry root rot using potential PGPMs in pot and field experiments

The role of plant growth-promoting microbes in management of plant diseases is considered as an environmental friendly alternative method to chemicals. PGPMs could enhance the supply of macro and micronutrients, promote plant growth and reduce the need of chemical fertilizers. Apart from this, they also used for management of diseases through their several mechanisms. The PGPMs are mainly inoculated to seeds, roots and soil (Lopes *et al.*, 2021). Hence, in the current study, four efficient PGPMs based on the *in vitro* bio efficacy tests (Dual culture technique, inverted plate technique and poisoned food technique) were selected for their pot as well as field performance. For this study, *T. asperellum*, *B. subtilis*, *T. harzianum* and *B. velezensis* were selected for the disease management studies in separate pot and field experiments.

4.6.1 *In vitro* efficacy of potential PGPMs on growth parameters in chickpea

The potential PGPMs such as, *T. asperellum*, *B. subtilis*, *T. harzianum* and *B. velezensis* were screened for their efficacy in plant growth promotion by roll towel method (ISTA, 1976) before taking them to the pot and field conditions (Plate 24).

With respect to per cent germination, results indicated that the seeds treated with *T. asperellum*, *B. subtilis*, *T. harzianum* and *B. velezensis* showed more than 90 per cent of germination compared to germination per cent in control (86.00). Among them, *T. asperellum* has shown maximum 99.33 germination per cent followed by *T. harzianum* and *B. velezensis* with germination per cent of 98.00 and 96.00, respectively. Whereas, *B. subtilis* showed the minimum germination per cent of 92.67 (Table 32).

Table 32: *In vitro* efficacy of PGPMs on growth parameters of chickpea in roll towel method

| Treatment | Per cent germination | Shoot length (cm) | Root length (cm) | Seedling vigour index |
|-------------------------------|-----------------------------|--------------------------|-------------------------|------------------------------|
| <i>Trichoderma asperellum</i> | 99.33 (87.29) | 14.53 | 16.17 | 3007.47 |
| <i>Bacillus subtilis</i> | 92.67 (74.32) | 9.35 | 10.47 | 1835.80 |
| <i>Trichoderma harzianum</i> | 98.00 (83.44) | 14.37 | 15.03 | 2825.47 |
| <i>Bacillus vazezensis</i> | 96.00 (78.72) | 13.23 | 13.33 | 2638.07 |
| Control (Water) | 86.00 (68.06) | 9.23 | 9.65 | 1621.19 |
| S.Em. ± | 1.01 | 0.75 | 0.94 | 10.21 |
| C.D. @ 1% | 4.80 | 3.54 | 4.44 | 33.31 |



T. asperellum



Control

Plate 24: Effect of *T. asperellum* on germination percent, root and shoot length of chickpea

The shoot length of the chickpea seedlings was ranging from 9.23 to 14.53. The maximum shoot length (14.53 cm) was observed in *T. asperellum* which was on par with *T. harzianum* (14.37 cm). While, minimum shoot length (9.35) was observed in *B. subtilis* (Table 32).

The results revealed that root length of the chickpea seedlings ranged from 9.65 to 16.17 cm. The maximum root length (16.17 cm) was observed in *T. asperellum*. While, minimum root length (10.47 cm) was observed in *B. subtilis*. The seedling vigour index of chickpea seedlings was significantly distinct and ranging from 3007.47 to 1621.19. The maximum vigour index (3007.47) was found in *T. asperellum* which is followed by *T. harzianum* with vigour index of 2638.07. Whereas, *B. subtilis* has showed minimum vigour index (1835.80) (Table 32).

From the present study it is clear that *T. asperellum*, *T. harzianum* and *B. velezensis* influenced the germination and plant growth compared to untreated control. The increase in plant growth is likely promoted by the enhanced IAA and availability of nutrients (phosphorus, potassium, siderophore, amino acids by protease activity and ammonia) to the host plant. Hence, only three plant growth influencing PGPMs were chosen for the further studies.

The present study was supported by Mukherjee *et al.* (2018) who explained the role of *Trichoderma* as a plant growth promoter. The enhancement of seed vigor parameters may be due to the production of phenolic compounds and secondary metabolite namely harzianolide by *Trichoderma* spp. In the present study also *T. asperellum* and *T. harzianum* showed considerable influence on growth parameters. Similarly, Shahid *et al.* (2021) reported the significant effects of *Bacillus* spp. as bioinoculants were noted on the growth of wheat plants. Results manifested that bacterial inoculation stimulated plant growth as compared to the un-inoculated controls. Plants inoculated with *B. amyloliquefaciens* SB-1 demonstrated maximum shoot lengths followed by *B. tequilensis* A-3, and *B. subtilis* A-2, respectively. Maximum root lengths were however noted for *B. subtilis* A-2 inoculated plants followed by *B. tequilensis* A-3. When compared for dry weight of shoot and root, most significant difference was recorded for the *B. amyloliquefaciens* SB-1 inoculated plants followed by *B. subtilis* A-2. Similar observations were recorded in the present investigation in which *B. velezensis*

showed significant influence on growth parameters. Hence these reports confirm the results of present investigation

4.6.2 Management of dry root rot using potential PGPMs in pot experiment

Three potential PGPMs viz., *T. asperellum*, *T. harzianum* and *B. velezensis* which were successful in enhancing the growth traits were used for the management of dry root rot under pot culture (Plate 25). The treatments imposed for the management studies were as like mentioned in section 3.6.2. in “Materials and Methods”. Before formulating the treatments, all the three isolates were tested for their compatibility for their consortium and as a result, they were compatible and were not affecting the growth of each other.

4.6.2.1 Growth parameters

With respect to growth parameters like plant height, number of branches and number of pods, the results indicated that, among nine treatments imposed, T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/L and *Beauveria bassiana* at 2 g /l.) showed the maximum plant height (27.53 cm), maximum number of branches (6) and maximum number of pods (9.52). The treatment was followed by T₈. Whereas, T₅ showed least plant height (21.63 cm) and minimum number of pods (3.02) (Table 33, Plate 26).

4.6.2.2. Yield parameters

Among nine treatments, T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/l and *Beauveria bassiana* at 2 g /l.) has shown maximum dry weight (13 g) and pod weight (3.24 g) followed by T₈. Minimum dry weight (6.67 g) and pod weight (1.24 g) was observed in the treatment T₅ (Table 33, Plate 26).

Percent disease incidence

The results showed significant difference among remaining treatments. T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar

Table 33: Impact of different treatment combinations on management of dry root rot under pot experiment

| Tr. No. | Treatment details | Growth Parameters | | | Yield Parameters | | Percent disease incidence | Percent reduction over control |
|----------------|---|---------------------|-------------------|-------------------|---------------------|--------------------|-------------------------------|--------------------------------|
| | | Plant height (cm) | No. of branches | No. of pods | Dry weight (g) | Pod weight (g) | | |
| T ₁ | Seed treatment with <i>Trichoderma harzianum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma harzianum</i> at 2.5 kg /100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. | 22.1 ^{bc} | 2.17 ^c | 4.63 ^e | 7 ^{cd} | 1.49 ^{bc} | 37.17 ^c (37.57) | 36.15 |
| T ₂ | Seed treatment with <i>Bacillus vazezensis</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Bacillus vazezensis</i> at 2.5 kg/100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. | 22.38 ^{bc} | 2.17 ^c | 5.76 ^d | 7.33 ^{bad} | 1.68 ^{bc} | 34.85 ^d (36.18) | 38.47 |
| T ₃ | Seed treatment with <i>Trichoderma asperellum</i> isolate at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma asperellum</i> at 2.5 kg/100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. | 23.61 ^{bc} | 2.29 ^c | 6.75 ^c | 9 ^{bad} | 1.83 ^{bc} | 28.58 ^f (32.32) | 44.74 |
| T ₄ | Seed treatment with <i>Trichoderma harzianum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma harzianum</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Trichoderma harzianum</i> at 10 g/Lit and <i>Beauveria bassiana</i> at 2 g/l. | 24.4 ^{abc} | 4 ^b | 7.98 ^b | 9.33 ^{bc} | 2.07 ^{bc} | 26.27 ^g (30.83) | 47.05 |
| T ₅ | Seed treatment with <i>Bacillus vazezensis</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Bacillus vazezensis</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Bacillus vazezensis</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g/l. | 21.63 ^{bc} | 2.53 ^c | 3.02 ^f | 6.67 ^{cd} | 1.24 ^c | 40.95 ^b (39.78) | 32.37 |

Contd.....

| Tr. No. | Treatment details | Growth Parameters | | | Yield Parameters | | Percent disease incidence | Percent reduction over control |
|-----------------|---|---------------------|-------------------|--------------------|---------------------|--------------------|-------------------------------|--------------------------------|
| | | Plant height (cm) | No. of branches | No. of pods | Dry weight (g) | Pod weight (g) | | |
| T ₆ | Seed treatment with <i>Trichoderma asperellum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma asperellum</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Trichoderma asperellum</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g/l. | 27.53 ^a | 6 ^a | 9.52 ^a | 13 ^a | 3.24 ^a | 20.79 ⁱ (27.13) | 52.53 |
| T ₇ | Seed treatment with <i>Trichoderma harzianum</i> (10 g/kg seed) + <i>Bacillus vazezensis</i> (10 g/kg seed) + <i>Trichoderma asperellum</i> (10 g/kg seed) followed by soil application of enriched vermicompost with <i>Trichoderma harzianum</i> , <i>Bacillus vazezensis</i> and each at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Trichoderma harzianum</i> (10 g/l) + <i>Bacillus vazezensis</i> (10 g/l) + <i>Trichoderma asperellum</i> (10 g/l) and <i>Beauveria bassiana</i> at 2 g/l. | 22.91 ^{bc} | 2.28 ^c | 6.17 ^{cd} | 8.67 ^{bd} | 1.71 ^{bc} | 32.53 ^e (34.77) | 40.79 |
| T ₈ | Seed treatment with <i>Trichoderma</i> at 5 g/kg seed followed by soil application of enriched FYM at 2.5 kg/100 kg per acre during sowing and foliar spray of 5% NSKE + 10% Cow urine 2 sprays (OF RPP). | 24.71 ^{ab} | 5 ^{ab} | 9.25 ^a | 10.67 ^{ab} | 2.61 ^{ab} | 23.66 ^h (29.10) | 49.66 |
| T ₉ | Untreated control | 20.65 ^c | 1.83 ^c | 2.41 ^f | 5.67 ^d | 1.07 ^c | 73.32 ^a (58.90) | - |
| S.Em ± | | 1.18 | 0.47 | 0.25 | 1.03 | 0.34 | 0.76 | |
| CD at 1% | | 4.87 | 1.93 | 1.03 | 4.04 | 1.39 | 3.16 | |



Plate 25: View of pot experiment on management of dry root rot using potential endophytic and rhizospheric PGPMs

application of *T. asperellum* at 10 g/l and *Beauveria bassiana* at 2 g/l.) was effective in the managing the disease with minimum PDI of 20.79 and 52.53 per cent reduction over control. This treatment was followed by T₈ with PDI of 23.66 and 55.63 per cent reduction over control. Whereas, the maximum PDI (40.95) was found in T₅ with 32.37 percent reduction over control (Table 33, Plate 26, Fig. 22).

The results of present investigation were in accordance with Sowmya *et al.* (2015) who studied the biocontrol efficacy of *Trichoderma* against dry root rot of blackgram caused by *M. phaseolina* (Tassi.) Goid which was evaluated in the pot culture infected with *M. phaseolina* and inoculated with *Trichoderma*. Among the 11 treatments imposed, the treatment T₉-seed treatment + soil application with combination of both *T. viride* isolates (TNAU and Thondamuthnur) significantly reduced the per cent disease incidence of 5.60 which accounted 88.79 per cent reduction over control followed by T₃ (Combination of both *Trichoderma* isolates) and T₅ (Thondamuthnur isolate of *Trichoderma*) that recorded 11.10 per cent of root rot incidence. Likely, Nirmalkar *et al.* (2017) reported that the seed treated with *T. harzianum* + *Rhizobium* along with soil application of *T. harzianum* enriched FYM found most effective to reduce the incidence of wilt complex of chickpea. All treatments showed significantly differences in grain yields. Maximum yield and highest benefit cost ratio was recorded in T₄ (Soil application with *T. harzianum* + seed treatment with *T. harzianum* followed by *Rhizobium* 10 % WP) with 8.44 q/ha and 1:2.95 respectively. Similar results were obtained in the present study, in which *T. asperellum* showed the maximum influence on growth and yield parameters and also on PDI.

4.6.3 Management of dry root rot using potential PGPMs under field conditions

The three potential PGPMs *viz.*, *T. asperellum*, *T. harzianum* and *B. velezensis* which were successful in enhancing the growth parameters and inhibited the pathogen, used for the management of dry root rot under field conditions (Plate 27). The treatments imposed for the management studies were as like mentioned in section 3.6.2. in “Materials and Methods”

4.6.3.1 Growth Parameters

All the treatments influenced the plant height, number of branches and number of pods compared to untreated control. Among nine treatments, T₆ (Seed treatment with



T₁: Seed treatment with *Trichoderma asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *Trichoderma asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *Trichoderma asperellum* at 10 g/l and *Beauveria bassiana* at 2 g/l.



T₂: Seed treatment with *Trichoderma* at 5 g/kg seed followed by soil application of enriched FYM at 2.5 kg/100 kg per acre during sowing and foliar spray of 5% NSKE + 10% cow urine 2 sprays (OF RPP).



T₀: Untreated control

Plate 26: View of effective treatments in comparison with untreated control in pot experiment on management of dry root rot

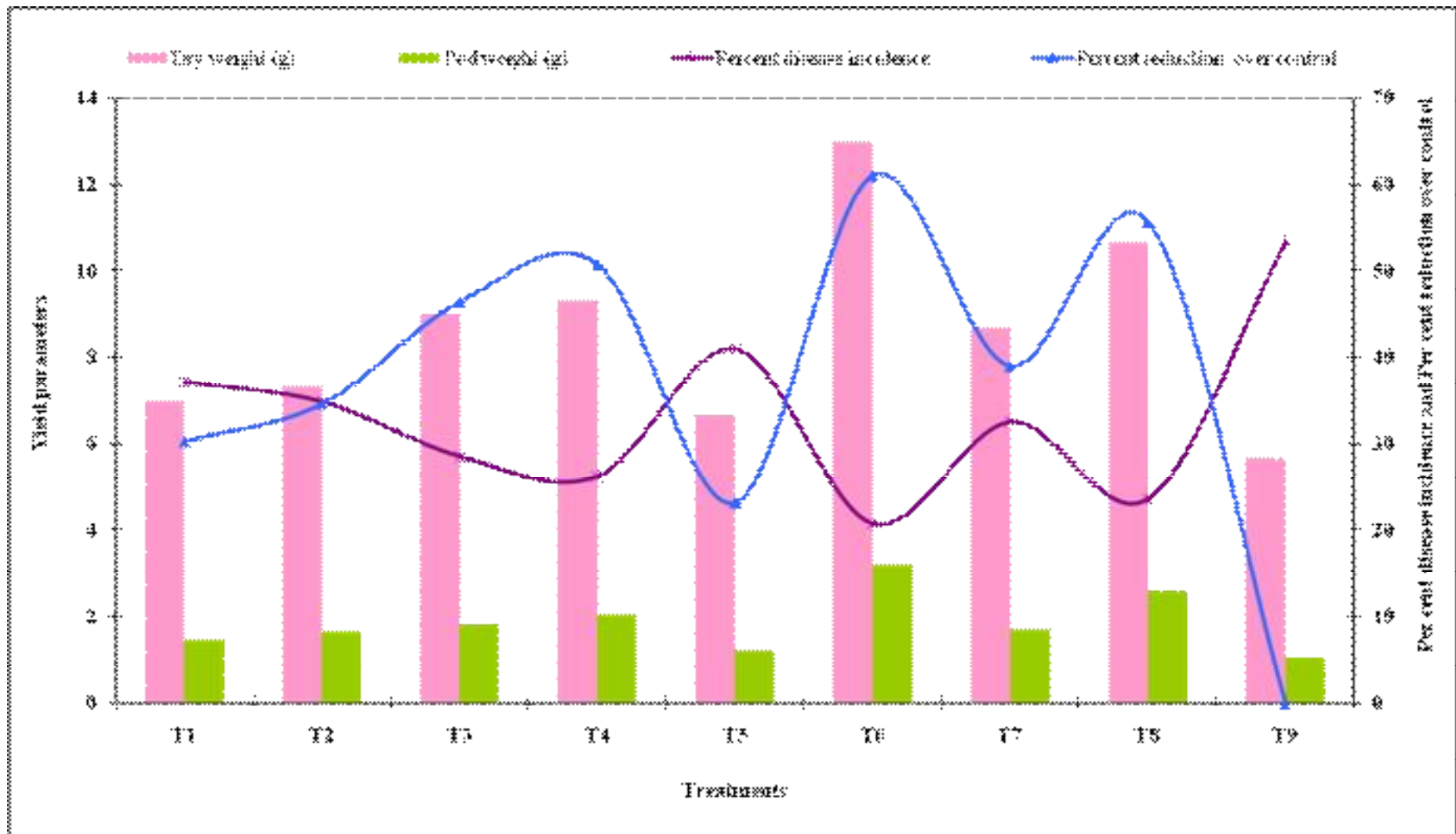


Fig. 22: Effect of different treatment combinations on chickpea yield parameters and PDI in pot experiment



Plate 27: View of field experiment on management of dry root rot using potential endophytic and rhizospheric PGPMs

T. asperellum at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/L and *Beauveria bassiana* at 2 g /l.) showed highest plant height (55.4 cm), maximum (12) number of branches and number of pods (106). T₆ was followed by T₈. Whereas, minimum influence on growth parameters was observed in T₅ (Table 34, Plate 28).

4.6.3.2. Yield parameters

The yield parameters like test weight and total yield were highly influenced in T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/L and *Beauveria bassiana* at 2 g /l.). The total yield was maximum (6.59 q/ha) in T₆ with maximum test weight (19.8 g) and it was statistically on par with T₈. While the minimum yield (3.84 q/ha) and test weight (16.68 g) was observed in the T₅ (Table 34, Plate 28).

4.6.3.3. Percent disease incidence

Among all the nine treatments imposed for the management of the disease, T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/L and *Beauveria bassiana* at 2 g /l.) showed the minimum PDI (20.78) with maximum per cent reduction over control (41.81). Among remaining treatments, T₁, T₂, T₃, T₇ were on par with each other with PDI of 37.52, 33.85, 32.44 and 32.76 respectively. While, the maximum PDI (44.79) was showed by T₅ with minimum per cent reduction over control (17.8) (Table 34, Plate 28, Fig. 23).

From the results of pot and field experiments, it is clear that, T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/L and *Beauveria bassiana* at 2 gm /l.) was the best treatment for the management of the dry root under *in vivo* and *in vitro*. The superiority of the treatment in inhibition of pathogen over all the treatments may be due to the antagonistic mechanisms exhibited by *T. asperellum* like mycoparasitism, hyper parasitism and exhibition of induced systemic resistance. With respect to growth parameters, the reason behind

Table 34: Effect of different treatment combinations on management of dry root rot under field conditions

| Tr. No. | Treatment details | Growth Parameters | | | Yield Parameters | | | Percent disease incidence | Percent reduction over control |
|----------------|--|---------------------|------------------|------------------|---------------------|-------------------------|---------------------|--------------------------------|--------------------------------|
| | | Plant height (cm) | No. of branches | No. of pods | 100 Seed weight (g) | Net plot yield (kg/ ha) | Total yield (q/ ha) | | |
| T ₁ | Seed treatment with <i>Trichoderma harzianum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma harzianum</i> at 2.5 kg /100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g/l. | 42.53 ^{cd} | 5 ^{ef} | 71 ^f | 17 ^b | 35.43 ^g | 3.91 ^c | 37.52 ^c (37.77) | 25.07 |
| T ₂ | Seed treatment with <i>Bacillus velezensis</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Bacillus velezensis</i> at 2.5 kg/100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g /l. | 43.69 ^{bc} | 6 ^{de} | 83 ^e | 17.5 ^b | 37.08 ^f | 4.01 ^c | 33.85 ^{cd} (35.58) | 28.74 |
| T ₃ | Seed treatment with <i>Trichoderma asperellum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma asperellum</i> at 2.5 kg/100 kg per acre at sowing and foliar application of <i>Beauveria bassiana</i> at 2 g /l. | 45.58 ^{bc} | 7 ^{bcd} | 88 ^{cd} | 17.79 ^{ab} | 42.26 ^d | 4.40 ^c | 32.44 ^d (34.72) | 30.15 |
| T ₄ | Seed treatment with <i>Trichoderma harzianum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma harzianum</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Trichoderma harzianum</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g /l. | 46.17 ^{bc} | 8 ^{bc} | 90 ^c | 18.21 ^{ab} | 48.36 ^c | 4.61 ^{bc} | 27.06 ^e (31.35) | 35.53 |
| T ₅ | Seed treatment with <i>Bacillus velezensis</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Bacillus velezensis</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Bacillus velezensis</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g /l. | 40.09 ^d | 4 ^{fg} | 68 ^f | 16.68 ^b | 38.40 ^e | 3.84 ^c | 44.79 ^b (42.01) | 17.8 |

Contd.....

| Tr. No. | Treatment details | Growth Parameters | | | Yield Parameters | | | Percent disease incidence | Percent reduction over control |
|----------------|---|---------------------|-----------------|------------------|---------------------|-------------------------|---------------------|--------------------------------|--------------------------------|
| | | Plant height (cm) | No. of branches | No. of pods | 100 Seed weight (g) | Net plot yield (kg/ ha) | Total yield (q/ ha) | | |
| T ₆ | Seed treatment with <i>Trichoderma asperellum</i> at 10 g/kg seed followed by soil application of enriched vermicompost with <i>Trichoderma asperellum</i> at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Trichoderma asperellum</i> at 10 g/l and <i>Beauveria bassiana</i> at 2 g/l. | 55.4 ^a | 12 ^a | 106 ^a | 19.8 ^a | 60.54 ^a | 6.59 ^a | 20.78 ^f (27.12) | 41.81 |
| T ₇ | Seed treatment with <i>Trichoderma harzianum</i> (10 g/kg seed) + <i>Bacillus vazezensis</i> (10 g/kg seed) + <i>Trichoderma asperellum</i> (10 g/kg seed) followed by soil application of enriched vermicompost with <i>Trichoderma harzianum</i> , <i>Bacillus vazezensis</i> and each at 2.5 kg/100 kg per acre at sowing followed by foliar application of <i>Trichoderma harzianum</i> (10 g/l) + <i>Bacillus vazezensis</i> (10 g/l) + <i>Trichoderma asperellum</i> (10 g/l) and <i>Beauveria bassiana</i> at 2 g/l. | 44.26 ^{bc} | 7 ^{cd} | 86 ^{de} | 17.75 ^{ab} | 42.06 ^d | 4.17 ^c | 32.76 ^d (34.92) | 29.83 |
| T ₈ | Seed treatment with <i>Trichoderma</i> at 5 g/kg seed followed by soil application of enriched FYM at 2.5 kg/100 kg per acre during sowing and foliar spray of 5% NSKE + 10% Cow urine 2 sprays (OF RPP). | 47.33 ^b | 8 ^b | 99 ^b | 19.74 ^a | 53.54 ^b | 5.78 ^{ab} | 23.65 ^{ef} (29.10) | 38.94 |
| T ₉ | Untreated control | 34.73 ^e | 3 ^g | 63 ^g | 16.06 ^b | 26.34 ^h | 3.23 ^c | 62.59 ^a (52.29) | - |
| | S.Em ± | 1.11 | 0.42 | 1.01 | 0.67 | 2.68 | 0.34 | 1.39 | |
| | CD at 5% | 3.34 | 1.25 | 3.03 | 2.02 | 8.03 | 1.01 | 4.15 | |



T₆: Seed treatment with *Trichoderma asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *Trichoderma asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *Trichoderma asperellum* at 10 g/l and *Beauveria bassiana* at 2 g/l.



T₅: Seed treatment with *Trichoderma* at 5 g/kg seed followed by soil application of enriched FYM at 2.5 kg/100 kg per acre during sowing and foliar spray of 5% NSKE + 10% cow urine 2 sprays (OFRPP).



T₀: Untreated control

Plate 28: Photograph showing effective treatment (T₆) in comparison with recommended package of practice and control in field experiment

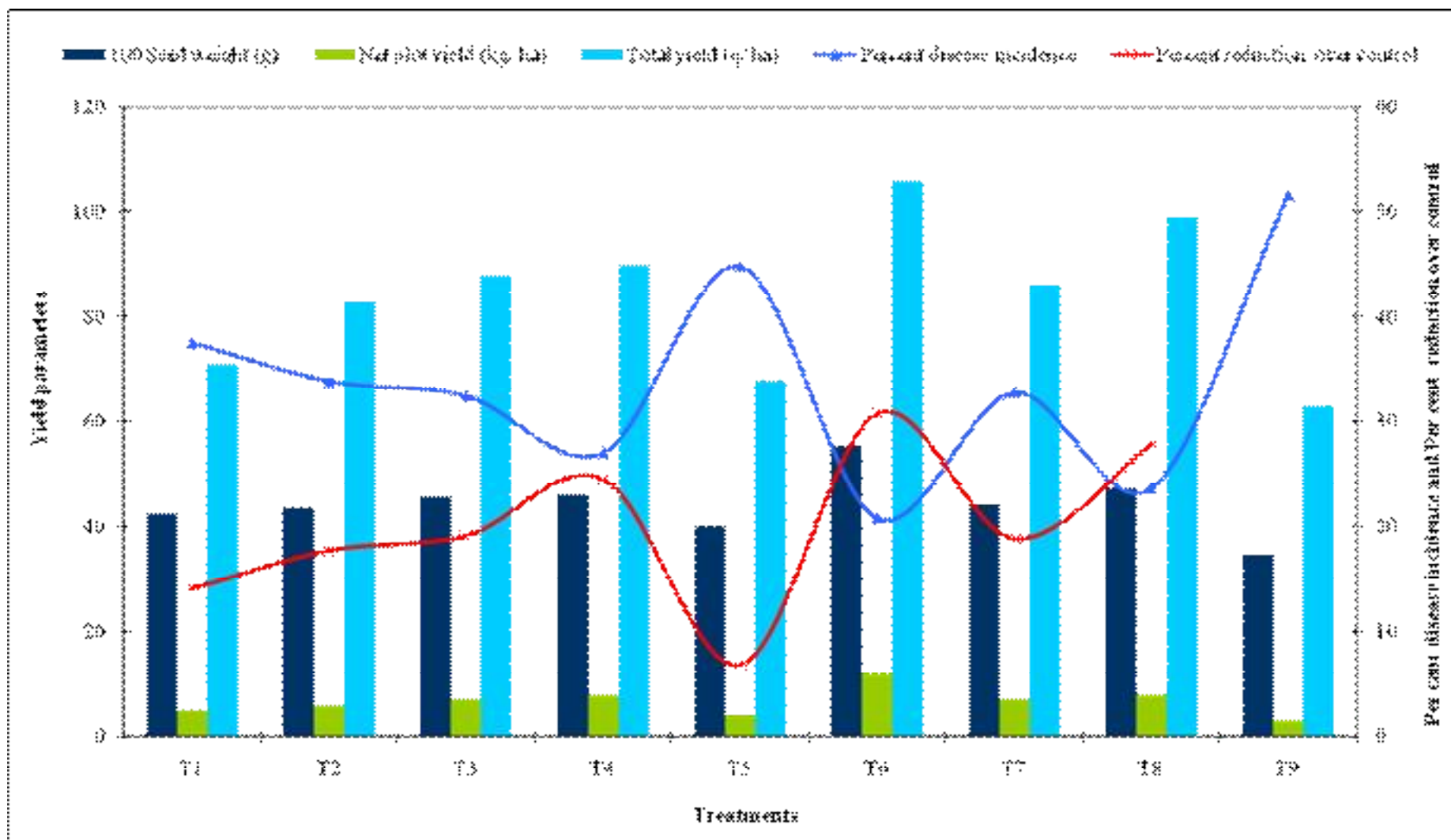


Fig. 23: Impact of different treatment combinations on chickpea yield parameters and PDI under field conditions

superiority of the treatment may be due to the production of IAA, ammonium and phosphate (growth promoting traits).

There has been a report that plants challenged with two fungal pathogens, *F. oxysporum* and *B. cinerea*, and pretreatment with *T. asperellum* resulted in less severe wilting and stunting symptoms than non-treated plants. Treatment with *T. asperellum* formulation inhibited reactive oxygen species production in response to the pathogens in comparison to plants that were only challenged with both pathogens. These results suggest that decrease in ROS levels contribute to the protective effects exerted by *T. asperellum* in tomato (Herrera-Télez *et al.*, 2019). In the current study also *T. asperellum* inhibited the pathogen *R. bataticola* under field conditions compared to control. Similarly, Khan and Gupta (1998) carried out an experiment to evaluate efficacy of five potential antagonistic (mycoparasitic) species of *Trichoderma*, namely *T. harzianum*, *T. hamatum*, *T. viride*, *T. koningii* and *T. polysporum* to control dry root rot of eggplant caused by *M. phaseolina* in field plots. Application of *T. hamatum*, *T. harzianum* or *T. viride* effectively checked the negative effect of *M. phaseolina* and subsequently, plant growth and yield of fungus-inoculated plants were enhanced by 36, 33 and 31 per cent, respectively. Similar observations were recorded in the present study, in which *T. asperellum* showed highest impact on growth and yield parameters, and also effective in managing the disease with least PDI. Likewise, Thilagavathi *et al.* (2007) also tested the biocontrol agents *T. viride* (strains Tv1 and Tv13), *P. fluorescens* (Pf1 and Py15) and *B. subtilis* (Bs16) individually and in combination for their effectiveness against root rot of green gram caused by *M. phaseolina*. The biocontrol agents tested *in vitro* against *M. phaseolina*, combinations of *P. fluorescens* + *T. viride* (Pf1+Tv1, Pf1+Tv13 and Py15+Tv1) inhibited mycelial growth of the pathogen and they also promoted the growth of the greengram seedlings. A combination of Pf1+Tv1 was most effective in reducing root rot incidence under glass-house and field conditions as compared with other single or combined treatments or the untreated control. Moreover, a combination of Pf1+Tv1 followed by Pf1+Tv13 and Py15+Tv1 significantly increased yield under glass house and field condition. This indicates that the combination of biocontrol agents *P. fluorescens* + *T. viride* also influences the growth and reduce the disease.

V. SUMMARY AND CONCLUSION

Dry root rot is an important disease in chickpea caused by pathogen *Rhizoctonia bataticola*. The disease is reported to cause up to 100 per cent yield loss under the favourable growth conditions of the pathogen. The role of PGPMs in plant disease management do not need any emphasis as they have twin advantages like plant growth promoting activity and antagonistic ability. In the present investigation, collection, isolation, identification of PGPMs and their antagonistic potential against *R. bataticola* was undertaken. Studies on plant growth promoting traits of potential PGPMs, molecular characterization and diversity analysis, screening for bio-active compound production against *R. bataticola* was also undertaken. Extraction, identification of bio-active compounds and screening of crude extract from potential PGPMs responsible for inhibition of *R. bataticola* was also another aspect of present study. Management of dry root rot using potential PGPMs in pot and field experiments. The results are summarized hereunder.

The dry root rot disease symptoms were initiated with yellowish discoloration of the foliage followed by premature drying and wilting at the seedling stage. In the advanced stage of the disease, brownish to black lesions were found in the taproot and lateral roots. Later, these lesions lead to the necrosis of the lateral roots. Hence, when we uprooted the affected plant, the plant comes off easily with blackish-brown taproot intact, whereas the lateral and finer roots were destroyed and shredded. The affected plant and roots were brittle and easily broken. In the field, foliage of the infected plants became straw coloured and were scattered in the patches.

The pathogen *R. bataticola* was successfully isolated by using hyphal tip isolation method from infected plants showing typical symptoms of dry root rot. Initially, the pathogen produced the white mycelial growth, later turned to brown to greyish black with growth. The fungus produced septate mycelium with right angle branching and constriction of the branch near the point of origin. The hypha was thin and hyaline and the sclerotial bodies were brown to black in color. Koch's postulates were successfully proved by carrying pathogenicity test on susceptible variety Annigeri-1 of chickpea

Totally, 126 isolates of PGPMs were collected from 11 different districts of northern Karnataka. Seventy isolates (40 fungal and 30 bacterial) were endophytic

PGPMs which were isolated from the plant tissues and fifty six isolates (26 fungal and 30 bacterial PGPMs) were rhizospheric PGPMs isolated from chickpea rhizosphere.

With respect to cultural characteristics of endophytic fungal PGPMs, the colony diameter of fungal PGPMs varied from 30.05-90.00 mm, colony color was very distinct, colony margin was categorized in to regular and irregular, colony surface varied from smooth to coarse and sectoring and zonation were observed in some of the isolates. In case of endophytic bacterial PGPMs, colony color was distinct, colony form was categorized in to circular and irregular form, colony margin varied from entire to undulate and most of the isolates were rod shaped and gram negative in reaction.

The colony diameter of the rhizospheric fungal PGPMs varied from 78.33-90.00 mm, colony color was very distinct, colony shape was categorized in to irregular and circular, most of the isolates had fluffy and raised mycelial growth and margin was categorized in to smooth and wavy margin. Whereas, in case of rhizospheric bacterial PGPMs, cultural characters were very distinct with respect to colony color, colony form (regular and irregular), colony elevation (flat and raised), colony margin (entire and undulated), fluorescence production and majority of the isolates were gram negative in reaction and rod shaped.

Eight fungal endophytic PGPMs (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34, FEPGPM-35) and eight bacterial endophytes (BEPGPM-5, BEPGPM-6, BEPGPM -9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29, BEPGPM-30) were identified as potential ones based on their inhibition of pathogen growth with more than 50 per cent inhibition with dual culture technique.

In case of rhizospheric PGPMs, three fungi (SFPGPM-3, SFPGPM-9, SFPGPM-13) with more than 70 per cent mycelial inhibition and nine bacteria (SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM-15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28 and SBPGPM-30) with more than 50 per cent mycelial inhibition were identified as potential in suppressing pathogen under *in vitro*.

Twenty eight potential PGPM isolates were studied for their plant growth promoting traits. All of them produced IAA and ammonium, while eleven fungal PGPMs

produced siderophore, twenty one isolates exhibited phosphate solubilization and twenty two isolates produced HCN as plant growth promoting traits.

Based on the BLAST results of ITS gene sequencing, eight endophytic fungal isolates such as FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34 were identified as *F. sulawesiense*, *F. incarnatum*, *F. nygamai*, *F. equeseti*, *F. proliferatum*, *Aspergillus nidulans*, *T. asperellum*, respectively. Among them, *F. sulawesiense*, *F. incarnatum*, *F. nygamai*, *F. equeseti* and *F. proliferatum* are new reports for pathogen inhibition and for growth promoting activity.

Three isolates of rhizospheric fungal namely, SFPGPM-3, SFPGPM-9 and SBPGPM-13 were identified as *T. asperelloides*, *T. asperellum* and *T. harzianum*, respectively with BLAST results of ITS gene sequencing.

With the result of BLAST analysis of 16S rRNA gene sequence, five potential endophytic bacterial PGPMs such as BEPGPM-5, BEPGPM-9 BEPGPM-27, BEPGPM-28, BEPGPM-30 were identified as *B. tropicus*, *B. tropicus*, *B. pacificus*, *B. cereus* and *B. subtilis*, respectively. Among them *B. tropicus* is a new report from which exhibited the antagonistic potential against *R. bataticola* along with plant growth promoting activity

Four potential rhizospheric bacterial isolates viz., SBPGPM-19, SBPGPM-21 SBPGPM-23 and SBPGPM-28 were identified *B. cereus*, *B. velezensis*, *B. subtilis* and *B. subtilis* sub sp. *subtilis* in the molecular identification based on BLAST results of 16S rRNA gene sequence. Among them, *B. velezensis* is a new report which inhibited *R. bataticola*, apart from exhibiting plant growth promoting traits *in vitro*.

Among nineteen PGPMs screened for bioactive compound production with invert plate technique, *T. asperellum* with per cent inhibition of 74.61, *T. harzianum* (67.22), *B. subtilis* (60.61) and *B. velezensis* (59.06) showed highest inhibition of pathogen which indicated the production of bio-active compounds by these microbes.

GC-MS/MS analysis of crude extract of *T. asperellum* showed the presence of 65 compounds at different retention time ranging from 3.166 to 30.91 min. Among them thirteen compounds were recorded as bio-active compounds having anti-microbial properties. Other species, *T. harzianum* showed the presence of 62 compounds in the GC-MS/MS analysis of its crude extract at different retention time ranging from 3.464 to

29.818 min. Among them, 20 were recorded as bio-active compounds processing antimicrobial activity.

The GC-MS/MS analysis revealed that the extract of *B. subtilis* showed the presence of 60 compounds at different retention time ranging from 3.139 to 29.737. Among them, 9 compounds with anti microbial property were regarded as bio-active compounds.

B. velezensis showed the presence of 61 compounds at different retention time ranging from 3.484 to 27.273 in the GC-MS/MS analysis. Among them, 11 were identified as bio-active compounds based on earlier reports on their anti-microbial property.

Plant growth promoting traits of chickpea seedlings (germination per cent, shoot length, root length) were influenced significantly by *T. asperellum*, *T. harzianum* and *B. velezensis* under *in vitro*.

In pot culture experiment, T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/l and *Beauveria bassiana* at 2 g/l) showed maximum plant height (27.53 cm), number of branches (6), number of pods (9.52), dry weight (13) and pod weight (3.24) with minimum PDI of 20.79.

Under field conditions, T₆ (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/l and *Beauveria bassiana* at 2 g/l) showed maximum plant height (55.4), number of branches (12), number of pods (106), test weight (19.8) and yield (6.59) with minimum PDI (20.78).

Conclusions:

- One hundred and twenty six PGPMs were successfully isolated from the samples collected from eleven districts.
- Out of 126 PGPM isolates, seventy isolates were endophytic (40 fungal and 30 bacterial PGPMs) and fifty six isolates were rhizospheric (26 fungal and 30 bacterial PGPMs).
- The cultural characteristics of fungal PGPMs varied with respect to the colony diameter, colony color, colony margin, colony surface and mycelial growth.
- In case bacterial PGPMs, the cultural characters were very distinct with respect to colony color, colony form, colony elevation, colony margin, cell shape and staining reaction.
- Eight endophytic fungi (FEPGPM-5, FEPGPM-11, FEPGPM-16, FEPGPM-24, FEPGPM-27, FEPGPM-28, FEPGPM-34 and FEPGPM-35) and eight endophytic bacteria (BEPGPM-5, BEPGPM-6, BEPGPM-9, BEPGPM-15, BEPGPM-25, BEPGPM-27, BEPGPM-29 and BEPGPM-30) were identified as potential PGPMs against *R. bataticola*.
- Three rhizospheric fungal (SFPGPM-3, SFPGPM-9, SFPGPM-13) and nine rhizospheric bacterial PGPMs (SBPGPM-4, SBPGPM-7, SBPGPM-9, SBPGPM-15, SBPGPM-19, SBPGPM-21, SBPGPM-23, SBPGPM-28 and SBPGPM-30) were considered as potential for inhibition of pathogen.
- Twenty eight potential PGPMs produced IAA and ammonium, eleven fungal PGPMs produced siderophore, twenty one isolates exhibited phosphate solubilization and twenty two isolates produced HCN which are growth promoting traits for chickpea development.
- *F. sulawesiense*, *F. incarnatum*, *F. nygamai*, *F. equeseti*, *F. proliferatum*, *Aspergillus nidulans*, *T. asperellum*, were identified as fungal endophytic PGPMs. Among them, *F. sulawesiense*, *F. incarnatum*, *F. nygamai*, *F. equeseti* and *F. proliferatum* are new reports for inhibition of *R. bataticola* with growth promoting activity

- *Trichoerma* sp. such as *T. asperelloides*, *T. asperellum* and *T. harzianum*, were identified as rhizospheric fungal PGPMs.
- *B. tropicus*, *B. pacificus*, *B. cereus*, *B. subtilis* were identified as bacterial endophytic PGPMs. Among them *B. tropicus* is a new report which exhibited the antagonistic potential against *R. bataticola* with plant growth promoting activity.
- *B. cereus*, *B. velezensis*, *B. subtilis* and *B. subtilis* sub sp. *subtilis* were identified as rhizospheric bacterial PGPMs. Among them *B. velezensis* is a new report which exhibited the antagonistic potential against *R. bataticola* with plant growth promoting activity.
- Among nineteen PGPMs screened for production of bio-active compounds, *T. asperellum*, *T. harzianum*, *B. subtilis* and *B. velezensis* have showed maximum per cent inhibition due to the production of bio-active compounds.
- The GC-MS/MS analysis of ethyl acetate extract of *T. asperellum* and *T. harzianum* showed the presence of 13 and 20 bio-active compounds, respectively. However, *B. subtilis* and *B. velezensis* showed 9 and 11 bio-active compounds which are reported to process antimicrobial property.
- Germination per cent, shoot length, root length and seedling vigour index which are growth promoting traits, were influenced significantly when the chickpea seedling were treated with *T. asperellum*, *T. harzianum* and *B. velezensis*.
- In pot and field experiment for the management of dry root rot of chickpea, the treatment combination (Seed treatment with *T. asperellum* at 10 g/kg seed followed by soil application of enriched vermicompost with *T. asperellum* at 2.5 kg/100 kg per acre at sowing followed by foliar application of *T. asperellum* at 10 g/l and *Beauveria bassiana* at 2 g/l) showed minimum per cent incidence and maximum growth and yield parameters.

Future line of work

1. The quantitative estimation and standardization of particular bio-active compound responsible for inhibition of *R. bataticola*.
2. The effective PGPM's formulations should be standardized for commercialization.
3. Cross evaluation of PGPMs can be done against other pathogens of other leguminous crops.

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APPENDICES

I. Sequences of endophytic fungal PGPMs

>FEPGPM -5 (*Fusarium sulawesiense*) (ON514084)

CAAACAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT
GTTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTGTGGGACTCGCGGTA
ACCCGCGTTCCCCAAATCGATTGGCGGTACAGTCGAGCTTCCATAGCGTAGTA
ATCATAACCTCGTTACTGGTAATCGTCGCGGCCACGCCGTAAAACCCCAACT
TCTGAATGTTGACCTCGGATCAGGT

> FEPGPM -11 (*Fusarium incarnatum*) (ON595690)

ACTCCCAAACCCCTGTGAACATACCTATACGTTGCCTCGGCGGATCAGCCCGC
GCCCCGTAAAACGGGACGGCCCGCCGAGGACCCCTAAACTCTGTTTTAGT
GGAACCTCTGAGTAAAACAAACAAATAAATCAAAACTTTCAACAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTG
GTGTTGGGACTCGCGGTAACCCGCGTTCCCCAAATCGATTGGCGGTACAGTCG
AGCTTCCATAGCGTAGTAATCATAACCTCGTTACTGGTAATCGTCGCGGCCA
CGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACC
CGCTGAA

>FEPGPM -16 (*Fusarium nygamai*) (ON564638)

CGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTCTGAGTAAA
ACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT
GTTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGTTTGGTGTGGGGATCGGC
GAGCCTCACGGCAAGCCGGCCCCGAAATACAGTGGCGGTCTCGCTGCAGCTT
CCATTGCGTAGTAGTAAAACCTCGCAACTGGTACGCGGCGCGGCCAAGCCG
TTAAACCCCAACTTCTGAATGTTG

>FEPGPM -24 (*Fusarium equeseti*) (ON514139)

CTCGGCGGATCAGCCCGCGCCCCGTAAAAAGGGACGGCCCGCCCGAGGACCC
CTAAACTCTGTTTTTAGTGGAACCTTCTGAGTAAAACAAACAAATAAATCAAA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAA
TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGTATTCTGGCTGGCATGCCTGTTTCGAGCGTCA

>FEPGPM -27 (*Fusarium proliferatum*) (ON514349)

GGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAAC
TCTGTTTCTATATGTAACCTTCTGAGTAAAACCATAAATAAATCAAAACTTTCA
ACAACGGATCTCTTGGTTCTGGATCGATGAAGAACGCAGCAAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACC

>FEPGPM-28 (*Aspergillus nidulans*) (ON564645)

TAAACTGTTGCTTCGGCGGGGAGCCCCCAGGGGCGAGCCCGCCGGGGACCA
CTGAACTTCATGCCTGAGAGTGATGCAGTCTGAGCCTGAATACAAATCAGTC
AAAACCTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCG
AACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGA
ACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATT
GCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCTGTCGTCCCCCCGGGGGACGG
GCCCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCCTCGAGCGTATGGGGCTTT
GTCACCCGCTCGATTAGGGCCGGCCGGGCGCCAGCCGGCGTCTCCAACCTTA
TTTTTCTCAGGTTGACCTCGGATCAGGT

>FEPGPM-34 (*Trichoderma asperillum*) (ON514144)

AACTGTTGCCTCGGCGGGGTACGCCCCGGGTGCGTCGCAGCCCCGGAACCA
GGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACG
TATTTCTTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCC
CGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACC
CCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCC

CTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAA
CTCGCACCGGGAGCGCGGGCGGTCCACGTCCGTAAAACACCCAACTTTCTGA
AATGTTGACCTCGGATCAGGTAGGAATACCCGCTGA
ACTTAAGCATATCAATAAGGCGGAGGAA

II. Sequences of rhizospheric fungal PGPMs

>SFPGPM-3 (*Trichoderma asperelloides*) (ON513885)

CGGCGGGGTACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCCGCCG
GAGGAACCAACCAAACCTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAG
CTCTGAGCAAAAATTCAAATGAATCAAACCTTCAACAACGGATCTCTTGGT
TCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGCCAGTATTCTG
GCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATC
GGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGG
CGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCCGGGAG
CGCGGCGCGTCCACGTCCGTAAAACACCC

>SFPGPM-9 (*Trichoderma asperellum*) (ON514143)

ACTGTTGCCTCGGCGGGGTACGCCCCGGGTGCGTCGCAGCCCCGGAACCAG
GCGCCCCCGGAGGAACCAACCAAACCTCTTTCTGTAGTCCCCTCGCGGACGT
ATTTCTTTACAGCTCTGAGCAAAAATTCAAATGAATCAAACCTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTGAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
GCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCC
CTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCC
TAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAAC
TCGCACCCGGGAGCGCGGCGCGT

>SFPGPM-13 (*Trichoderma harzianum*) (ON514140)

ACTGTTGCCTCGGCGGGGTACGCCCCGGGTGCGTCGCAGCCCCGGAACCAG
GCGCCCCCGGAGGAACCAACCAAACCTCTTTCTGTAGTCCCCTCGCGGACGT
ATTTCTTTACAGCTCTGAGCAAAAATTCAAATGAATCAAACCTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA

TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
GCCAGTATTCTGGCGGGCATGCCTG

III. Sequences of endophytic bacterial PGPMs

> BEPGPM-5 (*Bacillus tropicus*) (ON564730)

CATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTT
GAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGG
ACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGAT
GCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCT
GACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACCTCTGT
TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCT
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT
GGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTT
AAGTCTGATGTGAAAGCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGG
GAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG
CGTAGAGATATGGAGGAACACCAGTGGCCGAAGGCGACTTCTGGTCTGTAA
TGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGT
GCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCC

> BEPGPM-9 (*Bacillus tropicus*) (ON564907)

CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGC
ATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGT
CGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCC
GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAG
CAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACCTCTGTTGTTAGG
GAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGA
AACGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG
CGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTG
ATGTGGAAAGCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACT

TGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA
GATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTA ACTGACAC
TGAGGCGCGAAAGCGTGGGGAGCAAACA

> **BEPGPM-25** (*Bacillus pacificus*) (ON564773)

CCTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAA
CATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATG
GATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCA
ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACAC
GGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAA
AGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAAC
TCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGG
TACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
TAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGT
TTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAA
ACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTG
AAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCT
GTA ACTGACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTA AACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCC
TTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCA
AGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATG
TGGTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA
CAACCCTAGAGATAGGGCTTCTCCTTCCGGGAGCAGAGTGACAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGC
AACCCCTGATCTTAGTTGCCATCATTAGTTGGGCACTCTAAGGTGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG
ACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGC
GAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAAC
TCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGT
ACACCCGAAGTCGGTGGGGTAACCTTTT

> **BEPGPM-27** (*Bacillus cereus*) (ON564610)

ACCTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATA
ATATTTTGAAGTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTAT
GGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC
AACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACA
CGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA
CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACG
GTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGG
TTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAA
ACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCATGTGTAGCGGTG
AAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTCTGGTCT
GTAAGTACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCC
TTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCA
AGGCGAAACTCAAAGGAATTGACGGGGGCCCGC

> **BEPGPM-30** (*Bacillus subtilis*) (ON564689)

TGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTT
GTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAG
ATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG
GCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTTCGGATCGTAAAGCT
CTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGT
ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCGGCTCAACCGGGGAGGGTCATTGGAAA
CTGGGGAAGTCTGAGTGCAGAAGAGGAGAGTGGAATTCACGTGTAGCGGTGA
AATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTG
TAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCT
TAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAG
ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTG

GTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACA
ATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGG
TTGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA
CCCTTGATCTTA

IV. Sequences of rhizospheric bacterial PGPMs

> SBPGPM 19 (*Bacillus cereus*) (ON567448)

ATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTG
AACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGA
CCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATG
CGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTG
ACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAACTCTGTT
GTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTA
ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTA
AGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGG
AGACTTGAGTGCAAGAGAGGAAAGTGAATTCCATGTGTAGCGGTGAAATGC
GTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTCTGGTCTGTA
GACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGT
GCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCT
AGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTC
GTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
GATCTTAGTTGCCATCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG
CTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGG
AGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTA
CATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAAACCCCG
AAG

>SBPGPM 21 (*Bacillus velezensis*) (ON567373)

GGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCAT
GGTTCAGACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGC
GCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCG
ACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC
AACGCCGCGTGAGTGATGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTAGGG
AAGAACAAGTGCCGTTCAAATAGGGCGGCACCTTGACGGTACCTAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCG
TTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGAT
GTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACCTGA
GTGCAGAAGAGGAGAGTGGAATCCACGTGTAGCGGTGAAATGCGTAGAGAT
GTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAGTACTGACGCTGA
GGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCTTAGTGCTGCAGCT
AACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAA
GGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGC
AACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAG
GACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGAGCTC
GTGTGCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAG
TTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAG
GAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACA
CGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAA
TCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAA
GCTGGAATCGTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCG
GGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAAACACCCGAAGTC

>SBPGPM 23 (*Bacillus subtilis*) (ON566236)

GGGGATAGCCTTCGAAAGGAAGATTAATAACCGCATAACATAAGAGAATCGC
ATGATTTTCTTATCAAAGATTTATTGCTTTGAGATGGACCCGCGGGCGCATTAG
CTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAG
AGGGTGATCGGCCACATTGGAAGTACTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGC

GTGGGTGATGAAGGTCTTCGGATTGTAAAGCCCTGTTTTCTGGGACGATAATG
ACGGTACCAGAGGAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA
ATACGTAGGTGGCGAGCGTTGTCCGGATTACTGGGCGTAAAGGGTGCGTAG
GCGGATGTTAAGTGGGATGTGAAATCCCCGGGCTAACCTGGGGGCTGCAT
TCCAAACTGGATATCTAGAGTGCAGGAGAGGAAAGCGGAATTCCTAGTGTAG
CGGTGAAATGCGTAGAGATTAGGAAGAACACCAGTGGCGAAGGCGGCTTTCT
GGACTGTA ACTGACGCTGAGGCACGAAAGCGTGGGTAGCAAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGGATACTAGGTGTAGGGGGTATC
AACTCCCCCTGTGCCG CAGTTAACACAATAAGTATCCCGCCTGGGGAGTACG
GTCGCAAGATTA AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGG
AGCATGTGGTTTAATT CGAAGCAACGCGAAGAACCTTACCTGGACTTGACAT
CCCTTGCATAGCCTAGAGATAGGTGAAGCCCTTCGGGGCAAGGAGACAGGTG
GTGCATGGTTGTCGTCAGCTCGTGTCTGTGAGATGTTAGGTTAAGTCCTGCAAC
GAGCGCAACCCTTGTTATTAGTTGCTACCATT AAGTTGAGCACTCTAATGAGA
CTGCCTGGGTAACCAGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCC
TTATGTCCAGGGCTACACACGTGCTACAATGGTAGGTACAATAAGACGCAAG
ACCGTGAGGTGGAGCAAACTTATAAAACCTATCTCAGTTCGGATTGTAGGC
TGCAACTCGCCTACATGAAGCTGGAGTTGCTAGTAATCGCGAATCAGAATGT
CGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGA
GCTGGTAACACCCGAAGTCCGTGAGGTAACCGTAAGGAGCCAGCGGCCGAAG
GTGGGATTAGTGATTGGGGTGAAGTCGTAACAAGGT

>SBPGPM- 28 (*Bacillus subtilis* sub sp. *subtilis*) (ON566124)

GCGGCGGACGGGTGAGTAACACGTGGGT AACCTGCCTGTAAGACTGGGATAA
CTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAA
ACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCATTAT
CTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTATCCGACCTGAG
AGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG
CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCCG
GTGAGTGATGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAAAACAA
GTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGG
CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
AATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTTCTTAAGTCTGATGTGAAAG
CCCCCGGCTCAACCGGGGAGGGTCATTGGAAAACCTGGGGAACTTGAGTGCAG

AAGAGGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG
AGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAGTACGCTGAGGAG
CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA
ACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCTTAGTGCTGCAGCTAACG
CATTAAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA
TTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGTAAGCAAC
GCGAAGAACCTTACCAGGTCTT