EVALUATION OF NATIVE RHIZOSPHERE MICROFLORA FOR PLANT GROWTH PROMOTION AND MANAGEMENT OF FUSARIUM YELLOWS IN GINGER

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Evaluation of native rhizosphere microflora for plant growth promotion and management of Fusarium yellows in ginger"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Evaluation of native rhizosphere microflora for plant growth promotion and management of Fusarium yellows in ginger"** is a record of research work done independently by **Rekha, K.G. (**2012-11-182**)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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DEDICATION

This humble piece of work is dedicated

To My

Beloved & Respected Parents

&

To My Dear Husband

Whose affections inspired

To have mankind

Who did their best to uplift me up to

the heights of an ideal life.

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ABBREVIATIONS

CD	Critical Difference
cfu	Colony forming units
g	Gram
h	Hour (s)
HCN	Hydrogen Cyanide
IAA	Indole Acetic Acid
INM	Integrated nutrient managment
PSB	Phosphate Solubilizing Bacteria
MAP	Months After Planting
DAP	Days after planting
PGPR	Plant growth promoting rhizobacteria
CRD	Completely randomized design
WAP	Weeks after planting
bp	Base pair
Kb	Kilo Base
MSL	Mean Sea Level
NCBI	National Center for Biotechnology Information
NCFT	National Center for Fungal Taxonomy
M	Meter
N	Normality

PCR	Polymerase chain reaction
SDS	Sodium Dodecyl Sulphate
TAE	Tris Acetate EDTA Buffer
UV	Ultra violet
β	Beta

INTRODUCTION

1. INTRODUCTION

India is considered as '*The land of spices'* and enjoys a unique position in the production and export of ginger. Ginger is cultivated for use as a spice, confectionary product, and component of herbal remedies (Smith, 2004). It is a herbaceous, tropical perennial plant belonging to the family Zingiberaceae. It is usually grown as an annual crop. The whole plant is refreshingly aromatic, but it is the underground rhizome (raw or processed) which is valued as spice. Its medicinal value is increasingly being recognized now a days. Ginger originated in South- East Asia, probably in India (Purseglove, 1972).

Ginger is cultivated in several parts of the world and the most important countries being *viz.*, India, China, Indonesia, Bangladesh, Australia, Jamaica and Nepal. Among them, India and China are the dominant suppliers to the world market. Indian ginger is sold in the world market as 'Cochin' and 'Calicut' (Jhony *et al*., 2002).

At present, India is the largest producer of ginger in the world accounting for about one-third of the total world output. It is mainly cultivated in Meghalaya, Kerala, Arunachal Pradesh, Orissa, West Bengal and Mizoram. In Kerala, the area under ginger is 6088 ha with a production of 33197 MT and productivity of 5453 kg/ha (Selvan *et al*., 2002).

One of the major constraintsin ginger production is the complex of rhizome rot diseases. The important rhizome rot diseases are soft rot caused by *Pythium aphanidermatum* and *Fusarium* yellows (dry rot) caused by *Fusarium oxysporum* f. sp. *zingiberi*. Severe losses have been reported in ginger cultivation due to declining soil health and *Fusarium oxysporum* f. sp. *zingiberi* in Wayanad district of Kerala (Vijayaraghavan and Mathew, 2011). Hence, there is a need to enhance the yield as well as to manage the Fusarium yellows disease. Eventhough, the chemical fertilizers and fungicides can be used to enhance yield

as well as manage the Fusarium yellows disease, there will be residual problems and the pathogen may develop resistance due to its continous use.

Organic cultivation of ginger involves the use of microbial inoculants for enhancing growth, yield and reducing the disease incidence. Generally, interactions between plants and microorganisms help the plants to improve the uptake of nutrients and to restrict the entry of certain deleterious plant pathogens (Lynch, 1990). Plant Growth Promoting Rhizobacteria (PGPR) produces growth promoting substances to enhance yield and also protect them against diseases (Castro-Sowinski *et al*., 2009).

There are no studies on the effect of native beneficial isolates on growth promotion and disease management in ginger. With this background, the present studies were undertaken to screen native microbial isolates with an objectives to enhance the growth and yield of ginger using native beneficial microorganisms and also to manage Fusarium yellows disease in ginger by using antagonistic microorganisms from Wayanad district.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Sustainable agriculture is based on ecofriendly methods, tends to use Plant Growth Promoting Rhizobacteria (PGPR) as tools that could be the way to reduce the use of chemicals, incidence of diseases etc. Severe losses in productivity in ginger cultivation have been due to declining soil health and *Fusarium oxysporum* f. sp. *zingiberi* (*Foz*). Several studieshave demonstrated that plant growth promoting bacteria may increase yield, improve resistance to diseases and reduce the requirement for fertilisers (Compant *et al*., 2005).

2.1 GINGER CROP

Among all spices, ginger is an important cash crop supporting the livelihood and improving the economic level of many ginger growers. Ginger (*Zingiber officinale Rosc*) is an herbaceous perennial, the rhizomes of which are used as a spice. India is a leading producer of ginger in the world and the country produced 3.70 lakh tonnes of the spice from an area of 1.06 lakh hectares. Ginger is grown in almost all the states of the region (Ravindran *et al*., 2005).

Apart from improved varieties, a number of local cultivars exist. Some of the prominent indigenous cultivars are Maran, Kuruppampadi, Ernad, Wayanad, Himachal and Nadia. Exotic cultivars such as Rio-de-Janeiro have also become very popular among cultivators (Valsala,2004).

2.2 DISEASES

2.2.1 Fusarium yellows

It is common to find yellow, stunted above-ground shoots among apparently healthy green shoots. The plant finally dries out as the fungus invades entire vascular system of the underground rhizomes. The rhizomes show a creamy

brown discoloration of the water-conducting portion and a prominent black dry rot of the cortex tissues (the portion outside of water-conducting tissues). The rhizomes do not become soft and water-soaked as in bacterial wilt, and when cut they do not ooze bacterial slime (Trujillo, 1963).

Fusarium yellows is probably the most serious problem of ginger because it is more widely spread than bacterial wilt. Plants infected by the fungus, *Fusarium oxysporum* f. *zingiberi,* do not wilt rapidly as in bacterial wilt. Instead, infected ginger plants are stunted and yellow in colour. The lower leaves dry out over an extended period of time (Haware and Joshi, 1973).

In Kerala, a pilot survey for disease incidence showed that, out of 195 gardens visited, 8.84 % recorded *Fusarium* sp. (Dake and Edison, 1988;1989). In recent years it has become a serious disease in the ginger growing areas of Wayanad district in Kerala (Vijayaraghavan and Mathew, 2011).

2.2.2 Soft rot

Pythium aphanidermatum has been reported to cause soft rot of ginger in the field. Although, this problem was not considered very serious, it is possible that *Pythium* soft rot of ginger could be a problem under high rainfall and poorly drained soil (Sarma, 1994). The yellowing shown by the affected plants can be confused with symptoms of Fusarium yellows and with other problems such as mineral eficiencies. In Kerala, losses could be as high as 90 % during heavy incidence (Rajan and Agnihotri, 1989). Dake and Edison (1989) reported that 19.8 % of rhizome rot of ginger was associated with *P. aphanidermatum.*

2.2.3 Bacterial wilt

The disease was first reported from Madras state (Orian, 1953). The disease was endemic in majority of the ginger growing areas viz., Kerala, Sikkim

and many other north eastern regions of the country which caused yield loss up to 100 % under conducive conditions (Mathew *et al*., 1979). *Pseudomonas solanacearum* (Smith) Smith, biotype III has been identified as the causal agent of the disease (Sarma *et al*., 1978). The disease is typically seed and soil borne.It is generally noticed during June - July and maximum during August- September, ensuring higher soil moisture, relative humidity andlow temperature (Sarma *et al.,* 1978). Later, it was reported from Kerala (Mathew *et al*., 1979), Bihar (Ojha *et al*., 1986) and Himachal Pradesh (Dohroo, 1991).

2.2.4 Leaf spot

Leaf spot is caused by *Phyllosticta zingiberi* and the disease is noticed on the leaves during July to October. The disease starts as water soaked spot and later turns as a white spot surrounded by dark brown margins and yellow halo. The lesions enlarge and adjacent lesions coalesce to form necrotic areas (Ishii and Aragaki, 1963).

2.3 SOIL MICROFLORA FOR PLANT GROWTH PROMOTION

Kloepper *et al*. (1989) reported that wheat yield increased upto 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Because of the enormous numbers of microbial populations and species in the soil, especially in the rhizosphere, intensive and extensive interactions have been established between soil microorganisms and various other soil organisms, including plant roots, and plant growth promotion by rhizosphere microorganisms (Bashan, 1998).

In spite of the deleterious effects of some microorganisms on plants, the beneficial effects are usually greaterfor growth promotion and faster germination (Atlas and Bartha, 1998). Bacterial inoculants that augment populations of fluorescent pseudomonads, *Bacillus* sp., *Azospirillum* sp., phosphate solubilizing

bacteria and/or mycorrhizal helper bacteria have been applied to agronomically important crops in order to establish a "beneficial rhizosphere" (Atkinson and Watson, 2000).

Rhizosphere competence of rhizobacteria is strongly correlated with their ability to use organic acids as carbon sources, and the composition and quantity of root exudates (Goddard *et al*., 2001).Direct plant growth promotion includes symbiotic and non-symbiotic PGPR which function through production of plant hormones and the ability to produce a vital enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene in the root of developing plants thereby increasing the root length and growth (Glick and Penrose, 2001).

Significant increases in growth and yield of agronomical important crops due to inoculation with PGPR have been reported by various workers (Asghar *et al*., 2002). Although, there may be some artificial and trivial effects on plant growth promotion induced by the inoculation of some soil bacteria, overall evidence of significant plant growth induced by rhizosphere microorganisms was overwhelming (Gerhardson and Wright, 2002).

Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals (Bashan *et al*., 2004). Similar increases in plant height and leaf area were observed in different crops inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains (Shaukat *et al*., 2006). *Azospirillum*, *Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth (Roesti *et al*., 2006). *Azospirillum* and *Pseudomonas* have the potential for agricultural exploitation and could be used as natural fertilizers (Cakmack *et al*., 2006).

Beneficial effect of soil microorganisms on plant growth involves abilities to act as phytostimulators and biofertilizers. Plant growth promoting microorganisms (PGPR) could enhance crop yield through nutrient uptake and plant growth regulators and also as biocontrol agents by production of antibiotics, triggering induced local or systemic resistance, or preventing the deleterious effects of xenobiotics by degradation (rhizoremediators) by acting as rhizoremediators (Aseri *et al*., 2008).

In addition, soil microorganisms have great adaptation to high temperatures, dryness or heavy rainfalls in tropical countries (Da Mota *et al*., 2008), contaminated environments (Dell'Amico *et al*., 2008), harsh environments including drought stress (Arzanesh *et al*., 2011), indicating that they could contribute to ameliorate plant crops in areas with poor agricultural potential. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* increased root and shoot elongation in canola (Ahmad Gholami and Somayeh Nezarat, 2009).

Plant growth promotion and development is facilitated both directly and indirectly. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms achieved through the production of siderophores, synthesis of antibiotics, production of hydrogen cyanide (HCN) and fungal cell wall degrading enzymes such as chitinase and ß-1, 3-glucanase (Figueiredo *et al*., 2011).

PGPR has the ability to produce hormones like indole acetic acid (IAA) (Patten and Glick, 2002), abscisic acid (ABA) (Dobbelaere *et al*., 2003), gibberellic acid (GA) and symbiotic nitrogen fixation (Kennedy *et al*., 2004) antagonism against phytophatogenic bacteria by producing siderophores, ß-1, 3-glucanase, chitinases, antibiotic, fluorescent pigment and cyanide (Cattelan *et al*., 1999: Pal, 1999; Glick *et al*., 2003) solubilization and mineralization of nutrients, particularly mineral phosphates (de Freitas *et al*., 1997, Richardson, 2001; Banerjee and Yasmin, 2003) and improve soil structure and organic matter

content. PGPR also retain more soil organic N, and other nutrients in the plant–soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients.

2.3.1 Mechanism of plant growth promotion

Microorganism mediated plant growth promotion consist of direct and indirect growth promotion. The direct promotion by PGPR involves either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when PGPR prevent deleterious effects of one or more phytopathogenic microorganisms. Some PGPR may promote plant growth indirectly by affecting symbiotic N_2 fixation, nodulation or nodule occupancy (Burr, 1984).

2.3.1.1 Nitrogen fixation

Sen (1967), made one of the earliest suggestions that the activity of associated nitrogen fixing bacteria such as *Azospirillum* could meet the nitrogen nutrition of cereal crops. After establishing in the rhizosphere, *Azospirilla* usually, but not always, promotes the growth of plants. Although, they posses N_2-fixing capability (~1x10 kg N/ha), the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake (Dewan *et al.,* 1979).

Various crops in India have been inoculated with diazotroph particularly *Azotobacter* and *Azospirillum.* Reports have proved that application of *Azotobacter* and *Azospirillum* improved the yield of both annual and perennial grasses where *Azospirillum* strains have been isolated and used (Steenhoudt and Vanderleyden, 2000). A large number of bacterial genera are linked with rhizospheric like *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*,

Bacillus, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* (Tilak *et al.,* 2002).

Nitrogen is essential for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is also important for plant growth promotion. The process of biological nitrogen fixation (BNF) accounts for 65% of the nitrogen currently utilized in agriculture and will continue to be important in future sustainable crop production systems (Matiru and Dakora, 2004). The improvement of soil fertility is one of the most common strategies to increase agricultural production. The biological nitrogen fixation is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, phosphate solubilization is equally important in enhancing the soil fertility (Hafeez *et al*., 2006)

There is a great significance of non-symbiotic nitrogen fixation in agriculture though this process is facing some problems due to lack of carbon and energy used in the nitrogen fixation. However, the limitation could be decreased byendophytically growing diazotroph in rhizosphere such as *Acetobacter, Clostridium, Azomonas, Bacillus, Corynebacterium, Pseudomonas, Xanthobacter* (Saxena and Tilak, 1998) and *Azotobacter* sp. (Vessey, 2003; Barriuso *et al*., 2008). PGPR are generally used as fertilizer in the form of inoculants and should be an illustrative way of replacing chemical fertilizers, pesticides, and supplements (Ashrafuzzaman *et al.,* 2009).

2.3.1.2 Phosphate solubilization

Pseudomonas striata and *Bacillus polymyxa* solubilized 156 and 116 mg l-1 respectively (Rodríguez and Fraga, 1999). Direct application of rock phosphate is often ineffective with in short period for most of the annual crops (Goenadi and Kaushik, 2000). Acid producing microorganisms are able to enhance the solubilization of rock phosphate (Gyaneshwar *et al*., 2002). The PSB in

conjunction with single super phosphate and rock phosphate reduced the phosphorus dose by 25 and 50 %, respectively (Sundara *et al*., 2002). The PSB solubilized the fixed soil phosphorus and applied phosphates resulting in higher crop yields (Gull *et al*., 2004). Phosphorous is one of the major nutrients, second only to the nitrogen in requirement for plants. Most of the phosphorous in soil is present in the form of insoluble phosphates and cannot be utilized by plants (Pradhan and Sukla, 2005). The rhizospheric phosphate solubilizing bacteria could be a promising source for plant growth promoting agent in agriculture. The use of phosphate solubilizing bacteria as inoculants increases the phosphorous uptake by plants (Chen *et al*., 2006).

The PSB strains exhibited inorganic P-solubilization abilities ranging between 25-42 μg ml^{-1} and organic P mineralization abilities between 8-18 μg ml^{-1} (Tao *et al*., 2008). The *Pseudomonas putida* and *P. fluorescens* released 51, 29 and 62% phosphorus respectively with highest value of 0.74 mg/50 ml from Fe_2O_3 (Ghaderi *et al*., 2008). *P. fluorescens* solubilized 100 mg l^{-1} containing $Ca_3(PO_4)_2$ or 92 and 51 mg l^{-1} containing $AlPO_4$ and $FePO_4$ respectively (Henri *et al*., 2008).

2.3.1.3 Indol-3-Acetic Acid production

The most physiologically active auxins in plants is indole-3- acetic acid (IAA), which is known to stimulate both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants (Hagen, 1990). Bacteria synthesize indole acetic acid (IAA) predominantly by an alternate tryptophan-dependant pathway, through indole pyruvic acid. Promotion of root growth is one of the important parameters by which the beneficial effect of plant growth PGPR is measured (Glick, 1995). Indole-3-Acetic Acid is the most common and well characterized phytohormone. It has been estimated that 80% of bacteria isolated from the rhizosphere could produce IAA (Patten and Glick, 1996). In phytopathogenic bacteria, IAA seems to be mainly produced from

tryptophan via theintermediate indole-3-acetamide (IAM pathway),while in beneficial phytostimulatory bacteria, IAA appears to be synthesized predominantly via indole-3-pyruvic acid (IPyA pathway) (Patten and Glick 1996).

Sevcral microorganisms are capable of producing auxins, cytokinins, gibberilins, ethylene or abscisic acid (Lebuhn *et al.,* 1997). They are organic substances capable of regulating plant growth produced either endogeneously or applied exogeneously are called plant growth regulators. They also regulate growth by affecting physiological and morphological processes at very low concentrations (Arshad and Frankenberger,1998). Tien *et al.* (2000) reported that some of the P-solubilizing bacteria act as plant growth promoters due to their ability to produce IAA but there is a different IAA production potential among PSB and PSF isolates. *Bacillus megaterium* from tea rhizosphere is able to produce IAA and thus it helps in the plant growth promotion. The tryptophan increased the production of IAA in *Bacillus amyloliquefaciens* (FZB42).

In many PGPR, genes involved in IAA production are regulated by stress factors that commonly occur in soil and potentially within the rhizosphere (e.g. acidic pH and osmotic stress), and in some cases have been shown to be activated by plant exudates (Mahmoud *et al*., 2005). Earlier reports indicated that IAA can also be a signaling molecule in bacteria which, can have a direct effect on bacterial physiology (Costacurta *et al*., 1995; Tsavkelova *et al*., 2007).

Tsavkelova *et al*. (2007) screened a collection of root-associated bacteria from soybean for their ability to produce IAA and showed that it was present in 28% of isolates. *Pseudomonas fluorescens* (B16) is a PGPR which produces a plant growth promotion factor called pyrroloquinoline Quinone. The evidences indicate that PGPR influence plant growth and development by the production of phytohormone such as auxins, gibberellins, and cytokinins. The strains which produce the highest amount of auxins i.e. indole acetic acid (IAA) and indole acetamide (IAM) in non-sterilized soil, causes maximum increase in growth and

yield of the wheat crop. Even the strains, which create low amount of IAA, liberate it constantly. Indole-3-Acetic Acid (IAA) controls a wide variety of processes in plant development and growthand also plays a key role in shaping plant root architecturesuch as regulation of lateral root initiation, rootvascular tissue differentiation, polar root hair positioning, root meristem maintenance and root gravitrophism (Yasmin *et al*., 2007).The crop inoculated with the isolates capable of IAA production significantly increased the plant growth by the N, P, K, Ca and Mg uptake of sweetpotato cultivar (Aseri *et al*., 2008).

The impact of exogenous auxin on plant development ranges from positive to negative effects and occurs as a function of the amount of IAA produced, the number of auxin-producing rhizobacteria and on the sensitivity of the host plant to changes in IAA concentration (Spaepen *et al.,* 2008). Khakipour *et al.* (2008) evaluated the auxin productivity potential in *Pseudomonas* strains and reported variety of auxins like indole-3-acetic acid (IAA), indole-3-pyruvic acid, indole-3-butyric acid and indole lactic acid; cytokinins and gibberellins with auxin production being most important.

Karnwal *et al*. (2009) tested fluorescent pseudomonads isolates for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan and found that for both strains, indole production increased with increases in tryptophan concentration. Isolates producing IAA had stimulatory effect on the plant growth. The ability of *Azotobacter* to produce IAA is attributed more to yield improvement rather than to diazotrophic activity. *P. fluorescens* and *P. putida* are the most important PGPR which produces auxin and promote the yield. While working on chickpea, it was found that all the isolates of *Bacillus, Pseudomonas* and *Azotobacter* produced IAA, whereas only 85.7% of *Rhizobium* was able to produce IAA (Ghosh *et al*., 2008). Hariprasad and Niranjana, (2009) reported the highest concentration of IAA produced by *P. fluorescens*.

When cucumber, tomato and pepper were inoculated with different strains of PGPR, there was a significant increase in the growth of the vegetables due to produce IAA (Deusilin *et al*., 2011).

2.4 SOIL MICROFLORA FOR PLANT DISEASE MANAGMENT

Rhizobacteria could reduce the activity of pathogenic microorganisms not only through microbial antagonism but also by activating a phenomenon called, Induced Systemic Resistance (ISR) (Verma, 1993). Most important group of rhizobacteria for biological control is the *Pseudomonas* (Kremer and Kennedy, 1996). *Pseudomonas* sp. contains many characters that make them apt as biocontrol and growth-promoting agents (Weller, 1988). *Pseudomonas fluorescens* and *Bacillus* sp. are excellent examples of biocontrol agents. Plant growth promoting rhizobacteria produce HCN and siderophores. Bacteria used to target plant pathogens by antagonism (antibiosis or parasitism) or that induce systemic resistance have been referred to as biocontrol Plant Growth Promoting Bacteria (Bashan, 1998).

Soil microorganisms were isolated from the rhizosphere of healthy ginger plants among the rhizome rot affected plants in the diseased field and were quantitatively and qualitatively estimated. The population of bacteria was maximum followed by actinomycetes and fungi. *Rhizopus, Aspergillus, Trichoderma* and *Eupenicillium, Streptomyces* sp. and four species of bacteria B1, B2, B3 and B4 were found in the rhizosphere of ginger (Nybe, 2001). The ability to suppress disease by introduced *Pseudomonas* strains depends mainly on their rhizosphere population density (Raaijmakers *et al*., 1998) and their ability to colonize the roots (Chin-A-Woeng *et al*., 2000) and the lack of motility and consequently rhizosphere colonization, some *Pseudomonas* strains, producing the antibiotic phenazine, failed to suppress soil-borne pathogens (Chin-A-Woeng *et al*., 2003).

However, biocontrol by rhizobacteria could involve PGPR and non-PGPR bacteria for the suppression of plant diseases. Non-pathogenic rhizobacteria can antagonize pathogens through competition for nutrients, production of antibiotics and secretion of lytic enzymes (Van Loon and Bakker, 2003). Major application of PGPR is for biocontrol of the plant pathogens as well as biofertilization (Siddiqui, 2006). The strains of bacteria isolated from *Lolium perenne* rhizosphere soil were capable of acting as plant growth promoting bacteria and as an agent which controls the pathogens shows various plant growth promoting actions (Shoebitz *et al.,* 2007).

2.4.1 Mechanism of plant disease management

Generally, plant diseases cause 10–20 % loss in production (Jetiyanon *et al*., 1981). An alternative to chemical control of plant diseases is by the use of antagonists which are able to antagonize phytopathogenic are considered as a more environmentally friendly process. The important bacteria are *Pseudomonas, Bacillus*, and *Streptomyces*. Numerous studies on bacteria antagonistic to phytopathogens include fluorescent pseudomonads and *Bacillus subtilis* (Kloepper *et al*., 1989). Biological control of soil-borne pathogens with antagonistic bacteria has been intensively investigated. In this mode of action, direct interaction between PGPR and the endogenous microflora is necessary. PGPR can promote plant growth by suppressing diseases caused by soil-borne pathogens (Van Loon and Glick, 2004).

2.4.1.1 Hydrogen Cyanideproduction

One group of microorganisms which acts as a biocontrol agents include the Deleterious Rhizobacteria (DRB) which can colonize plant root surfaces and able to suppress plant growth (Suslow and Schroth, 1982). Cyanide is a dreaded chemical produced by them as it has toxic properties.

Hydrogen cyanide (HCN), a volatile compound is produced by certain fluorescent pseudomonads in presence of glycine and Fe^{3+} ions (Keel *et a*l., 1989). A secondary metabolite produced commonly by rhizosphere pseudomonads is hydrogen cyanide (HCN), a gas known to negatively affect root metabolism and root growth (Schippers *et al.,* 1990). Although, cyanide acts as a general metabolic inhibitor, it is synthesized, excreted and metabolized by hundreds of organisms, including bacteria, algae, fungi, plants and insects. The host plants are generally not negatively affected by inoculation with cyanide-producing bacterial strains and host-specific rhizobacteria can act as biological agents (Zeller *et al.,* 2007).

The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soils of different agronomic crops (Ahmad *et al.,* 2008). It was shown in some literature that few species of *Pseudomonas* with HCN may improve the plant establishment (Wani *et al.,* 2007) when the rhizospheric isolates were tested in laboratory. Similarly, the HCN production in *Azotobacter* sp. and phosphate solubilizing bacteria such as *Pseudomonas aeruginosa* and *Bacillus* sp. were found to be positive (Ravikumar *et al.*, 2010).

2.4.1.2 Siderophore Production

Siderophores produced by PGPR could contribute to enhanced growth (Kloepper *et al*., 1980b). Addition of *Pseudomonas* pyoverdine to soils conducive to Fusarium wilts confer them suppressiveness (Kloepper *et al*., 1980a). Actinomycete also produces siderophore. Endogenous siderophore (ferrioxamine) and exogenous siderophore (ferrichrome) have been studied in *Streptomyces pilosus* (Anupama *et al*., 1984).

In addition, when soil was treated either by *Pseudomonas* or its pyoverdine, reduced chlamydospore germination of pathogenic *F. oxysporum*

(Elad and Baker, 1985a), suggesting a possible role of pyoverdines in soil fungistasis and suppressiveness. They include pyoveridins produced by *Pseudomonas*, catechols produced by *Agrobacterium tumefaciens*, hydroxamates produced by *Erwinia carotovora*, *Enterobacter cloacae*, and various fungi; and rhizobactin produced by *Rhizobium meliloti*. Pyoverdine which is a yellow-green, water-soluble fluorescent pigment is the major class of siderophores produced by fluorescent pseudomonad. However, strains of *P. aeruginosa, P. syringae*, and *P. putida* could also produce pyoverdine (Scher *et al*., 1986).

In addition, the ability to produce siderophores is likely to contribute to the root-colonizing ability of *Pseudomonas* strains, their antagonistic properties, and their usefulness in biocontrol (Leong, 1986). The role of these microorganisms in disease-suppressive soils particularly to fusarium wilts was shown to be related to siderophore-mediated iron competition. Duffy *et al*. (1988) reported that, iron is important for plant health and metabolism. It is found in proteins such as nitrogenase, ferredoxins, cytochromes, and leghemoglobin. PGPR bacteria could help in uptake of iron from soil and provide it to plant. The most widely studied rhizospheric bacteria with respect to the production of siderophores are fluorescent pseudomonads.

Fluorescent pseudomonads exclusively recognize the ferric complex and the differences in structure affect the biological activity of the siderophores (Hohnadel and Meyer, 1988). Wild-types of *Pseudomonas putida* (WCS358) which relatively suppressed *F. oxysporum* in roots were found to depend only on siderophore-mediated competition for iron. Subsequently, its mutant defective in siderophore biosynthesis was ineffective. This fact provides the proof that siderophores were implicated in the suppressivness of Fusarium wilt by this strain (Duijff *et al*., 1993). *Streptomyces violaceusniger* strain (YCED9) was reported as able to chelate iron under limiting conditions (Buyer *et al*., 1989). Siderophores are low-molecular-weight microbial compounds with high affinity for iron. They possess an iron uptake system (iron binding ligand) able to chelate Fe^{3+} molecules.

They are often induced under limiting Fe^{3+} concentrations to allow bacteria to partially fulfill their iron requirement. Siderophores represent a large biochemically diverse group produced by either plants or plant associated microorganisms (Loper and Buyer, 1991). Siderophore-producing microbes could contribute to various alterations in plants via the action of siderophores on ferric nutrition (Bar-Ness *et al*., 1991). In addition, *in vitro* assays showed that the inhibition of pathogens based on competition for iron tends to decrease with increasing ironcontent of the medium (Duijff *et al*., 1993).

When various plants growing in soil or nutrient solution were supplemented with pyoverdin or ferriopyoveridne, chlorophyll content, iron content in the roots and ferric reductase activity were enhanced (Duijff *et al*., 1994b,c). Siderophores are produced by *Pseudomonas* bacteria to compete for iron and consequently impairing growth of soil-borne phytopathogens, and thus are considered as a control mechanism for many pathogens (Schippers *et al*., 1987; Duijff *et al*., 1994a). Suppressive soils to Fusarium wilts are known to have a very low solubility of ferric iron (Garbeva *et al*., 1996). Consequently, a strong iron competition occurs in these soils. Siderophores can be used not only by the producing bacteria (Ongena *et al*., 1999), but also by other microorganisms.

In addition, some siderophores like pyocyanin and pyoveridin are essential for the induction of systemic resistance (Audenaert *et al*., 2002). *Streptomyces lydicus* (WYEC108) was found to produce hydroxamate-type siderophores (Tokala *et al*., 2002). Similar results were obtained in *P. putida* (WCS358) for Fusarium wilt in radish caused by *F. oxysporum f.* sp. *raphani* (de Boer *et al*., 1999). However, as disease suppression does not rely only on siderophore production, lack of evidence in the use of mutant-derived strains has been reported.

2.4.1.3 Antagonistic activity against plant pathogens

Pseudomonas contains many characters that make them suitable for biocontrol and growth-promoting agents (Weller, 1988). Rhizobacteria can restrain a wide range of bacterial, fungal and nematode diseases. PGPR can also offer shelter against viral diseases. Rhizobacteria can reduce the activity of pathogenic microorganisms not only through microbial antagonism, but also by activating the plant to produce Induced Systemic Resistance (ISR) (Peer *et al.,* 1991). Most important group of rhizobacteria for biological control is the *Pseudomonas* (Kremer and Kennedy, 1996). *Pseudomonas* sp. is present everywhere in the soil. Marvelous progress has been made in characterizing the procedure of root colonization by *Pseudomonas*, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere ability, and the way of pathogen inhibition. Among various biocontrol agents, fluorescent pseudomonads have several mechanisms for control of phytopathogens and plant growth promotion (Banasco *et al.,* 1998).

2.4.1.4 Fusarium oxysporum f.sp. *zingiberi*

Infection of ginger by *Fusarium oxysporum* f. sp. *zingiberi* (*Foz*) might manifest as a severe rhizome rot, stunting of plants, yellowing of leaves and a vascular wilt that resulted in plant death (Pegg *et al.,*1974). As the disease progresses, rhizomes become shrivelled until only a shell and fibrous tissue remain (Pegg *et al.,* 1974). Sharma and Jain (1979) reported growth promotion and reduced incidence of *Fusarium oxysporum* in ginger following the application of *B. subtilis* strain-1. Fluorescent pseudomonads and *Bacillus* species have been reported to improve resistance against diseases caused by *Fusarium oxysporum*, which causes severe losses in crop production worldwide.

While biocontrol activity of *Bacillus* and fluorescent pseudomonads strains against *Fusarium oxysporum* havebeen demonstrated, only one account of

the use of plant growth promoting bacteria against *Fusarium oxysporum* f. sp. *zingiberi* has been reported. Sharma and Joshi (1979) reported antagonism and reduced incidence of *Fusarium oxysporum* f. sp. *zingiberi* in ginger with the application of a *B. subtilis* strain to ginger seed pieces and soil.

The informal designation of *Fusarium oxysporum* into species is based the on specificity of the host plant infection that is *Fusarium oxysporum* f. sp. *zingiberi* is known to only infect ginger (Burgess *et al.,* 1981). Infection by *Fusarium oxysporum* diseases often occurs through roots, entry of *Fusarium oxysporum* f. sp. *zingiberi* is reported to be via rhizome cracks or wounds in ginger (Pegg *et al.,*1974; Burgess *et al.,*1981). Infected rhizomes display an internal brown discolouration.

Sahare and Asthana (1988) reported that *Trichoderma harzianum* and *Trichoderma viride* were the most effective in reducing mycelial growth of *Fusarium oxysporum* f. sp. *Zingiberi* and *Pythium aphanidermatum*, causal agents of yellows and rhizome rot of ginger, respectively. Rajan *et al*. (2002), reported constraints associated with *Ralstonia solanacearum*, *Pythium* spp., *Fusarium oxysporum* and *Pratylenchus coffeae*. *Trichoderma harzianum*, from Sikkim was found effective in control of ginger diseases substantially.

The colonisation of hyphae of *Fusarium oxysporum* f. sp. *lycopersici* by certain fluorescent pseudomonads produced antifungal metabolites and lytic enzymes, which contributed to the biocontrol traits of these bacteria (Bolwerk *et al.,* 2003). It was also shown that fusaric acid produced by *Fusarium oxysporum* f. sp. *lycopersici* served as a chemoattractant stimulating motility of *P. fluorescens* (WCS635) and colonisation of the hyphae of the fungus (de Weert *et al.,*2004).

The pathogen *Fusarium oxysporum* f. sp. *zingiberi,* causes a rhizome rot and vascular wilt of ginger (Stirling, 2004). Shanmugam *et al*. (2013) reported

that rhizobacteria and *Trichoderma* sp. were antagonistic against *Fusarium oxysporum* f. sp. *zingiberi* and *Pythium splendens* inciting rhizome rot of ginger. Ram *et al*. (2013) reported that rhizome rot complex in ginger caused by *Ralstonia solanacearum*, *Pythium* spp., *Fusarium oxysporum* is managed through rhizomes dipped in Curzate M8 (0.2 %) + Streptocycline (0.05 %) for 30 min along with soil application of neem cake @ 5q/ha, *Pseudomonas fluorescens* and *Trichoderma viride* @ 10 Kg mixed with 25t FYM/ha @ One drench with Curzate M8 (0.2 %) immediately at the onset of the disease. It was found effective in reducing disease incidence and population density of pathogens along with increasing germination percentage, number of tillers per hill, plant height, yield and B:C ratio.

Vinayaka *et al*. (2014) conducted a study of determining biocontrol potential of a macrolichen (*Usneapictoides*) against *Fusarium oxysporum* f. sp. *zingiberi* and *Pythium aphanidermatum* isolated from rhizome rot of ginger. The extract exhibited inhibition of *Fusarium oxysporum* f. sp. *zingiberi* under *in-vitro* conditions. Among fungi, susceptibility was higher in case of *P. aphanidermatum*. The lichen could also be used as a potential candidate in the biocontrol of rhizome rot.

2.4.1.5 Pythium aphanidermatum

Important diseases have been controlled with antagonistic fungi, bacteria and actinomycetes. In several cases, extents of disease control obtained were similar to those of standard fungicides (Whipps, 1991). Among fungal antagonists, the genus *Trichoderma* species are the most studied. Among bacteria and actinomycetes, fluorescent pseudomonads, *Bacillus* sp. and *Streptomyces* sp. have received maximum attention. Two years of field trials showed that *T. harzianum* was efficient in controlling the disease both in solarized and non-solarized plots (Usman, 1996).

Usman *et al*. (1997) reported that, out of 8 species of *Trichoderma* and 2 isolates of *Gliocladium virens,* only *T. hamatum* (ISO.2) showed inhibition of *P. aphanidermatum* in dual culture tests. *T. harzianum* (ISO.2), *T. hamatum* (ISO.2) and *T. polysporum* (*Tolypocladium niveum*) were efficient in hyphal interactions. Rhizome rot caused by *Pythium aphanidermatum* in ginger was effectively controlled by *T. hamatum* on seed ginger (Bhardwaj *et al*., 1998). Shanmugam *et al*. (2000) reported that only *T. harzianum* and *T. viride* were potential antagonists and further pot culture experiments established the efficacy of *T. harzianum* for control of rhizome rot of ginger.

Jayasekhar *et al.* (2001) reported that seeds of ginger, dipped in 0.2% copper oxychloride, 1% Bordeaux mixture, 0.1% chlorothalonil, 0.01% Metalaxyl MZ, 0.25% mancozeb or 0.1% emison and treated with biological control agents (*Trichoderma harzianum* + neem cake, *Laetisaria arvalis*+ neem cake or *T. viride* + neem cake), exhibited effectiveness in the reduction of ginger rhizome rot caused by *P. aphanidermatum*, and increased crop yield. Sagar *et al*. (2007) reported that out of nine biological control agents evaluated under *in vitro* conditions against *Pythium aphanidermatum* in ginger, the maximum inhibition of mycelial growth of *Pythium aphanidermatum* was noticed in *T. harzianum* (Sirsi isolate) (77.77%), which was at par with *T. harzianum* (Dharwad isolate) (76.40%). Among the nine biocontrol agents evaluated against *Fusarium solani*, *T. harzianum* (Sirsi and Dharwad isolates) inhibited the maximum mycelial growth (78.51 and 76.29%), which was at par with *T. virens* (77.03%).

2.4.1.6 Ralstonia solanacearum

Rhizome wilt has been an important threat to the cultivation of ginger since, it was reported from Surat area in Gujarat (Butler, 1907). The disease is usually caused by a fungus, bacterium and plant parasitic nematode, *Meloidogyne* spp. The wilts caused by fungus and bacterium ultimately lead to rhizome wilt which is reported to be caused by *Fusarium oxysporum* f. sp. *zingiberi* (Trugello)

and bacteria, *Ralstonia solanacearum* (Smith) in early stage of the crop. The strains *Bacillus subtilis* (1JN2), *Myroides odoratimimus* (3YW8), *Bacillus amyloliquefaciens* (5YN8) and *Stenotrophomonas maltophilia* (2JW6) showed more than 50% biocontrol efficacies under greenhouse condition. Among the biocontrol agents, *Pseudomonas fluorescens* resulted in maximum inhibition of *Ralstonia solanacearum.*

Chao Hui *et al*. (2011) reported that three strains LW-4, LW-7 and LW-32 of *Bacillus* sp. had strong antagonistic effect against *R. solanacearum.* Bandyopadhyay *et al*. (2012) reported that rhizome treatment with hot water at 51°C for 10 m. along with soil application of *T. harzianum* inoculum (mixed with 1 kg neem cake at the time of planting) resulted in the lowest disease incidence (27.14%) and highest rhizome yield (6.46 kg $plot^{-1}$). Wei *et al*. (2013) reported that, out of 420 bacterial strains isolated from rhizosphere soil, the plant surface stem, leaf, and root tissue of ginger, 19 antagonists were effective against *Ralstonia* wilt of ginger and recorded disease control between 26.09-69.17%.

2.4.1.7 Rhizoctonia solani

Bunker *et al*. (2001) reported *T. harzianum* as the most effective in causing significant suppression of growth and sclerotia formation of *R. solani* causing dry root rot in chilly through production of volatile and non-volatile antibiotics followed by *G. virens* and *T. aureoviride*. Chavarria *et al*. (2005) reported the population of *T. viride*, and *Streptomyces* sp. as 4.5×10^{8} and 2×10^{1} conidia g^{-1} respectively. *Bacillus subtilis* was 6.1×10^{10} cfu ml^{-1}. The biological treatment showed a significantly higher yield compared to the chemical treatment in ginger.

2.5 EFFECT OF MICROBIAL INOCULANTS ON PLANT GROWTH ON SPICES

Kloepper *et al.* (1980) used the term "plant growth promoting rhizobacteria" to describe bacteria having a stimulatory effect on plant growth. Dobbelaere *et al.* (2003) reported that plant associated bacteria may have beneficial, inhibitory or neutral effects on plant growth. Such bacteria may increase the availability of plant nutrients and stimulate root proliferation, resulting in increased water and nutrient uptake and yield of crops.

Increased plant vigour, induced systemic resistance and antagonism of plant pathogens by PGPB may reduce disease incidence and further improve yields in crop production (Vessey, 2003).A consortia of B_9 (*Bacillus* from Kolavayal of Wayanad) FP_8 (Fluorescent pseudomonads from Ambalavayal of Wayanad) were the most efficient consortia for the growth and establishment of micropropagated ginger under field conditions (Gopal, 2008).

2.5.1 Nutrient availability and uptake

Bolwerk *et al.* (2003) used fluorescently labelled *Pseudomonas* spp. and *Fusarium oxysporum* f. sp. *lycopersici* to demonstrate that both microorganisms occupied the same niche on tomato root cells (intercellular junctions), a site of plant exudation.Production of organic acids or phosphatases by certain bacteria may mobilise nutrients such as phosphorus (Rodriguez *et al.,* 2006). Bioinoculants controls the availability of iron, nutrient mobilization and biological nitrogen fixation. Certain bacteria, including strains of fluorescent pseudomonads and *Azospirillum* species, produce sideophores that sequester iron in the rhizosphere, making it unavailable to other microbes but often also usable by plants (Mercado- Blanco and Bakker, 2007).

2.5.2 Plant stimulating hormones and metabolites

Specific strains of *Azospirillum brasilense* and *Pseudomonas fluorescens* have demonstrated the capacity to excrete indole acetic acid, an important auxin in most plants (Dobbelaere *et al.,* 1999). Production of gibberellins by strains of *Bacillus macroides, Bacillus subtilis, Azospirillum brasilense* and *Azospirillum lipoferum* have been documented (Bottini *et al.,* 1989, Janzen *et al*., 1992; Joo *et al*., 2004). Plant growth promoting bacteria may produce hormones, such as auxins (indole -3- acetic acid) and gibberellins, which stimulate plant growth (Steenhoudt and Vanderleyden, 2000; Yanni and El-Fatttah*,* 2001; Woodward and Bartel, 2005). Ryu *et al.* (2003) demonstrated the bacterial volatile metabolites, acetonin and 2,3- butanediol, produced by *Bacillus subtilis* GB03 and *Bacillus amyloliquiefaciens* (IN937a) promoted the growth of *Arabidopsis thaliana* seedlings.

Cytokinins are known to stimulate plant cell division (Lynch,1985). Growth promotion induced by *B. amyloliquefaciens* (IN937a) was independent of known pathways (ethylene, gibberellic acid, cytokinins and brassinosteroids) although studies with auxin deficient mutants were not conclusive. These volatiles were later demonstrated to confer resistance to disease in *Arabidopsis* (Ryu *et al.,* 2004). Many processes in plants are regulated by the volatile hormone ethylene, including seed emergence, fruit ripening, senescence and defence responses against plant pathogens (Abeles *et al.,* 2007).

2.5.3 Plant growth promotion by nitrogen fixers

Yield of cotton (Iruthayaraj *etal*., 1981), rice (Yanni and El-Fattah, 1999), and wheat (Barassi *et al*., 2000) increased with the application of *Azotobacter.* Knosop *et al.* (2010) found that *A. chroococcum* produced siderophores which act as growth factors and as phytopathogenic suppressive agents. Narula and Tauro (1986) reported that the beneficial effects of *Azotobacter* were attributed to their

ability to produce ammonia, vitamins, growth substances like IAA and auxins, gibberlins and cytokinins. These bacteria are able to fix nitrogen independently in contrast to *Rhizobium* species (Sprent and Sprent 1990). *Azotobacter* species (*Azotobacter vinelandii* and *Azotobacter chroococcum*) are free-living heterotrophic diazotrophs that depend on an adequate supply of reduced carbon compounds such as sugars for their energy source (Kennedy and Tchan, 1992).

Mali *et al*. (1993) reported production of siderophores by *A. chroococcum* which solubilized Fe^{3+} and suppressed plant pathogens through iron deprivation.The beneficial effect of *Azotobacter* is attributed to production of plant growth hormones, improved nutrient uptake and antagonistic effect on plant pathogens (Parmar and Dadarwal, 1999). The strains of *Azotobacter chroococcum* have demonstrated the potential of the bacteria to promote plant growth and enhance the yield of crops in different soils and in different climatological conditions (Kumar *et al*., 1998). Dobbelaere *et al.* (1999) demonstrated that an increase in root hair formation in wheat following inoculation with auxin producing *Azospirillum brasilense* (Sp7), could be mimicked by the application of indole acetic acid.

Kumar *et al*. (2001) found that seed inoculation of wheat varieties with phosphorus solubilizing and phytohormone producing *Azotobacter chroococcum* showed better response compared to control. Verma (2001) reported that *Azotobacter* inhibited phytopathogenic fungi through antifungal substances.Other free-living diazotrophic bacteria include *Bacillus* spp., *Azotobacter* sp*., Klebsiella* sp., *Azoarcus* sp., *Acetobacter* sp. and *Azospirillum* sp. (Dobbelaere *et al.,* 2003). The main reasons associated with the failure to demonstrate nitrogen fixation in mechanisms of plant growth promotion by *Azospirillum* species included inactivation of nitrogenase enzymes due to attachment to root surfaces, and also ammonia derived from nitrogen fixation is not typically excreted by *Azospirillum* spp. (Bashan *et al.,* 2004).

A range of plant growth promoting rhizobacteria (PGPR) participates in interaction with crop plants and significantly increases their vegetative growth and yield (Kennedy *et al*., 2004). The biological fixation of atmospheric nitrogen (and conversion into plant available ammonia) is carried out by nitrogenase enzymes of diazotrophic bacteria (Bashan *et al.,* 2004). The increase in plant growth due to inoculation of wheat with microaerophilic *Azospirillum* species was often linked to the production of plant growth promoting substances such indole acetic acid rather than nitrogen fixation.

Growth promotion due to the introduction of *Azospirillum brasilense* and *Azospirillum lipoferum* in cereal and other plants have been extensively documented (Okon and Labandera-Gonzalez, 1994, Dobbelaere *et al*., 2003). Upto fifty percent nitrogen fertiliser input was sufficient following inoculation of monocots, grains, grasses and a variety of vegetable crops with *Azospirillum* species, *Herbaspirillum* spp. or *Gluconacetobacter* spp. (Okon and Labandera-Gonzalez, 1994; Kennedy *et al.* 2004; Cocking,2005).

Ahamad and Holl (2005) found that *A. paspali* secreated IAA into the culture media and significantly increased the dry weight of leaves and roots of several plant species following root treatment. Behal *et al*. (2006) demonstrated the contribution of *A. chroococcum* on the solubilization of calcium phosphates. *Azotobacter* sp. played a significant role in mobilizing phosphorous from the native soil pool, as well as from the added insoluble phosphates such as rock phosphates for plants to use.

Dinesh *et al*. (2013) isolated plant growth-promoting rhizobacteria (PGPR) from soils under ginger and the promising PGPR (*Burkholderia cepacia, Klebsiella* sp*., Serratia marcescens,* and *Enterobacter* sp.) were either applied alone or in combination with varying rates of NPK fertilizers to determine their effect on sensitive biochemical and microbial properties of soils under ginger (*Zingiber officinale Rosc.*). Results revealed that combined application of PGPR

and fertilizers positively influenced microbial biomass-P (PMIC) and net N mineralized (NMIN) rates compared to sole application of PGPR or NPK. It was also revealed that activities of dehydrogenase (DHA) was more than 49.0 %, urease (UR) by 15 %, acid phosphatase (AcP) by 40 %, and β-glucosidase (bG) by 35 % in PGPR +NPK treatments compared to NPK alone.

2.5.4 Plant growth promotion by phosphate solubilizing bacteria

Considerable evidence supports the specific role of phosphate solubilizing bacteria in the enhancement of plant growth. *Bacillus megaterium* var. *phosphaticum* was applied successfully in the former Soviet Union and India, but it did not show the same efficiency in soils in the United States. Simultaneous increases in phosphorus uptake and crop yields have been observed after inoculation with *Bacillus polymyxa* (Gaur and Ostwal, 1972), *Bacillus firmus* (Datta *et al*., 1982), and *Bacillus cereus*.

Undoubtedly, the efficiency of the inoculation varies with the soil type, specific cultivar and other parameters. The P content of the soil is probably one of the crucial factors in determining the effectiveness of the product. The production of chelating substances by microorganisms as well as the production of inorganic acids, such as nitric and carbonic acid has been considered as the mechanisms involved in phosphate solubilization. The plant growth promotion by PSB is considered to be related to their ability to synthesize plant growth promoting substances (Sattar and Gaur, 1987).

A strain of *Pseudomonas putida* stimulated the growth of roots and shoots and increased 32P-labeled phosphate uptake in canola. Complexing of cations is an important mechanism in P solubilization, if the organic acid structure favors complexation (Fox *et al*., 1990). Rock phosphates are often too insoluble to provide sufficient phosphorus for crop uptake. Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid, which is present

in *Bacillus firmus* (Banik and Dey 1982), *Rhizobium meliloti* (Halder and Chakrabarty 1993).

Use of PSMs can increase crop yields up to 70 per cent (Verma, 1993). Chabot *et al*. (1993) demonstrated growth stimulation of maize and lettuce by several microorganisms capable of mineral phosphate solubilization. Production of organic acids results in acidification of the microbial cell and its surroundings. Consequently, Pi may be released from a mineral phosphate by proton substitution for Ca^{2+} (Goldstein, 1994). Phosphorus (P) is one of the important macronutrients for plant growth and development. It is present as organic and inorganic phosphates in soils. Among the diverse and naturally abundant microbes inhabiting the rhizosphere, the Phosphate Solublising Microorganisms (PSM) not only provides phosphorous to plants, but also assists plant growth by other mechanisms (Young, 1994).

The production of organic acids by phosphate solubilizing bacteria has been well documented. Type and position of the ligand in addition to acid strength determine its effectiveness in the solubilization process (Kpomblekou and Tabatabai, 1994).The concentration of soluble P in soil is usually very low (Goldstein, 1994). Phosphate solubilization takes place through various microbial processes or mechanisms including organic acid production and proton extrusion (Surange *et al.,* 1995; Dutton and Evans,1996; Nahas, 1996). Organic acids produced by PSB solubilized insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996).

Carboxylic anions produced by PSB, have high affinity to calcium, solubilized more phosphorus than acidification alone (Staunton and Leprince, 1996). Strains of *B. subtilis, B. megaterium*, *B. polymxa, B. sphaericus, B. brevis*, *B. thuringiensis, Enterobacter* spp. and *Agrobacterium radiobacter* have demonstrated the *in vitro* ability to solubilize phosphates, which may be related to

the production of phosphatases or organic acids (De fretis *et al.* 1997). A strain of *Burkholderia cepacia* showed no indole acetic acid production, but displayed significant mineral phosphate solubilization and moderate phosphatase activity which improved the yield of tomato, onion, potato, banana and coffee. Higher crop yields resulted from solubilization of fixed soil phosphorus and applied phosphates by PSB (Zaidi *et al*., 2009).

The most efficient PSM belongs to genera *Bacillus, Rhizobium* and *Pseudomonas*. The production of organic acids especially gluconic acid seems to be the most frequent agent of mineral phosphate solubilization by bacteria such as *Pseudomonas* sp. *Pseudomonas cepacia* and *Burkholderia cepacia* (Rodríguez and Fraga, 1999). The application of phosphorus biofertilizers in the form of plant growth promoting microorganisms can facilitate the availability of accumulated phosphates for plant growth and development by solubilization. The bacteria involved in phosphorus solubilization as well as better scavenging of soluble forms can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements (Gyaneshwar *et al*., 2002).

To convert insoluble phosphates (both organic and inorganic) compounds in available form accessible to the plant is an important trait in a plant growth promoting bacteria for increasing plant yields (Rodriguez *et al.,* 2006). The plant takes up several P forms but major part is precipitated in soil is generally highly dependent on pH and soil type. The capacity of some microorganisms to convert insoluble phosphorus (P) to orthophosphate is an important trait in a plant growth promoting bacteria for increasing plant yields (Rodriguez *et al.,* 2006).

Strains of *Bacillus licheniformis* and *B. amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Other organic acids, such as glycolic acid, oxalic acid, malonic acid, succinic acid, citric acid and propionic acid, have also been identified among phosphate solubilizers (Chen

et al., 2006). Secretion of organic acids and phosphatase enzymes are common mechanisms that facilitate the conversion of insoluble forms of phosphorous to plant accessible forms (Kumari *et al*., 2009). The inorganic phosphate mobilization is realised due to organic acid production, proton release or production of chelating substances by the bacteria (Zaidi *et al*., 2009). Some soil bacteria with phytase activity contribute to the phosphorus release from organic phosphates (Singh and Satyanarayana, 2011).

2.6 EFFECT OF MICROBIAL INOCULANTS ON THE DISEASE MANAGEMENT IN GINGER

Effective suppression of rhizome rot under field conditions has been obtained in Rajasthan when *T. viride*, *T. harzianum* was applied to soil in combination with wood sawdust (Anon, 1994). Good control of rhizome rot caused by *P.aphanidermatum* was obtained in Kerala when *T. harzianum* or *T. hamatum* was applied to soil along with neem oil cake (Anon, 1994). Besides foliar diseases, rhizome diseases are very important that result in severe crop losses. The rhizome diseases appear as soft rot caused by *Pythium aphanidermatum* and dry rot caused by *Fusarium* spp. (Sharma and Jain, 1979).

Incidence of rhizome rot was low when *T. viride* was applied to soil along with wood sawdust (Dataram, 1988). *Trichoderma harzianum, T. virde, Azadirachta indica* and *Agave americana*were the most effective in reducing mycelial growth of *Fusarium oxysporum f. sp.zingiberi* and *P. aphanidermatum* causing yellows and rhizome rot of ginger respectively (Sharma, 1998). Ram *et al.* (2000) evaluated native isolates of biocontrol agents *viz., T. harzianum, T. aureoviride* and *T. virens* for control of ginger rhizome rot. All the four bioagents could establish and reduced the population density of both *F. solani* and *P. aphanidermatum*.

Efficacy of different fungal and bacterial biocontrol agents against rhizome diseases of ginger and plant growth parameters was determined under field conditions. Combined applications of bioagents were more effective in reducing the disease incidence than the individual treatments. *Trichoderma harzianum*+ *Pseudononas fluorescens* + *Bacillus subtilis* gave minimum disease incidence on rhizomes (8.64 %) as well as on tillers (12.50 %). Combined treatment also proved more effective in increasing the plant growth parameters i.e. number of tillers, plant height, fresh rhizome weight along with more recovery of old rhizome (Meena and Mathur 2003).

Orhan and Esitken, (2006) reported the experiment on nutrient mobilization with four isolates namely *Burkholderia cepacia* (GRB 25), *Klebsiella* sp. (GRB 36), *Serratia marcescens* (GRB 38) and *Enterobacter* sp. (GRB 70) and graded levels of NPK. Field experiments revealed that *B. amyloliquifaciens* (GRB 35) and *S. marcescens* (GRB 68) were effective for disease control and plant growth promotion. Both the isolates (GRB 35 and GRB 68) recorded more than 75% sprouting. Soft rot incidence was also significantly less (<10%) compared to control. The rhizobacterial treatment recorded significantly higher yield.

Mathew (2006) reported the antagonistic activity of *T. aureoviride, T. virens* and an endophytic bacteria *P. schulzeri* inoculated against the pathogens causing rhizome rot and bacterial wilt in ginger. Consortia of *T. harzianum* + *P. fluorescens* and *T. harzianum* + *T. virens* provided better management of rhizome rot and bacterial wilt disease and also enhanced the plant growth in ginger (Mathew, 2009).

2.6.1 *Bacillus* sp. as biocontrol agent

Bacillus subtilis inhibited *S. cepivorum* under *in vitro* and were able to suppress the incidence of onion white rot, leading to an increased onion

emergence and yield (Utkhede and Rahe, 1980). *Bacillus* sp. were reported as producers of antibiotics inhibiting various phytopathogens including *F. oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* (Asaka and Shoda, 1996). *B. subtilis* strain (A13) was antagonistic to a wide range of fungal pathogens under*in vitro* and due to moderate amounts of gibberellins production (Broadbent *et al.,* 1977). Sharma and Jain (1979) reported growth promotion and reduced incidence of *Fusarium oxysporum* f. sp. *zingiberi* in ginger following the application of *B. subtilis* (strain-1). The bacterium was applied to the soil and rhizomes in greenhouse conditions.

Grosch *et al*. (1998) reported that the formation of antifungal metabolites did not contribute significantly when compared with different isolates of *Bacillus subtilis*. No correlation was found between the ability to form metabolites that are effective against *Fusarium oxysporum* on various media *in vitro. B. subtilis* (A13) was one of the first commercialised biopesticides in the United States, where it was applied as a seed treatment (along with fungicides) for suppression of soil-borne fungal pathogens (Zehnder *et al.,*2001).

Bacillus is most abundant in the rhizosphere, and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved (Gutierrez *et al*., 2003). Different *Bacillus* species were reported to be effective biocontrol agents in greenhouse or field trials (Kloepper *et al*., 2004).The eliciting ISR and antibiotic production are mechanisms by which *Bacillus* spp. may reduce disease and increase yield in crop production.

Certain strains have been shown to also produce volatiles, lytic enzymes and/or gibberellins that may be involved in growth promotion or disease resistance (Schallmey *et al.,* 2004). *Bacillus* sp. produces highly resistant endospores and have therefore been formulated with relative ease in commercial biopesticide preparations (Schisler *et al.,* 2004). *Bacillus licheniformis* when

inoculated on tomato and pepper showed considerable colonisation and can be used as a biofertiliser without altering normal management in greenhouses (Garcia *et al*., 2004). *B. subtilis* endospores have been used extensively in cotton crops for the suppression of *Fusarium oxysporum* f. sp. *vasinfectum* (Backman *et al.,*1994; Jacobsen *et al.,* 2004).

Mechanisms involved in *Bacillus* eliciting plant growth promotion include biocontrol abilities (Asaka and Shoda, 1996) and induction of systemic resistance (Zehnder *et al*., 2000), increased uptake availability of phosphorus, auxin production (Idris *et al*., 2004).The ability of certain strains of *B. subtilis, B. pumilus, B.cereus, B. amyloliquefaciens, B. licheniformis, B. simplex, B. firmus* and *B. sphaericus* to inhibit plant pathogens and promote plant growth have been reported earlier (Manjula and Podile, 2005). *Bacillus* also has potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions (Orhan *et al*., 2006).

The *Bacillus megaterium* var. *phosphaticum* and potassium solubilising bacteria (KSB) *Bacillus mucilaginosus* when inoculated in nutrient limited soil showed that rock materials and both the bacterial strains consistently increased mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer (Han *et al*., 2006). The *Bacillus pumilus* 8N-4 can also be used as a bio-inoculant to increase the crop yield (Hafeez *et al*., 2006). *Bacillus megaterium* is very consistent in improving different root parameters (rooting performance, root length and dry matter content of root) in mint (Kaymak *et al*., 2008). *Bacillus* species have been reported as plant promoting bacteria in a wide range of plants (Deepa *et al*., 2010).

2.6.2 *Pseudomonas fluorescens* as biocontrol agent

Increased population of fluorescent pseudomonads was noticed in the rhizosphere of diseased plants (Mazzola and Cook, 1991). Sequestration of iron

by these siderophores may result in suppression of plant disease by certain strains of fluorescent pseudomonads (O'Sullivan and O'Gara, 1992). *Pseudomonas* sp. is ubiquitous bacteria in agricultural soils and has many traits that make them a suitable PGPR. The most effective strains of *Pseudomonas* have been Fluorescent Pseudomonads. Considerable research is underway globally to exploit the potential of fluorescent pseudomonads (FLPs). FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lata *et al*., 2002).

Field trials of a pseudomonad strain (GRP3) increased yield of legumes (Johri, 2001). Raaijmakers (2002) demonstrated that bacterial strains with different 2, 4-diacetylphloroglucinol (DAPG) genotypes varied in their rhizosphere colonization efficiency and ability to inhibit *Fusarium*. Meena and Mathur (2003) tested the ability of a fluorescent pseudomonads spp. to reduce rhizome rot of ginger caused by *Fusarium oxysporum*. The bacteria were applied to seed pieces and the plants were grown in an autoclaved garden soil. They reported the ability of fluorescent pseudomonadsto reduce infection of different plants by *F. oxysporum*.

Specific strains of the *Pseudomonas fluorescens* and*Pseudomonas putida* have been used as seed inoculants for crop plants to promote growth and increase yield. These pseudomonads rapidly colonize plant roots of potato, sugar beet and radishand caused statistically significant yield increases up to 144% in field tests (Kloepper *et al*., 2004).Competitive exclusion and antibiosis may also be involved in the biocontrol activity of fluorescent pseudomonads (Haas and Defago, 2005).

Fluorescent pseudomonads (RNA group 1) produce siderophores and yellow-green pigments that fluoresce under UV light (Elliot, 1958) and include *Pseudomonas fluorescens, Pseudomonas putida* and *Pseudomonas aeruginosa* (Bakker, 2007). It was also shown that fusaric acid produced by *Foz* served as a chemoattractant stimulating motility of *P. fluorescens* (WCS635) toward and colonisation of the hyphae of the fungus (de Weert *et al.,*2004). The lysis of

fungal hyphae released nutrients which might have been used by the bacterium as a substrate (Ahn *et al.,* 2007).

The presence of *Pseudomonas fluorescens* inoculant in combination of microbial fertilizer is effective in stimulating yield and growth of chickpea (Rokhzadi *et al*., 2008). Sivasakthi *et al*., (2014) reported fluorescent pseudomonad with biocontrol activity against different *Fusarium oxysporum* diseases. The colonisation of hyphae of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) by certain fluorescent pseudomonads, produced antifungal metabolites and lytic enzymes, which contributed to the biocontrol traits of these bacteria (Bolwerk *et al*., 2003).

2.6.3 *Streptomyces* sp. as biocontrol agent

The genus *Streptomyces* is representedby the largest number of species and varieties among the family Actinomycetaceae. They differ greatly in their morphology, physiology, and biochemical activities and majority produce, producing the majority of antibiotics. Generally, population density of actinomycetes is largely higher in rhizosphere in comparison with non-rhizosphere soils (Miller *et al*., 1990). El-Abyad *et al*. (1993) described *S. pulcher*, *S. canescens,* and *S. citreofluorescens*as, effective in thecontrol of tomato diseases by *F. oxysporum* f.sp. *lycopersici*. Most studied PGPR actinomycetes possess antibacterial or antifungal activity to suppress a plant disease (de Vasconcellos and Cardoso, 2009; El-Tarabily and Sivasithamparam, 2006, Hamby and Crawford, 2000).

El-Tarabily (2008) reported that the plant growth promotion was most pronounced with *S. filipinensis* than with *S. atrovirens* in greenhouse experiment, due to the ability of *S. filipinensis* to produce both ACC deaminase and IAA while *S. atrovirens* produced only ACC deaminase. One *Streptomyces* isolate is genetically close to *Streptomyces kasugaensis* and inhibited the growth of

Fusarium rot and showed plant growth promotion under greenhouse experiment in Brazil (de Vasconcellos and Cardoso, 2009). Martinez *et al*. (2014) reported that out of 164 strainsonly six of them, inhibited the growth of *Fusarium* sp. A growing reduction was observed from 50 to 83 % under *in vitro* antagonism assays.

Actinomycetes could be potentially tool to prevent several fungal plants diseases. Srividya *et al*. (2012) reported a potent actinomycete isolate with broad spectrum antifungal property against *Rhizoctonia solani* MTCC 4633. The isolate was identified as *Streptomyces* sp. based on colony morphology, microscopy and 16s rDNA. The isolate produced 2 most important hydrolytic enzymes- chitinase and β-1, 3 glucanase along with cellulase, lipase and protease. Concurrent production of protease, lipases, siderophore, IAA production coupled with anti-fungal activity suggests the plant growth promotion and broad spectrum biocontrol potential of *streptomyces* sp. isolate.

Kekuda *et al.* (2013) studied six biocontrol potential of *Streptomyces* sp. (SSC-MB-01 to SSC-MB-06) against *Fusarium oxysporum* f. sp. *Zingiberi* and reported inhibitory activity of *Streptomyces* sp.(SSC-MB-02) for the protection of the ginger rhizomes from soft rot symptoms. Sreeja and Gopal (2013) studied antagonistic effect of endophytic actinomycetes from tomato plants against bacterial wilt pathogen under *in vivo* conditions. The studies revealed that the Ozhalapathy isolate (EAOP) (*Streptomyces* sp.) was the best in plant growth promotion as well as in the management of bacterial wilt in tomato.

2.6.4 *Trichoderma* sp. as biocontrol agent

The genus *Trichoderma* is widely distributed in soils and on decaying wood and vegetables. This may be attributable to the diverse metabolic capability of *Trichoderma* species and their aggressively competitive nature. Increased rhizome yield due to suppression of ginger rhizome rot caused by *F. oxysporum* or

P. aphanidermatum or both wereobserved by using *T. harzianum, T. aureoviride* and a non-resident isolate of *T. viride*. Weindling (1932) first demonstrated that *T. viride* was parasitic and antagonistic to *Rhizoctonia solani*.

Eighty per cent control of *F. oxysporum* was obtained when stored rhizomes were treated with *T. viride* (Dohroo and Sharma, 1983). Baker *et al*. (1984) first reported the growth improvement of important crops by application of *Trichoderma harzianum* and *Trichoderma viride* in soil. According to Windham *et al*. (1986), diffusible growth regulating factors produced by *Trichoderma* spp. induced growth enhancement in plants. The fungus *T. harzianum* which was applied to pathogen free soil increased the emergence of seedlings, plant height, leaf area, and dry weight.

In vitro tests using *T. viride, T. harzianum* and *T. hamatum* against *P. aphanidermatum,* and *F. oxysporum* showed that these antagonists were inhibitory to the pathogens (Bharadwaj and Gupta, 1987). Good control of storage rot caused by *P. aphanidermatum* and *F. oxysporum* were obtained when *T. viride* and *T. hamatum* were applied to ginger rhizomes either by dipping them in spore suspension or smearing them with the antagonists (Bharadwaj *et al*., 1988).

According to Harman (2000), *Trichoderma* spp. increased the plant growth and also produced more robust roots. According to Phuwiwat and Soy tong (2001) the application of *T. harzianum* strain (PC01) at $5x10^9$ and $10x10^9$conidia/ml gave significantly better growth and yield. Ram *et al.* (2000) evaluated resident isolates of biocontrol agents *viz., T. harzianum, T. aureoviride* and *T. virens* for control of ginger rhizome rot. All the four bioagents could establish and reduced the population density of both *F. oxysporum* and *P. aphanidermatum*.

Rajan *et al*. (2002) reported that ginger *(Zingiber officinale* Rosc.) diseases are important production constraints and often associated with *Ralstonia*

(Pseudomonas) solanacearum, Pythium spp. and *Fusarium oxysporum.*A biocontrol agent, *T. harzianum* isolated from Sikkim was found to be effective in control of ginger diseases substantially. *Trichoderma harzianum* when applied against rhizome rot of turmeric is the field (*F. solani*), resulted in reduced disease incidence and increased yield (Reddy *et al*., 2003). Mukhopadhay (2005) reported that root colonization by *Trichoderma* spp. enhanced root growth and the uptake and use of nutrients. They found that *Trichoderma* spp. added directly to the rhizosphere or as seed treatment protected the plant against numerous plant pathogens.

Gupta *et al*. (2010) reported *Trichoderma* spp. are among the most promising biocontrol fungi against many fungal disease and growth parameters of gingeron rhizome pathogens. Rekha *et al*. (2015) reported that, *Trichoderma harzianum* could be a potential biocontrol agent for the management of Fusarium yellows disease. The most efficient plant growth promoting rhizobacteria (PGPR) and antagonists were PSBKh (from Kappikunnu) and TrKUh (from Kurumankotta) respectively.

According to Dohroo *et al.* (2014), efficacy of different fungal and bacterial biocontrol agents used in combination against rhizome diseases of ginger under field conditions were more effective in reducing the disease incidence than the individual treatments. *Trichoderma harzianum* + *Pseudomonas fluorescens* + *Bacillus subtilis* gave minimum disease incidence on rhizomes (8.64%) as well as on tillers (12.50%). Combined treatment also proved more effective in increasing the plant growth parameters i.e. number of tillers, plant height and fresh rhizome weight.

Eventhough, there is a lots of literature on individual biofertilizers and biocontrol agents like nitrogen fixers, phosphate solubilizing bacteria, *Bacillus* sp., *Pseudomonas fluorescens*, *Streptomyces* sp. and *Trichoderma* sp., not much information is available on growth promotion and fusarium yellows disease

management in ginger by native isolate. Also currently, very little information is available on disease management and mechanisms by native microbial inoculants and their effect on growth promotion and yield of several crops including ginger.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study on"Evaluation of native rhizosphere microflora for plant growth promotion and management of Fusarium yellows in ginger" was carried out at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara from 2012 to 2014. The experimental materials used and methods followed in the study are given below.

3.1 COLLECTION OF SOIL SAMPLES FROM DIFFERENT GINGER GROWING AREAS OF WAYANAD DISTRICT OF KERALA

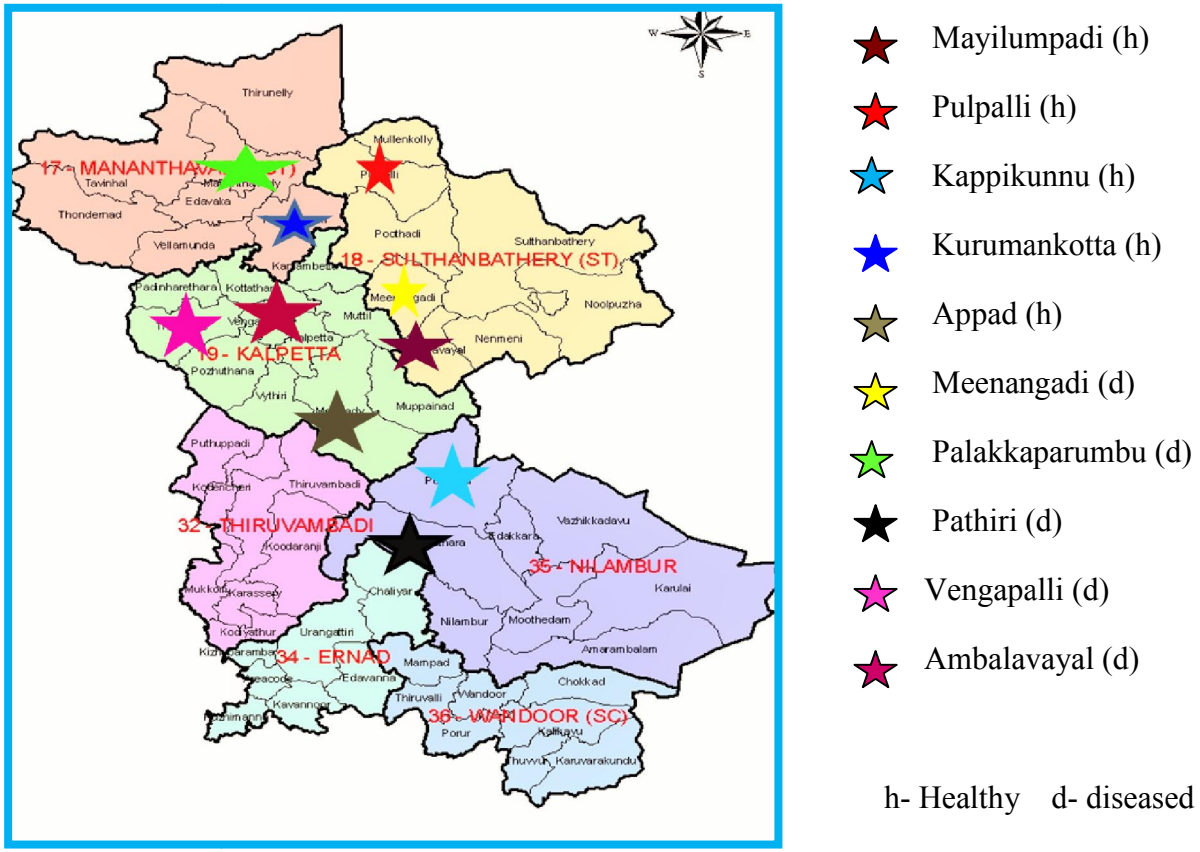
The rhizosphere soil samples were collected from both healthy and Fusarium yellows infected ginger from ten different locations of Wayanad district (Fig. 1). The location is a plot which is selected at random, based on the nature of crop growth and disease incidence. Soil samples were collected at a depth of 0-15 cm using standard protocols. From each location, five random samples were collected and pooled together to get a representative composite soil sample by quartering technique. About 100 g of soil from each location were properly labelled, sealed and stored in refrigerator till further studies.

3.2 ISOLATION AND ENUMERATION OF PREDOMINANT ISOLATES

Samples from ginger plots of Mayilumpadi, Pulpalli, Kappikunnu, Kurumankotta, Appad, Meenangadi, Palakkaparambu, Pathiri, Vengapalli, and Ambalavayal areas of Wayanad district were collected (Fig. 1). Total microflora was isolated by serial dilution plate technique.

One mililitre of the appropriate dilution was plated for the enumeration in respective media; Jensen's nitrogen free agar medium(Appendix-Ia) for nitrogen fixing bacteria (10^4), Pikovskaya's agar medium(Appendix-Ib)for phosphorous solubilizing bacteria (10^3), nutrient agar medium(Appendix-Ic)for *Bacillus* sp.

Fig. 1. Locations of soil samples collected from Wayanad district



Mayilumpadi (h)

Pulpalli (h)

Kappikunnu (h)

Kurumankotta (h)

Appad (h)

Meenangadi (d)

Palakkaparumbu (d)

Pathiri (d)

Vengapalli (d)

Ambalavayal (d)

h- Healthy d- diseased

(10^4), King's B medium(Appendix-Id)for fluorescent pseudomonads (10^4), Kenknights agar medium (Appendix-Ie)for *Streptomyces* sp. (10^4) and Trichoderma selective medium (Appendix-If) for *Trichoderma* sp. (10^3) were used.

Three replications for each dilution were maintained and Petri dish containing medium mixed with sterile water were kept as control. Petri dishes were incubated for 2-7 days at 28±2°C for the growth of microbial colonies. After incubation, predominant colonies formed were counted and expressed as the mean of three replications in terms of ($CFUg^{-1}$). The typical colonies of nitrogen fixers, phosphate solubilizing bacteria, *Bacillus* sp., *Pseudomonas fluorescens*, *Streptomyces* sp. and *Trichoderma* sp. developed on the media were repeatedly purified using standard protocols and the pure cultures were maintained for further studies.

3.3. CULTURAL AND BIOCHEMICAL CHARACTERIZATIONOF THE BACTERIAL ISOLATES

3.3.1 Cultural characterization of bacterial isolates

For cultural studies, 24 h old culture of the predominant bacterial isolates was used. Gram staining and endospore staining were carried andthe shape and gram reaction of the bacteria were observed under oil immersion objective of the microscope.

3.3.1.1 Gram's staining

Gram's staining was done as per the procedure described by Hucker and Conn (1923). Smear of the isolates were prepared on a clean glass slide and heat fixed by passing over a flame. The smear was then flooded with Hucker's ammonium crystal violet solution for one minute and then washed in a gentle stream of running tap water. It was then applied with Gram's iodine solution for

one minute and again washed. Later the smear was decolorized with 95 per cent ethyl alcohol for one minute. After the washing, the smear was again stained with saffranin for one minute and the excess stain was washed off in running water. The smear was then blot dried and examined under oil- immersion under light microscope.

3.3.1.2 Endospore staining

Air dried and heat fixed smear on a glass slide was covered with a square of blotting paper (Rangaswami and Bagyaraj, 1993). The blotting paper was saturated with malachite green stain solution (5%) and steamed for about ten minutes, dye is added to keet the paper wet. Washed the slide in tap water and counterstained with safranin for 30 seconds. The slides were examined under the oil immersion lens for the presence of endospores. Endospores were bright green and vegetative cells were brownish red to pink.

3.3.2 Biochemical characterization of bacterial isolates

3.3.2.1 Citrate utilization test

Twenty four hour old cultures were streaked on the surface of the slants of Simmon's citrate agar and observed for colour change of the medium (Schaad, 1992). The colour change from green to blue indicated positive for citrate utilization test.

3.3.2.2 Oxidase Test

Oxidase test was carried out by smearing a colony of isolates on oxidase disc (Hi-media DD018-1VL). The reaction was observed within 5-10 seconds at 25-30^{0}C. Appearance of violet colour indicated positive reaction. A colour change later than 60 seconds or no change at all was considered as negative reaction.

3.3.2.3 Urease Test

The urea broth medium (Appendix-Ig) was inoculated with bacterial culture. The culture was incubated at 30±2^{0}C for 48 h. The phenol red indicator turned

pink due to alkaline nature of the medium because of ammonia production (Stuart *et al*., 1945). Yellow colour indicated acidic pH which is a negative reaction for urease production.

3.3.3.4 Catalase Test

Few drops of an overnight broth culture of the isolates were taken on a microscope slide and a drop of hydrogen peroxide (3.0%) was added to it. Cultures which immediately showed effervescence were treated as positive for catalase activity (Taylor and Achanzar, 1972).

3.3.3.5 Starch Hydrolysis

Starch agar (Appendix-1h) plates were prepared and streaked with isolates. The isolates were incubated at 32^0C for 48 h. Iodine solution was poured on to the each plate and the blue-black colour appeared due to formation of starch-iodine complex (Priest, 1977). The clear zone around the colony indicated the degradation of starch, which occurred due to production of amylase.

3.3.3.6 Glucose fermentation test

Glucose fermentation broth was prepared in test tube. A durham's tube was put in an inverted position into the broth. The test bacteria were inoculated into the broth and the inoculated tubes were incubated at 37^0C for 48 h. A change in colour from red to yellow and appearance of bubbles indicated positive for glucose fermentation (Cowan, 1974).

3.3.3.7 Methyl Red Test and Voges-Proskauer Test

The glucose phosphate broth was inoculated with 48 h old bacterial culture and incubated for 48 h at room temperature, one mililitre of methyl red reagentwas added to it. Development of red colour indicated positive and yellow colour indicated negative result (Cappuccino *et al*., 1992).

For V-P test, inoculated the 5 mililitre glucose phosphate broth containing tubes with 48 h old bacterial culture and incubated for 48h at room temperature. After incubation 1 milititre 40% potassium hydroxide and 3 mililitre 5% α-naphtanol solution were added and observed for development of pink colour in 2-5 minutes. VP test positive result was indicated by wine red color and negative reaction by brown color development (Cappuccino *et al*., 1992).

3.4 MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF FUNGAL ISOLATE

Morphological and cultural characters of fungal isolates were studied on potato dextrose agar medium (Appendix-1i). Observations shapes and colours of conidia, the branching patterns of conidiophores were observed under microscope. Cultural characters like colony form, colony colour were studied in potato dextrose agar medium (Chet, 1987).

3.5 SCREENING OF NITROGEN FIXING BACTERIA, PHOSPHATE SOLUBILIZING BACTERIA, *Bacillus* sp., FLUORESCENT PSEUDOMONADS, *Streptomyces* sp. AND *Trichoderma* sp.FOR PLANT GROWTH PROMOTION ACTIVITIES UNDER *IN VITRO* CONDITION

3.5.1 Nitrogen Fixation

The nitrogen fixers developed on the Jensen's nitrogen free agar medium were screened for their efficiency in nitrogen fixation. The nitrogen fixing ability of different isolates was estimated by method of Jackson (1973) and Bremner (1960). A loopful of 48 h old culture of different isolates of nitrogen fixing bacteria was inoculated in 5ml of Jensen's broth containing glasstubes and incubated for 48h. One ml of this culture was inoculated in 50ml of respective medium in 250 ml conical flasks and three replicates were maintained for each isolate. After 15 days of incubation, the cultures were homogenized by shaking. 10 ml of the homogenized culture was drawn and mixed with10 ml concentrated H_2So_4 and 1g of digestion mixture containing K_2So_4 and Catalyst mixture ($CuSo_4$:

selenium in the ratio 20:1) in the ratio 10:1. The mixture was kept for digestion overnight at room temperature and then in a block digester for 2 hours at 300°C till it become clear. The clear digest was cooled, and transferred to Kjeldhal's distillation unit. 10 mlof 40% NaOH was added and condensed NH_3 was collected in10 ml boric acid-indicator mixture (4% boricacid solution was prepared in hot water). 4ml of mixed indicator solution (0.2% bromocresolgreen +0.2% methylredinalcoholin 5:1 ratio) was added to 1000 ml of 4% boric acid solution. The colour changed from reddish pink to bluish green as the NH_3 entered. After this process was over, it was titrated against 0.01 N HCl till the solution turned black to reddish pink. A blank was also used for titration. Total nitrogen content of the cultures were determined and the results were expressed among N fixed per gram of carbon source utilized.

$$\text{Mg of N/g of C source} = \frac{\text{TV-BVxNx0.014x1000}}{\text{Y}}$$

Where,

TV=Titrevalue
BV=Blankvalue
N=NormalityofH_2SO_4
Y=WeightofCsource

3.5.2 Phosphate Solubilization

Ten microlitres of 24 h old bacterial isolates and *Trichoderma* sp. was inoculated on Pikovskaya's agar plate and incubated for seven days at 28 $\pm 2^0$C. The halo-zone and colony diameter were measured 2, 5 and 7 days after incubation. The results were expressed as percentage of solubilization efficiency (SE) (Nguyen *et al.*, 1992).

$$\text{Solubilization Efficiency (\%)} = \frac{\text{SD}}{\text{CD}} \text{ X } 100$$

Where, SD- Solubilization diameter (mm) CD- Colony diameter (mm)

3.5.3 Indole-3- Acetic Acid (IAA) production

In vitro auxin production by the bacterial isolates and *Trichoderma* sp. were determined as indole acetic acid (IAA) equivalent in the presence of L-tryptophan (Khalid *et al.,* 2004). For this purpose, 10 ml nutrient broth (Appendix-1j) were taken in 100 ml flasks, autoclaved and cooled. L-tryptophan was filter sterilized by passing though 0.2 µm membrane filters and added at the rate of 1.0 mg/ml to the liquid medium. The contents in the flask were inoculated with 1.0 ml of 3 days old bacterial broth adjusted to a population of 10^7-10^8 cfu/ml. The flasks were plugged tightly and incubated at 28±2^{0}C for 10 days with un-inoculated media as control. After incubation, the contents were filtered through Whatman No.2 filter paper. Auxin compounds (IAA equivalent) were determined by spectrophotometer. While measuring IAA equivalents, 2.0 ml filtrate was mixed with 2 drops of orthophosphoric acid and 4.0 ml of Salkowski reagent (2.0 ml of 0.5 M $FeCl_3$ + 98.0 ml of 35 % $HClO_4$). The contents in the test tubes were allowed for colour development.The intensity of colour was measured at 530 nm by using spectrophotometer. Standard curve was used for comparison to calculate auxin production by the isolates.

3.6 SCREENING OF PREDOMINANT ISOLATES FOR HYDROGEN CYANIDE AND SIDEROPHORE PRODUCTION

3.6.1 Hydrogen cyanide production

HCN production by the bacterial isolates and *Trichoderma* sp. was detected by the method of Bakker and Schipper (1987). For HCN production, the bacterial isolates were grown on an HCN induction medium (30 g tryptic soy broth, 4.4 g glycine, 15 g agar l–1) at 28°C for four days. For each bacterial isolate, 100 µl of inoculum was dropped in the centre of the plates. Then a disk of Whatman filter paper dipped in 0.5% picric acid and 2% Na_2CO_3 (Appendix II2)was placed in the lid of the Petri dish, which was then sealed with parafilm.

After four days of incubation at 28°C, the orange-brown discoloration of the paper indicated HCN production. The reaction was scored on a 1-4 scale depending on the colour gradation.

3.6.2 Siderophore production

The analysis of siderophore production by bacterial isolates and *Trichoderma* sp. was screened by following the chrome azurol S (CAS) method of Schwyn and Neilands (1987). For each bacterial isolate was spotted in the centre of Petri dishes containing CAS agar. The dishes were incubated at 28°C for four days and were observed daily. The discoloration of the medium (blue to orange) indicated siderophore production by the bacterial isolates.

3.7 *IN VITRO* SCREENING OF BACTERIAL AND FUNGAL ISOLATES FOR ANTAGONISTIC ACTIVITY AGAINST THE SELECTED PATHOGENS

In vitro antagonistic effect of all the predominant bacterial isolates were tested against the major soilborne pathogens of ginger such as, *Fusarium oxysporum, Pythium aphanidermatum, Rhizoctonia solani, Ralstonia solanacearum* by dual culture method (Dhingra and Sinclair, 1995). For screening, antagonistic isolates were streaked through the centre of potato dextrose agar media (Appendix-1i) plates with a sterile inoculation loop. The plates were then incubated at 28^{o}C±2 overnight. The next day, an agar disc from the hyphal tip of the selected fungal pathogen was placed opposite side of the bacterial streak. The plates were then incubated at 24^{o}C for 2-7 days. Three replications of each isolates were maintained.

For testing the antagonism against *Ralstonia solanacearum*, nutrient agar medium seeded with 48 h old culture of the pathogen in Petri dishes was spot inoculated with the antagonistic isolates at the center. Three replications were maintained for each antagonist. The plates with pathogen alone served as control. Observations on the zone of inhibition were recorded after 48 h onwards.

3.8 SELECTION OF EFFICIENT ISOLATES OF BENEFICIAL AND ANTAGONISTIC ORGANISMS FOR POT CULTURE STUDIES

Three most efficient nitrogen fixers (NFMh, NFPh and NFKh), phosphate solubilizing bacteria (PSBAh, PSBKh and PSBPh), *Bacillus* sp. (BSMh, BSAh and BSKUh), fluorescent pseudomonads (PFPh, PFKh and PFAh), *Streptomyces* sp. (StrPh, StrKh and StrMh) and *Trichoderma* sp. (TrAh, TrKUh and TrPh) were selected based on the *invitro* screening. These selected isolates were used for evaluation of their efficiency in plant growth promotion and management of Fusarium yellows in ginger. The pot culture experiments were conducted as two experiments.

3.9 EVALUATION OF EFFICIENT NITROGEN FIXERS AND PHOSPHATE SOLUBILIZING BACTERIA FOR GROWTH ENHANCEMENT OF GINGER

The three most promising isolates of nitrogen fixers and phosphate solubilizing bacteria were evaluated for its efficiency of enhancing growth and yield in ginger under pot culture condition. The treatment details of the experiment were as follows:

Season : May to December
Variety : Rio- De jenario
Design : CRD
Replication : 3
Treatments : 7
Number of plants/treatment: 9

Treatment details

T_1 : NF_1 (NFMh isolate from Mayilumpadi)

T_2 : NF_2 (NFPh isolate from Pulpalli)

T_3 : NF_3 (NFKh isolatefrom Kappikunnu)

T_4 : PSB_1(PSBPh isolate from Pulpalli)

T_5 : PSB_2 (PSBKh isolate from Kappikunnu)

T_6 :PSB_3 (PSBAh isolate from Appad)

T_7 : Control

NF- Nitrogen fixing bacteria PSB- Phosphate solubilizing bacteria h- healthy

The isolates were applied at the time of planting as seed treatment.

3.9.1 Prepration of inoculum

For the prepration of bacterial inoculums, two loop full of bacterial culture were inoculated into 50 ml broth specific for its growth. After 48h of inoculation, 5 ml inoculum was taken from 50 ml and transferred to 300 ml of respective broth media at 28 ± 2^0C for 3 days (Ziadi *et al*., 2014).

3.9.2 Preparation of potting mixture and sowing

The potting mixture was prepared with sand: soil: cowdung in the ratio of (1:1:1) and fumigated with 2 % formaldehyde and covered with polythene film for 7 days. Potting mixture was then raked thoroughly and left open for 15 days before use. Disease free ginger rhizomes procured from farmers' field were used for planting.

3.9.3 Seed treatment

Inoculum was then uniformly adjusted to 10^6 cfu $ml^{-1}$and used for seed treatment. Ginger rhizomes weighing 20 g were dipped in the respective culture suspension for 30 minutes and air dried under shade for 30 m before planting it.

3.9.4. Biometric observations

Observations on sprouting per centage, plant height, number of tillers, rhizome yield, pest and disease incidence were recorded.

3.9.4.1. Per cent sprouting

Per cent sprouting was calculated using the following formula:

$$\text{Per cent sprouting} = \frac{\text{No. of rhizomes sprouted}}{\text{Total no. of plants per treatment}} \times 100$$

3.9.4.2. Number of tillers

Number of tillers was recorded by counting the numbers as and when they sprouted.

3.9.4.3. Plant height

The distance from the base of the plant to the tip was taken as plant height at 30 days interval and expressed in centimeters.

3.9.4.4. Rhizome yield

The fresh rhizome yield per pot recorded at the time of harvest which was expressed in g/plant.

3.10 EVALUATION OF EFFICIENT ANTAGONISTIC ISOLATES FOR THE MANAGEMENT OF FUSARIUM YELLOWS DISEASE IN GINGER

The three most promising isolates of antagonists obtained under *in vitro* screening were evaluated for the management of Fusarium yellows in ginger under pot culture studies. The treatment details of experiment were as follows:

Season : Mayto December
Variety : Rio- De jenario
Design : CRD

Replication : 3

Treatments : 14

Number of plants/treatment: 9

Treatment details

T_1 : Bs_1 (BsMh isolate from Mayilumpadi)

T_2 :Bs_2 (BsAh isolate from Appad)

T_3 : Bs_3(BsKUh isolate from Kurumankotta)

T_4 : PF_1 (PFKh fluorescentpseudomonadsfrom Kappikunnu)

T_5 : PF_2 (PFPh isolatefrom Kappikunnu)

T_6 : PF_3 (PFAh isolatefrom Appad)

T_7 : Str_1 (StrPh isolate from Pulpalli)

T_8 :Str_2(StrMh isolate from Mayilumpadi)

T_9 : Str_3(StrKh isolate from Kappikunnu)

T_{10} :Tr_1(TrAh isolate from Appad)

T_{11} : Tr_2 (TrKUh isolate from Kurumankotta)

T_{12} :Tr_3 (TrPh isolatefrom Pulpalli)

T_{13} : Control (Pathogen alone)

T_{14} : Absolute control

Bs: *Bacillus* sp. PF: fluorescentpseudomonadsStr: *Streptomyces* sp.

Tr: *Trichoderma* sp. h- healthy plot

The selected antagonistic isolates were applied at the time of planting and four months after planting as soil drenching.

3.10.1 Prepration of inoculum

For the prepration of bacterial inoculum two loop full of bacterial culture were inoculated into 50 ml of broth specific for its growth. After two days of inoculation, 5 ml of inoculum was taken from 50 ml and transferred to 300 ml of respective broth media at 28±2^{0}C for 3 days. In the case of fungus, 2 discs (10 millimeter) were transferred into 50 ml PDA broth and incubated for two days.

Five milliliter of inoculums was transferred into 300 ml of PDA broth and incubated for five days for incubation (Ziadi *et al*., 2014).

3.10.2 Preparation of potting mixture and sowing

The potting mixture was prepared as mentioned in 3.9.2

3.10.3 Soil application of antagonists

Earlyculture suspension of antagonists @ 10^8cfu/ml for bacteria, @10^5 for actinomycetes and fungi @ 10^6 cfu/ml were applied @ 30 ml/ plant at the time of planting and also four months after planting.

3.10.4. Challenge inoculation of the pathogen

For challenge inoculation, the inoculum of the pathogen was prepared by mixing the culture of *Fusarium oxysporum* with sterilized soil + healthy ginger rhizomes or bits and incubated for two weeks (Plate 1). Challenge inoculation was done two weeks after second application (4 months) of the antagonists. Inoculum was applied to the soil @ 10 g/plant. Plants were watered regularly with sterilized water.

3.10.5. Biometric observations

Observations on per cent sproting, number of tillers, plant height, rhizome yield, pest and other diseases were recorded as mentioned in 3.7.4.

3.10.5.1. Fusarium yellows disease incidence

Per cent disease incidence of Fusarium yellows on plants were recorded after the artificial inoculation of the pathogen. Drying up of lower leaves, stunted growth, yellowing, falling of tillers and rhizome infection were recorded.

$$\text{Per cent disease incidence in tillers} = \frac{\text{Total no. of infected tillers}}{\text{Total no. of tillers}} \text{ X } 100$$

$$\text{Per cent disease incidence in rhizome} = \frac{\text{Total weight of infected rhizome}}{\text{Total weight of rhizome}} \text{ X } 100$$

Plate 1. Mass multiplication of *Fusarium oxysporum*in sterilized soil containing ginger rhizomes (2 weeks old inoculum)

3.11 16S rDNA SEQUENCE ANALYSIS OF EFFICIENT PSB ISOLATE

The most efficient isolates obtained under pot culture studies was identified using 16S rDNA sequence analysis.

3.11.1 Amplification of 16S rDNA

A colony was taken by micropipette tip, mixed with 10 µl sterile water and 2µl of the culture suspension as template for amplification of 16S rDNA. The details of primer used are given below.

Table 1. Details of primer used

Primer details	Sequence 5'- 3'	Base pair
8 F	AGAGTTTGATCCTGGCTCAG	20
1522 R	AAG GAG GTG ATC CAG CCG CA	20

Polymerase chain reaction was carried out in Eppendorf Master Cycler (Gradient) using PCR master mix 'Emerald Amp GT PCR'. The composition of the reaction mixture for PCR was as follows:

Table 2. Composition of the reaction mixture for PCR

Component	Per reaction volume required
Master Mix	12.5 µl
Template	2.0 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
dH_2O	9.5 µl
Total	25.0 µl

The reaction was set in 200 µl microfuge tube chilled over ice flakes. A momentary spin was given to mix completely all reagents and set in master cycler for amplification. The details of master cycler programme are as follows:

Table 3. Details of master cycler programme

No.	Step	Temperature (^{0}C)	Time (min)
1	Initial denaturation	95	3.00
2	Denaturation	94	1.30
3	Annealing	55	0.40
4	Primer extension	72	01.30
5	Steps 2 – 4	34 cycles	-
6	Final extension	72	20.00
7	Final hold	4	10.00

3.11.2 Agarose Gel Electrophoresis

The quality of isolated DNA was evaluated though agarose gel electrophoresis (Sambrook *et al*., 1989). 1X TAEbuffer was prepared from the 50X TAE (pH 8.0) stock solutions. Agarose (Genei, Low EEO) (1%) was weighed and dissolved in TAE buffer by boiling. Ethidium bromide prepared from a stock of 10 mg $ml^{-1}$was added to it at a concentration of 0.5 µg ml^{-1} and mixed well. The comb was placed properly and dissolved agarose was poured into the tray. The gel was placed in the electrophoresis unit after 30 min with well side directed towards the cathode. 1X TAE buffer was added to the buffer tank (Genei, Bangalore) so as to cover the well with a few mm of buffer. 2 µl of the PCR product was carefully loaded into the wells using a micro pipette. The GeneRuler 1 kb DNA Ladderwas used as the molecular weight marker. The cathode and anode of the electrophoresis unit were connected to the power pack and the gel was run at constant voltage of 100 V. The power was turned off when the tracking dye reached at 3 cm from the anode end.

3.11.3 Gel Documentation

The DNA bands separated by electrophoresis were viewed and photographed using gel documentation imaging system.

3.11.4 Purification and Sequencing of PCR Product

The PCR product was purified and sequenced at Scigenome Pvt. Ltd. Cochin, using the primers 8F and 1522r.

3.11.5 Nucleotide Sequence Analysis

The Blastn programme (http://blast.ncbi.nlm.nih.gov/Blast.) was used to find out the homology of the nucleotide sequences.

3.12 STATISTICAL ANALYSIS

Analysis of variance was done on the data collected using the statistical package MSTAT (Freed, 1986). Multiple comparisons among the treatment means were done using DMRT.

RESULTS

4. RESULTS

A study was conducted on the "Evaluation of native rhizosphere microflora for plant growth promotion and management of Fusarium yellows in ginger"at the College of Horticulture, Vellanikkara during 2012-2014. The results of the study are presented below.

4.1 COLLECTION OF SOIL SAMPLES FROM DIFFERENT GINGER GROWING AREAS OF WAYANAD DISTRICT IN KERALA

Rhizosphere soil samples were collected from ten different locations of healthy and infected ginger growing areas of Wayanad district (Table 4, Plate 2). Outof ten soil samples collected, five were from healthy rhizosphere and five were from the rhizosphere of Fusarium yellows infected ginger field.

4.2 ENUMERATION OF PREDOMINANT ISOLATES OBTAINED FROM DIFFERENT GINGER GROWING AREAS

Of the ten locations, highest population of nitrogen fixers($1.4x10^4$cfu g^{-1})were recorded from Pulpalli (Ph) (Table 5, Plate 3) whereas, Ambalavayal (Ad),Kurumankotta (KUh) and Pathiri (PAd) recorded lowest population. A total of 8 PSB isolates were obtained from ten soil samples. The population of PSB ranged from $1x10^3$cfu g^{-1} to $9x10^3$cfu g^{-1}. Highest population of PSB isolates were obtained from Appad (Ah) and lowest from Kurumankotta (KUh) and Pathiri (PAd).

Highest population of *Bacillus* sp. ($2.3x10^4$ cfu g^{-1}) were obtained from Mayilumpadi and the lowest from ($1x10^4$ cfu g^{-1}) from Vengapalli (Vd). Among the fluorescent pseudomonads, Pulpalli (Ph) recorded highest population ($9x10^4$ cfu g^{-1}) and was absent in Meenangadi (Md), Vengapalli (Vd) and Ambalavayal (Ad) locations. Highest population of *Streptomyces* sp. ($6x10^4$cfu g^{-1}) was obtained from Kappikunnu (Kh) were as it was absent in Meenangadi (Md) and Pathiri (PAd).

Table 4. Geographical position of different locations of soil samples collected from Wayanad district

Sl. No.	Location	Code	Geographical position		
1. Healthy plot			North	East	Elevation
1	Mayilumpadi	Mh	11. 69891^0	076. 1733^0	2617ft
2	Pulpalli	Ph	11. 69783^0	076. 1699^0	2572ft
3	Kappikunnu	Kh	11. 65678^0	076. 0535^0	2573ft
4	Kurumankotta	Kuh	11. 67841^0	076. 1478^0	2624ft
5	Appad	Ah	11. 66133^0	076. 1491^0	2559ft
2. Diseased plot					
6	Meenangadi	Md	11. 67859^0	076. 2718^0	2547ft
7	Palakkaparumbu	Pd	11. 69569^0	076. 2139^0	3065ft
8	Pathiri	Pad	11. 69053^0	076. 2385^0	3063ft
9	Vengapalli	Vd	11. 69471^0	076. 2385^0	3706ft
10	Ambalavayal	Ad	11. 61062^0	076. 2109^0	3072ft

A. Healthy garden (Kurumankotta in Wayanad district)

B. Diseased garden (Pathiri in Wayanad district)

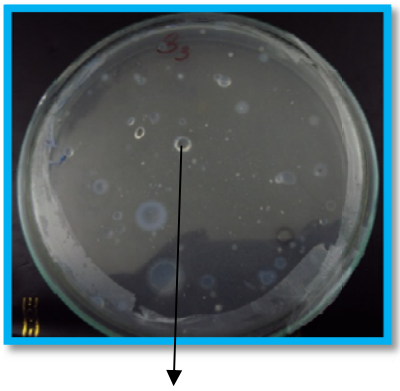
Plate 2. Healthy and diseased ginger growing areas of Wayanad district

Table 5. Enumeration of predominant isolates from healthy plot (cfu/g of soil)

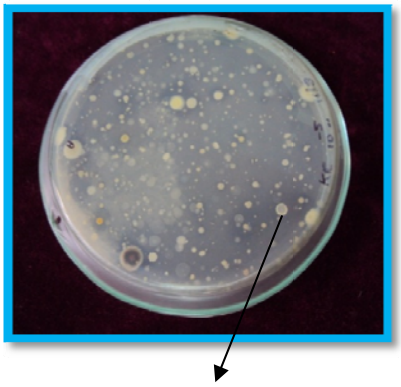
Sl. No.	Location	Code	Nitrogen fixers($x10^4$)	Phosphate solubilizing bacteria($x10^3$)	*Bacillus* sp. ($x10^4$)	Fluorescent pseudomonads ($x10^4$)	*Streptomyces* sp. ($x10^4$)	*Trichoderma* sp. ($x10^3$)
1	Mayilumpadi	Mh	1.0	4.0	2.3	7.0	5.0	4.0
2	Pulpalli	Ph	1.4	8.0	1.4	9.0	3.0	6.0
3	Kappikunnu	Kh	1.1	9.0	1.2	8.0	6.0	3.0
4	Appad	Ah	0.9	3.0	0.9	6.0	2.0	3.0
5	Kurumankotta	KUh	1.3	ND	1.5	4.0	4.0	2.0
6	C.D		1.843	1.764	1.843	1.843	1.843	1.843

Each value represents mean of three replications

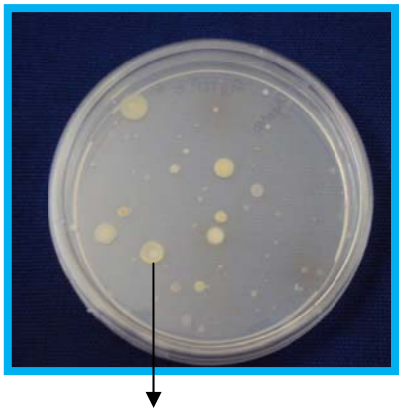
ND- Not detected



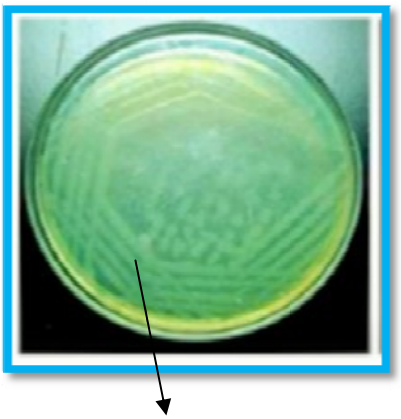
A. Nitrogen fixers



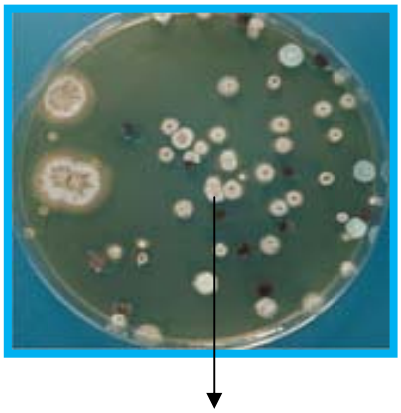
B. Phosphate solubilising bacteria



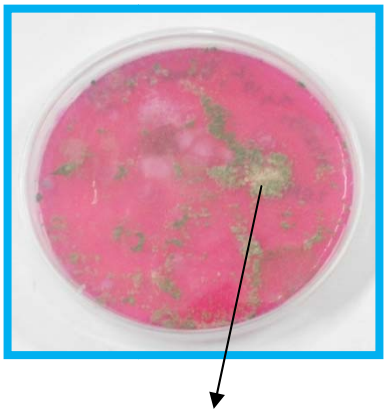
C. *Bacillus* sp.



D. Fluorescent pseudomonads



E. *Streptomyces* sp.



F. *Trichoderma* sp.

Plate 3. Isolation of predominant isolates

The highest population of *Trichoderma* sp. ($6x10^3$cfu g^1) wasrecorded from Pulpalli (Ph), and was absent in Palakkaparambu (Pd) and Ambalavayal (Ad) of Wayanad district.The nitrogen fixers, phosphate solubilizing bacteria, *Bacillus* sp., flurescent pseudomonads, *Streptomyces* sp. and *Trichoderma* sp. recorded higher population in the healthy ginger growing areas as compared to the disease infected plots in Wayanad district (Table 6). *Bacillus* sp. recorded highest population when compared to other microorganisms.

4.3 CHARACTERIZATION OF PREDOMINANT BENEFICIAL AND ANTAGONISTIC MICROORGANISMS

All the predominant colonies of the beneficial and antagonistic isolates were characterized based on their cultural, morphological and biochemical characters.

4.3.1 Cultural and biochemical characterization of nitrogen fixing bacteria

The ten isolates were appearedgram negative (Plate 4), motile, and positive fornitrogen fixation in Jensen's broth (Table 7). Endospore formation in bacteria was positive when observed under phase contrast microscope. All the isolates were positive to citrate utilization (Plate 4), glucose, oxidase, and catalasewhereas negative to starch hydrolysis. The isolate were tentatively identified as *Azotobacter* sp.

4.3.2 Cultural and biochemical characterization of phosphate solubilizing bacteria

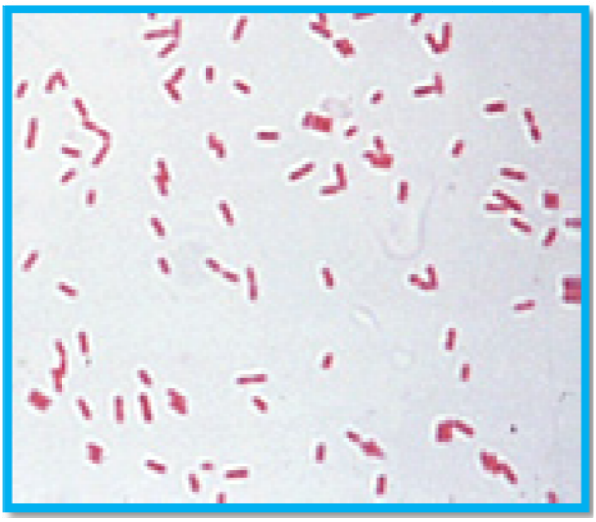
All the eight isolates obtained were gram negative, rodsand motile. The isolates were positive for catalase (Plate5) and citrate utilization whereas negative for indole, urease and methyl-red tests. Oxidase test was positive only for PSBMd. The isolates PSBMh, PSBPh, PSBKh and PSBMd were positive for Voges-Proskauer test (Table 8). The isolates were tentatively identified as *Bacillus* sp.

Table 6. Enumeration of predominant isolates from diseased plot (cfu/g of soil)

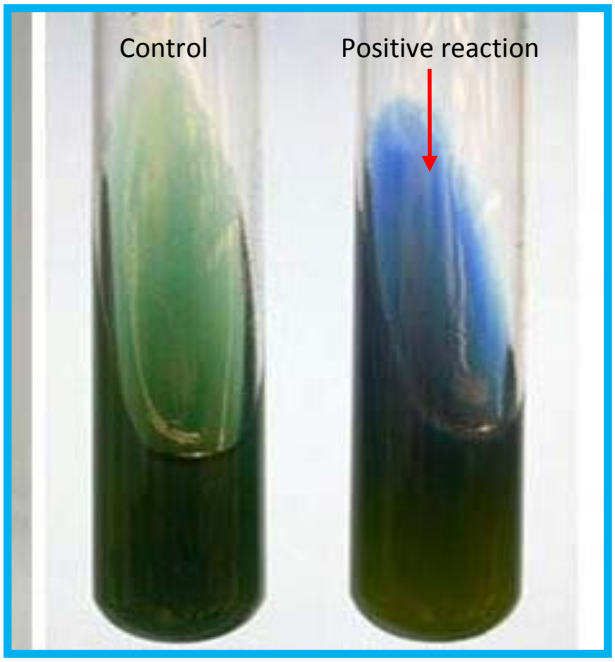
Sl. No.	Location	Code	Nitrogen fixers($x10^4$)	Phosphate solubilizing bacteria($x10^3$)	*Bacillus* sp. ($x10^4$)	Fluorescent pseudomonads ($x10^4$)	*Streptomyces* sp. ($x10^4$)	*Trichoderma* sp. ($x10^3$)
1	Meenangadi	Md	4.0	4.0	7.0	ND	ND	2.0
2	Palakkaparumbu	Pd	7.0	2.0	4.0	3.0	2.0	ND
3	Pathiri	PAd	5.0	ND	8.0	2.0	ND	2.0
4	Vengapalli	Vd	9.0	1.0	1.0	ND	4.0	3.0
5	Ambalavayal	Ad	6.0	3.0	5.0	ND	2.0	ND
6	C.D		1.65	1.91	1.65	NS	NS	NS

Each value represents mean of three replication

ND- Not detected NS- Not significant



A. Gram negative microscopic image of NFMh isolate



B. Citrate activity of NFPh isolate

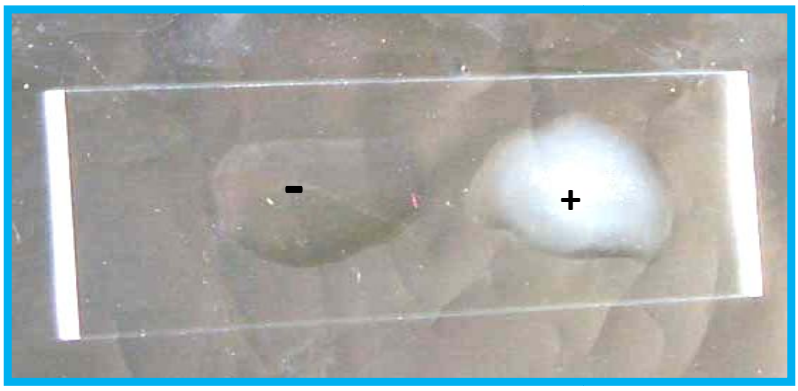
Plate 4. Characterization of nitrogen fixing bacteria

Table 7. Cultural and biochemical characterization of predominant isolates of nitrogen fixers (NF) from healthy and diseased ginger plots

Characteristics	**Isolates**									
	NFMh	NFPh	NFKh	NFAh	NFKUh	NFMd	NFPd	NFPTd	NFVd	NFAd
Gram reaction	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Nitrogen fixation on Jensen's broth	+	+	+	+	+	+	+	+	+	+
Cyst formation	+	+	+	+	+	+	+	+	+	+
Citrate activity	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+
Oxidase activity	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+

+ Positive, – Negative h- healthy d- diseased

NFMh isolate from Mayilumpadi NFPh isolate from Pulpalli NFKh isolate from Kappikunnu NFAh isolate from Appade NFKUh isolate from Kurumankotta NFMd isolate from Meenangadi NFPh isolate from Palakkaparambu NFPTd isolate from Pathiri NFVd isolate from Vengapalli NFAd isolate from Ambalavayal



Catalase activity of PSBKh isolate

Plate 5. Characterization of phosphate solubilising bacteria

4.3.3 Cultural and biochemical characterization of *Bacillus* sp.

All the ten isolates were gram positive, rods (Plate 6) and motile (Table 9). They produced endospore and positive for catalase, Voges- Proskauer, starch hydrolysis (Plate6) and citrate utilization test. All the isolates were negative to indole and urease test. Hence, it was tentatiely identified as *Bacillus* sp.

4.3.4 Cultural and biochemical characterization of fluorescent pseudomonads

The seven isolates obtained were gram negative, motile, straight rods and produced fluorescens under UV- light (Table 10). All the isolates were positive to oxidase (Plate7), glucose and citrateutilization and negative for starch hydrolysis. Out of seven isolates, only three isolates namely FPMh, FPPh, and FPKUh were positive to sucrose test. They produced fluorescent pigments which were visible under ultra violet light.

4.3.5 Cultural and biochemical characterization of *Streptomyces* sp.

Out of ten samples, only eight predominant isolates of *Streptomyces* sp. were obtained (Table 11). All the eight isolates were gram positive, filamentous (Plate8), coloured spores which ranged from grey to white and were non motile spores. The morphology of the spore chains varied from rectiflexible to spirales and was positive to glucose test (Plate8). Four isolates namely SM, SP, SPD, and SV were negative to sucrose test. Hence, it was tentatively identified as *Streptomyces* sp.

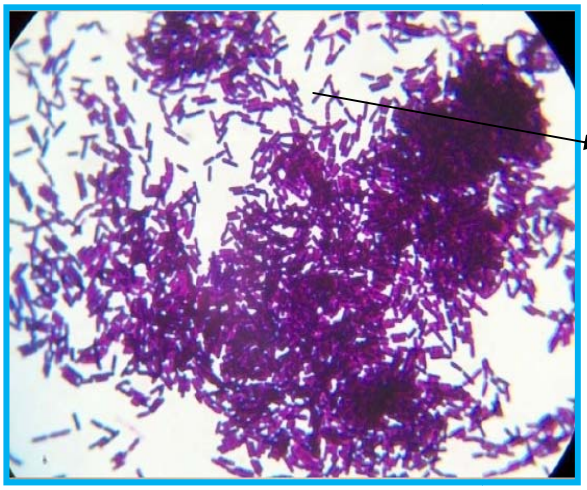
Table 8. Cultural and biochemical characterization of phosphate solubilizing bacteria(PSB) obtained from healthy and diseased plots of ginger in Wayanad district

Characters	Isolates							
	PSBMh	PSBPh	PSBKh	PSBAh	PSBMd	PSBPd	PSBVd	PSBAd
Gram reaction	-	-	-	-	-	-	-	-
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	+	-	-	-
Catalase	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-	-
Voges- Proskauer	+	+	+	-	+	-	-	-
Citrate utilization	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-

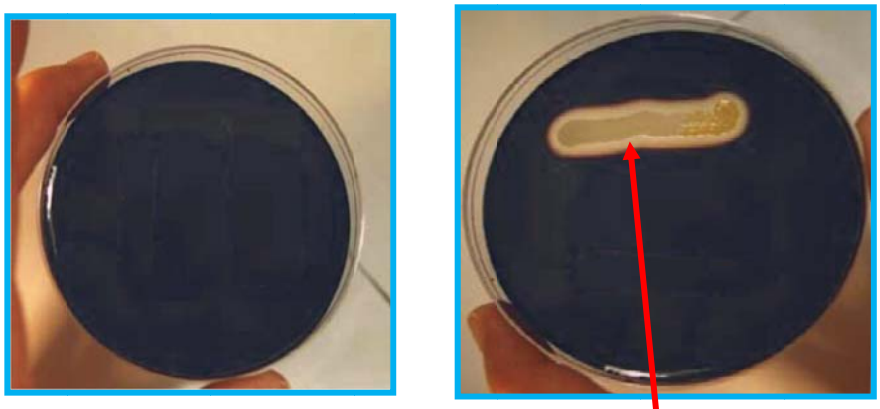
+ Positive, – Negative h- healthy d- diseased

PSBMh isolate from Mayilumpadi PSBPh isolate from Pulpalli PSBKh isolate from Kappikunnu PSBAh isolate from Appade

PSBMd isolate from Meenangadi PSBPd isolate from Palakkaparambu PSBVd isolate from Vengapalli PSBAd isolate Ambalavayal



A. Gram Positive microscopic image of BsMh isolate



B. Starch Hydrolysis of BsPh isolate

Plate 6. Characterization of *Bacillus* sp.

Table 9. Cultural and biochemical characterization of *Bacillus* sp.(Bs) from healthy and diseased plots of ginger in Wayanad district

Characters	Isolates									
	BsMh	BsPh	BsKh	BsAh	BsKUh	BsMd	BsPd	BsPTd	BsVd	BsAd
Gram reaction	+	+	+	+	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-
Voges-Proskauer	+	+	+	-	+	-	+	-	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
Citrate Utilization	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-

+ Positive, – Negative, h- healthy, d- diseased

BsMh isolate from Mayilumpadi BsPh isolate from Pulpalli BsKh isolate from Kappikunnu BsAh isolate from Appade BsKuh isolate from Kurumankotta BsMd isolate from Meenangadi BsPd isolate from Palakkaparambu BsPTd isolate from Pathiri BsVd isolate from Vengapalli BsAd isolate from Ambalavayal

Table 10. Cultural and biochemical characterization of fluorescent pseudomonads(Pf) from healthy and diseased plots of ginger in Wayanad district

Characters	**Isolates**						
	PfMh	**PfPh**	**PfKh**	**PfAh**	**PfKUh**	**PfPd**	**PfPTd**
Gram reaction	-	-	-	-	-	-	-
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	-	-	+	-	-
Starch hydrolysis	-	-	-	-	-	-	-
Citrate Utilization	+	+	+	+	+	+	+

+ Positive, – Negative, h- healthy, d- diseased

PfMh isolate from Mayilumpadi PfPh isolate from Pulpalli PfKh isolate from Kappikunnu PfAh isolate from Appade
PfKUh isolate from Kurumankotta PfPd isolate from Palakkaparambu PfPTd isolate from Pathiri

Control Positive

Oxidase activity of PfPh isolate

Plate 7. Characterization of fluorescent pseudomonads

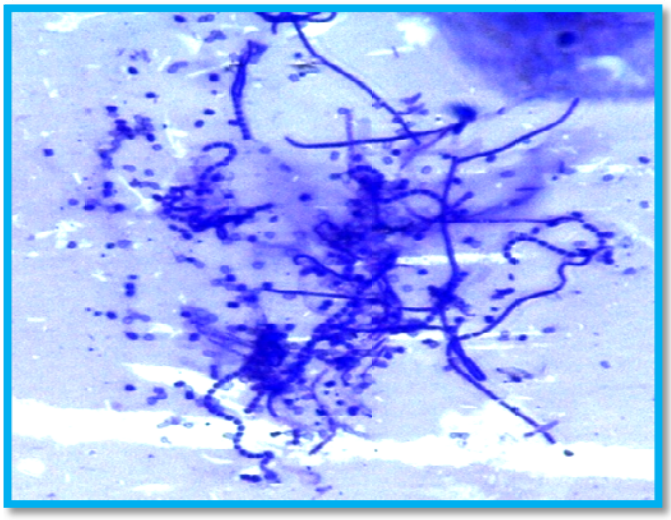
Table 11. Cultural and biochemical characterization of *Streptomyces* sp.(Str) from healthy and diseased plots of ginger in Wayanad district

Characters	**Isolates**							
	StrMh	**StrPh**	**StrKh**	**StrAh**	**StrKUh**	**StrPd**	**StrVd**	**StrAd**
Gram reaction	+	+	+	+	+	+	+	+
Shape	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous
Motility	-	-	-	-	-	-	-	-
Spore colour	Grey	Grey	Grey	White	Grey	White	White	Grey
Glucose	+	+	+	+	+	+	+	+
Sucrose	-	-	+	+	+	-	-	+

+ Positive, – Negative, h-healthy, d-diseased

StrMh isolate from Mayilumpadi StrPh isolate from Pulpalli StrKh isolate from Kappikunnu StrAh isolate from Appade

StrKuh isolate from Kurumankotta StrPd isolate from Palakkaparambu StrVd isolate from Vengapalli StrAd isolate from Ambalavayal



Gram positive and filamentous (StrPh isolate)

Plate 8. Characterization of *Streptomyces* sp.

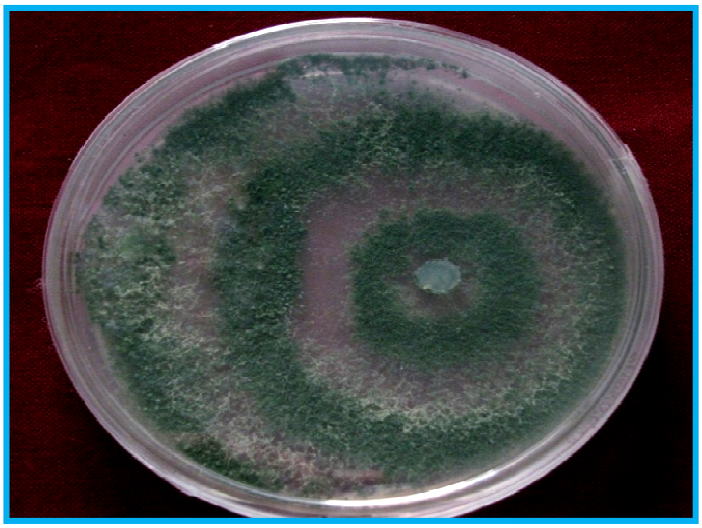


Plate 9. Characterization of *Trichoderma* sp. TrAh isolate

4.4 MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *Trichoderma* sp.

Morphological and cultural characters were studied on potato dextrose agar media (Plate9). Isolates TrMh, TrAh, TrMd, TrAd and TrVd were dark green coloured, septate, hyaline, hyaline conidiophores and conidia were green in colour. Isolates TrPh, TrKh and TrKUh were light green, septate, hyaline hyphae, conidiophores are hyaline and conidia pale green colour. Hence,the isolates were tentatively identified as *Trichoderma* sp. (Table 12).

4.5 SCREENING OF PREDOMINANT ISOLATES FOR NITROGEN FIXATION, PHOSPHATE SOLUBILIZATION, IAA, HCN AND SIDEROPHORE PRODUCTION UNDER *in vitro* CONDITION

All the predominant isolates of nitrogen fixing bacteria and phosphate solubilizing bacteriawere tested for their efficiency in nitrogen fixation, phosphate solubilization, IAA, HCN and siderophore production under *in vitro* condition.

4.5.1 Nitrogen fixation, IAA, HCN and siderophore production by nitrogen fixers.

All the ten isolates of nitrogen fixing bacteria fixed nitrogen in the range of 0.11/g to 0.43 mg/g of sucrose utilized (Table 13). The highest (0.43 mg/g of sucrose utilized) nitrogen fixed was in NFMh isolate and lowest (0.11 mg/g of sucrose utilized) by the isolate NFMd. Except two isolates NFPTd and NFMd,all other isolates produced IAA under *in vitro* condition (Plate10). Highest IAA production was (35.02 µg/ml) by the isolate NFAh from Appad and the lowest (16.07 µg/ml) by NFMDh isolate. Hydrogen cyanide production of all the ten isolates were tested and that isolates NFPh and NFAh produced highest HCN under *in vitro* screening

Table 12. Characterization of *Trichoderma* sp. (Tr)

Sl. No.	Location	Isolates	Characters
1	Mayilumpadi	TrMh	Dark green coloured colonies, septate, hyaline hyphae, branched hyaline conidiophores, conidia green colour, no pigmentation
2	Pulpalli	TrPh	Light green, distinct concentric rings, septate, hyaline hyphae, conidia pale green colour, no pigmentation
3	Kappikunnu	TrKh	Colour of the colonies varied from whitish green to dull green, septate, hyaline hyphae, conidia pale green colour, no pigmentation
4	Appade	TrAh	Dark green, septate, hyaline hyphae, conidiophores are hyaline, conidia green colour, no pigmentation
5	Kurumankotta	TrKUh	Light green, septate, hyaline hyphae, conidiophores are hyaline, conidia pale green colour, no pigmentation
6	Meenangadi	TrMd	Dark green coloured colonies, septate, hyaline hyphae, branched hyaline conidiophores, conidia green colour, no pigmentation
7	Pathiri	TrPd	Dark green coloured colonies, septate, hyaline hyphae, conidiophores are hyaline, conidia green colour, no pigmentation
8	Vengapalli	TrVd	Dark green coloured colonies, septate, hyaline hyphae, branched hyaline conidiophores, conidia green colour, no pigmentation

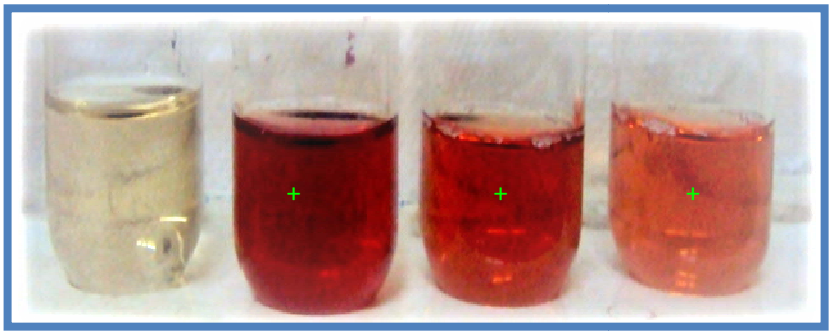
h- healthy, d-diseased

Table 13. Nitrogen fixation, IAA, HCN and siderophore production by nitrogen fixers (NF)

Sl. No.	Isolates	Nitrogen fixation(mg / g of sucrose utilized)	Indole-3-Acetic Acid production(µg/ml)	Hydrogen Cyanide production	Siderophore production
1	NFMh	0.43	27.09	+++	-
2	NFPh	0.35	32.43	+++	-
3	NFKh	0.27	25.74	++	+
4	NFAh	0.32	35.02	++++	+
5	NFKUh	0.40	18.39	++	+
6	NFMd	0.11	16.07	-	-
7	NFPd	0.14	18.91	-	-
8	NFPTd	0.13	-	-	-
9	NFVd	0.20	21.02	++	-
10	NFAd	0.12	-	-	-
	CD	0.06	1.63		

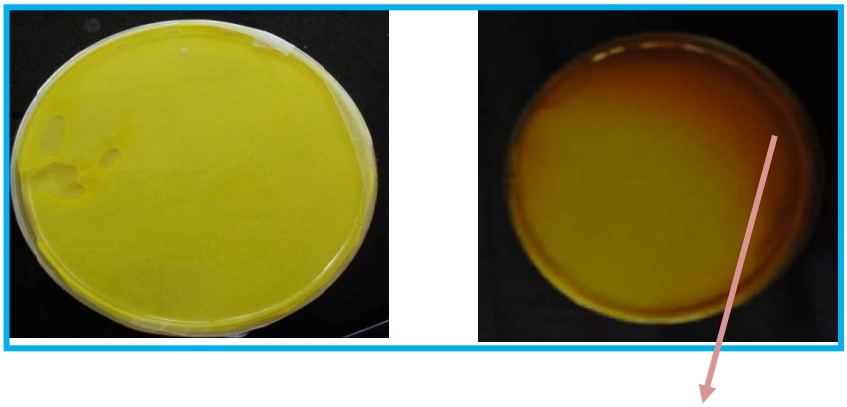
h- healthy d- diseased - Negative ++++ Excellent +++ Good ++ Moderate + poor

NFMhisolate from Mayilumpadi NFPhisolate from Pulpalli NFKh isolate from Kappikunnu NFAh isolate from Appade NFKUh isolate from Kurumankotta NFMd isolate from Meenangadi NFPd isolate from Palakkaparambu NFPTd isolate from Pathiri NFVd isolate from Vengapalli NFAd isolate from Ambalavayal



Control **NFAh** **NFPh** **NFMh**

A. IAA production by nitrogen fixing bacteria



Control Positive- NFAh isolate

B. HCN production by nitrogen fixing bacteria

Plate 10. IAA & HCN Production by nitrogen fixing bacteria

whereas isolates NFMd, NFPd, NFPTd, and NFAddid not produce hydrogen cyanide (Plate10). Siderophore production was produced only by three isolates NFKh, NFAh, and NFPd. The microbial isolates from healthy ginger plots recorded higher efficiency in plant growth promotion as well as antagonistic activities when compared to the isolates from diseased plot.

4.5.2 Phosphate solubilization, IAA, HCN and siderophore production by phosphate solubilizing bacteria.

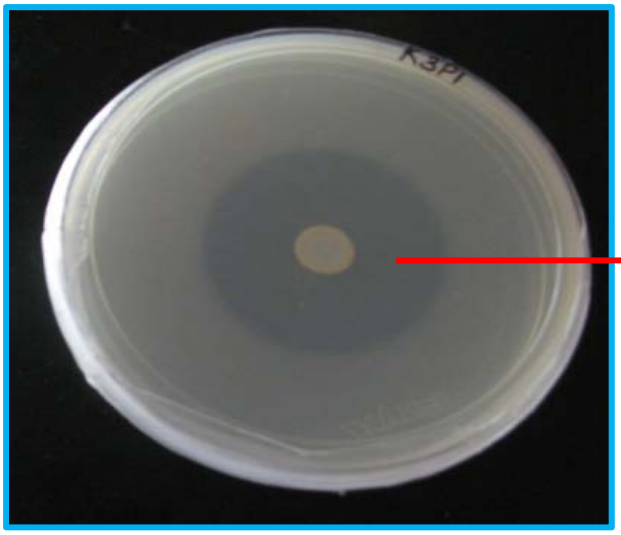
A total of eight isolates of phosphate solubilizing bacteria were tested for their phosphate solubilization efficiency (PSE) and it ranged from 150 to 450 per cent (Table 14, Plate 11). The maximum P- solubilization index (450.0%) was recorded by PSBAh isolate and the least (151.84%) by PSBMd isolate. Indole-3-Acetic Acid production was recorded by all the isolates except for PSBVd isolate (vengapalli).Highest IAA (33.07µg/ml) was produced by PSBMh isolate (Mayilumpadi) and the least (12.92 µg/ml) by PSBPd isolate (Palakkaparambu). Out of the eight isolates tested for HCN production, only one PSBVd isolate did not produce hydrogen cyanide whereas the PSBMh isolate recorded as highest HCN production. Only two isolates, PSBKh and PSBAh were positive for siderophore production (Plate 11).

Table 14. Phosphate solubilization, IAA, HCN and siderophore production by phosphate solubilizing bacteria (PSB)

Sl.No.	Isolates	Phosphate solubilization efficiency (%)	IAA (µg/ml) production	HCN production	Siderophore production
1	PSBMh	183.33	33.07	++++	-
2	PSBPh	262.50	19.57	++	+
3	PSBKh	247.62	31.19	+++	-
4	PSBAh	450.00	17.20	+	+
5	PSBMd	151.84	14.17	+	-
6	PSBPd	158.33	12.92	+	-
7	PSBVd	164.70	-	-	-
8	PSBAd	185.18	16.03	+	-
	CD	1.74	1.94		

h- healthy d- diseased - Negative ++++ Excellent +++ Good ++ Moderate + Low

PSBMh isolate from Mayilumpadi PSBPh isolate from Pulpalli PSBKh isolate from Kappikunnu PSBAh isolate from Appade
PSBMd isolate from Meenangadi PSBPd isolate from Palakkaparambu PSBVd isolate from Vengapalli PSBAd isolate Ambalavayal



A. P- solubilisation by PSBAh isolate

B. Siderophore production by PSBAh isolate

Plate 11. Phosphate solubilisation and siderophore production by phosphate solubilizing bacteria

4.5.3 Phosphate solubilization, IAA, HCN and siderophore production by *Bacillus* sp.

A total of ten isolates of *Bacillus* sp.were tested for their phosphate solubilization efficiency (PSE) and only three isolates BsMh (Mayilumpadi), BsAh (Appade) and BsKUh (Kurumankotta) resulted P- solubilization (Table 15). The maximum P- solubilization index (182.99%) was recorded by BsAh isolate and the least (137.43%) by BsKUh isolate. Indole-3-Acetic Acid production was recorded for theabove mentioned three isolates except.Highest IAA (14.54µg/ml) was produced by BsMh isolate (Mayilumpadi) and the least (12.92 µg/ml) by BsAh isolate (Appade). Out of the ten isolates tested for HCN production, only three BsMh, BsKuh and BsPTd isolateresulted moderate level of HCN production. Only one isolates, BsMh positive for siderophore production.

4.5.4 Phosphate solubilization, IAA, HCN and siderophore production by fluorescent pseudomonads

All the seven isolates of fluorescent pseudomonads solubilized phosphorus in the range of 190.11%to 241.57% (Table 16). The highest (241.57%) p-solubilization was in PfMh isolate and lowest (190.11%) by the isolate PfKh. Two isolates PfPh and PfKh produced IAA under *in vitro* condition. Highest IAA production was (16.75µg/ml) by the isolate PfKh from Kappikunnu and the lowest (12.13µg/ml) by PfPh isolate. Hydrogen cyanide production of all the isolates were tested and that isolates PfPh and PfKUh produced highest HCN under *in vitro* screening. Siderophore production was produced only by three isolates PfKh, PfMh, and PfPh.

Table 15. Phosphate solubilization, IAA, HCN and siderophore production by *Bacillus* sp. (Bs)

Sl.No	Isolates	Phosphate solubilization efficiency (%)	IAA (µg/ml)	HCN	Siderophore
1	BsMh	149.62	14.54	++	+
2	BsPh	-	-	-	-
3	BsKh	-	-	-	-
4	BsAh	182.99	12.11	-	-
5	BsKUh	137.53	12.27	++	-
6	BsMd	-	-	-	-
7	BsPd	-	-	-	-
8	BsPTd	-	-	++	-
9	BsVd	-	-	-	-
10	BsAd	-	-	-	-
	CD	2.04	1.66		

h- healthy d- diseased - Negative ++++ Excellent +++ Good ++ Moderate + Low

BsMh isolate from Mayilumpadi BsPh isolate from Pulpalli BsKh isolate from Kappikunnu BsAh isolate from Appade BsKuh isolate from Kurumankotta BsMd isolate from Meenangadi BsPd isolate from Palakkaparambu BsPTd isolate from Pathiri BsVd isolate from Vengapalli BsAd isolate from Ambalavayal

Table 16. Phosphate solubilization, IAA, HCN and siderophore production by fluorescentpseudomonads (Pf)

Sl.no	Isolates	Phosphate solubilization efficiency (%)	IAAproduction (µg/ml)	HCN production	Siderophore production
1	PfMh	-	-	-	+
2	PfPh	241.57	12.13	+++	+
3	PfKh	190.11	16.75	-	+
4	PfAh	-	-	-	-
5	PfKUh	235.84	-	++	-
6	PfPd	-	-	-	-
7	PfPTd	-	-	-	
	CD	2.03	1.65		

h- healthy d- diseased - Negative ++++ Excellent +++ Good ++ Moderate + Low

PfMh isolate from Mayilumpadi PfPh isolate from Pulpalli PfKh isolate from Kappikunnu PfAh isolate from Appade
PfKUh isolate from Kurumankotta PfPd isolate from Palakkaparambu PfPTd isolate from Pathiri

4.5.5 Phosphate solubilization, IAA, HCN and siderophore production by *Streptomyces* sp.(Str)

A total of eight isolates of *Streptomyces* sp.were tested for their phosphate solubilization efficiency (PSE) and only two isolates StrPh (Pulpalli) and StrAh (Appade) resulted P- solubilization (Table 17). The maximum P- solubilization index (176.99%) was recorded by StrAh isolate and the least (153.76%) by StrPh isolate. Indole-3-Acetic Acid production was recorded for three isolates, StrMh, StrPh and StrKUh. Highest IAA (11.63µg/ml) was produced by StrMh isolate (Mayilumpadi) and the least (10.41 µg/ml) by StrKUh isolate (Kurumankotta). Two isolates StrMh and StrPh resulted moderate level of HCN production. Out of the eight isolates tested for siderophore production, four StrMh, StrPh, StrAh and StrAd isolates resulted siderophore production.

4.5.6 Phosphate solubilization, IAA, HCN and siderophore production by *Trichoderma* sp.

All the eight isolates of *Trichoderma* sp. did not solubilized phosphorus and IAA under *in vitro* screening (Table 18). Two isolates TrPh and TrKUh produced moderate HCN under *in vitro* screening. Siderophore production was produced only by three isolates TrKh, TrMh, and TrAh.

4.6 SCREENING OF *Bacillus* sp., fluorescent pseudomonads, *Streptomyces* sp., and *Trichoderma* sp. FOR ANTAGONISTIC ACTIVITY AGAINST FOUR SELECTED PATHOGENS UNDER *IN VITRO* CONDITION

All the predominant isolates of *Bacillus* sp., fluorescent pseudomonads, *Streptomyces* sp., and *Trichoderma* sp. were tested for their antagonistic efficiency against four major soil borne pathogens *viz*: *Fusarium oxysporum*, *Pythium aphanidermatum*, *Rhizoctonia solani*, and *Ralstonia solanacearum* under *in vitro* condition.

Table 17. Phosphate solubilization, IAA, HCN and siderophore production by *Streptomyces* sp. (Str)

Sl.No.	Isolates	Phosphate solubilization efficiency (%)	IAA production (µg/ml)	HCN production	Siderophore production
1	StrMh	-	11.63	++	+
2	StrPh	153.76	10.41	++	+
3	StrKh	-	-	-	-
4	StrAh	176.99	-	-	+
5	StrKUh	-	10.16	-	-
6	StrPd	-	-	-	-
7	StrVd	-	-	-	-
8	StrAd	-	-	-	+
	CD	1.05	NS		

h- healthy d- diseased - Negative ++++ Excellent +++ Good ++ Moderate + Low

StrMh isolate from Mayilumpadi StrPh isolate from Pulpalli StrKh isolate from Kappikunnu StrAh isolate from Appade
StrKuh isolate from Kurumankotta StrPd isolate from Palakkaparambu StrVd isolate from Vengapalli StrAd isolate from Ambalavayal

Table 18. Phosphate solubilization, IAA, HCN and siderophore production by *Trichoderma* sp. (Tr)

Sl.no	Isolates	Phosphate solubilization efficiency (%)	IAA Production (µg/ml)	HCN Production	Siderophore Production
1	TrMh	-	-	-	+
2	TrPh	-	-	++	-
3	TrKh	-	-	-	+
4	TrAh	-	-	-	+
5	Truth	-	-	++	-
6	TrMd	-	-	-	-
7	TrPd	-	-	-	-
8	TrVd	-	-	-	-

h- healthy d- diseased - Negative ++++ Excellent +++ Good ++ Moderate + Low

TrMh isolate from Mayilumpadi TrPh isolate from Pulpalli TrKh isolate from Kappikunnu TrAh isolate from Appade
TrKUh isolate from Kurumankotta TrMd isolate from Meenangadi TrPd isolate from Palakkaparambu TrVd isolate from Vengapalli

4.6.1 *Invitro* screening of *Bacillus* sp. for antagonistic activity against the selected pathogens

All the ten isolates of *Bacillus* sp. were screened for their antagonistic activity against selected pathogens (Table 19). Only three isolates of BsMh (39.05per centinhibition), BsAh (64.08per centinhibition), and BsKUh (43.93per centinhibition) showed antagonism against *Fusarium oxysporum.* The BsAh (15.90per centinhibition) is the only one isolate antagonistic against *Pythium aphanidermatum.* Four isolates namely BsMh (26.06per centinhibition), BsKh (47.09per centinhibition), BsAh (23.01per centinhibition), and BsKUh (22.51per centinhibition) showed antagonism against *Rhizoctonia solani.*

The isolates BsMh (43.81per centinhibition), BsPh (15.30per centinhibition), and BsAh (39.09per centinhibition) showed antagonism against *Ralstonia solanacearum.* It was observed that the isolates obtained from the healthy locations had more antagonistic activity as compared to those from diseased area. Isolates BsMh, BsAh, and BsKUh were the best isolates agaist plant pathogens.

Only BsAh isolate recorded antagonistic activity against *Fusarium oxysporum* (64.08per centinhibition), *Pythium aphanidermatum* (15.90 per centinhibition), *Rhizoctonia solani* (23.01per centinhibition) and *Ralstonia solanacearum* (39.09 per centinhibition) under *in vitro* condition.

Table 19. Antagonistic activities of *Bacillus* sp. (Bs) against soil borne plant pathogens

Sl.No.	Isolates	Per cent inhibition			
		Fusarium oxysporum	***Pythium aphanidermatum***	***Rhizoctonia solani***	***Ralstonia solanacearum***
1	BsMh	39.50	-	26.06	43.81
2	BsPh	-	-	-	15.30
3	BsKh	-	-	47.09	-
4	BsAh	64.08	15.90	23.01	39.09
5	BsKUh	43.93	-	22.51	-
6	BsMd	-	-	-	-
7	BsPd	-	-	-	-
8	BsPTd	-	-	-	-
9	BsVd	-	-	-	-
10	BsAd	-	-	-	-
	CD	1.66		1.656	2.04

Each value represents the mean of three replication, (-) No inhibition

BsMh isolate from Mayilumpadi BsPh isolate from Pulpalli BsKh isolate from Kappikunnu BsAh isolate from Appade BsKuh isolate from Kurumankotta BsMd isolate from Meenangadi BsPd isolate from Palakkaparambu BsPTd isolate from Pathiri BsVd isolate from Vengapalli BsAd isolate from Ambalavayal

Isolates PfAh (33.71 per cent inhibition), PfKh (33.02 per cent inhibition), PfPd (25.7 per cent inhibition) and PfPh (20.01 per cent inhibition) showed antagonism against *Pythium aphanidermatum.* The isolates PfAh (27.2 per cent inhibition), PfKh (19.20 per cent inhibition), and PfPTd (16.9 per cent inhibition) showed antagonism against *Rhizoctonia solani.*

The isolates PfPh (31.32 per cent inhibition), PfKh (30.33 per cent inhibition), and PfKUh (28.57 per cent inhibition) showed antagonism against *Ralstonia solanacearum.* The isolates PfPh, PfKh, and PfAh were the best antagonistic isolates. Among these, of PfMh isolate did not show any antagonistic activity against the four selected pathogens.

4.6.3. *In vitro* screening of *Streptomyces* sp. for antagonistic activity against selected plant pathogens

Eight isolates of *Streptomyces* sp. were tested for their antagonistic activity against selected pathogens (Table 21). Only three isolates namely StrPh (67.78 per cent inhibition), StrKh (42.85 per cent inhibition) and StrMh (25.78 per cent inhibition) showed antagonism against *Fusarium oxysporum.*

Isolates StrKh (34.28 per cent inhibition), StrKUh (31.21 per cent inhibition), StrPh (30.63 per cent inhibition), StrMh (16.51 per cent inhibition), and StrPd (12.09 per cent inhibition) recorded antagonism against *Pythium aphanidermatum.* The isolates StrMh (23.14 per cent inhibition), StrKh (53.11 per cent inhibition), StrKUh (40.22 per cent inhibition), and StrAd (13.48 per cent inhibition) were showed antagonism against *Rhizoctonia solani* (Plate 12).

The isolates StrPh (34.77 per cent inhibition), StrAh (34.23per cent inhibition), StrPd (15.89 per cent inhibition) and StrVh (23.13 per cent inhibition) showed antagonism against *Ralstonia solanacearum.*

Table 20. Antagonistic activities of fluorescent pseudomonads (Pf)against soil borne plant pathogens

Sl.No.	Isolates	Per cent inhibition			
		Fusarium oxysporum	***Pythium aphanidermatum***	***Rhizoctonia solani***	***Ralstonia solanacearum***
1	PfMh	-	-	-	-
2	PfPh	28.89	20.01	-	31.32
3	PfKh	59.43	33.02	19.2	30.33
4	PfAh	28.57	33.71	27.2	-
5	PfKUh	-	-	-	28.57
6	PfPd	-	25.7	-	-
7	PfPTd	23.1	-	16.9	-
	CD	1.92	1.91	2.04	2.15

Each value represents the mean of three replication, (-) No inhibition

PfMh isolate from Mayilumpadi PfPh isolate from Pulpalli PfKh isolate from Kappikunnu PfAh isolate from Appade
PfKUh isolate from Kurumankotta PfPd isolate from Palakkaparambu PfPTd isolate from Pathiri

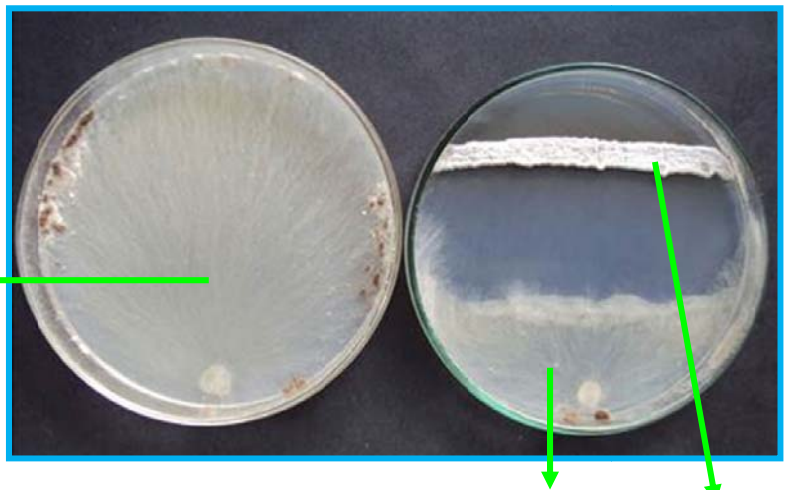
Table 21. Antagonistic activity of *Streptomyces* sp. (Str) against soil borne plant pathogens of ginger

Sl.No.	Isolates	Per cent inhibition			
		Fusarium oxysporum	***Pythium aphanidermatum***	***Rhizoctonia solani***	***Ralstonia solanacearum***
1	StrMh	25.78	16.51	23.14	-
2	StrPh	67.78	30.63	53.11	34.77
3	StrKh	42.85	34.28	-	-
4	StrAh	-	-	-	34.23
5	StrKUh	-	31.21	40.22	-
6	StrPd	-	12.09	-	15.89
7	StrVd	-	-	-	23.13
8	StrAd	-	-	13.48	-
	CD	1.66	1.84	1.91	1.91

Each value represents the mean of three replication, - No inhibition

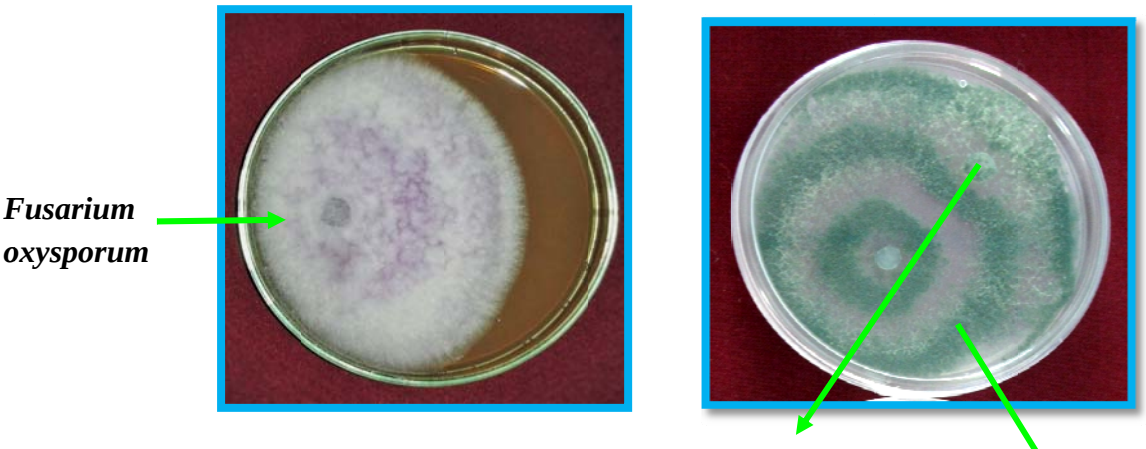
StrMh isolate from Mayilumpadi StrPh isolate from Pulpalli StrKh isolate from Kappikunnu StrAh isolate from Appade

StrKuh isolate from Kurumankotta StrPd isolate from Palakkaparambu StrVd isolate from Vengapalli StrAd isolate from Ambalavayal



Control ***Rhizoctoniasolani x Streptomyces* sp.**

Plate 12. Antagonistic activity of *Streptomyces* sp. Against *Rhizoctonia solani* under *in vitro* condition



Control ***Fusarium oxysporum x Trichoderma* sp. (TrKUh isolate)**

Plate 13. Antagonistic activity of *Trichoderma* sp. against *Fusarium oxysporum* under *in vitro* condition

4.6.4. *In vitro* screening of *Trichoderma* sp. for antagonistic activity against selected plant pathogens

Eight isolates of *Trichoderma* sp. were tested for their antagonistic activity against selected pathogens (Table 22). Only three isolates namely, TrAh (78.93 per cent inhibition), TrKUh (73.91 per cent inhibition) and TrPh (71.08 per cent inhibition) showed antagonism against *Fusarium oxysporum* (Plate 13). Isolates of TrKh (51.30 per cent inhibition), TrKUh (30.01 per cent inhibition), TrMd (27.33 per cent inhibition), TrPh (23.14 per cent inhibition), TrPd (20.55 per cent inhibition) and TrMh (12.90per cent inhibition) showed antagonism against *Pythium aphanidermatum.* The isolates TrAh (43.11 per cent inhibition), TrVd (34.63 per cent inhibition), TrKh (23.14 per cent inhibition) and TrMd (13.48 per cent inhibition) showed antagonism against *Rhizoctonia solani.* The isolates TrMh (15.96 per cent inhibition) was the only isolate which showed antagonism against *Ralstonia solanacearum.*

4.6.5. Isolates antagonistic against the selected plant pathogens

The isolate BsAh (64.08per cent inhibition) from Appad, PfKh (59.43 per cent inhibition) from Kappikunnu, StrPh (67.78 per cent inhibition) from Pulpalli and TrAh (78.91 per cent inhibition) from Kurumankotta were antagonistic against *Fusarium oxysporum* (Table 23).The isolates BsAh (15.90 per cent inhibition) from Appad, PfAh (33.71 per cent inhibition) from Appad, StrKh (34.28 per cent inhibition) from Kappikunnu, and TrAh (51.30 per cent inhibition) from Appad showed maximum antagonism against *Pythium aphanidermatum.* Isolates BsKh (47.09 per cent inhibition) from Kappikunnu, PfAh (27.20 per cent inhibition) from Appad, StrPh (53.11 per cent inhibition) from Pulpalli, and TrKUh (43.27 per cent inhibition) from Kurumankotta were found to be highly antagonistic against *Rhizoctonia solani.* The isolates viz; BsMh (43.81 per cent inhibition) from Mayilumpadi, PfPh (31.32 per cent inhibition) from Pulpalli, and StrPh (34.23 per cent inhibition) Pulpalli were antagonistic against *Ralstonia solanacearum.* Isolates from Appad, Kappikiunnu and Pulpalli locations were good in antagonistic activity.

Table 22. Antagonistic activities of *Trichoderma*sp. (Tr) against soil borne plant pathogens of ginger

Sl.No.	Isolates	Per cent inhibition			
		Fusarium oxysporum	***Pythium aphanidermatum***	***Rhizoctonia solani***	***Ralstonia solanacearum***
1	TrMh	-	12.90	-	15.96
2	TrPh	71.08	23.14	-	-
3	TrKh	-	-	31.38	-
4	TrAh	78.93	51.30	35.09	-
5	TrKUh	73.91	30.01	43.27	-
6	TrMd	-	27.33	19.42	-
7	TrPd	-	20.55	-	-
8	TrVd	-	-	34.63	-
	CD	1.66	1.84	1.64	

Each value represents the mean of three replication, - No Per cent inhibition

TrMh isolate from Mayilumpadi TrPh isolate from Pulpalli TrKh isolate from Kappikunnu TrAh isolate from Appade
TrKUh isolate from Kurumankotta TrMd isolate from Meenangadi TrPd isolate from Palakkaparambu TrVd isolate from Vengapalli

Table 23. Effect of different isolates on the antagonistic activity against the selected plant pathogens of ginger

Sl. No.	Pathogens	Isolates	Per cent inhibition	CD
1	*Fusarium oxysporum,*	BsAh PfKh StrPh TrKUh TrAh	64.08 59.43 67.78 73.91 78.93	1.65
2	*Pythium aphanidermatum*	BsAh PfAh StrKh TrAh	15.90 33.71 34.28 51.30	1.91
3	*Rhizoctonia solani*	BsKh PfAh StrPh Truth	47.09 27.20 53.11 43.27	1.91
4	*Ralstonia solanacearum*	BsMh PfPh StrPh	43.81 31.32 34.23	2.04

4.7 SELECTION OF ISOLATES BASED ON PLANT GROWTH PROMOTION AND ANTAGONISTIC ACTIVITES UNDER *invitro*

Finally based on the *invitro* studies for plant growth promotion and disease management activities, three most promising isolates of three most efficient nitrogen fixers (NFMh, NFPh and NFKh), phosphate solubilizing bacteria (PSBAh, PSBKh and PSBPh), *Bacillus* sp. (BSMh, BSAh and BSKUh), fluorescent pseudomonads (PFPh, PFKh and PFAh), *Streptomyces* sp. (StrPh, StrKh and StrMh) and *Trichoderma* sp. (TrAh, TrKUh and TrPh) were selected for pot culture experiment. These selected isolates were used for evaluation of their efficiency in plant growth promotion and management of Fusarium yellows.

4.8 EVALUATION OF SELECTED NITROGEN FIXERS AND PHOSPHATE SOLUBILIZING BACTERIA FOR PLANT GROWTH PROMOTION IN GINGER

Three most promosing isolates of nitrogen fixers and phosphate solubilizing bacteria were evaluated for their efficiency in plant growth promotion under pot culture experiment (Plate 14).

4.8.1 Effect of nitrogen fixers and phosphate solubilizing bacteria on sprouting percentage

After four weeks of planting, only T_4 (PSBPh) isolate from Pulpalli recorded cent percent sprouting and the least sprouting percentage (36.31) was recorded in T_7 (Control) (Table 24). However, all the treatments showed sprouting at five weeks after planting. T_4 (PSBPh) was the promising treatment in terms of sprouting percentage.

Plate 14. An overview of pot culture experiment for plant growth promotion in ginger by nitrogen fixers and phosphate solubilizing bacteria

Table. 24. Effect of selected nitrogen fixers and phosphate solubilizing bacteria on sprouting percentage

Treatments	4 WAP	5 WAP
T_1 (NFMh)	76.17[d]	100
T_2 (NFPh)	62.68[e]	100
T_3 (NFKh)	88.38[b]	100
T_4 (PSBPh)	100[a]	100
T_5 (PSBKh)	77.57[c]	100
T_6 (PSBAh)	54.35[f]	100
T_7 (Control)	36.31[g]	100

Each value represents mean of three replicationsWAP- Weeks after planting

NF-Nitrogen fixers PSB- Phosphate solubilizing bacteria

NFMh isolate from Mayilumpadi

NFPh isolate from Pulpalli

NFKh isolate from Kappikunnu

PSBPh isolate from Pulpalli

PSBKh isolate from Kappikunnu

PSBAh isolate from Appad

Control

4.8.2 Effect of nitrogen fixers and phosphate solubilizing bacteria on plant height (cm)

Observations on plant height were recorded two, three, four and five months after planting (Table 25). The plant height showed significant difference at two months after planting among the treatments. The control recorded minimum (9.27 cm) and the highest (36.73 cm) was in T_4 (PSBPh).

Three months after planting, T_4 (PSBPh) recoded maximum plant height (46.73 cm) and the minimum was in T_7 (Control) (12.67 cm). Four and five months after planting also showed similar results. T_4(PSBPh) was found to be on par with T_2 (NFPh). The plants in T_4 (PSBPh) showed the maximum plant height of (69.22 cm) and (81.08 cm) at four and five months after planting respectively. The plants in T_7 (Control) showed the minimum plant height 20.01 cm and 29.93 at four and five months after plantingrespectively.

Significant differences among the treatments were observed five months after planting with the highest plant height in T_4 (PSBPh) with 80.08 cm whereas the minimum was recorded in T_7 (Control) with plant height of 29.93 cm.

4.8.3 Effect of nitrogen fixers and phosphate solubilizing bacteria on number of tillers

The number of tillers after two, three, four and five months of planting showed significant differences among the treatments (Table 26).After two months of planting, maximum number of tillers (4.33/plant) were recorded in T_3 (NFKh from Kappikunnu) and the least number was inT_7 (Control) (1.91/plant).

Three months after planting, the highest number of tillers (4.99) werenoticed in T_3 (NFKh from Kappikunnu) and the least number was in T_7 (Control) (2.93).

Table 25. Effect of nitrogen fixers and phosphate solubilizing bacteria on plant height (cm)

Treatments	2 MAP	3MAP	4MAP	5 MAP
T_1 (NFMh)	33.27^{bc}	39.38^{ab}	54.92^{bc}	68.33^{bc}
T_2 (NFPh)	26.32^{b}	36.17^{ab}	62.36^{ab}	77.21^{ab}
T_3 (NFKh)	35.63^{a}	44.22^{a}	56.35^{bc}	69.43^{bc}
T_4 (PSBPh)	36.73^{a}	46.73^{a}	69.22^{a}	80.08^{a}
T_5 (PSBKh)	26.32^{b}	38.13^{ab}	57.18^{bc}	70.42^{bc}
T_6 (PSBAh)	24.77^{b}	36.97^{ab}	60.07^{b}	72.11^{b}
T_7 (Control)	9.27^{c}	12.67^{b}	20.01^{c}	29.93^{c}

Each value represents mean of three replications MAP- Months after planting
NF-Nitrogen fixers PSB- Phosphate solubilizing bacteria

NFMh isolate from Mayilumpadi

NFPh isolate from Pulpalli

NFKh isolate from Kappikunnu

PSBPh isolate from Pulpalli

PSBKh isolate from Kappikunnu

PSBAh isolate from Appad

Table 26. Effect of nitrogen fixers and phosphate solubilizing bacteria on number of tillers (no./plant)

Treatments	2MAP	3MAP	4MAP	5 MAP
T_1 (NFMh)	2.77^{ab}	3.02^{bc}	3.99^{bc}	4.09^{bc}
T_2 (NFPh)	2.91^{ab}	3.71^{ab}	3.80^{ab}	3.87^{ab}
T_3 (NFKh)	4.35^{a}	4.99^{a}	6.80^{a}	6.85^{a}
T_4 (PFPh)	3.98^{a}	4.87^{ab}	4.99^{ab}	5.10^{ab}
T_5 (PFKh)	3.20^{ab}	3.35^{abc}	3.43^{abc}	3.49^{ab}
T_6 (PFAh)	3.58^{ab}	4.09^{ab}	4.12^{ab}	4.25^{ab}
T_7 (Control)	1.91^{b}	2.93^{c}	2.94^{c}	3.07^{c}

Each value represents mean of three replications MAP- Months after planting NF-Nitrogen fixers PSB- Phosphate solubilizing bacteria

NFMh isolate from Mayilumpadi

NFPh isolate from Pulpalli

NFKh isolate from Kappikunnu

PSBPh isolate from Pulpalli

PSBKh isolate from Kappikunnu

PSBAh isolate from Appad

Four months after planting, T_3 (NFKh from Kappikunnu) recorded the highest number of tillers (6.80) and the least number was T_7 (Control) (2.94).After five months of planting, all the treatments showed increase in the number of tillers compared to control. The highest number of tillers (6.85) were observed in T_3 (NFKh from Kappikunnu) and the least number of tillers (3.07) inT_7 (Control).

4.8.4 Effect of nitrogen fixers and phosphate solubilizing bacteria on rhizome yield

The data on the average yield of rhizomes per plant are presented (Table 27). Plants in T_4 (PSBPh) produced the highest yield of 128.92 g/plant which was on par with T_3(NFKh) 125.74 g/plant and the lowest yield (49.06 g/plant) was in T_7 (Control).

4.9 EVALUATION OF THE SELECTED ISOLATES OF *Bacillus* sp., FLUORESCENT PSEUDOMONADS, *Streptomyces* sp. and *Trichoderma* sp. FOR DISEASE MANAGEMENT IN GINGER

Three most promising isolates of *Bacillus* sp., Fluorescent pseudomonads, *Streptomyces* sp. and*Trichoderma* sp. were evaluated for their efficiency against Fusarium yellows disease management under pot culture experiment (Plate 15).

4.9.1 Effect of antagonists on sprouting percentage

After four weeks of planting, only T_4 (PfKh) isolate from Kappikunnu recordedcent percent sprouting and the least sprouting percentage (33.20) were recorded in T_{13} (Control; Pathogen alone) (Table 28). However, all the treatments sprouted in five weeks after planting. Treatment T_4 (PfKh) was the promising treatment in terms of sprouting percentage.

.Plate 15. An overview of pot culture experiment for Fusarium yellows disease management in ginger by *Bacillus* sp., fluorescent pseudomonads, *Streptomyces* sp. and *Trichoderma* sp.

Table 27. Effect of nitrogen fixers and phosphate solubilizing bacteria on rhizome yield

Treatments	Rhizome yield (g/plant)
T_1 (NFMh)	118.26[d]
T_2 (NFPh)	120.98[c]
T_3 (NFKh)	127.34[a]
T_4 (PSBPh)	128.92[a]
T_5 (PSBKh)	123.47[b]
T_6 (PSBAh)	122.03[b]
T_7 (Control)	49.06[e]

Each value represents mean of three replications NF- Nitrogen fixers PSB- Phosphate solubilizing bacteria

NFMh isolate from Mayilumpadi

NFPh isolate from Pulpalli

NFKh isolate from Kappikunnu

PSBPh isolate from Pulpalli

PSBKh isolate from Kappikunnu

PSBAh isolate from Appad

Table 28. Effect of antagonists on sprouting percentage

Treatments	4 WAP	5 WAP
T_1 (BsMh)	72.15[e]	100
T_2 (BsAh)	67.34[g]	100
T_3 (BsKUh)	84.59[b]	100
T_4 (PfKh)	100 [a]	100
T_5 (PfPh)	76.70[d]	100
T_6 (PfAh)	53.42[j]	100
T_7 (StrPh)	55.90[ij]	100
T_8 (StrMh)	69.53[f]	100
T_9 (StrKh)	56.05[i]	100
T_{10} (TrAh)	76.87[d]	100
T_{11} (TrKUh)	78.22[c]	100
T_{12} (TrPh)	61.66 [h]	100
T_{13}(Control;pathogen alone)	33.20[l]	100
T_{14}(Absolute control)	44.80[k]	100

Each value represents mean of three replications

WAP: Weeks after planting Bs-*Bacillus* sp. Pf- fluorescentpseudomonads

Str- *Streptomyces* sp. Tr- *Trichoderma* sp.

BsMh isolate from Mayilumpadi BsAh isolate from Appad

BsKUh isolate from Kurumankotta PFKh isolate from Kappikunnu

PFPh isolatefrom KappikunnuPFAh isolate from Appad

StrPh isolate from PulpalliStrMh isolate from Mayilumpadi

StrKh isolate from KappikunnuTrAh isolate from Appad

TrKUh isolate from KurumankottaTrPh isolate from Pulpalli

4.9.2 Effect of antagonists on plant height (cm)

The plant height recorded at monthly interval till harvestsshowed significant differences among the treatments (Table 29).After two months of planting,T_{11}(TrKUh) isolate from Kurumankotta recorded maximum plant height (20.82 cm) and minimum plant height (10.25 cm) was observed in T_{13} (Control; pathogen alone).

Three months after planting, treatment T_{11} (TrKUh from Kurumankotta) is on par with T_{12} (TrPhfrom Pulpalli) and T_5 (PfPhfrom Pulpalli). The treatment T_{13} (Control; pathogen alone) recorded minimum plant height (10.38 cm). Among the treatments, T_{11} (TrKUh from Kurumankotta) recorded a maximum height (37.93 cm). After four months, plants in T_{11} (TrKUh from Kurumankotta) showed the maximum plant height (65.60 cm) and the minimum (10.41 cm) was in T_{13} (Control; pathogen alone).

As a result of challenge inoculation of the pathogen, significant differences were observed five months after planting. T_{11} (TrKUh from Kurumankotta) recorded maximum height (69.98 cm) whereas the minimum was recorded in T_{13} (Control; pathogen alone).It was found that T_{11} (TrKUh from Kurumankotta) was the most promising isolate for enhancing the plant height.

4.9.3 Effect of antagonists on number of tillers

The number of tillers after two, three, four and five months of planting showed significant differences among the treatments (Table 30). The number tillers were increasing up to four months after planting but five months after planting it showed a senescence in number of tillers.

Table 29. Effect of antagonists on plant heightof ginger (cm)

Treatments	2 MAP	3 MAP	4 MAP	5 MAP
T_1 (BsMh)	18.38^{abc}	21.58^{ab}	52.89^{bc}	61.94^{ab}
T_2 (BsAh)	12.65^{de}	13.93^{cd}	52.64^{bc}	47.03^{c}
T_3 (BsKUh)	16.01^{bc}	17.22^{bc}	40.73^{de}	39.42^{de}
T_4 (PfKh)	15.94^{cd}	17.49^{bc}	59.10^{ab}	53.15^{bc}
T_5 (PfPh)	19.88^{ab}	22.60^{a}	59.88^{ab}	63.65^{ab}
T_6 (PfAh)	13.22^{d}	14.36^{c}	49.37^{c}	58.10^{b}
T_7 (StrPh)	16.02^{bc}	17.84^{bc}	24.53^{e}	28.06^{e}
T_8 (StrMh)	12.14^{de}	13.09^{cde}	43.38^{c}	40.98^{d}
T_9 (StrKh)	11.23^{e}	12.30^{d}	49.17^{b}	46.20^{cd}
T_{10} (TrAh)	15.80^{c}	17.51^{bc}	56.71^{ab}	62.90^{ab}
T_{11} (TrKUh)	20.82^{a}	22.53^{a}	65.60^{a}	69.98^{a}
T_{12} (TrPh)	17.46^{b}	18.48^{b}	43.77^{d}	46.96^{cd}
T_{13}(Control;pathogen alone)	10.25^{g}	10.38^{f}	10.41^{f}	0.00^{g}
T_{14}(Absolute control)	$11.18d^{f}$	11.29^{e}	19.53^{g}	8.23^{f}

Each value represents mean of three replications MAP: Months after planting Bs- *Bacillus* sp. Pf- fluorescent pseudomonads Str- *Streptomyces* sp. Tr- *Trichoderma* sp.

BsMh isolate from Mayilumpadi BsAh isolate from Appad BsKUh isolate from Kurumankotta PFKh isolate from Kappikunnu PFPh isolatefrom Kappikunnu PFAh isolate from Appad StrPh isolate from Pulpalli StrMh isolate from Mayilumpadi StrKh isolate from Kappikunnu TrAh isolate from Appad TrKUh isolate from Kurumankotta TrPh isolate from Pulpalli

After two months of planting, the maximum number of tillers (4.0) was recorded inT_{11} (TrKUh) and the least number (2.87) was with treatment T_{13}(Control; pathogen alone). Third month after planting, the highest number of tillers (4.16) was found in T_{11} (TrKUh) which was on par with T_4 (PfKh) and the least number was in T_{13} (Control; pathogen alone) which was also on par with T_{14} (Absolute ontrol). Four months after planting, T_{11} (TrKUh) recorded highest number of tillers (6.21) which was on par with T_{10} (TrAh from Appad) and the least number was in T_{13} (Control; pathogen alone) which was on par with T_{14}(Absolute ontrol).

As a result of the challenge inoculation of *Fusarium oxysporum* five months after planting, significant difference among the treatments was observed number of tillers. T_{11} (TrKUh) had a positive influence on the number of tillers compared to T_{13} (Control; pathogen alone). The maximum number of tillers (6.44) was observed in T_{11} (TrKUh) which was on par with T_6 (PfAh) and minimum was recorded in T_{13} (Control; pathogen alone). Treatment T_{11} (TrKUhfrom Kurumankotta) was fund to be the best treatment.

4.9.4. Effect of different treatments on rhizome yield of ginger

The data on the average yield of ginger rhizomes per plant are presented (Table 31). Plants in T_{11} (TrKUh) produced maximum yield (120.35 g/plant) which was on par with T_4(PfKh) (119.10 g/plant) and the lowest yield was in T_{13}(Control; Pathogen alone).

4.9.5 Incidence of Fusarium yellows disease

After four months of planting second application of the antagonists was done. Two weeks after the second application of antagonists challenge inoculation of the pathogen *Fusarium oxysporum* was also done.

Table 30. Effect of antagonists on number of tillers in ginger

Treatments	2 MAP	3 MAP	4 MAP	5 MAP
T_1 (BsMh)	3.48^{bcd}	3.62^{bc}	4.70^{d}	2.75^{ef}
T_2 (BsAh)	3.51^{bc}	3.82^{b}	5.02^{c}	2.32^{f}
T_3 (BsKUh)	3.49^{bcd}	3.87^{b}	5.07^{c}	2.96^{e}
T_4 (PfKh)	3.83^{ab}	4.04^{ab}	5.26^{bcd}	3.00^{de}
T_5 (PfPh)	3.34^{cd}	3.41^{c}	5.55^{bc}	5.68^{ab}
T_6 (PfAh)	3.17^{e}	3.35^{cd}	5.57^{bc}	6.05^{a}
T_7 (StrPh)	3.28^{d}	3.65^{bc}	4.92^{cd}	4.92^{b}
T_8 (StrMh)	3.52^{bc}	3.58^{bc}	4.64^{d}	2.15^{fg}
T_9 (StrKh)	3.38^{c}	3.45^{c}	4.52^{de}	3.52^{c}
T_{10} (TrAh)	3.78^{b}	3.83^{b}	5.88^{b}	5.89^{ab}
T_{11} (TrKUh)	4.00^{a}	4.16^{a}	6.21^{a}	6.44^{a}
T_{12} (TrPh)	3.27^{d}	3.33^{cd}	3.39^{e}	3.39^{cd}
T_{13}(Control;pathogen alone)	3.07^{f}	3.13^{e}	3.16^{f}	0.00^{g}
T_{14}(Absolute control)	2.87^{g}	3.09^{f}	3.13^{g}	3.17^{d}

Each value represents mean of three replications MAP: Months after planting Bs- *Bacillus* sp. Pf- fluorescentpseudomonads Str- *Streptomyces* sp. Tr- *Trichoderma* sp.

BsMh isolate from Mayilumpadi BsAh isolate from AppadBsKUh isolate from Kurumankotta PFKh isolate from Kappikunnu PFPh isolatefrom KappikunnuPFAh isolate from AppadStrPh isolate from PulpalliStrMh isolate from Mayilumpadi StrKh isolate from KappikunnuTrAh isolate from AppadTrKUh isolate from KurumankottaTrPh isolate from Pulpalli

Table 31. Effect of antagonists on rhizome yield of ginger

Treatments	Total yield of rhizome (g/plant)
T_1 (BsMh)	115.695 ^{ab}
T_2 (BsAh)	81.750^{e}
T_3 (BsKUh)	112.293^{b}
T_4 (PfKh)	119.10^{a}
T_5 (PfPh)	106.326^{bc}
T_6 (PfAh)	95.357^{d}
T_7 (StrPh)	116.115^{ab}
T_8 (StrMh)	101.95^{cd}
T_9 (StrKh)	104.037^{c}
T_{10} (TrAh)	117.129^{ab}
T_{11} (TrKUh)	120.35 ^{a}
T_{12} (TrPh)	114.735^{ab}
T_{13}(Control;pathogen alone)	0.00^{g}
T_{14}(Absolute control)	43.07^{f}

Each value represents mean of three replicationsBs- *Bacillus* sp.
Pf- fluorescentpseudomonadsStr- *Streptomyces* sp. Tr- *Trichoderma* sp.

BsMh isolate from Mayilumpadi BsAh isolate from AppadBsKUh isolate from Kurumankotta PFKh isolate from KappikunnuPFPh isolatefrom KappikunnuPFAh isolate from AppadStrPh isolate from PulpalliStrMh isolate from MayilumpadiStrKh isolate from KappikunnuTrAh isolate from AppadTrKUh isolate from KurumankottaTrPh isolate from Pulpalli

4.9.5.1 Number of tillers infected due to the challenge inoculation of Fusarium oxysporum

After the challenge inoculation of the pathogen, the symptoms were stunted growth of tillers, yellowing, drying up, falling of tillers and percentage of rhizome rot were recorded (Plate 16).

Fusarium yellows incidence in the tillers were found except in T_5 (PfPh from Pulpalli), T_6 (PfAh from Appad), T_7 (StrPh from Pulpalli), T_{10} (TrAh from Appad),T_{11} (TrKUh from Kurumankotta), T_{12} (TrPh from Pulpalli) and T_{14} (Absolute control) (Table 32).All other treatments were infected with Fusarium yellows disease. Yellowing, drying up and total falling of tillers were observed in T_{13} (Control; Pathogen alone).

4.9.5.2 Per cent disease incidence of Fusarium yellows in ginger rhizome

Percent disease incidence of Fusarium yellows in rhizome were also recorded (Table33). Except T_3 (BsKUh from Kurumankotta), T_7 (StrPh) Pulpalli, T_9 (StrKh) Kappikunnu, T_{10} (TrAh from Appad), T_{11} (TrKUh from Kurumankotta), T_{12} (TrPh from Pulpalli) and T_{14} (Absolute control), all other treatments were infected with Fusarium yellows disease. Highest per cent disease incidence was recorded in the case of T_{13} (Control; Pathogen alone). Fusarium yellows disease was effectively controlled in T_{11} (TrKUh isolate from Kurumankotta) and the same isolate also recorded higher yield. Hence the treatment T_{11} (*Trichoderma harzianum*) was most effective biocontrol agent for the management of Fusarium yellows in ginger. (Plate 17).

4.10 16S rDNA SEQUENCE ANALYSIS OF EFFICIENT PSB ISOLATE

Based on the evaluation of nitrogen fixers and phosphate solubilizing bacteria, the most promising isolate was found to be PSBPh (phosphate solubilizing bacteria) (Plate 18). It was identified by 16S rDNA sequence analysis using polymerase chain reaction (PCR)

Plate 16. An overview of pot culture experiment for Fusarium yellows disease management in ginger after the challenge inoculation of the pathogen

4.10.1 Amplification of 16S rDNA gene

Amplification of 16S rDNAgene was carried out by colony PCR. The Product was checked on 1.0% (w/v agarose gel and documented. Only one amplicon of about 1500 bp was obtained (Plate 19).

4.10.2 Purification and Sequencing of PCR Product

PCR product of the PSB isolate PSBPh were purified and sequenced. The nucleotide sequences of the isolate are given in Appendix-III.

4.11.3 Nucleotide Sequence Analysis

Homology search of nucleotide sequence obtained from the isolates PSBPh with other reported 16S rRNA gene sequences was carried out. PSBPh showed homology with *Burkholderia cepecea* (Accession number U96927). The sequence analyses plate 20 and phylogenetic tree are given in plate21.

Table 32. Effect of treatments on Fusarium yellows infected tillers after challenge inoculation of *Fusarium oxysporum*

Treatments	Number of infected tillers	Per cent infected tillers
T_1 (BsMh)	1.95	41.48(6.67 ^{de})
T_2 (BsAh)	2.7	53.78 (7.20 ^{b})
T_3 (BsKUh)	2.11	41.62 (6.71 ^{d})
T_4 (PfKh)	2.26	42.97 (6.82^{c})
T_5 (PfPh)	ND	ND
T_6 (PfAh)	ND	ND
T_7 (StrPh)	ND	ND
T_8 (StrMh)	2.49	53.66(7.13 ^{bc})
T_9 (StrKh)	1.52	33.63(5.84 ^{e})
T_{10} (TrAh)	ND	ND
T_{11} (TrKUh)	ND	ND
T_{12} (TrPh)	ND	ND
T_{13}(Control;pathogen alone)	3.16	100(8.5 ^{a})
T_{14}(Absolute control)	ND	ND

Each value represents mean of three replications

Square root transformed values are given in parenthesis

Bs- *Bacillus* sp. Pf- fluorescentpseudomonads

Str- *Streptomyces* sp. Tr- *Trichoderma* sp.

BsMh isolate from Mayilumpadi BsAh isolate from AppadBsKUh isolate from Kurumankotta PFKh isolate from KappikunnuPFPh isolatefrom Kappikunnu PFAh isolate from AppadStrPh isolate from PulpalliStrMh isolate from MayilumpadiStrKh isolate from KappikunnuTrAh isolate from AppadTrKUh isolate from KurumankottaTrPh isolate from Pulpalli

Table 33. Per cent infected rhizome at harvest due to infection by *Fusarium oxysporum*

Treatments	Per cent infected rhizome
T_1 (BsMh)	34.36 (7.38^{c})
T_2 (BsAh)	61.61 (8.55^{b})
T_3 (BsKUh)	33.01 (6.72^{f})
T_4 (PfKh)	ND
T_5 (PfPh)	33.12 (6.97^{e})
T_6 (PfAh)	33.33 (7.15^{d})
T_7 (StrPh)	ND
T_8 (StrMh)	33.29 (7.03^{d})
T_9 (StrKh)	ND
T_{10} (TrAh)	ND
T_{11} (TrKUh)	ND
T_{12} (TrPh)	ND
T_{13}(Control;pathogen alone)	100.00 (9.71^{a})
T_{14}(Absolute control)	ND

Each value represents mean of three replications

Square root transformed values are given in parenthesis

Bs-*Bacillus* sp. Pf- fluorescent pseudomonads Str- *Streptomyces* sp. Tr- *Trichoderma* sp.

BsMh isolate from Mayilumpadi BsAh isolate from AppadBsKUh isolate from Kurumankotta PFKh isolate from KappikunnuPFPh isolatefrom KappikunnuPFAh isolate from AppadStrPh isolate from PulpalliStrMh isolate from MayilumpadiStrKh isolate from KappikunnuTrAh isolate from AppadTrKUh isolate from KurumankottaTrPh isolate from Pulpalli

Plate 18. Effect of T_4 treatment (PSBPh isolate) on plant height (cm) of ginger

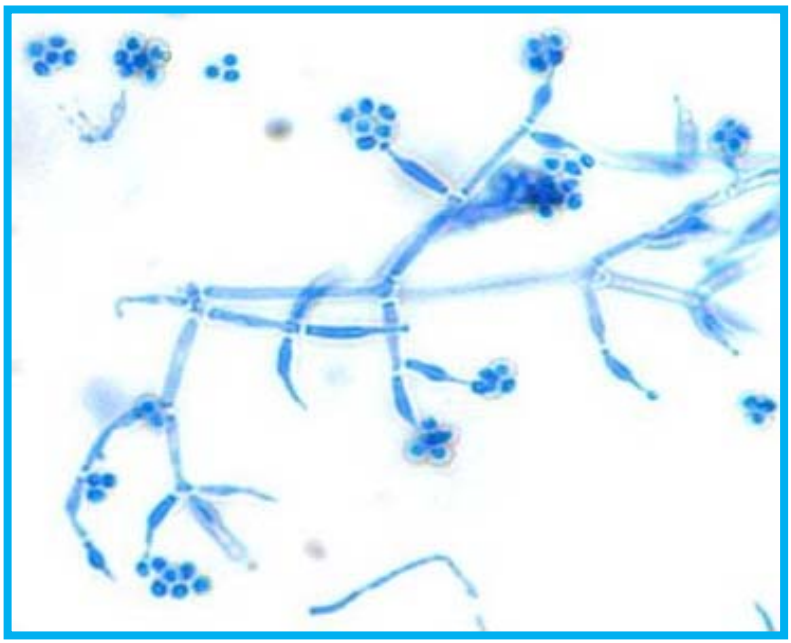


Plate 17.*Trichoderma harzianum*- (NCFT id: 6034.14) (TrKUhisolate)

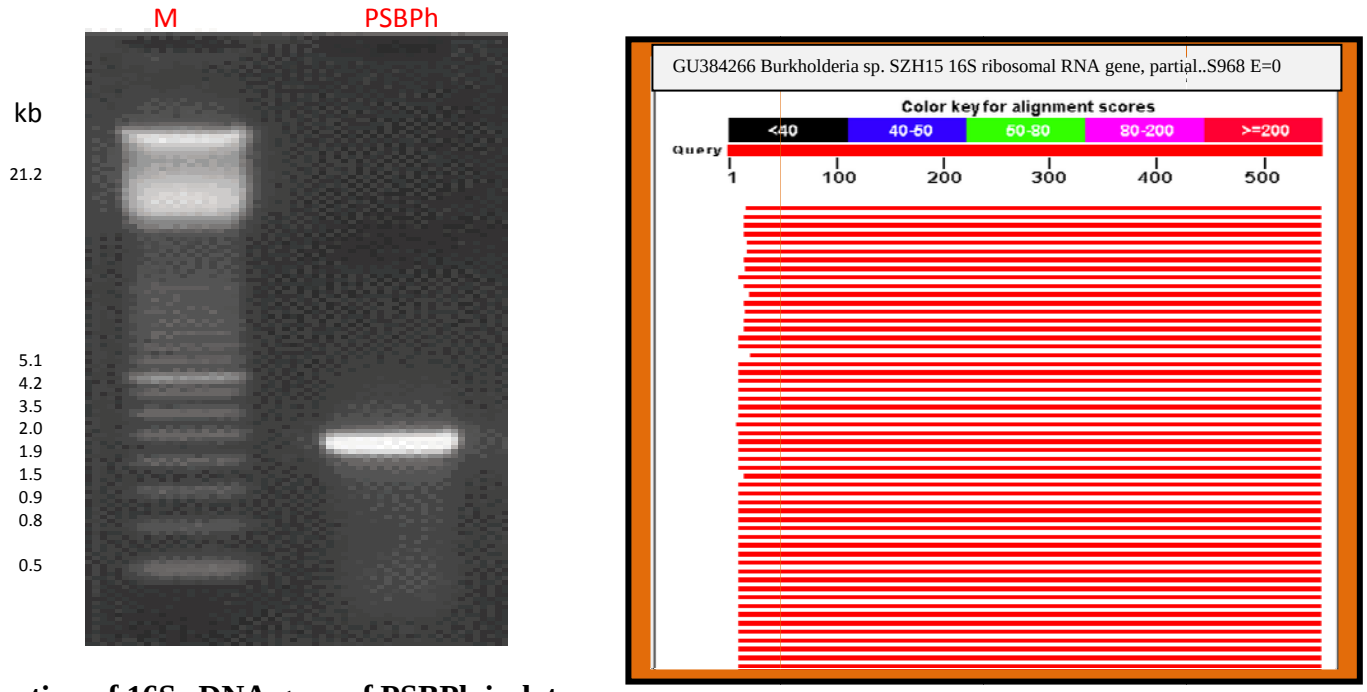


Plate 19. Amplification of 16S rDNA gene of PSBPh isolate

Description	Max score	Total score	Query cover	E value	Ident	Accession
Burkholderia sp. AB101 16S ribosomal RNA gene, partial sequence	970	970	97%	0.0	99%	AF219126
Burkholderia sp. AB2 16S ribosomal RNA gene, partial sequence	968	968	96%	0.0	99%	AF219125
Burkholderia sp. CEB 01056 16S ribosomal RNA gene, partial sequence	968	968	98%	0.0	99%	AJ491304
Burkholderia M35 b 16S ribosomal RNA gene, partial sequence	968	968	97%	0.0	99%	U96937
Burkholderia pyrocinia 16S ribosomal RNA gene, partial sequence	966	966	96%	0.0	99%	AB021396
Burkholderia cepacia 16S ribosomal RNA gene, partial sequence	966	966	97%	0.0	99%	U96927
Burkholderia vietnamiensis 16S ribosomal RNA gene, partial sequence	966	966	96%	0.0	99%	U96929
Burkholderia multivoram 16S ribosomal RNA gene, partial sequence	966	966	97%	0.0	99%	AB092606
Burkholderia gladioli BJAB0715, complete genome	965	5790	98%	0.0	99%	AB024491
Burkholderia plantarri 16S ribosomal RNA gene, partial sequence	965	965	98%	0.0	99%	U96933
Burkholderia glumae 16S ribosomal RNA gene, partial sequence	965	965	98%	0.0	99%	U96931

Plate 20. Sequence analysis of PSBPh isolate

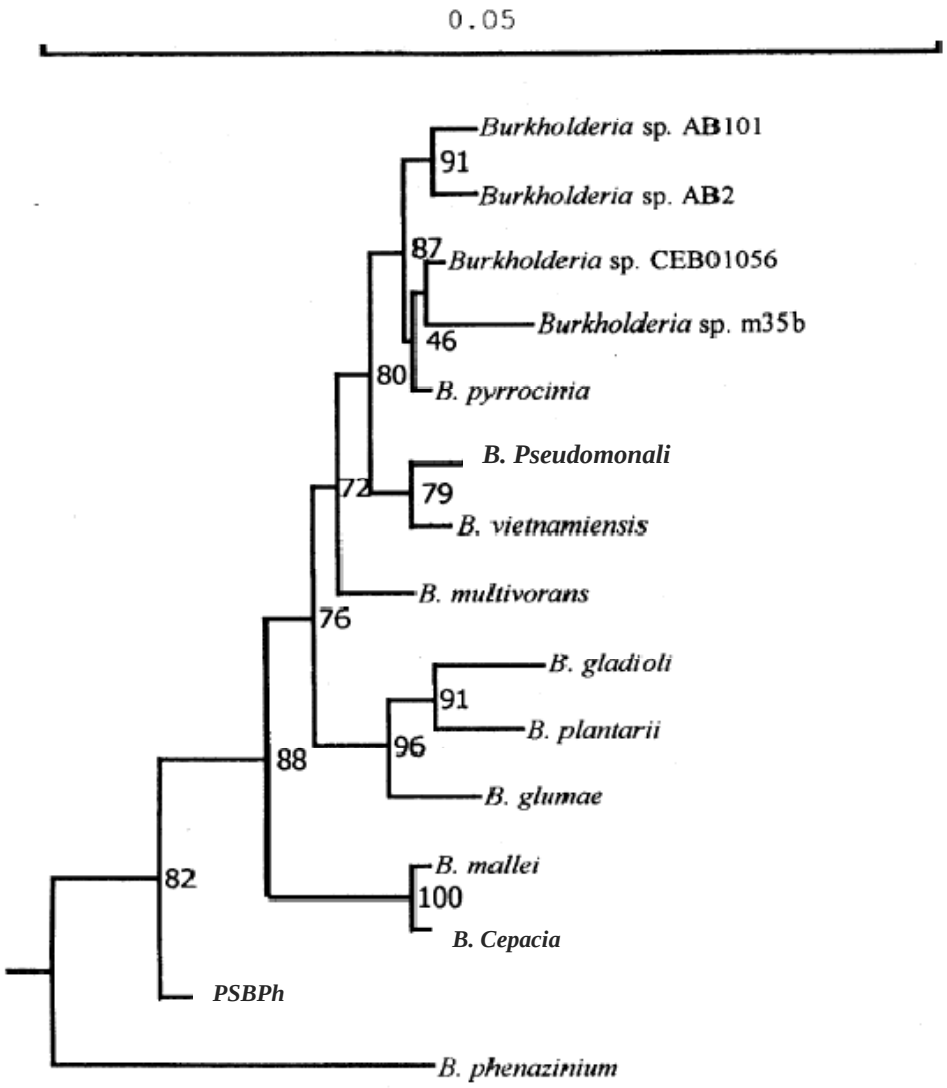


Plate 21. Phylogenetic tree of PSBPh isolate

DISCUSSION

5. DISCUSSION

Ginger is an important commercial cash crop of Kerala. The major ginger growing areas of Kerala are Wayanad, Palakkad, Ernakulam, Kottayam and Idukki districts (Selvan *et al*., 2002). At present, ginger is being exported to other countries which fetches very attractive price. Since, it is directly consumed, organic ginger has more demand in the market.

One of the major constraints in ginger cultivation is the occurrence of different diseases at various stages of plant growthsuch as *Pythium aphanidermatum*, *Rhizoctonia solani* and *Ralstonia solanacearum* (Nada *et al*., 1996). However, a new disease called "Fusarium yellows"was reported in the Wayanad district of Kerala, which sevearly affected the growth and yield of ginger (Vijayaraghavan and Mathew, 2011). As the ginger is directly consumed, it is not advisable to use agro-chemicals for the management of diseases. Moreover, ginger is a nutrient exhausting crop which results in nutrient deficiency in the soil (Pimentel and Greiner, 1997). Hence, ginger crop needs nutrient supplement particularly nitrogen and phosphorous and the diseases have to be managed effectively in a sustainable manner. One of the alternatives to chemicals is the use of bioinoculants which are not only cost effective, but also eco- friendly.

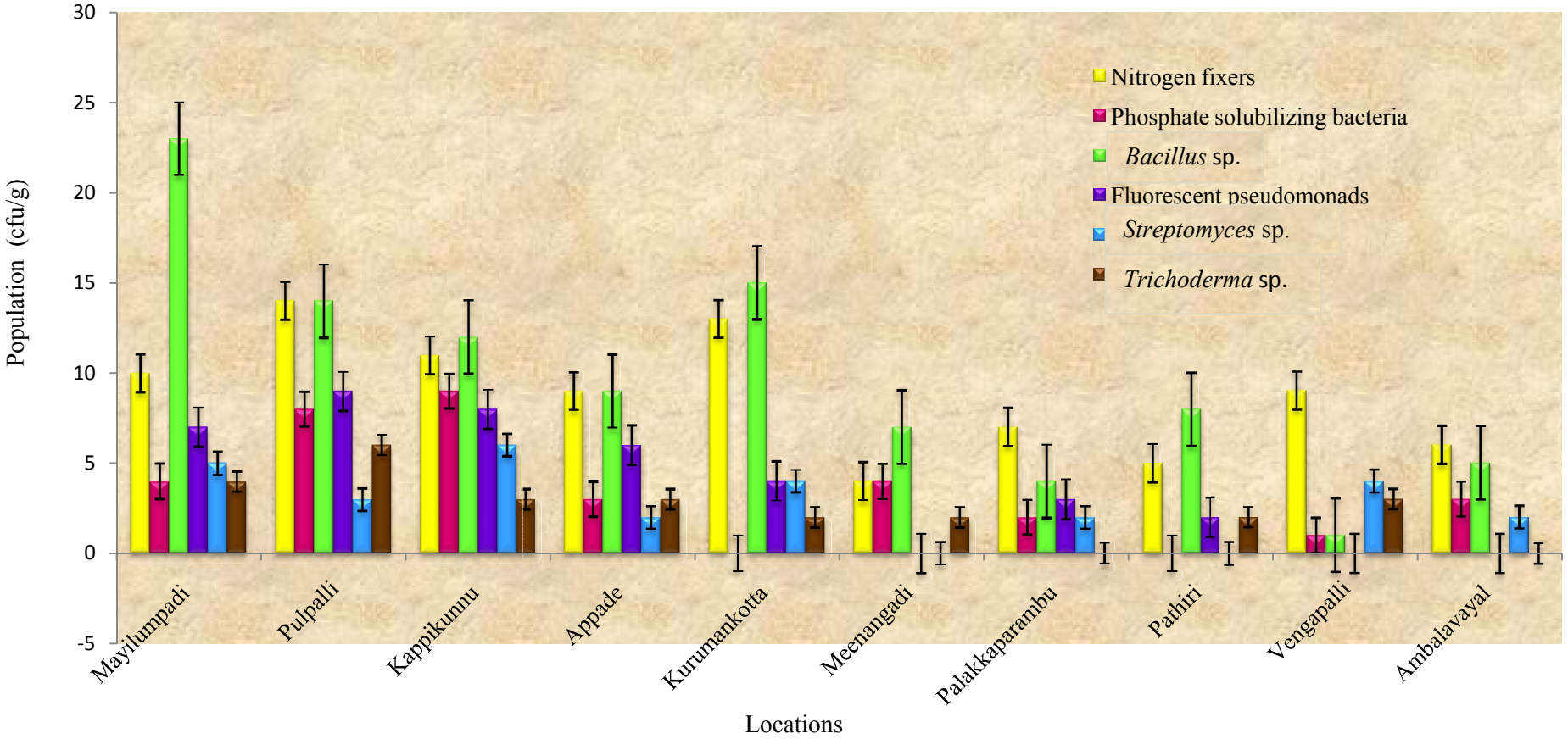
There are several studies to indicate that bioinoculants not only supplies nutrients but also control various plant pathogens. Application of Plant Growth Promoting Rhizobacteria (PGPR) in agriculture have been reported for biocontrol of the plant pathogens as well as biofertilization (Fravel, 2006). As these are eco-friendly and cost effective, microbial inoculants can be used for organic cultivation of ginger. Therefore, a study was undertaken to identify a suitable native isolate for the growth promotion and management of Fusarium yellows disease in ginger.

In order to determine the native isolates for growth promotion and management of disease in ginger,ten predominant isolates of nitrogen fixers,

phosphate solubilizing bacteria, *Bacillus* sp., fluorescent pseudomonads, *Streptomyces* sp. and *Trichoderma* sp. were isolated and enumerated from healthy and diseased plots of ginger in Wayanad district. Each of these predominant isolates were enumerated in order to determine the initial status of native population. Among all the isolates, *Bacillus* sp. recorded highest ($2.3x10^4$ cfu/g) population followed by nitrogen fixers ($1.4x10^4$ cfu/g) (Fig. 2). The lowest population was recorded in the case of phosphate solubilizing bacteria ($9x10^3$cfu/g) and *Trichoderma* sp. ($6x10^3$cfu/g). In general, the healthy ginger recorded higher population of beneficial microflora than the diseased plot (Fig. 2). However in similar studies, it has been reported that, soil microorganisms are influenced by high temperatures, dryness or heavy rainfalls in tropical countries (Da Mota *et al*., 2008), and contaminated environments (Dell'Amico *et al*., 2008), harsh environments including drought stress (Arzanesh *et al*., 2011). It indicates thatpopulation of soil microflora varies with different location.The present studies indicated lower population of beneficial microflora viz; phosphate solubilizing bacteria, fluorescent pseudomonads, *Streptomyces* sp. and *Trichoderma* sp. which might have been influenced by soil adaphic factors as well as the host plant.The acidic pH is known to affect the microbial population and its ability to function but some bacterial isolates are known to tolerate acidic pH. Wardle *et al.*(1967) reported that the *Azotobacter* failed to fix nitrogen when the pHwas below 5.5 and above 9.0. This indicates that the soil pH is the single most important factor affecting the quality and quantity of microflora. Similarly, Pal (1999) reported that PSB isolate (PAS-2) obtained from pasture and wasteland with pH 4.8 had highest P- solubilizing capacity and tolerated a very wide range of soil acidity. The results of the present study indicated that the population of soil microflora might have been influenced by the soil pH and nutrient status.

The predominant isolates obtained from different locations were characterized using morphological and biochemical characters as described in Bergey's manual of Systematic Bacteriology (Kreig and Dobereiner, 1984) and Laboratory Manual of Basic Microbiology (Kanwar, 1997). The nitrogen fixing

Fig. 2. Population of predominant isolates from ten different locations of Wayanad district



bacteria were found to be gram negative, motile, positive for nitrogen fixation, cyst formation, citrate activity, glucose utilization, oxidase and catalase activity. However, starch hydrolysis reaction was negative. The isolate were tentatively identified as *Azotobacter* sp. In a similar study by Ahmad *et al*. (2008) reported that, *Azotobacter* sp. isolated from the rhizospheric soil of different crops were characterized by their cultural conditions, morphological and biochemical characteristics using standard methods. Cappuccino and Sherman, (1992) who reported that the isolates were raised transparent milky and watery colonies, gram negative rods, growth in nitrogen free medium, citrate and catalase tests positive. These results are in agreement with the present studies where the isolates were tentatively identified as *Azotobacter*.

All the isolates, which solubilized phosphorus in the selective media were characterized further to confirm the identity (Kreig and Dobereiner, 1984).The isolates were gram negative, rod, motile, and positive for catalase, Voges-Proskauer and citrate utilization whereas oxidase, indole production, methylred and urease test showed negative reaction. The isolates were tentatively identified as *Burkholderia* sp. whichis in agreement with the characters described in a similar study by Islam *et al*. (2007). The colonies which exhibited the charecteristics of *Bacillus* sp. were gram positive rod, motile and positive for endospore, catalase, Voges-Proskauer, starch-hydrolysis and citrate utilization test. However, indole production and urease test were negative. These results are in agreement with the keys described for the identification of *Bacillus* sp. (Pavan Kumar and Sruthy Agarwal, 2013).

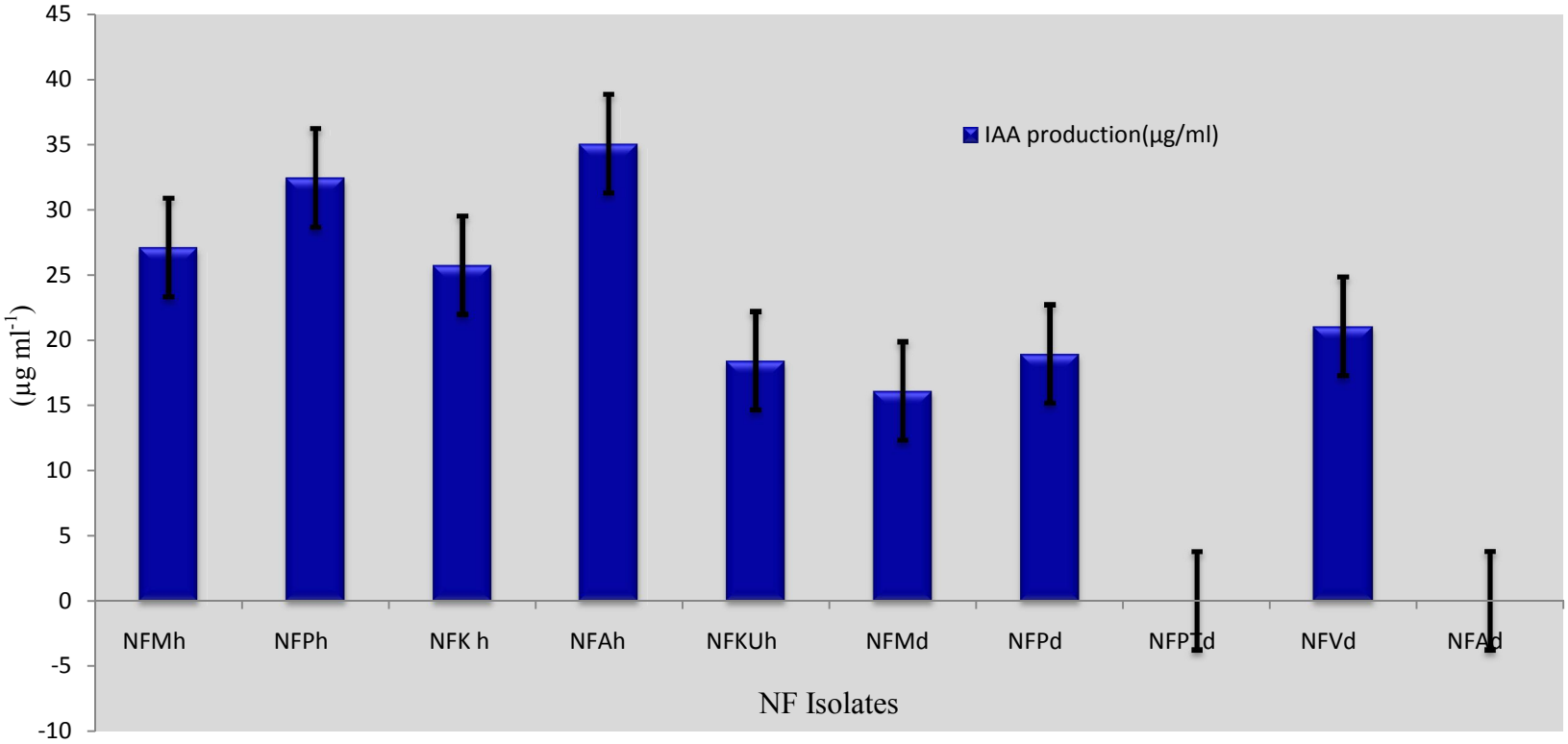
The fluorescent pseudomonds were gram negative rod and positive for all test conducted except for starch hydrolysis which was negative. Ahmad *et al*. (2008) reported that a total of 9 bacterial isolates were isolated from the rhizosphere soils of different agronomic crops. The isolates were gram negative rods, produced fluorescent green pigment, and showed positive to catalase and citrate test.The colonies exhibiting the characters of *Streptomyces* sp. were gram

positive, filamentous, non-motile, grey-white coloured and positive for glucose utilization where as the isolates showed variation in the utilization of sucrose. These characters mentioned were in agreement with the keys described for the identification and the of *Streptomyces* sp. (Locci, 1989).The fungal isolates exhibiting the characters of *Trichoderma* sp. were subjected to morphological and cultural characterization on potato dextrose agar media in order to confirm its identity. The isolates were found to be light-dark green coloured colonies, septate, hyaline hyphae, branched hyaline conidiophores, conidia green colour and no pigmentation were recorded. These characters of different predominant isolates were compared with the standard keys (Chet, 1987) and tentatively identified as *Trichoderma* sp.

These characters of different predominant isolates were compared with the standard keys, described in Bergey's Manual of Determinative Bacteriology. Based on the standard keys, the predominant isolates were tentatively identified as *Azotobacter* sp., *Burkholderia* sp., *Bacillus* sp., fluorescent pseudomonds and *Streptomyces* sp.

The identified isolates were subjected to screening for functional efficiency under *in vitro* condition. Among the nitrogen fixers, the nitrogen fixation ranged from 0.11 to 0.43 mg/g of sucrose utilized. The highest nitrogen was fixed by the NFMh isolate (Mayilumpadi) where as the least amount was in the case of NFMd isolate (Meenanagadi).In a similar study, the results indicated that the nitrogen fixation varied depending up on the location from which the isolate was obtained. The variation in the nitrogen fixation might be due to the variation in the efficiency of bacterial isolates. The nitrogen fixing ability of different isolates might be associated with soil type, environmental factors, nitrogen status of soil, crop and its varieties (Lakshmikumari *et al*., 1976, Rai, 1991). The IAA production ranged from 0 to 35.02 µg/ml (Fig. 3). Highest (35.02 µg/ml) IAA production was recorded in the case of NFAh isolate (Appad) where as isolates NFPTd (Pathiri) and NFAd (Ambalavayal) did not produce IAA. Indole-3-Acetic Acid is very important phytohormone for the plant growth

Fig. 3. Indole Acetic Acid production by predominant isolates of nitrogen fixers (NF)

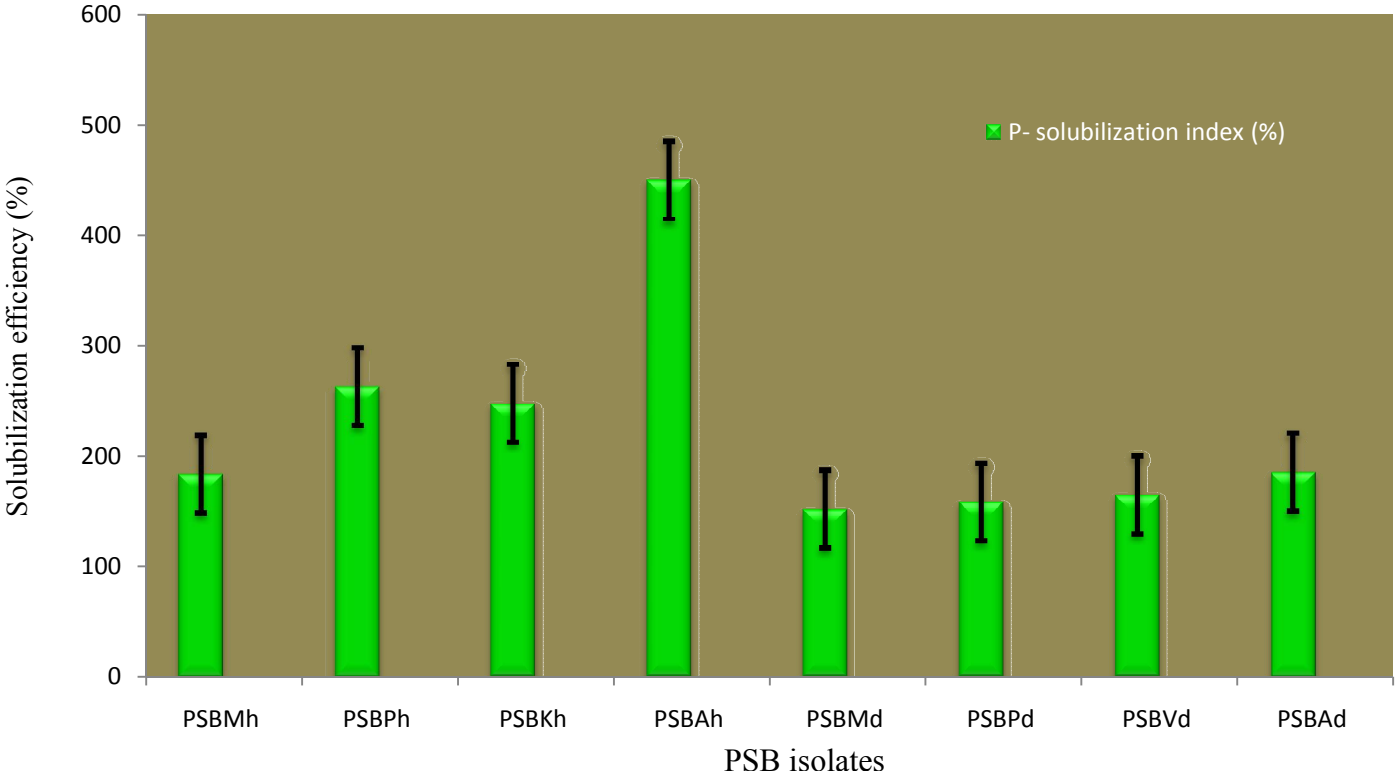


NFMh isolate from Mayilumpadi NFPh isolate from Pulpalli NFKh isolate from Kappikunnu NFAh isolate from Appade NFKUh isolate from Kurumankotta NFMd isolate from Meenangadi NFPd isolate from Palakkaparambu NFPTd isolate from Pathiri NFVd isolate from Vengapalli NFAd isolate from Ambalavayal

promotion and has a direct beneficial effect in enhancing the root, cell division and elongation. Narula and Tauro (1986) reported the beneficial effects of Indole-3-Acetic Acid and auxins, gibberlins and cytokinins for growth promotion by *Azotobacter* sp. However, the HCN production was moderate to excellent. In the present study, the bacterial isolates showed variation in HCN production, with highest HCN production in the case of NFAh (Appade). These results are in agreement with Ravikumar *et al*. (2010) who reported that *Azotobacter* sp. produced HCN. Among all the isolates ofnitrogen fixers, siderophore production was excellent in the case of NFAh (Appade) isolate. Siderophores are known to act as growth factor as well as suppressive agents for plant pathogens (Knosop *et al*., 2010). In the present study, NFMh (Mayilumpadi) and NFAh (Appade) were found as the best isolates.

The predominant isolates of phosphate solubilizing bacteria were screened for P-solubilization and itranged from 151.84% to 450.0% (Fig. 4). Highest P-solubilisation (450.0%) was in PSBAh (Appade) isolate. Kumari *et al*., (2009) reported that PSB solubilized the unavailable P in to available form which can be easily absorbed by the plants. The conversion of insoluble P in to available form is due to the production of organic acids and phosphatase enzymes which resulted in increased plant growth and yield. Similarly, the PSB strains exhibited inorganic P-solubilization ranging between 25-42 µg ml^{-1} and organic P mineralization between 8-18 µg ml^{-1} (Tao *et al*., 2008). The IAA production ranged from 0 to33.07 µg/ml in the present studies. The highest (33.07 µg/ml) IAA production was by PSBMh (Mayilumpadi) isolate. In a similar study, a PSB (*Bacillus* sp.) showed indole acetic acid production, significant mineral phosphate solubilization and moderate phosphatase activity which improved the yield of tomato, onion, potato, banana and coffee. (Zaidi *et al*., 2009). HCN production was low to excellent with highest in the case of PSBMh isolate (Mayilumpadi). In a similar study, out of 8 isolates from back pepper, only 6 isolates produced HCN. The bacterial strain (IISR-310) produced maximum amount of HCN.Out of 8 isolatesin the present studies, PSBPh and PSBAh isolatesproduced siderophore at

Fig. 4. P-solubilization by predominant isolates of PSB



PSBMh isolate from Mayilumpadi PSBPh isolate from Pulpalli PSBKh isolate from Kappikunnu PSBAh isolate from Appade

PSBMd isolate from Meenangadi PSBPd isolate from Palakkaparambu PSBVd isolate from Vengapalli PSBAd isolate Ambalavayal

moderate level. These results are in agreement with Ahmad *etal*. (2008) who reported that, important trait of PGPR indirectly influence the plant growthdue to siderophore production. The fluorescent pseudomonad (Ps5) showed multiple PGP activities including siderophore productionin moderate level. In the present study, PSBMh, PSBAh and PSBPh isolates were the mostpromising isolates.

Among the isolates of *Bacillus* sp., the maximum P- solubilization index (182.99%) was recorded by BsAh isolate and the least (137.43%) by BsKUh isolate. Highest IAA (14.54µg/ml) was produced by BsMh isolate (Mayilumpadi) and the least (12.92 µg/ml) by BsAh isolate (Appade). In a similar study, Tien *et al.* (2000) reported that *Bacillus megaterium* from tea rhizosphere was able to produce IAA and thus it helped in the plant growth promotion. The tryptophan increased the production of IAA in *Bacillus amyloliquefaciens* (FZB42). Out of the ten isolates tested for HCN production in the present studies, only three BsMh, BsKuh and BsPTd isolate resulted moderate level of HCN production. Only one isolates, BsMh was positive for siderophore production. In a similar study, HCN production was found to be a common trait of *Bacillus* (50%) in the rhizospheric soils of different agronomic crops (Ahmad *et al.,* 2008).

Among the isolates of fluorescent pseudomonads, the highest (241.57%) p- solubilization was in PfMh isolate and lowest (190.11%) by the isolate PfKh. In a similar study, *Pseudomonas striata* and *Bacillus polymyxa* solubilized 156 and 116 mg l-1 respectively (Rodríguez and Fraga, 1999). Similarly, direct application of rock phosphate is often ineffective with in short period for most of the annual crops (Goenadi *et al*., 2000). In the present studies, highest IAA production was (16.75 µg/ml) by the isolate PfKh from Kappikunnu and the lowest (12.13 µg/ml) by PfPh isolate. Karnwal *et al*. (2009) tested fluorescent pseudomonads isolates for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan and found that for both strains, indole production increased with increasing in tryptophan concentration. Isolates producing IAA had stimulatory effect on the plant growth. Hydrogen cyanide production in the

present studies were tested and PfPh and PfKUh produced highest HCN under *in vitro* screening. In a similar study, HCN production was found to be a common trait of *Pseudomonas* (88.89%) in the rhizospheric soils of different agronomic crops (Ahmad *et al.,* 2008). Siderophore production was produced only by three isolates PfKh, PfMh, and PfPh. In a similar study, *Pseudomonas* strains had the ability to produce siderophores which contributed to the root-colonization, antagonistic properties, and in biocontrol (Leong, 1986).

Among the eight isolates of *Streptomyces* sp., the maximum P-solubilization index (176.99%) was recorded by StrAh isolate and the least (153.76%) by StrPh isolate. Highest IAA (11.63µg/ml) was produced by StrMh isolate (Mayilumpadi) and the least (10.41µg/ml) by StrKUh isolate (Kurumankotta). StrMh and StrPh isolates resulted moderate level of HCN production whereas StrPh (Pulpalli) isolate showed maximum siderophore production. In a similar study by Srividhya *et al*. (2012), *Streptomyces violaceusniger* strain (YCED9) was positive for IAA, siderophore and HCN production under *in vitro* condition.

Only two isolates of TrPh and TrKUh produced moderate HCN under *in vitro* screening. Siderophore production was produced by TrKh, TrMh, and TrAh. In a similar study by Meenu *et al*. (2010), *Trichoderma* was found to be a major biocontrol agent which reduced soilborne diseases of various crops.

The predominant isolates of antagonists obtained in the present study viz; *Bacillus* sp., fluorescent pseudomonads*, Streptomyces* sp. and *Trichoderma* sp. were evaluated against four major pathogens (*Fusarium oxysporum, Pythium aphanidermatum, Rhizoctonia solani and Ralstonia solanacearum*) of ginger under *in vitro* condition. All the ten isolates of *Bacillus* sp. were screened for their antagonistic activity against selected pathogens (Table 19). Only BsAh isolate recorded antagonistic activity against *Fusarium oxysporum* (64.08 per cent inhibition). In a similar study, Sharma and Jain (1979) reported growth promotion

and reduced incidence of *Fusarium oxysporum* in ginger following the application of *B. subtilis* (strain-1). *Bacillus* sp. have been reported to improve resistance against diseases caused by *Fusarium oxysporum*, which causes severe losses in crop production worldwide.While biocontrol activity of *Bacillus* strains against *Fusarium oxysporum* havebeen demonstrated, only one account of the use of plant growth promoting bacteria against *Fusarium oxysporum* f. sp. *zingiberi* has been reported. Sharma and Joshi (1979) reported antagonism and reduced incidence of *Fusarium oxysporum* f. sp. *zingiberi* in ginger with the application of *B. subtilis*.

Seven isolates of fluorescent pseudomonads were tested for their antagonistic activity against selected pathogens. The isolates PfKh (59.43 per cent inhibition) showed maximum inhibition against *Fusarium oxysporum. Pseudomonas* characters make them suitable for biocontrol and growth promotion activities (Weller, 1988). Most important group of rhizobacteria for biological control is the *Pseudomonas* (Kremer and Kennedy, 1996). The colonisation of hyphae of *Fusarium oxysporum* f. sp. *lycopersici* by certain fluorescent pseudomonads produced antifungal metabolites and lytic enzymes, which contributed to the biocontrol traits of these bacteria (Bolwerk *et al.,* 2003). It was also shown that fusaric acid produced by *Fusarium oxysporum* f. sp. *lycopersici* served as a chemo attractant stimulating motility of *P. fluorescens* (WCS635) and colonisation of the hyphae of the fungus (de Weert *et al.,* 2004). Meena and Mathur (2003) tested the ability of a fluorescent pseudomonads spp. to reduce rhizome rot of ginger caused by *Fusarium oxysporum*. Sivasakthi *et al*. (2014) reported fluorescent pseudomonadwith biocontrol activity against different *Fusarium oxysporum* diseases.

Eight isolates of *Streptomyces* sp. were tested for their antagonistic activity against selected pathogens (Table 21). Isolate StrPh (67.78per cent inhibition) highest inhibition against *Fusarium oxysporum.* In a similar study, *Streptomyces* isolate inhibited the growth of *Fusarium* rot and showed plant growth promotion under greenhouse experiment in Brazil (de Vasconcellos and

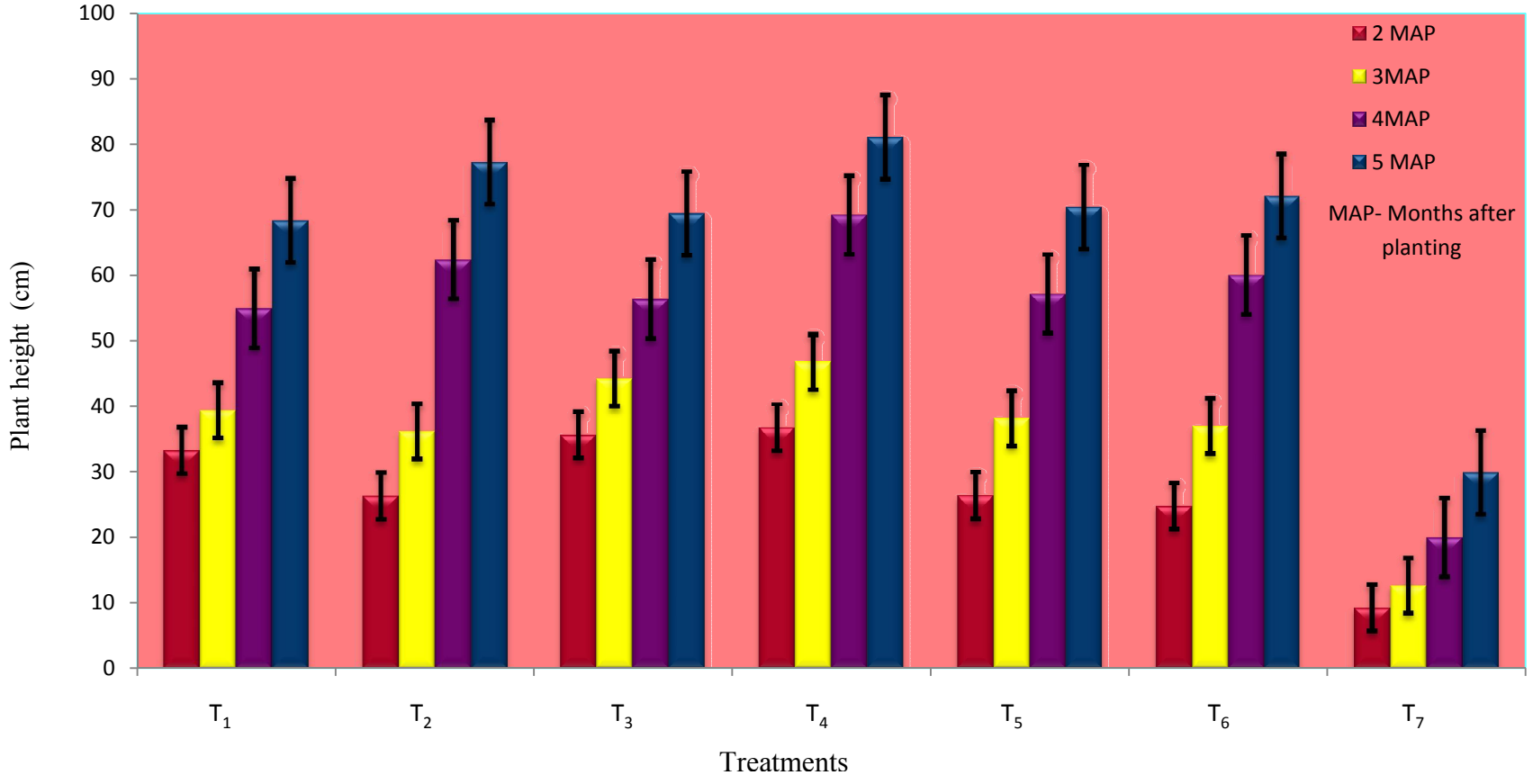
Cardoso, 2009). Kekuda *et al.* (2013) studied six biocontrol potential of *Streptomyces* sp. (SSC-MB-01 to SSC-MB-06) against *Fusarium oxysporum* f. sp. *zingiberi.* The inhibitory activity was observed in the case of *Streptomyces* sp. (SSC-MB-02) isolates for the protection of ginger rhizomes from soft rot.

Similarly, all the ten isolates of *Trichoderma*sp. were tested for their antagonistic activity against the selected pathogens. Isolate TrAh (Appad) recorded highest inhibition percentage (78.93 per cent) against *Fusariumoxysporum*and *Pythium aphanidermatum* (73.91 percent). These results are in agreement with several studies (Elad and Baker, 2000; El-Katatny *et al*., 2001; Howell *et al*., 2002), *Trichoderma* sp. are well known biocontrol agents for the management of plant diseases particularly in the spice crop. Gupta *et al*. (2010) reported *Trichoderma* spp. as the most promising biocontrol fungi against many fungal disease and growth parameters of gingeron rhizome pathogens. Antagonism of *Trichoderma* sp. against several pathogens have been reported. In the present studies, the TrAh isolate of *Trichoderma* sp. (78.93per cent) from Appad was the most promising antagonist against *Fusarium oxysporum* under *in vitro* condition. Rajan *et al*. (2002) reported that ginger *(Zingiber officinale* Rosc.) diseases are the major production constraint which isassociated with *Pythium* spp., *Ralstonia solanacearum,* and *Fusarium oxysporum.*One of the aim in biocontrol of plant diseases is to develop an antagonist which is effective against wide range of plant pathogens. In the present studies, TrKUh isolate (Kurumankotta) was the most effective against three major pathogens of ginger namely *Pythium aphanidermatum, Fusarium oxysporum* and *Rhizoctonia solani* under *in vitro* condition. Weindling (1932) demonstrated that, *T. viride* was antagonistic to *Rhizoctonia solani* and biocontrol efficacy of *in vitro* selected antagonistic *Trichoderma* spp. against rhizome rot ofginger were also reported.Many researchers have already reported that *Trichoderma* is a very effective biocontrol agent for the management of soil borne fungal pathoges. The present studies indicated that, TrAh (Appade), TrKUh (Kurumankotta) and TrPh (Pulpalli) isolates was effective against *Fusarium oxysporum.*

The efficiency of the beneficial microflora in soil varies under field conditions. Based on the *in vitro* evaluation, three most promising isolates of nitrogen fixing bacteria (NFMh, NFPh and NFKh) and Phosphate solubilizing bacteria (PSBAh, PSBKh and PSBPh) were further screened for plant growth promotion under pot culture experiment. The T_4 (PSBPh from Pulpalli) recorded cent per cent sprouting, highest plant height (80.08 cm) and highest yield of (128.74 g/plant). Whereas, the highest number of tillers (6.85) were observed in T_3 (NFKh from Kappikunnu). Hence, T_4 (PSBPh from Pulpalli) was found to be the most promising isolate for plant growth promotion in ginger, based on the sprouting percentage, plant height and rhizome yield (Fig. 5).The isolate PSBPh (from Kappikunnu) was identified as *Burkholderia cepacia* by 16srDNA sequencing. In a similar study, a strain of *Burkholderia cepacia* (PSB) displayed significant mineral phosphate solubilization and moderate phosphatase activity which improved the yield of tomato, onion, potato, banana and coffee. Higher crop yields were reported by solubilization of fixed soil phosphorus and applied phosphates by PSB (Zaidi *et al*., 2009). Pandey *et al*. (2005) reported that a bacterial strain (MSSP) solubilized phosphate, produced IAA, siderophore, HCN and was antagonistic against different phytopathogens. The 16S rDNA demonstrated that MSSP belonged to the genus *Burkholderia*. Similarly, phosphate-solubilizing bacteria have been reported forplant growth promotion and enhancementof yield (Rodrigez and Fraga, 1999; Khan *et al*., 2009). The present studies indicated that *Burkholderia cepacia* (PSBPh isolate) was the most promising isolate for plant growth promotion in ginger

One of the major constaints in ginger production is the complex of rhizome rot disease. The important rhizome rot disease is Fusarium yellows (dry rot) caused by *Fusarium oxysporum* f. sp. *zingiberi.* Hence, the most promising isolates of *Bacillus* sp. fluorescent pseudomonads, *Streptomyces* sp. and *Trichoderma* sp. obtained under *in vitro* screening were evaluated for the management of Fusarium yellows in ginger under pot culture. Based on the higest number of tillers (6.44), plant height (69.98 cm), maximum rhizome yield (Fig. 6)

Fig. 5a. Effect of most promising nitrogen fixers and phosphate solubilizing bacteria on plant height (cm) of ginger



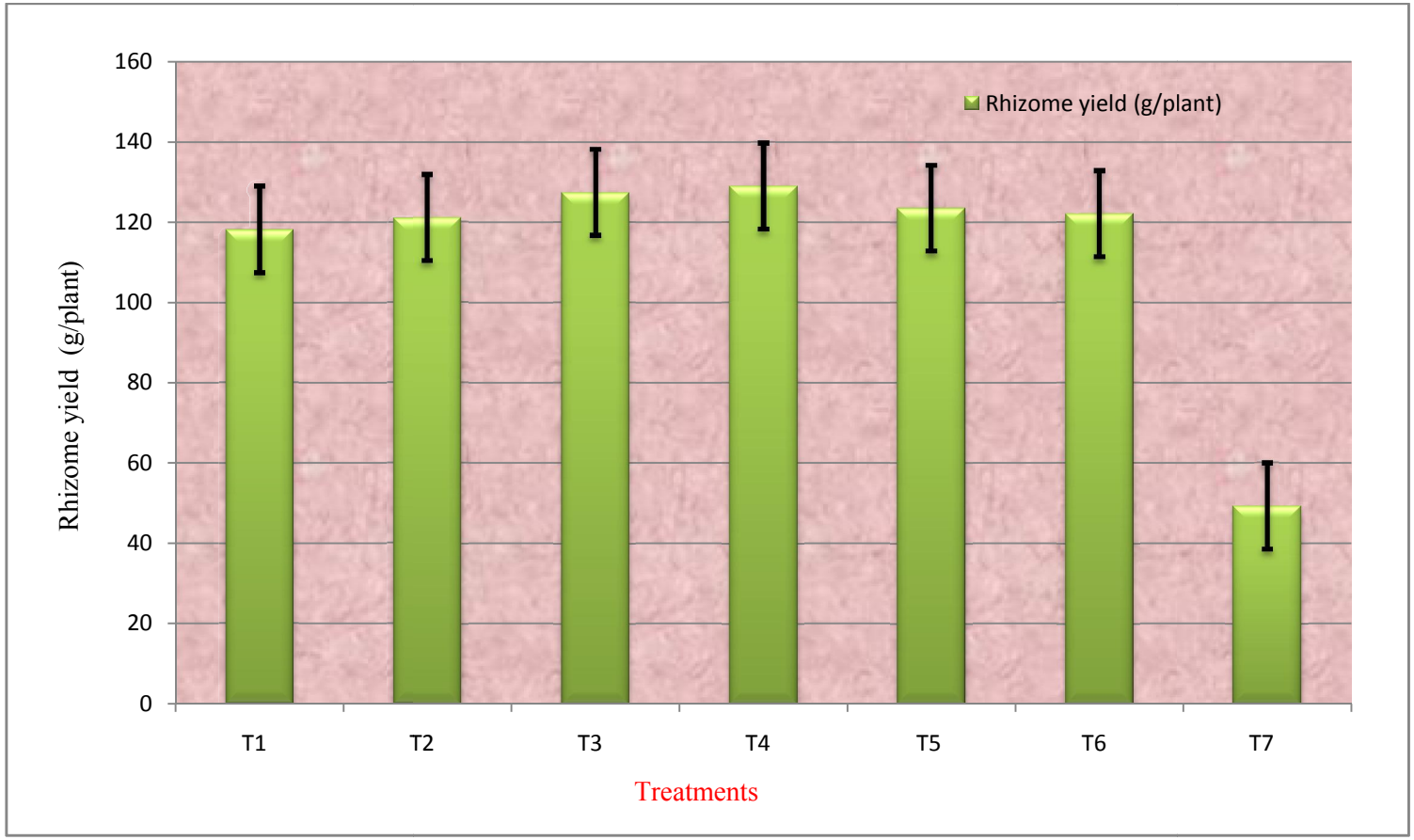
NF- Nitrogen fixers PSB- Phosphate solubilising bacteria h- healthy

T_1 : NFMh isolate from Mayilumpadi T_2 : NFPh isolate from Pulpalli

T_3 : NFKh isolate from Kappikunnu T_4 : PSBPh isolate from Pulpalli

T_5 : PSBKh isolate from Kappikunnu T_6 : PSBAh isolate from Appad T_7 : Control

Fig. 5b. Effect of most promising nitrogen fixers and phosphate solubilizing bacteria on rhizome yield



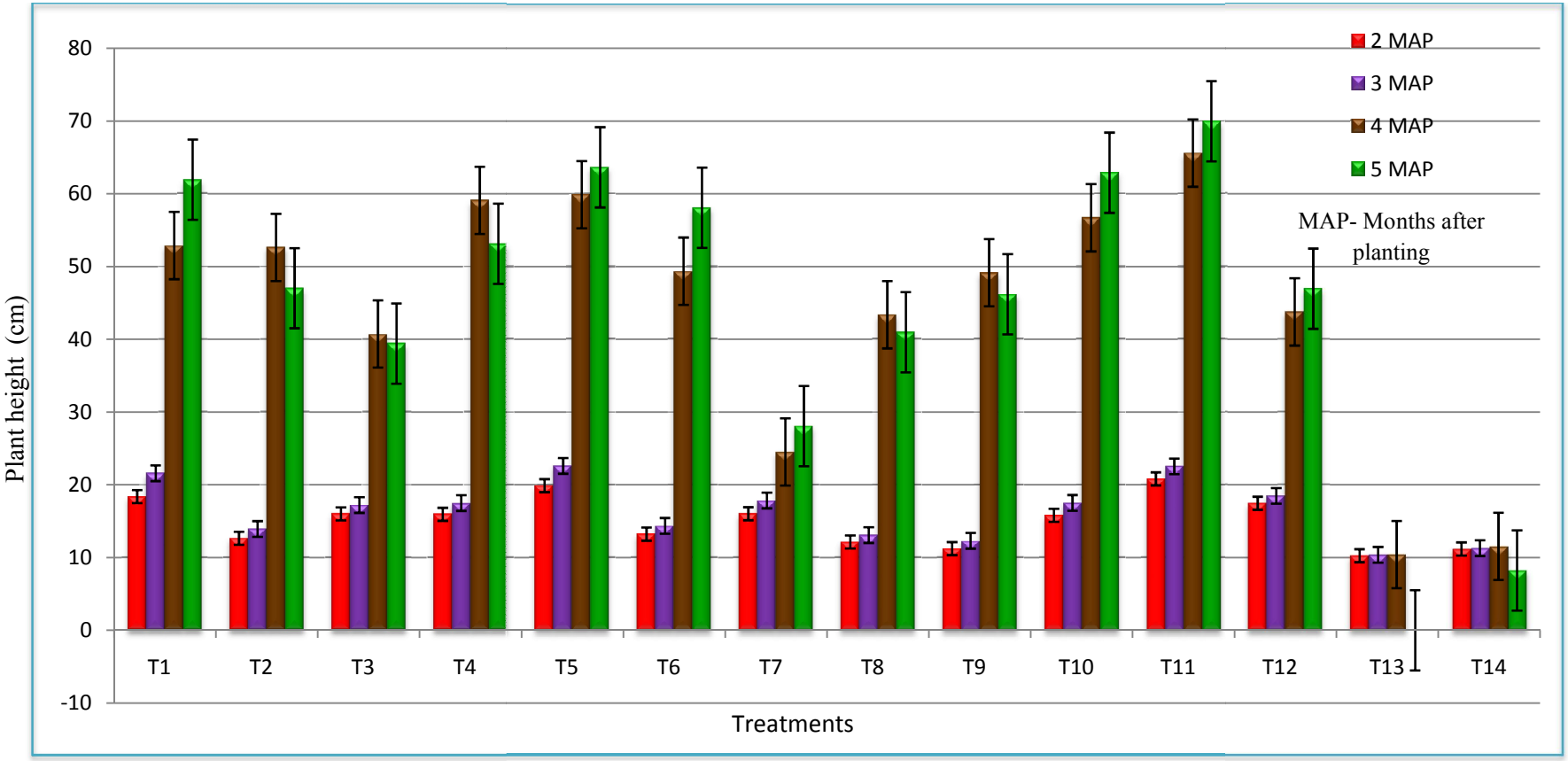
NF- Nitrogen fixers PSB- Phosphate solubilising bacteria h- healthy

T_1 : NFMh isolate from Mayilumpadi T_2 : NFPh isolate from Pulpalli

T_3 : NFKh isolate from Kappikunnu T_4 : PSBPh isolate from Pulpalli

T_5 : PSBKh isolate from Kappikunnu T_6 : PSBAh isolate from Appad T_7 : Control

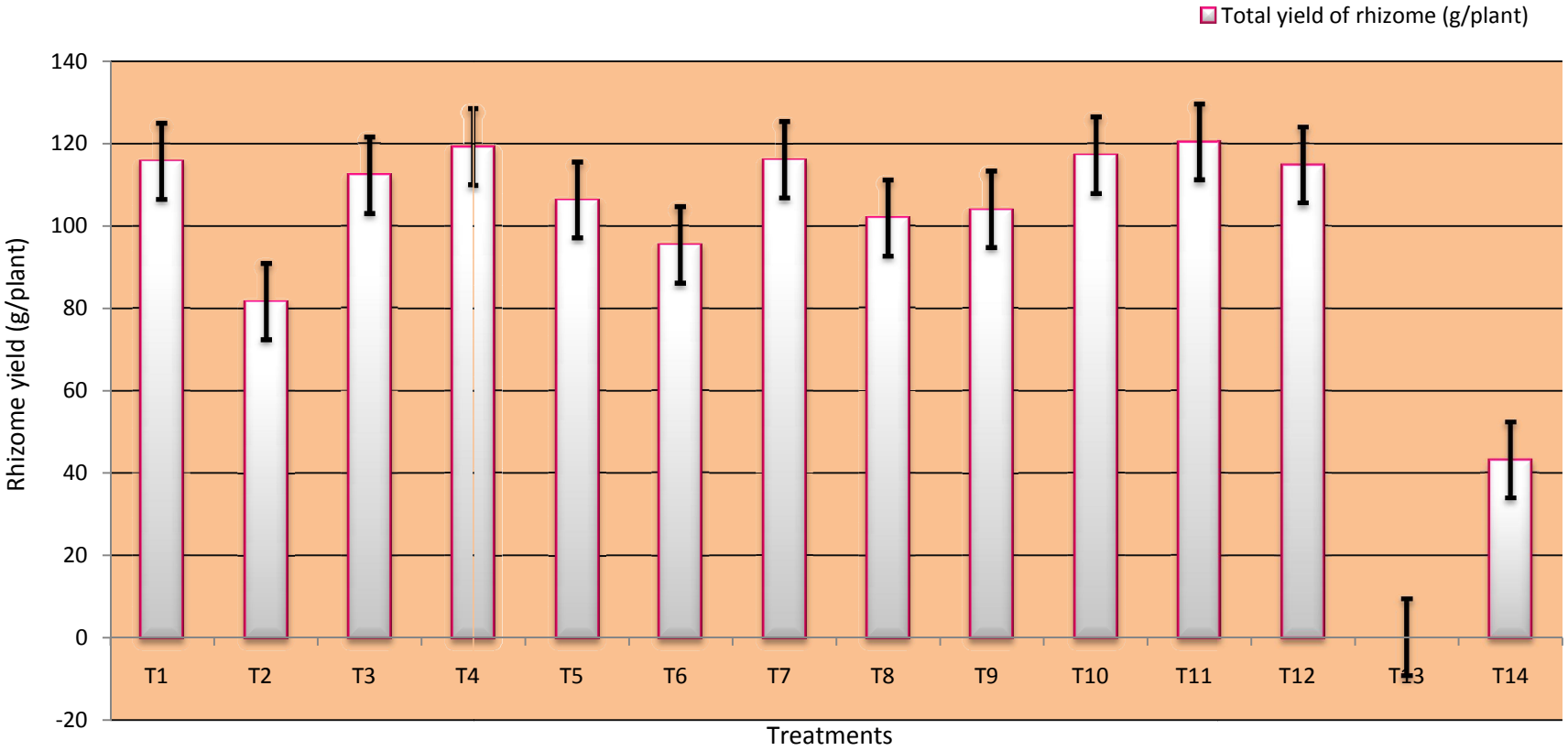
Fig. 6a. Effect of selected antagonists on plant height (cm) of ginger



Bs- *Bacillus* sp. Pf- fluorescent pseudomonads Str- *Streptomyces* sp. Tr- *Trichoderma* sp. h- healthy

T_1: BsMh isolate from Mayilumpadi T_2: BsAh isolate from Appad T_3: BsKUh isolate from Kurumankotta T_4: PFKh isolate from Kappikunnu
T_5: PFPh isolate from Kappikunnu T_6: PFAh isolate from Appad T_7: StrPh isolate from Pulpalli T_8: StrMh isolate from Mayilumpadi
T_9: StrKh isolate from Kappikunnu T_{10}: TrAh isolate from Appad T_{11}: TrKUh isolate from Kurumankotta T_{12}: TrPh isolate from Pulpalli
T_{13} Control (Pathogen alone T_{14} :Absolute control

Fig. 6b. Effect of selected antagonists on rhizome yield of ginger

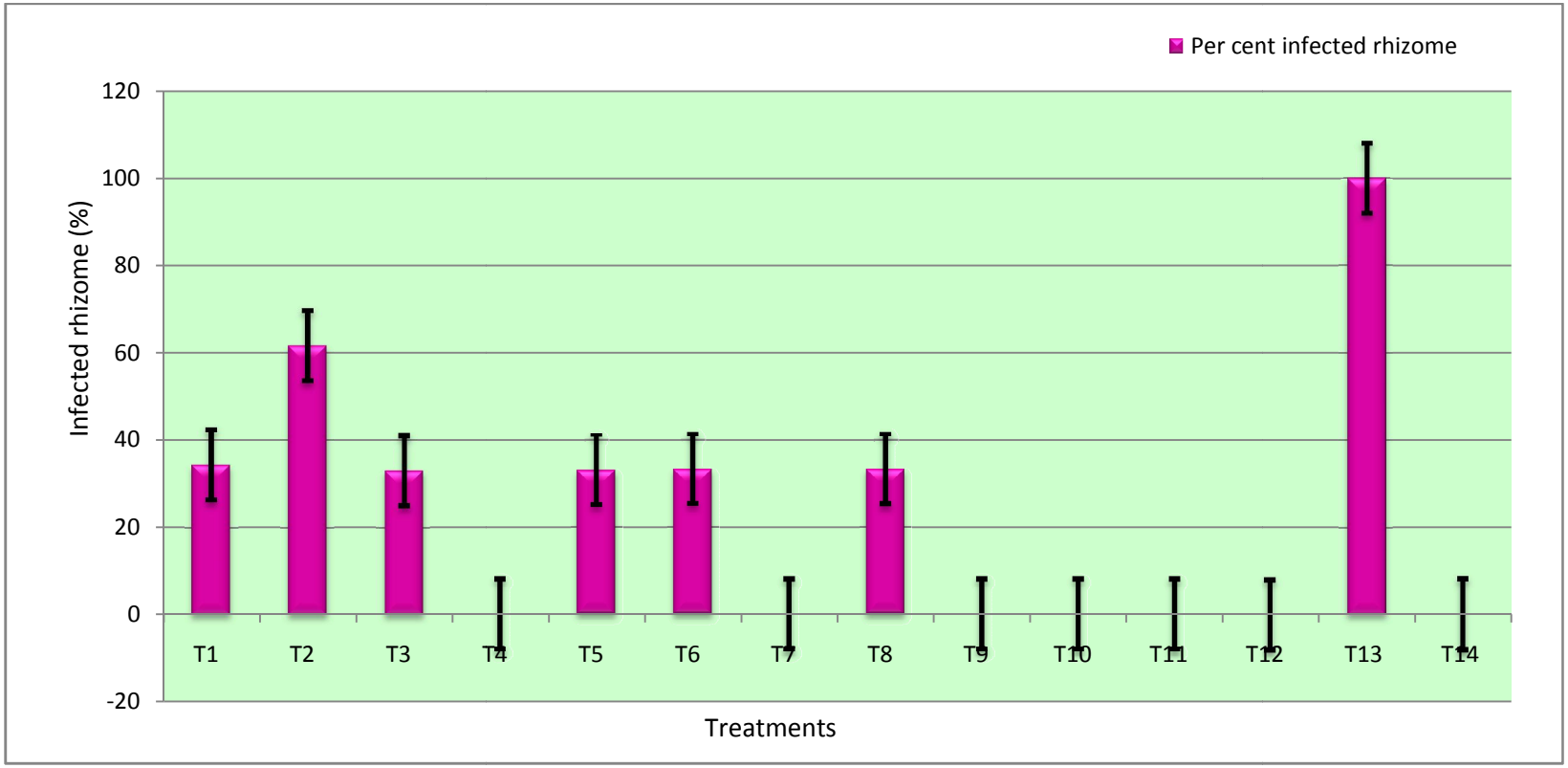


Bs- *Bacillus* sp. Pf- fluorescent pseudomonads Str- *Streptomyces* sp. Tr- *Trichoderma* sp. h- healthy

T_1: BsMh isolate from Mayilumpadi T_2: BsAh isolate from Appad T_3: BsKUh isolate from Kurumankotta T_4: PFKh isolate from Kappikunnu
T_5: PFPh isolate from Kappikunnu T_6: PFAh isolate from Appad T_7: StrPh isolate from Pulpalli T_8: StrMh isolate from Mayilumpadi
T_9: StrKh isolate from Kappikunnu T_{10}: TrAh isolate from Appad T_{11}: TrKUh isolate from Kurumankotta T_{12}: TrPh isolate from Pulpalli
T_{13} Control (Pathogen alone T_{14} :Absolute control

and Fusarium yellows disease incidence (Fig. 7), treatment TrKUh isolate (Kurumankotta) was the most effective isolate. In a similar study, Sharma and Joshi (1979) reported that, *T. harzianum* and *T. viride* were most effective in reducing mycelial growth of *Fusarium oxysporum* f. sp. *zingiberi* and *Pythium aphanidermatum*, which are causal agents of yellows and rhizome rot of ginger respectively. Similarly, Mathew *et al*. (2006) reported that *Trichoderma* sp. have been very extensively used as the biocontrol agent for the management of various soil borne fungal diseases. Increased rhizome yield was noticed due to suppression of gingerrhizome rot caused by *F. oxysporum* by using *T. harzianum, T. aureoviride* and a non-resident isolate of *T. viride*,whereas T_4 (PfKh from Kappikunnu) recorded cent per cent sprouting. In a similar study, Raaimakers (2002) demonstrated that pseudomonad strains with different 2, 4-diacetylphloroglucinol (DAPG) genotypes varied in their rhizosphere colonization efficiency and ability to inhibit *Fusarium*. Fusarium yellows disease incidence in the tillers were noticed except in the case of T_5 (PfPh from Pulpalli), T_6 (PfAh from Appade), T_7 (StrPh from Pulpalli), T_{10} (TrAh from Appade), T_{11} (TrKUh from Kurumankotta), T_{12} (TrPh from Pulpalli) and T_{14} (Absolute control). Per cent disease incidence in rhizome were found except in T_3 (BsKUh from Kurumankotta), T_7 (StrPh from Pulpalli), T_9 (StrKh from Kappikunnu), T_{10} (TrAh from Appade), T_{11} (TrKUh from Kurumankotta), T_{12} (TrPh from Pulpalli) and T_{14} (Absolute control). Highest per cent disease incidence was recorded in the case of T_{13} (Control: Pathogen alone).In the present study, *Trichoderma harzianum* (T_{11}TrKUh from Kurumankotta) was found to be the most potential biocontrol agent for the management of Fusarium yellows which is in conformity with the earlier studies. In similar studies, Sahare and Asthane (1988) reported that *T. harzianum* and *T. viride* were the most effective in reducing the mycelial growth of *F. oxysporum* f. sp. *zingiberi.* Shanmugam *et al*. (2000) reported that only *T. harzianum* and *T. viride* were potential antagonists against *Pythium aphanidermatum* and further pot culture experiments established the efficacy of *T. harzianum* for control of rhizome rot of ginger. Reddy *et al*., (2003) reported that *Trichoderma harzianum* application against rhizome rot of turmeric (*F. solani*)

Fig. 7. Per cent infected ginger rhizome at the time of harvest



Bs- *Bacillus* sp. Pf- fluorescent pseudomonads Str- *Streptomyces* sp. Tr- *Trichoderma* sp. h- healthy

T_1: BsMh isolate from Mayilumpadi T_2: BsAh isolate from Appad T_3: BsKUh isolate from Kurumankotta T_4: PFKh isolate from Kappikunnu
T_5: PFPh isolate from Kappikunnu T_6: PFAh isolate from Appad T_7: StrPh isolate from Pulpalli T_8: StrMh isolate from Mayilumpadi
T_9: StrKh isolate from Kappikunnu T_{10}: TrAh isolate from Appad T_{11}: TrKUh isolate from Kurumankotta T_{12}: TrPh isolate from Pulpalli
T_{13} Control (Pathogen alone T_{14} :Absolute control

resulted in reduced disease incidence and increased yield. However, Dohroo *et al.* (2014) reported that, combined applications of bioagents were more effective in reducing the disease incidence than the individual treatments under field conditions. *Trichoderma harzianum*+ *Pseudononas fluorescens* + *Bacillus subtilis* gave minimum disease incidence on rhizomes (8.64%) as well as on tillers (12.50%). Rekha *et al*. (2015) reported that *Trichoderma harzianum* could be a potential biocontrol agent for the management of Fusarium yellows disease. The most efficient plant growth promoting rhizobacteria (PGPR) and antagonists were PSBPh (Pulpalli) and TrKUh (Kurumankotta) respectively.

The present studies clearly indicated the potential of *Burkholderia cepacia*for plant growth promotion and *Trichodermaharzianum*as the antagonist for the management of Fusarium yellows in ginger. However, further studies are needed to evaluate its efficiency under field condition before being recommended for the commercial production of these bioinoculants.

Future line of work

- Compatibility between PSBPh and TrKUh isolate have to be evaluated for development of consortial inoculum.
- Compatability between isolates and agrochemicals have to be evaluated
- Field level evaluation of the isolates.
- Multilocational trial and commercial production of either single or consortia of inoculant.

SUMMARY

6. SUMMARY

The present study on "Evaluation of native rhizosphere soil microflora for plant growth promotion and management of Fusarium yellows in ginger" were carried out in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara during 2012-2014. The major objectives were to enhance the growth and yield of ginger using native beneficial microorganisms isolated from Wayanad district soils of Kerala and to manage Fusarium yellows disease in ginger by using antagonistic microorganisms.

The important findings of the study are summarized below:

- A total of 10 isolates of nitrogen fixers, 8 PSB isolates, 10 isolates of *Bacillus* sp., 8 isolates of Fluorescent pseudomonads, 8 isolates of *Streptomyces* sp. and 8 isolates of *Trichoderma* sp. were obtained.The highest population of nitrogen fixing bacteria ($1.4x10^4$ cfu/g) were obtained from Pulpalli (PH), Phosphate solubilizing bacteria ($9x10^3$) from Kappikunnu (KH), *Bacillus* sp. ($2.3x10^4$ cfu/g) from Mayilumpadi (MH), *Fluorescent pseudomonads* ($9x10^4$ cfu/g) from Pulpalli (PH), *Streptomyces* sp. **(**$6x10^4$ cfu/g) from Kappikunnu (KH), and *Trichoderma* sp. **(**$6x10^3$ cfu/g) from Pulpalli (PH).

- Among the isolates of nitrogen fixing bacteria, the maximum (0.43 mg/g of sucrose utilized) nitrogen fixation was obtained by NfMh isolate and minimum (0.11 mg/g of sucrose utilized) by the isolate NfMd. The isolates showed no phosphate solubilization efficiency under *in vitro* condition. Highest IAA (35.02 µg/ml) was produced by the isolate NfAh from Appad and the least (16.07 µg/ml) by NfMd isolate. Isolates of NfPh and NfAh produced highest HCN under *in vitro* screening whereas isolates NfMd, NfPd, NfPtd, and NfAd did not produced HCN.Siderophore production was positive only for three isolates of nitrogen fixers viz; NfKh, NfAh, and NfPd.

- Among all the isolates of phosphate solubilizing bacteria, the highestP-solubilization index (450.00%) was recorded by the PSBAh isolate and the least (151.84%) by the isolate PSBMd. IAA production of all the eight isolates were tested. Except for the PSBVd isolate (vengapalli), all other isolates were positive in IAA production. The highest IAA (33.07µg/ml) was produced by PSBMh isolate (Mayilumpadi) and the least (12.92 µg/ml) by PSBPd isolate (Palakkaparambu). The PSBMh isolate of phosphate solubilizing bacteria recorded the maximum HCN production. Only two isolates, PSBKh and PSBAh were positive for siderophore production.

- Among the isolates of *Bacillus*sp.The maximum P- solubilization index (182.99%) was recorded by BsAh isolate and the least (137.43%) by BsKUh isolate. Highest IAA (14.54µg/ml) was produced by BsMh isolate (Mayilumpadi) and the least (12.92 µg/ml) by BsAh isolate (Appade). Out of the ten isolates tested for HCN production, only three BsMh, BsKuh and BsPTd isolate resulted moderate level of HCN production. Only one isolates, BsMh positive for siderophore production.

- All the seven isolates of fluorescent pseudomonadsthe highest (241.57%) p-solubilization was in PfMh isolate and lowest (190.11%) by the isolate PfKh. Highest IAA production was (16.75 µg/ml) by the isolate PfKh from Kappikunnu and the lowest (12.13 µg/ml) by PfPh isolate. Hydrogen cyanide production of all the isolates were tested and that isolates PfPh and PfKUh produced highest HCN under *in vitro* screening.Siderophore production was produced only by three isolates PfKh, PfMh, and PfPh.

- Among the eight isolates of *Streptomyces*sp.the maximum P- solubilization index (176.99%) was recorded by StrAh isolate and the least (153.76%) by StrPh isolate. Highest IAA (11.63µg/ml) was produced by StrMh isolate (Mayilumpadi) and the least (10.41 µg/ml) by StrKUh isolate (Kurumankotta). Two isolates StrMh and StrPh resulted moderate level of

HCN production. Four isolates StrMh, StrPh, StrAh and StrAd isolates resulted siderophore production. StrPh (Pulpalli) isolate resulted excellent siderophore production.

- All the isolates of *Trichoderma* sp. did not solubilized phosphorus and IAA under *in vitro* screening. Two isolates TrPh and TrKUh produced moderate HCN under *in vitro* screening. Siderophore production was produced only by three isolates TrKh, TrMh, and TrAh.

- The isolates BsAh (64.08 per cent) from Appad, PfKh (59.43per cent) from Kappikiunnu, StrPh (67.78 per cent) from Pulpalli, and TrAh (78.93 per cent) from Appad were highly antagonistic against *Fusarium oxysporum* under *invitro.*

- The isolates BsAh (15.90 per cent) from Appad, PfAh (33.71 per cent) from Appad, StrKh (34.28 per cent) from Kappikiunnu, and TrAh (78.93 per cent) Appad showed maximum antagonism against *Pythium aphanidermatum.*

- Isolates BsKh (47.09 per cent) from Kappikiunnu, PfAh (27.20 per cent) from Appad, StrPh (43.11 per cent) from Pulpalli, and TrKUh (43.27 per cent) from Kurumankotta were found to be highly antagonistic against *Rhizoctonia solani.*

- The isolates BsMh (43.81 per cent) from Mayilumpadi, PfPh (31.32 per cent) from Pulpalli, and StrPh (34.23 per cent) Pulpalli were antagonistic against *Ralstonia solanacearum.*

- T_4 (PSBPh) from Pulpalli was the best treatment in terms of sprouting percentage under pot culture for plant growth promotion. The plant height was highest T_4 (PSBPh) with 81.08 cm. The maximum number of tillers (6.85) was observed in T_3 (NfKh) from Kappikunnu. Plants inT_4 (PSBPh)

from Pulpalli produced the maximum yield 128.92 g/plant which was on par withT_1 (NfMh) compared to T_7 (control).

- T_4 (PfKh) from Kappikunuwas the best treatment in terms of sprouting percentage under pot culture for disease managment. The plant height was highest T_{11} (TrKUh) from Kurumankotta with 69.98 cm. The maximum number of tillers (4.44) was observed in T_{11} (TrKUh) from Kappikunnu.Plants in T_{11}(TrKUh) produced the maximum yield 120.35 g/plant which was on par with T_4 (PfKh) compared to T_{13} (control; Pathogen alone).

- After four months of planting second application of the antagonists was done. Two weeks after the second application of the antagonists challenge inoculation of the pathogen *Fusarium oxysporum* f. sp. *zingiberri* was done and recorded the observation.

- After the challenge inoculation of the pathogen the following symptomps were recorded stunted growth of tillers, yellowing, drying up, falling of tillers and percentage of rhizome rot were recorded.

- Fusarium yellows disease incidence in the tillers was recorded except in treatments T_5 (PfPh from Pulpalli), T_6 (PfAh from Appad), T_7 (StrPh from Pulpalli), T_{10} (TrAh from Appad), T_{11} (TrKUh from Kurumankotta), T_{12} (TrPh from Pulpalli) and T_{14} (Absolute control).All other treatments were infected with Fusarium yellows disease.

- Percent disease incidence in rhizome yield were also recorded. Except for the treatments T_3 (BsKUh) Kurumankotta, T_7 (StrPh) Pulpalli, T_9 (StrKh) Kappikunnu, T_{10} (TrAh) Appad, T_{11} (TrKUh) Kurumankotta, T_{12} (TrPh) Pulpalli and T_{14}(Absolute control) all other treatments were infected with Fusarium yellows disease.

- 16S rDNA sequence analysis were carried out for the identification of most efficient PSB isolate (PSBPh from Pulpalli). The isolate (PSBPh from Pulpalli) was identified as *Burkholderia cepacia*. The most efficient isolate (TrKUh from Kurumankotta) for disease management was identified as *Trichoderma harzianum.*

- The present studies revealed the potential of *Burkholderia cepacia* as a plant growth promoting rhizobacteria and *Trichoderma harzianum* for the management of Fusarium yellows in ginger.

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APPENDICES

APPENDIX – I

MEDIA USED AND COMPOSITION

a. Jensens agar media

Sucrose	– 20g
K_2HPO_4	– 0.1g
$MgSO_4$	–0.50g
NaCl	– 0.50g
$FeSO_4$	– 0.10g
Sodium Molybdate	– 0.005g
$CaCo_3$	– 2.00g
Agar	- 20.00g
Distilled water	- 1000 ml

b. Pikovskaya's agar media

Glucose	– 10g
$Ca_3(PO_4)_2$	– 5g
$(NH_4)_2SO_4$	– 0.5g
NaCl	– 0.2g
$MgSO_4.\ 7H_2O$	– 0.1g
KCl	– 0.2g
Yeast extract	– 0.5g
$MnSO_4.H_2O$	– 0.002g
$FeSO_4.7H_2O$	– 0.002g
Distilled water	– 1000 ml
pH	– 7.0

c. Nutrient agar media

Beef extract	– 3g
Peptone	– 5g
NaCl	– 5g
Agar	– 20g
Distilled water	– 1000 ml

d. Kings B agar media

Proteose peptone	- 20g
K_2HPO_4	- 1.50g
Magnesium sulphate heptahydrate	- 1.50g
Agar	- 20.00g
Glycerol	- 15 ml
Distilled water	- 1000 ml
pH	- 7.2 ± 0.2

e. Kenknight & Murinaiers agar media

Dextrose	- 1.00g
Mono potassium di hydrogen phosphate	- 0.10g
Potassium chloride	– 0.10g
Magnesium sulphate	– 0.10g
Agar	– 20.00g
Distilled water	– 1000 ml.

f. Trichoderma selective media

Magnesium sulphate heptahydrate	- 0.200
Dipotassium hydrogen phosphate	- 0.900
Ammonium nitrate	- 1.000
Potassium chloride	- 0.150
Glucose	- 3.000
Rose Bengal	- 0.150
Agar	- 20.000

g. Urea broth

Urea	– 20g
Yeast extract	– 0.1g
KH_2PO_4	– 9.0g
K_2HPO_4	– 9.5g
Phenol red	– 0.01g
Distilled water	– 1000 ml
pH	– 6.8

h. Starch agar

Starch	– 20g
Beef extract	– 3g
Peptone	– 3g
Agar	– 20g
Distilled water	– 1000 ml

i. Potato Dextrose Agar

Potato infusion – 200.00g
Glucose – 20.00g
Agar – 20.00g
Distilled water – 1000ml
pH – 5.1

j. Nutrient broth

Beef extract	– 3g
Peptone	– 5g
NaCl	– 5g
Distilled water	– 1000 ml

APPENDIX – II

REAGENTS USED

1. **Boric acid- indicator mixture**

   4 % boric acid solution in hot water

2. **Picric acid solution**

   2.5 g of picric acid

   12.5g of Na_2Co_3

   1000 ml of distilled water

3. **Salkowski reagent**

   2% of 0.5 M $FeCl_3$ in 35% perchloric acid

APPENDIX – III

Nucleotide sequences of PSBPh isolate

CACACGGCCACTCGTTGATTCGACTTCACCCCAATCATCTGTCCCA
CCTTAGGCGGCTAGCTCCTTACGGTTACTCCACCGACTTCGGGTGT
TACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGA
ACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAG
CTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAATGGTT
TTATGGGATTGGCTTGACCTCGCGGTCTTGCAGCCCTTTGTACCATC
CATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTT
GACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTA
GAGTGCCCAACTAAATGCTGGCAACTAAGATCAAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAA
CCATGCACCACCTGTCACTCTGTCCCCCGAAGGGGAACGCTCTATC
TCTAGAGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGT
TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGT
CAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAG
TGCTTAATGCGTTAGCTGCAGCACTAAAGGGCGGAAAACCCTCTAA
CACTTATCACTCATCGTTTACGGCGTGGACTACCCAGGGTATCTAA
TCCTGTTTGCTCCCCACGCTTTCTCGCCTCAGCGTCAGTTCAGACCA
AAAAGCCGCCCTTCGCCAACTGGTGTTCCTCCACATCTTCTACGCA
TTTCACCGCTACCCGTGGAATTCCGCTTTTCTCTTCTGACCTCAAGT
TTCCCAAGTTTCCA

ABSTRACT

EVALUATION OF NATIVE RHIZOSPHERE MICROFLORA FOR PLANT GROWTH PROMOTION AND MANAGEMENT OF FUSARIUM YELLOWS IN GINGER

By

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was undertaken on "Evaluation of native rhizosphere microflora for plant growth promotion and management of Fusarium yellows in ginger". The main objectives were to enhance the growth and yield of ginger using native beneficial microorganisms isolated from wayanad district and to manage Fusarium yellows disease in ginger by using antagonistic microorganisms.

Rhizosphere soils were collected from ten different locations of healthy and diseased ginger fields of Wayanad district. The predominant beneficial microorganisms namely nitrogen fixing bacteria, phosphate solubilizing bacteria, *Bacillus* sp., *Pseudomonas fluorescens*, *Streptomyces* sp., and *Trichoderma* sp. were isolated.

The highest population of nitrogen fixers ($1.4x10^4$ cfu/g), fluorescent pseudomonads ($9x10^4$ cfu/g), and *Trichoderma* sp. ($6x10^3$ cfu/g) were obtained from Pulpalli location. Phosphate solubilizing bacteria ($9x10^3$ cfu/g) and *Streptomyces* sp. ($6x10^4$cfu/g) were highest in Kappikunnu and maximum population of *Bacillus* sp. ($2.3x10^4$ cfu/g) was recorded in Mayilumpadi.

The isolates were screened for plant growth promotion and antagonistic activity under *in vitro* condition. Maximum nitrogen fixation was recorded in NFMh isolate (0.43 mg/g of sucrose utilized) among nitrogen fixers. Highest IAA (35.02 µg/ ml), HCN and siderophore production were recorded by NFAh (nitrogen fixer) isolate.

Among the phosphate solubilizers, highest P- solubilization (450.00%) was by PSBAh isolate where as, IAA (33.07 µg/ ml) and HCN was shown by PSBMh isolate. Among the isolates of *Bacillus* sp. The maximum P- solubilization index (182.99%) was recorded by BsAh isolate and the least (137.43%) by BsKUh isolate. Highest IAA (14.54 µg/ml) was produced by BsMh isolate (Mayilumpadi) and the least (12.92 µg/ml) by BsAh isolate (Appade). Out of the ten isolates

tested for HCN production, only three BsMh, BsKuh and BsPTd isolate resulted moderate level of HCN production. Only one isolates, BsMh positive for siderophore production.

All the seven isolates of fluorescent pseudomonads the highest (241.57%) p-solubilization was in PfMh isolate and lowest (190.11%) by the isolate PfKh. Highest IAA production was (16.75 µg/ml) by the isolate PfKh from Kappikunnu and the lowest (12.13 µg/ml) by PfPh isolate. Hydrogen cyanide production of all the isolates were tested and that isolates PfPh and PfKUh produced highest HCN under *in vitro* screening. Siderophore production was produced only by three isolates PfKh, PfMh, and PfPh.

Among the eight isolates of *Streptomyces* sp. the maximum P- solubilization index (176.99%) was recorded by StrAh isolate and the least (153.76%) by StrPh isolate. Highest IAA (11.63 µg/ml) was produced by StrMh isolate (Mayilumpadi) and the least (10.41 µg/ml) by StrKUh isolate (Kurumankotta). Two isolates StrMh and StrPh resulted moderate level of HCN production. Four isolates StrMh, StrPh, StrAh and StrAd isolates resulted siderophore production. StrPh (Pulpalli) isolate resulted excellent siderophore production.

All the isolates of *Trichoderma* sp. did not solubilized phosphorus and IAA under *in vitro* screening Two isolates TrPh and TrKUh produced moderate HCN under *in vitro* screening. Siderophore production was produced only by three isolates TrKh, TrMh, and TrAh.

Selected isolates of *Bacillus* sp., *Pseudomonas fluorescens*, *Streptomyces* sp., *Trichoderma* sp., were tested for their antagonistic activity against the major soil borne pathogens of ginger *viz., Fusarium oxysporum, Pythium aphanidermatum, Rhizoctonia solani* and *Ralstonia solanacearum* under *in vitro* condition.

The isolate BsAh showed maximum antagonistic activity against *F. oxysporum* (64.08 per cent) and *P. aphanidermatum* (15. 09 per cent) where as, BsKh isolate was antagonistic against *R. solani* (47.09 per cent) and BsMh isolate showed 43.81per cent inhibition against *R. solanacearum*.

Among *Pseudomonas fluorescens,* PfKh showed 59.43 per cent inhibition against *F. oxysporum* where as PfAh isolate recorded 33.71 per cent inhibition against *P. aphanidermatum*, 27.20 per cent aginst *R. solani* and PfPh isolate showed 31.32 per cent inhibition against *R. solanacearum*.

Among the isolates of *Streptomyces* sp., StrPh isolate recorded maximum inhibition (67.78 per cent) against *F. oxysporum*, 43.11 per cent inhibition against *R. solani* and 34.23 per cent against *R. solanacearum*. TrAh isolate (*Trichoderma* sp.,) showed highest (78.93 per cent) inhibition against *F. oxysporum,* 51.30 per cent inhibition against *P. aphanidermatum* and 43.27 per cent against *R. solani.* However, TrMh isolate shown 15.96 per cent inhibition against *R. solanacearum.*

Three most efficient isolates of nitrogen fixers (NFMh, NFPh, NFKh), phosphate solubilizing bacteria (PSBPh, PSBKh, PSBAh), *Bacillus* sp., (BSMh, BSAh, BSKUh), fluorescent pseudomonads (PFKh, PFPh, PFAh), *Streptomyces* sp., (StrPh, StrMh, StrKh) and *Trichoderma* sp., (TrAh, TrKUh, TrPh) obtained under *in vitro* studies were further screened under pot culture studies.

Among the isolates PSBPh (T_4) was found to be the most efficient in enhancing the growth and yield of ginger where as the isolate TrKUh (T_{11}) was the most efficient isolate for the management of Fusarium yellows disease. These two isolates were identified as *Burkholderia cepacia* (PSBPh) and *Trichoderma harzianum* (TrKUh).

The present study clearly showed that *Burkholderia cepacia* (PSBPh) and *Trichoderma harzianum* (TrKUh) were effective for plant growth promotion and management of Fusarium yellows in ginger respectively. However, these isolates have to be evaluated for efficiency under field condition.