Introduction

Agricultural crops suffer heavily due to diseases and pests. It is essential to have a basic knowledge about plant pathogens, different kinds of diseases caused by them on various crops and the methods of managing the diseases to avoid the enormous losses caused by the diseases.

Modern agriculture relies a lot on recent innovations in technology for betterment of agricultural produce. Fungicides are still in vogue for crop protection purposes, but greater consumer health-consciousness is driving demand for products completely free of, or only minimally contaminated with chemicals. There is, thus demand for novel and viable strategies which are in complete harmony with the environment for crop protection, which has led to increased research into biocontrol methods, offering benefits of greater biodiversity and cost saving to growers. In this context, use of plant growth-promoting fungi and rhizobacteria and induction of systemic resistance against diseases are viewed as novel and potential tools, which are known to present substantial benefits to agriculture.

Microorganisms constitute a major component of the rhizosphere. Rhizosphere, the narrow zone of soil directly adjacent to plant roots, containing root exudates, leaked and secreted chemicals, sloughed root cells, and mucilages of a plant has a major influence on the health and productivity of crops. However, it is a complex system wherein a series of interaction take place. Many studies show much higher numbers of bacteria and fungi in the rhizosphere an in bulk soil which is referred to as the 'rhizosphere effect' under the influence of both biotic and abiotic factors. (Jeffrey *et al.,* 2002). The composition often differs as a result of diverse plant- microbe interaction. The rhizosphere of established tea bushes has some specific characteristics which are associated with the long lived nature of tea plants, viz. negative rhizospheric effect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species (Sood *et al.,* 2007). The overall interactions between tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seem to favor the growth of microbes, which are known to produce strong antibiotics with potential use as biocontrol agents. Till now very limited and isolated efforts were made for tapping of microbial diversity, identification, evaluation and preserving them for different applications.

It has been reported that natural rhizosphere is often inimical to pathogens, because antagonists from a part of the rhizosphere community (Lynch, 1987). Microorganisms present in the soil play an important role in nutrient solubilization, mobilization and recycling. They have very wide potentialities by controlling soil-borne pathogens, stimulating plant growth, increasing nutrient availability and accelerating decomposition of organic materials, and are anticipated to increase crop productions as well as maintain sound environment for crop productions. Its resident microbial flora chiefly determines the health of the soil. Despite the many achievements of modern agriculture certain cultural practices or continued release of new resistant cultivars and pesticides, pathogens still cause crop damages and losses that exceed 12% worldwide and have actually enhanced the destructive potential of disease. In addition, disease often compromises the quality of the harvest by producing toxins that seriously threaten human and livestock health. Thus, an understanding of how pathogens cause disease and how plants defend themselves are crucial research issues for global food security. Almost 30% of the yield in agriculture is lost because of combined effects of biotic and abiotic stresses, with pathogenic fungi alone responsible for reduction of about 12% plant disease.

Management of root disease through the application of beneficial soil microorganisms has been considered as a highly valuable tool to improve productivity without damaging the soil environment. The rhizosphere population may have either a favorable or a detrimental influence upon plant development, because the micro flora is so intimately related with the root system, partially covering its surface any beneficial or toxic substance produced can cause an immediate and profound response.

Tea is an important commercial crop; it is cross pollinated and shows high levels of heterogeneity in the progenies. Because of the out breeding nature, seedling population of tea is extremely heterogenous. For vegetative propagation, type of soil required is neither too sandy nor too clayey. Commercial tea population lies under three principle taxa, 'China' *Camellia sinessis*, L.O.Kuntse), 'Assam' (*C.assamica spp.assamica* (Master) Wight and "Cambod" (*C.assamica spp.lasiocalyx* (Planch ex watt) Wight. They exhibit considerable variability in morphological characters and yield. Repeated out-breeding through natural hybridization over a prolonged period between the taxa and

their alleles had contributed to the evolution of the present day commercial population of tea plant (Mohanan and Sharma,1981).*Camellia sinensis* generally consists of two variants, *C. sinensis var.sinensis* and *C. sinensis var. assamica.* Tea plants belonging to variety sinensis are characterized by a bush type with small leaves, are resistant to the cold and suitable for making green tea and semi- fermented tea. In a broad sense, they are referred to as the china variety. On the other hand, tea plants belonging to var. *assamica* are characterized by a tall tree type with large leaves, are less resistant to the cold and are suitable for making black tea.

The earliest knowledge of the tea plant is derived from China where tea as a beverage was known for about 3000 years. It is generally believed that it originated somewhere in south East Asia kingdom- (Ward, 1950). However, the current distribution patterns of the tea varieties suggest that the probable centre of origin of the tea is Burma region, and from there it dispersed to South Eastern China, Indonesia and Assam.

In India, tea seeds from China were brought and sown at Botanical Garden, Calcutta in 1780 (Bezbaruah, 1999). Tea (China type) was introduced in North East India in 1836, although in 1823, Major Robert Bruce discovered tea plants growing wild in some hills near Ragpur/ Sibsagar, the then capital of Assam. In South India, one Dr. Christy has experimented on growing tea in Nilgiris in 1832 (Muraleedharan, 1991). Currently, India is having 1.7 million hectares of area under certified organic farming. The organic market in the country is valued at Rs 100 crores with an annual growth rate steadily going up from 35 per cent which will farther expected to grow 50 per cent by 2010. India is fast becoming a major base for production and supply of organically produced agricultural products to the world market. The global market for organic farm produce is expected to touch US$ 100 billion by 2010. As we are aware, India is the largest producer of tea and its share in global production is 28 percent. It is the second most widely consumed drink worldwide, after water. So tea industry supports, directly and indirectly, almost 2% of the population of the country.

Plate 1 : Plantation site of Temi Tea Garden , Sikkim

In context to global scenario Sikkim is small mountainous state in northeast India with uneven topography. Temi Tea Garden (TTG) is the only tea estate existing in the state of Sikkim (South Sikkim), India at present (Plate 1). Under the assistance of Temi Tea Board, tea growers society like Sang-Martam Tea Growers' Cooperative Society was established in the year 1998 area of tea estate covering 75 acres has started flourishing which will further increase the products. Efforts are underway to source direct buyers in foreign markets for Sikkim's tea and the field is open for investors interested in increasing Sikkim's tea production capabilities. Some tea plantations in South Sikkim (Ravangla) have been also reported. Tea grown in Sikkim is famous by its brand name 'Temi Tea'. Focusing on branding of its products, Temi tea also sells 30 per cent of its annual production in small packets under four brands - Temi Tea, Sikkim Solja, Sikkim Kanchendzonga Tea and Mystique (Plate 2). The Garden also touches the bottom of the renowned Tendong Hill in West Sikkim district. The Garden is one of the best tea producing estate in the country and produces one of the top quality teas in the international tea market. It was established in the year 1965 with an estimated area of 435 acres. It employees a total number of 406 workers and 43 staff members approximately. It produces about 100 MT of tea annually. The garden received All India Quality Award; from Tea Board of India for the two consecutive years i.e. 1994 and 1995. During the year 1997-98 the production figure of garden drastically improved by producing 1,16,000 kgs of tea leaves which is a record achieved till date. Consequently, the garden had directly exported 100 kg of bulk loose tea to Canada and Japan at a record price of Rs 2500 per kg. But apart from these progresses made by the garden it still suffers some losses due to unexpected problems, fungal disease being one of them.

Among fungal diseases brown root rot (*F.lamaoensis*), red root rot (*P. hypobrunnea*,), black root rot (*Rosellinia arcuata*), and charcoal stump rot (*U. zonata*), is common, while few other disease also occur. Weeds like *Ageratum conyzoides, Amaranthus viridis, Drymaria cordata, Euoatorium odoratum, mimosa pudica, oxalis sp., Polygonium sp., cherry tree, Eragrostis unilodes, colocasia* sp. etc. are also found.

Tea plants are subjected to attacks by a large number of insect pests and fungal diseases, also by bacteria, algae and viruses. Worldwide, there are 380 fungal pathogens which attack roots, leaves and branches of tea plants. Traditionally, the diseases in the tea

have been classified as primary and secondary diseases. In primary root diseases, healthy section of tea without previous history of any root diseases, the infection may occur through the air borne contact of the root with infected material in the soil. The infected material might have come into otherwise healthy section of tea inadvertently through infected wooden posts, chips of firewood with the pathogen dropped in the field by labour or other such physical methods. The group of diseases which comes under this are brown root rot of tea, red root rot, charcoal stump rot, black root rot, root split disease. In case of secondary root diseases the fungi that cause the secondary root disease of tea are very common and abundant in nature and they are seen either in soil or above ground or in both these habitats. They attack the tea bush under certain predisposing conditions like adverse soil conditions, impaired health and vigour of the plants due to several causes and usually it take a longer time to kill bushes completely then do primary ones, e.g. violet root rot, wood or branch canker etc. *Ustulina zonata* which is one of the primary root diseases of tea causes charcoal stump rot. The fungus remains in the root system for several years and the plants appear normal up till the time of death, which takes place suddenly. The foliage wilts and dies but the withered leaves remain attached to the branches for some time before they drop off. Gradually the disease extends along the roots killing the host tissues until it reaches the collar, the water conducting cells which make up the wood which become gradually blocked by the mycelium or threads of the fungus which weave in and out of the tissues. Eventually the fungus completely blocks the water conducting tissues at or near the collar, so that passage of water from the roots to the leaves is stopped and the bush suddenly dies. The process may take about 1-4 years, according to age and size of the bush.

Soil and plant associated environment harbor a wide variety of microorganisms that play an integrated role in plant growth and disease management. Biological control of pathogens by application of specific antagonistic microorganisms to seeds or planting material has been studied intensively, however, only few of these biocontrol agents have been effective from years to year, and over a broad range of condition very few are successful. Few commercial preparations based on *Trichoderma, Pseudomonas, Bacillus* sp. are available in the market for biocontrol of phytopathogens. The last decade has witnessed a tremendous break through in the research efforts on biological control of plant disease in India especially by using species of *Gliocladium,Trichoderma harzianum*

Plate 2(A-C): Panoramic view of Temi Tea Estate (A), Tea factory (B), Packed tea leaves for commercial use (C)

and viride,, Beauveria bassiana, Verticillium lecanii, Paecilomyces lilacinus, and bacteria like *Bacillus subtilis* which proved their potentiality as bio-control agents. These were found to be hyper parasitic/ antagonistic/pathogenic against some of the major tea pathogens and pest.

The production and use of pesticides have increased during the last few decades. Use of these pesticides has benefited the modern society by improving the quality and quantity of world's food supply. Some of these pesticides are highly toxic and considered a potential risk to both human health and environments and recently the problem of pesticide residue has become an alarming issue among the consumers. Wide spread applications of chemical pesticides/ fungicides inundates the agro-ecosystem with toxic compounds that affect the balance of natural food chain.

To escape from these problems, presently attention has been given towards the alternative plant protection tools. The use of biofertilizers or microbial inoculants for replacing the efficacy of chemical fertilizers has been found to be effective in reducing the cost of cultivation and maintaining the natural fertility of soil. Microorganisms have been used over the past 50 years to advance medical technology, food processing, food safety and quality, environmental protection, and agricultural biotechnology many of these technological advances would not have been possible using straightforward chemical and physical energy methods.

Considering the importance of the use of eco-friendly microorganisms for plant growth promotion and disease protection in tea and the fact that little or no work has been done on tea rhizosphere of Sikkim. The present study was undertaken with the following objectives giving special emphasis on:

- Isolation of phosphate solubilizing fungi (PSF) and phosphate solubilizing bacteria (PSB) from tea rhizosphere
- Screening of PSF and PSB on growth promoting activity of tea seedlings.
- *In vitro* testing of PSF and PSB isolates for antagonism against *Ustulina zonata* (test pathogen).
- *In vivo* testing of selected microorganisms (PSF,PGPR,AMF) for suppression of charcoal stump rot disease of tea.

- Evaluation of combined effect of PSF,PGPR and AMF as well as selected commercial formulation of VAM (JOSH), and bioperotectant (Kalisena) for disease suppression of tea.
- Determination of biochemical changes in tea plants due to applications of various formulations of bioinoculants.
- Determination of population level of antagonist as well as pathogen in soil following application.
- Preparation of formulation of these bioinoculants and their field trial.

In agricultural management two of the most important goals are to ensure the crop of enough nutrients and to prevent it from diseases. Traditionally, these goals have been achieved by using pesticides and high inputs of fertilizers. However, these management practices lead to a high loss of biodiversity all over the landscape. Therefore, other approaches should be investigated. Soil organisms play an important role in nutrient cycling and can reduce diseases; a new approach is to manage the system by increasing the soil biodiversity. Since the below ground organisms are for a large part dependent on input from above ground organisms, this may be achieved by modifying the rhizosphere.

The rhizosphere is a micro-zone at the root-soil interface that is under the influence of the plant root. A plethora of mutually interacting physical, chemical and biological processes operate within this zone. Although attempts have been made to unravel some of these processes, the understanding of the intricacies of this unique 'twilight zone' is still in its infancy. The structural and functional diversity of the rhizosphere is maintained by input of root derived carbon sources. Available evidence suggests that plants and rhizosphere organisms function in an interdependent fashion. Rhizosphere organisms depend on plants for continuous supply of reduced carbon and play a significant role in nutrient cycling, thus exerting an influence on plant growth. The increase in soluble carbon in soil sections close to the root surface is related to the rhizodeposition of root exudates that include low- molecular weight organic acids, carbohydrates, nucleic acid derivatives and amino acids. Microorganisms in turn contribute to the availability and mobilization of nutrients, production of growth regulators, phytotoxic substances or by suppression of pathogens and pollutants added to soils.

The plant rhizosphere represents a highly complex ecosystem that is influenced by a number of abiotic and biotic factors. The bacterial/ cyanobacterial communities of the rhizosphere have strong influence on the growth and health of the plant as well as on their ability to adapt to changed environmental conditions. The enhanced degradation of pesticides in soil sections close to the root surface is related to the rhizosphere- induced co- metabolism of pesticides. The actively growing plant roots provide an excellent environment for intensive microbial activity, resulting in enhanced biodegradation of organic contaminants. Selective enrichment of microorganism is likely to have a

significant impact on the rhizo-remediation, rhizoextraction or rhizo-filtration of recalcitrant organic contaminants in soils. Attempts may be given to provide nutrient and plant growth promoting substances through microorganisms for sustainable crop production.

The microbial biomass in soil also contains a significant quantity of immobilized P that is potentially available to plants. This aspect has been reviewed by Greaves and Webley, 1965; Raghu and Mac Rae, 1966; Hedley and Steward, 1982; Rao, 1982; Brookes *et al.*, 1984; Goldstein, 1986; Tandon, 1987; Kucey *et al.*,1989; Richardson, 1994; Bishop *et al.*, 1994; Narula *et al.*, 2000; Oberson *et al.*,2001.

Considering the importance of using biological agents for growth promotion and disease suppression in plants by reducing the use of chemicals, the present review describe observation of previous workers in connection with the potential role of biofertilizers in the growth and development of plants, use of rhizosphere microorganisms and biocontrol agents and their role in disease suppression

Plant growth promoting rhizobacteria

Soil microorganisms can be classified into major divisions, such as the bacteria, actinomycetes, fungi, algae, protozoa, worms and arthropods. Soil is abode for several micro-biota - a few beneficial and some antagonistic. While the former are characterized by their positive effects on the plants, the latter are either parasites or pathogens of the plant-host. The groups of beneficial microbes which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms and these bacteria are collectively known as plant growth promoting rhizobacteria, (PGPR). In soil there are certain inorganic compounds like tricalcium phosphate, which is water insoluble and unavailable to crop plants. Some phosphate solubilizing bacteria solubilize such insoluble phosphates into form which are available to plants. Another bacterium *Bacillus siliceous* degrades silicate minerals and makes potassium available to plants. In the similar way, different microbes affect the solubility of boron, sulphur, iron etc. and make them available to crops.

Siderophore production was also postulated to be an important mechanism for the biocontrol activity of PGPR which was confirmed by Neillands (1886), Loper and Buyer (1991), Bakker *et al.* (1993).

Selected PGPR strains belonging to diverse Gram- positive and Gram- negative genera can, upon seed treatment or soil drench treatment to plant root system, reduce the incidence of distally infecting pathogens Kloepper *et al.*, (1998). Single PGPR strains have been shown to reduce pathogen infection and symptoms of multiple diseases on cucumber and tomato. Cucumber diseases affected in greenhouse and field studies in multiple years include foliar diseases (angular leaf spot, caused by *Pseudomonas syringae* pv. *lachrymans; and anthracnose,* caused by *Colletotrichum orbiculare)*; systemic wilt disease (cucurbit wilt, caused by *Erwinia tracheiphila;* fusarium wilt, caused by *Fusarium oxysporum f. sp. cucumerinum)*, and the systemic viral disease caused by cucumber mosaic virus(CMV). In case of cucurbit wilt, disease control is linked to PGPR mediated reductions in plant preference by the insect vectors, the striped and spotted cucumber beetles. In field and greenhouse studies, PGPR treatments led to significant reduction in beetle feeding attractant. With tomato, protection has been noted in the greenhouse or field against CMV; bacterial spot, caused by *Xanthomonas axonopodis* pv. *Vesicatoria*; tomato mottle geminivirus; and bacterial speck, caused by *P. syringae* pv. *tomato*. Mode of action studies support the conclusion that the observed systemic biocontrol results from ISR, since measurable biochemical and cytological changes occur in the plant in relation to host recognition of the inducing PGPR strains and are the focus of intense current investigation. They have observed enhanced peroxidase activity and lignification in cucumber and induction of PRla promoter in transgenic tobacco containing a GUS reporter gene. In this tobacco system, all PGPR strains which enhanced protection against wildfire disease- caused by *P. syringae* pv. *tabaci in* the greenhouse induced GUS activity, whereas control strains lacking disease protecting activity did not induce GUS activity significantly relative to controls.

PGPR mediated induced systemic resistance has been demonstrated against fungi, bacteria, and viruses by Van Loon *et al.* (1998). Bacterial determinants of ISR include lipopolysaccharides, siderophores, and salicylic acid. The induced disease resistance was enhanced by the simultaneous activation of ISR and the systemic acquired resistance

pathway which resulted in additive effects on the level of induced protection (Van Wees *et al.*, 2000). To protect themselves from the disease, plants have evolved sophisticated defense mechanisms in which the signal molecules salicylic acid, jasmonic acid and ethylene often plays crucial roles Pieterse *et al.*, (2002).

Munimbazi *et al.* (1998) antifungal metabolites produced by *Bacillus pumilus* in potato dextrose broth were isolated from culture supernatant fluid by precipitation with ammonium sulphate. The antifungal metabolites inhibited mycelial growth of many species of *Aspergillus, Penicillum* and *Fusarium.* They also inhibited production of aflatoxins, cyclopiazonic acid, ochratoxin A and patulin. Their activity was stable over wide range of temperature and pH (2-10). The metabolites were also resistant to hydrolysis by various proteases, peptidases and other enzymes, so it has the potential to use as fungicide but more investigations is needed with regard to their inexpensive large scale production, evaluation for toxicity and degradation in the environment.

The aim of study of Jacques *et al.* (1999) was to evaluate four *pseudomonas* strains for their intrinsic properties conferring their ability to protect long English cucumber against *pythium aphanidermatum* in hydrophonic culture. Two of the strains, BTPI and its siderophores negative mutant M3, increased plant yield as compared with the non-inoculated control plants. Strain BTP7 was intermediate in its biocontrol activity while strain ATCC 17400 failed to reduce disease development. The role of pyoverdines could not be confirmed since treatment with either BYPI or its siderophores- negative mutant M3 provided similar suppression of pythium disease. In addition, no siderophores were detected in the nutrient solution. BTPI did not inhibit pathogen growth in vitro o several media, suggesting that antibiosis was not a mechanism of suppression. Quantification of root bacterial population did not indicate differences among the strains. On the other hand, roots treated with either BTP1 contained more antifungal phenolics then roots from any other treatments including controls. These results suggest that antifungal phenolics compounds induced by inoculation of cucumber roots with the fluorescent pseudomonas strains BTP1 and M3 participate actively in the protection of cucumber plants against *P. aphanidermatium.*

Wee-Scm-Van *et al.* (1999) selected strain of non- pathogenic rhizobacteria from the genus *Pseudomonas* are capable of eliciting broad-spectrum ISR in plant that is

phenotypically similar to pathogen-induced systemic acquired resistance. In *Arabidopsis thaliana* , the ISR pathway function independently of salicylic acid (SA) but known defense- related gene, i.e. SA responsive genes PR-1,PR-2 and PR-5 ethylene-inducible gene Hel, ethylene and jasmonate inducible genes ATUSP, Lox1, Lox2,Pal1 and Pal2, are neither induced locally in roots nor systemically in leaves open inductions of ISR by *P. fluorescens* with WCS4178. in contrasts plants infected with the virulent leaf pathogen *P. syringe* Pv. tomato (Ps1) or expressing SAR induced by preinfecting lower leaves with the avirulant pathogen Pst(arrRp+2) exhibited elevated expression levels of most of the defense related genes studiedly upon challenge inoculation with Pst, PR gene transcripts accumulated to a higher levels in SAR- expressing plants than is control treated and ISR expressing plants, indicating that SAR involves potentiation of SA responsive PR gene expression. In contrast pathogen challenge of ISR expressing plants, let to an enhanced level of ATVSP transcripts accumulation. The other jasmonate responsive defense related genes studied were not potentiated during ISR, indicating the ISR is associated with the potentiation of specific jasmonate responsive genes.

Cattelan *et al.* (1999) conducted a study to identify the specific traits by which plant growth promoting rhizobacteria (PGPR) promote plant growth. They selected 116 isolates from bulk soil and the rhizosphere of soybean and examined them for a wide array of traits that might increase early soybean growth in non sterile soil (PGPR traits). A sub sample of 23 isolates, all but one of which tested positive for or one or more of these PPGR traits, was further screened for traits associated with biocontrol, brady-rhizobial inhibition, and rhizosphere competence. Six of eight isolates positive for 1-aminocyclopropane-1- carboxylate (ACC, a precursor of ethylene) deaminase production, four of seven isolates positive for siderophore production, three of four isolates positive for β -1,3-glucanase production and two of five isolates, positive for P solubilization increased at least one aspect of early soybean growth. One isolate which did not share any of the PGPR traits tested *in vitro* except antagonism to *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, also promote soybean growth. One of the 23 isolates changed bradyrhizobial nodule occupancy. Although the presence of a PGPR trait *in vitro* does not guarantee that a particular isolate is a PGPR, the result suggest that rhizosphere able to produce ACC deaminase and to a lesser extent, β -1,3- glucanase or siderophores or those able to solubilize P *in vitro* may increase soybean growth in non sterile soil.

Bolemberg and Lutenberg (2001) suggested that PGPR- mediated induced systemic resistance ISR is an important mechanism of biological disease control. They can hold soil aggregates, creating channels through which roots grow, soil fauna move and water percolates. Efficient PGPR should survive in the rhizosphere, make use of nutrients exuded by the plant roots, proliferate, be able to colonize the entire root system and compete with indigenous microbes. Ramamoorthy *et al.* (2001) confirmed that PGPR mediated ISR results in the reinforcement of plant cell wall by lignin, callose and phenolic compounds, alteration of the physiological and biochemical reaction of the plant cell and productions of antimicrobial substances, such as pathogenesis- related protein and phytoalexin. The prominent plant growth regulators (PGRs) and their analogues produced by PGPR are auxins, cytokinins, and gibberellins. Some PGPRs also release a blend of volatile components that promote growth (Ryu *et al.*, 2003).

Plants develop an enhanced defensive capacity against a broad spectrum of plant pathogens after colonization of the roots by selected strains of nonpathogenic biocontrol bacteria. In *Arabidopsis thaliana*, this induced systemic resistance (ISR) functions independently of salicylic acid but requires an intact response to the plant hormones jasmonic acid (JA) and ethylene. Pieterse *et.al.* (2000) in their investigation found that upon treatment of the roots with ISR inducing WCS417r bacteria, neither the JA content, nor the level of ethylene evolution was altered in systemically resistant leaves. These results indicate that rhizobacteria- mediated ISR is not based on the induction of changes in the biosynthesis of either JA or ethylene. However, in ISR-expressing plants the capacity to convert 1-aminocyclopropane-1-carboxylate (ACC) to ethylene was significantly enhanced, providing a greater potential to produce ethylene upon pathogen attack.

Strains isolated from naturally disease - suppressive soils, mainly fluorescent *Pseudomonas* Spp. have been demonstrated to reduce plant diseases by suppressing soil-borne pathogens. Some of these biological control strains are also able to reduce disease caused by foliar pathogens by triggering a plant mediated resistance mechanism called induced systemic resistance (ISR). The potential of PGPR in relation to improved tea growth has also been recognized by Pandey *et al.* (2000), Pandey and Palni (2002).

Rhizosphere colonization is one of the first steps in the parthenogenesis of soil borne microorganisms. It can also be crucial for the action of microbial inoculants used as biofertilizers, biopesticides, phytostimulators and bioremediayors. *Pseudomonas*, one of the best root colonizers is therefore used as a model root colonizer. Lugtenberg *et al.* (2001) focused on (a) the temporal spatial description of root colonizing bacteria as visualized by confocal laser scanning microscopical analysis of autofluorescent microorganisms, and (b) bacterial genes and the traits used for the colonization of root and of animal tissues, indicating the general importance of a study.

Plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonas were isolated from the rhizosphere of rice and sugarcane by Kumar *et al.* (2002). Among 40 strains that were confirmed as *Pseudomonas fluorescens, 18* exhibited strong antifungal activity against *Rhizoctonia bataticola* and *Fusarium oxysporum,* mainly through the production of antifungal metabolites. Genotyping of these *P. fluorescens* strains was made by PCR-RAPD analysis, since differentiation by biochemical methods was limited.

Mikanova and Novakova (2002) reported that Microbial solubilization of hardly soluble mineral phosphates in soil is an important process in natural ecosystem and in agricultural soil. Regulation of the P-solubilizing activity by the presence of soluble phosphates in medium was determined. For this reason they decided to test a number of soil bacteria showing a high P-solubilizing activity for its sensitivity to the presence of soluble dihydrogen potassium phosphate in medium. At these studies, the direct determination of the solubilized phosphate in medium was masked by the presence of relatively high concentration of soluble phosphate added. Therefore, we have modified the method, determining the residual tricalcium phosphate. The effect of soluble phosphate in medium on the P-solubilizing activity of rhizosphere isolates and strains of Rhizobium were tested in liquid cultures with the addition of various concentration of soluble KH2 PO4. The medium was filtered after incubation and the remaining tricalcium phosphate was separated by filtrations. Filter papers with the remaining tricalcium phosphate were hydrolyzed with 2N H2SO4. Phosphorus was determined spectrophotometrically. The P-solubilizing activity was expressed as a difference between the tricalcium phosphate added and its reminder after the incubation. These

results fully confirmed that there exist the strains, whose P-solubilizing activity is inhibited and other strains, whose P-solubilizing activity is not inhibited or is inhibited very little in the presence of soluble phosphate. The use of our adapted method was much more suitable for this type of experiments.

Rhizosphere bacteria are excellent agents to control soil-borne plant pathogens. Bacterial species like *Bacillus, Pseudomonas, Serretia* and *Arthrobacter* have been proved in controlling the fungal diseases. As they have chitinolytic activity. Non pathogenic soil *Bacillus* species offer several advantages over other organisms as they form endosphores and can tolerate extreme pH, temperature and osmotic conditions reported by Basha and Ulaganathan (2002).

Thus it is very important that organic manures should be supplied with effective strains of beneficial microorganisms. Competition for colonization sites and nutrients, scavenging nutrients, niche exclusion, production of anti- fungal compounds, excretion of sideriophores, growth inhibition via the production of bacteriocins and antibiotics, and induction of plant resistance have been the mechanisms involved in the disease suppressions reported by Ongena *et al.* (2002). Some of the characterized antibiotics produced are phenazines (Phz), Pyouteorin(Plt), Phloroglucinols(Phl), pyrrolitrin (Prn) and hydrogen cyanide (HCN).

Pieterse *et al.* (2002) confirmed that to protect themselves from the disease, plants have evolved sophisticated defense mechanisms in which the signal molecules salicylic acid, jasmonic acid and ethylene often plays crucial roles The phenomenon of systemic acquired resistance (SAR) suggests that there is a signal that originates at the site of elicitor (biotic or abiotic) application and moves throughout the plant. The activation of SAR turns the compatible plant–pathogen interaction into an incompatible one, reported by Uknes *et al.* (1992). This resistance was correlated with the accumulation of pathogenesis related (PR) proteins, generally assumed to be markers of defense response added by Ward *et a*l. (1991).

Experiments were conducted during 2000 and 2001 to determine the effects of floral and foliar application of the bacterial strain *Bacillus* OSU 142 on the yield., growth and nutrient element composition of leaves of the apricot *cultivar Hacihaliloglu* grown in the Malatya province of turkey. In 2000, trees were sprayed with a bacterial suspension at

full bloom, and 30 and 60 days after full bloom. This experiment demonstrated significant differences in yield, shoot length and nutrient element composition of leaves only on trees treated at the full bloom stage. In view of this, the bacterial application was performed only at full bloom in 2001. The average increase in yield in 2000 and 2001 was 30% and 90% respectively, compared with the untreated control. Shoot length development was significantly higher when trees were treated with OSU 142 at full bloom stage in both years. Similarly, N, P, K, Ca and Mg contents of leaves were higher on OSU 142-treated trees then on the untreated control. The results of this study by Esitken *et al.* (2003) suggest that OSU 142 has the potential to increase the yield of apricot trees.

PGPRs play important role in phytostimulation, phytoremediation and biofertilization. PGPR improve plant growth in two different ways, directly or indirectly. The direct promotion of plant growth by PGPR is through production of plant growth promoting substances or facilitation of uptake of certain nutrients from the soil. On the other hand, PGPR can also prevent the proliferation of phytopathogens and thereby support plant growth. One of the mechanisms involved here is through their ability to produce sideriophores for sequestering iron. The secreted sideriophore binds to the Fe^{3+} that is available in the rhizosphere and thereby effectively prevent growth of pathogen in that region which was confirmed in the work of Kumar *et al.*(2002). Important traits of PGPR include production of EPS (Exopolysachharide), plant hormones, siderophores, bacteriocins, solubilisation of phosphorus and calcium and antibiotic resistance.

Borling *et al.* (2001) observed that, unfavorable pH and high reactivity of aluminum and iron in soil decrease P availability as well as added P- fertilizer; however there is a possibility of greater utilization of unavailable P form by the action of phosphate mobilizing micro-organism. Previous works have shown the potential of local beneficial microorganisms for nutrient uptake and crop production in central Africa.

Soil microbiota communities have demonstrated their crucial role in maintaining the soil ecological balance and therefore the sustainability of either natural ecosystem or agroecosystem. Rhizospheric microbe plant interactions have a great influence on plant health and soil quality since these root- associated microorganisms are able to help the host plant to deal with drought, nutritional and soil- borne pathogens stress conditions.

Plant growth promoting rhizobacteria (PGPR) can be considered among rhizosphere-beneficial microorganisms. In a micropropagated plant system, bacterial inoculation at the beginning of the acclimatization phase must also be observed from the perspective of the establishment of the soil microbiota rhizosphere (Hao *et al.*, 2002).

Three strains of plant growth promoting fluorescent Pseudomonas (HPR6, RRLJ008 and RRLJ134) were studied for their effects on growth and yield of French bean (*Phaseolus vulgaris* L.) under field conditions by Boruah *et al.* (2003). They examined the effects of these strains on nature of root developments and leaf palisade tube length. The strains induced positive response on growth and physiological parameters resulting in higher yield in *P. vulgaris*. Strain HPR6 produced the most promising results in thickening of leaf palisade layer, spreading of lateral roots and production of root hairs. The increase in specific leaf weight (SLW), net assimilation rate (NAR) and relative growth rate (RGR) by these strains was 68%, 152% and 167% respectively. The growth and yield parameters were also significantly improved compared to the uninoculated control. Antibiotic resistant mutant strains demonstrated that these bacteria effectively colonized the rhizosphere of French bean. The results suggest that the strains could be developed for field application on a large scale.

Bacterial species employ complex communication mechanisms termed quorum-sencing (QS) that link cell density with gene expression. In this process diffusible signal molecules, auto inducers like acyl-homoserine lactones, accumulate in the extra cellular environment, attain a critical threshold concentration and trigger the response which leads to gene expression. Besides operation of QS in the rhizosphere, it is apparent that some cross- talk between bacterial forms also occurs: plant growth promoting bacteria such as *Pseudomonads, Bacilli*, etc. can influence the operation of QS systems in plant pathogenic forms. At threshold cell-density level, bacteria produce substances that inhibit proliferation of pathogens; beneficial bacteria responsible for nitrogen fixation on the other hand, use QS to optimize nodule formation on plants roots, reported in the review of Sharma *et al.* (2003).

Colonization of the rhizosphere by microorganisms results in modifications in plant growth and development. The review by Persello *et al.* (2003) examined the mechanisms involved in growth promotion by plant growth promoting rhizobacteria

which are divided into indirect and direct effects. Direct effects include enhanced provision of nutrients and the production of phytohormones. Indirect effects involve aspects of biological control, the production of antibiotics and iron chelating siderophores and the induction of plant resistance mechanisms. The study of the molecular basis of growth promotion demonstrated the important role of bacterial traits (motility, adhesion, and growth rate) for colonization. New research areas emerge from the discovery that molecular signaling occurs through plant perception of eubacterial flagellins. Recent perspectives in the molecular genetics of cross–talking mechanisms governing plant-rhizobacteria interactions were also discussed.

Tank and Saraf (2003) isolated nine different bacteria from the rhizosphere of field grown Trigonella and further identified as *Bacillus, Pseudomonas, Azotobacter, Rhizobium* and *Azospirillum.* They were examined for solubilization of phosphate and production of exopolysaccharide and indole acetic acid. In addition to pseudomonas isolate (TP2), Rhizobium also showed high tricalcium phosphate solubilizing activity both solid and liquid medium. Increased incubation time and shake conditions improved the level of phosphate solubilizing activity in *Bacillus* isolate TB3. *Azospirillum* produced more exopolysaccharide and its hexose content was also higher in comparison to other isolates. Indole acetic acid production was maximum in *Rhizobium* after 96 h of incubation. The study supports that *Rhizobium* can be considered as an important PGPR for legume as well as non- legumes.

A series of laboratory, greenhouse and field experiments were conducted by Niranjan *et al.* (2003) on the strains of plant growth promoting rhizobacteria. The PGPR were tested as suspension of fresh cultures and talc- based powder formulation. Evaluations were conducted on pearl millet (*pennisetum glaucum)* for growth promotion and management of downy mildew caused by *Sclerospora graminicola.* All treatments with fresh suspension and powdered formulation showed enhancement in germination and vigor index over the respective untreated controls. With fresh suspensions, maximum vigor index resulted from treatments by *Bacillus pumilus* strain INR7 followed by *B. subtilis* strain IN937b (64 and 38% higher then the untreated control, respectively) with powdered formulation, treatment with strain INR7 also resulted in the highest germination and vigor indexes, which were 10 and 63% , respectively, over the untreated

control. Under experimental plot conditions, prominent enhancement in growth also was observed in the disease tests. Yield was enhanced 40 and 37% over the untreated control by seed treatment with powdered formulations of strains INR7 and SE34, respectively. The same strains also increased yield by 36 and 33%, respectively. When applied as fresh suspensions. Studies on downy mildew management resulted in varied degrees of protection by the PGPR both under greenhouse and field conditions. With fresh suspensions, treatment with INR7 resulted in the highest protection (57%), followed by *B. pumilus* strain SE34 and *B. subtilis* strain GBO3, which resulted in 50 and 43% protection, respectively, compared with the untreated control. With powdered formulation, PGPR strain INR7 suppressed downy mildew effectively, resulting in 67% protection, while SE34 resulted in 58% protection, followed by GBO3 with 56% protection. Treatment with Apron (Metalaxyl) resulted in the highest protection against downy mildew under both greenhouse and field conditions, thus, the present study suggest that the tested PGPR, both as powdered formulations and fresh suspensions, can be used within pearl millet downy mildew management strategies and for plant growth promotion.

The search for PGPR and investigation of their modes of action are increasing at a rapid pace as efforts are made to exploit them commercially as biofertilizers. These microorganisms have important contributions towards the growth and development of plants. Plant growth promoting rhizobacteria increase plant growth directly by producing hormones, siderophores or by solubilizing phosphates or indirectly either by the suppression of well known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens. Considering the importance of the role of PGPRs in agriculture and understanding their mechanisms of action, several authors (Lugtenberg *et al.*,2001; Whipps,2001; Haas and Keel, 2003;, Morris and Monier, 2003; Morgan *et al.*, 2005) have reviewed this topic exhaustively.

Efficacy of seven strains of *Pseudomonaas fluorescens (Pfs* 17), plant growth promoting rhizobacteria (PGPR), were tested by Sharma *et al.* (2002) under field condition for their ability to protect *Cicer arietinum* against *Sclerotium rolfsii* infection. Best protection was observed in strains *pfs3* where 23% seedling mortality was recorded in comparision to 44% in non- treated control. To correlate the induction of phenolic

compounds by the PGPR with disease resistance, qualitative and quantitative alterations of phenolic conpounds in different parts of *C. arietinum* were estimated following PGPR application as seed trratment. High performance liquid chromatographic (HPLC) analysis of the leaves, collars and roots of the PGPR –treated and nontreated (control) plants showed the presence of gallic, ferulic, chlorogenic and cinnamic acids with varied amounts in the PGPR treated as well as non treated plants. Maximum accumulation of cinnamic acid was observed in plants treated with *pfs*3 strain (1660ng^{1} fresh wt) which was almost 19.5 times higher then untreated control plants and also significantly high when compared to other PGPR treatments. *Pfs*3 also caused maximum accumulation of total phenolics and gallic acid in all chickpea plant as compared to other treatments and untreated control. A direct relationship between the level of total phenolics and seedling survivability was observed. PGPR –mediated induction of phenolic compounds as a biochemical barrier in *C. arietinum* against *S. rolfsii* infection was envisaged

Tank and Saraf (2003) reported that addition of PGPR and their biomolecules, help in crop production without adversely affecting the soil health. Phosphorus directly affects nitrogen fixation which is a principal yield limiting nutrient in many areas of the world, EPS production provides to the producing organisms several ecological advantages especially in ecosystem like soil and finally microbially released plant growth promoting substance like IAA have been found to show positive influence on plant growth.

According to Penrose and Glick (2003) one of the major mechanisms utilized by plant growth promoting rhizobacteria (PGPR) to facilitate plant growth and development is the lowering of ethylene levels by deamination of l-aninocyclopropane-1-carboxylic acid (ACC) the immediate precursor of ethylene in plants.the enzyme catalyzing this reaction, ACC deaminase, hydrolyses ACC to alpha-ketobutyrate and ammonia. Several bacterial strains that can utilize ACC as a sole source of nitrogen were isolated from rhizosphere soil samples. All of these strains were considered to be PGPR based on the ability to promote canola seedling root elongation under gnotobiotic conditions. The treatment of the plant seeds or roots with these bacteria reduced the amount of ACC in plants, thereby lowering the concentration of ethylene.

In order to select potential plant growth promoting rhizobacteria, a selection of strain from the predominant genera in the rhizosphere of four lupine species, based on genetic divergence criteria was carried out in a study by Gutierrez-Manero *et al.* (2003) this yielded 11 *Aureobacterium* (Aur), 4 *Cellulomonas* (Cell), 2 *Arthrobacter* (Arth), 2 *Pseudomonas* (Ps) and 6 *Bacillus* (Be) strains. Cell free culture filtrates of each bacterium were assayed for effects on germination, growth and biological nitrogen fixation (BNF) of *Lupinus albus* L.cv Multolupa seeds, or seedling. Four (Aur6,Aur9, Aur11 and Cell 1) of the twenty five strains assayed promoted germination. *Aureobacterium* 6 and Aur 9 also increased root surface total nitrogen content, and BNF. As a result of screening, and considering the entire variable studied, author suggested that Aur 6 can be considered a plant growth promoting rhizobacterium suitable for futher field trials in other plants and in different production system

Ryu *et al.* (2003) showed that some PGPRs release a blend of volatile components that promote growth of *Arabidopsis thaliana;* in particular, the volatile components 2.3-butanediol acetoin were released exclusively from two bacterial strains that trigger the greatest level of growth promotion. Furthermore, pharmacological applications of 2,3-butanediol enhanced plant growth whereas bacterial mutants blocked in 2.3- butanediol and acetion synthesis were devoid in this growth promotion capacity. The demonstration that PGPR strains release different volatile blends and that plant growth is stimulated by differences in these volatile blends establishes an additional function for volatile organic compounds as signaling molecules mediating plant – microbe interactions.

A large number of microorganisms were isolated from the rhizosphere of tea bushes ranging in age from 10-90 yrs. growing in tea estates of Darjeeling and Dooars regions, which are important tea growing regions of India. Maximum population was observed in forty year old bushes and minimum in ten year old ones. All the isolates, including both fungi and bacteria were tested for antagonism against three tea root pathogens- *Poria hypobrumea , Fomes lamaoensis and Sphaerostilbe repens* causing root rot, brown root rot and violet root rot, respectively of 105 bacterial and 50 fungal isolates tested, four bacterial isolates were antagonistic to all three pathogen. These bacteria were identified as *Serratia marcescens, Ochrobactrum anthropi, Bacillus megaterium and Bacillus pumilus.* The bacteria inhibited the growth of the pathogen in both solid and

liquid media. These bacteria, when applied as soil drench to tea seedling and potted plants of two three varieties respectively, significantly increased growth rate of both seedling and older bushed , in terms of increase in plant height, number of branches and number of leaves. *In vitro* tests with these bacteria revealed their ability, in varying degrees, to produce siderophore, indole acetic acid, volatiles and to solubilize phosphate. None of the bacteria produced hydrogen cyanide Chakraborty *et al.*, (2004)

Suryakala *et al.* (2004) isolated plant growth promoting rhizobacteria belonging to fluorescent pseudomonas from rhizosphere of rice, wheat, pigeon pea, groundnut and chilli crops. The isolates belonged to *Pseudomonas fluorescens* and produced extracellular siderophores when grown in casamino acid medium under iron deficiency. The siderophores were found to be of trihydroxamate type pyoverdines forming hexadentate legends with Fe+ 3 ions. These siderophoers were antagonistic to fungal pathogen like *Fusarium oxysporum, Alternaria sp. and Colletotricum capsicii.* Although significant control of plant pathogens or direct enhancement of plant development has been demonstrated by PGPRs in the laboratory and in the greenhouse, results in the field have been less consistent. Because of these and other challenges in screening, formulation, and application, PGPRs have yet to fulfill their promise and potential as commercial inoculants. Recent progress of their diversity, colonization ability, and mechanisms of action, formulation, and application should facilitate their development as reliable components in the management of sustainable agricultural systems. Although significant control of plant pathogens or direct enhancement of plant development has been demonstrated by PGPR in the laboratory and in the greenhouse, results in the field have been less consistent Nelson (2004).

Talc–based formulations containing cells of *Pseudomonas fluorescens, Bacillus subtilis and Saccharomyces cerevisae* were evaluated for their potential to attack the mango (*Mangifera indica*) anthracnose pathogen *Colletotricum Gloeosporiodes* under endemic conditions by Vivakananthan *et al.* (2004). The preharvest aerial spray was given at fortnightly and monthly intervals. The plant growth promoting rhizobacteria *Pseudomonas fluorescens* (FP7) amended with chitin sprayed at fortnightly intervals gave the maximum induction of flowering, a yield attribute in the preharvest stage, consequently reduced latent symptoms were recorded at he post harvest stage. An

enormous induction of the defense – mediating lytic enzymes chitinase and β- 1,3-glucanase was recorded in colorimetric assay and the expression of discrete bands in native PAGE analysis after FP7 + chitin treatment.

In greenhouse experiments conducted by Lavania *et al.* (2006)., plant growth promoting rhizobacteria (PGPR) *Serratia marcescens* NBR1213 was evaluated for plant growth promotion and biological control of foot and root rot of Betelvine caused by *Phytophthora nicotianae.* Bacterization of betelvine (*Piper betle* L.) cutting with *S. marcescens* NBRI1213 induced phenylalanine ammonia-lyase, peroxidase, and polyphenoloxidase activities in leaf and root. Qualitative and quantitative estimation of phenolic compounds was done through high- performance liquid chromatography (HPLC) in leaf and root of betelvine after treatment with *S. marcescens* NBI1213 and infection by *P. nicotianae.* Major phenolics detected were gallic, protocatechuic, chlorogenic, caffeic, ferulic, and ellagic acids by comparison of their retention time with standards through HPLC. In all of the treated plants, synthesis of phenolic compounds was enhanced compared with control. Maximum accumulation of phenolics was increased in *S. marcescens* NBRI1213-treated plants infected with *P. nicotianae.* In a greenhouse test, bacterization using *S. marcescens* NBRI1213 decreased the number of diseased plants compared to nonbacterized controls. There were significant growth increases in shoot length, shoot dry weight, root length, and root dry weight, averaging 81%, 68%, 152% and 290%, respectively,greater then untreated controls. This is the first report of PGPR- mediated induction of phenolics for biologic control and their probable role in protecting betelvine against *P. nicitianae,* an important soil- borne phytopathogenic fungus.

Field trials were conducted by Kokalis- Burelle *et al.* (2006) in Florida on bell pepper (*Capsicum annuun*) to monitor the population dynamics of two plant growth promoting rhizobacteria strains (*Bacillus substilis* strain GBO3 and *Bacillus anyloliquefaciens* strain IN937a) applied in the potting media at seeding and at various times after transplanting to the field during the growing season. In field drenches aqueous bacterial formulation were used for the mid- season applications. The effects of the applied PGPR and application methods on bacterial survival, rhizosphere colonization, plant growth and yield, and selected indigenous rhizosphere microorganisms were

assessed. The Gram- positive PGPR applied to the potting media established stable population in the rhizosphere that persisted throughout the growing season. Additional aqueous applications of PGPR during the growing season did not increase the population size of applied strains compared to treatments only receiving bacteria in the potting media; however, they did increase plant growth compared to the untreated control to varying degrees in both trials. Most treatments also reduced diseases incidence in a detached leaf assay, indicating that systemic resistance was induced by the PGPR treatments. However, treatments did not adversely affect population of beneficial indigenous rhizosphere bacteria including Fluorescent *Pseudomonas* and siderophore producing bacterial strains. Treatment with PGPR increased root diseases incidence. This fungal response to the PGPR product was likely due to an increase in nonpathogenic chitinolytic fungal strains resulting from the application of chitosan, which is a component of the PGPR formulation applied to the potting media.

In search of efficient PGPR strains with multiple activities, a total of 72 bacterial isolates belonging to *Azotobacter,* fluorescent *Pseudomonas, Mesorhizobium and Bacillus* were isolated from different rhizospheric soil and plant root nodules in the vicinity of Aligarh by Ahmad *et al.* (2006). These test isolates were biochemically characterized and were screened *in vitro* for their plant growth promoting traits like production of indoleacetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore, phosphate solubilization and antifungal activity. More then 80% of the isolates of *Azotobacter,* fluorescent *Pseudomonas* and *Mesorhizobium ciceri* produced IAA, whereas only 20% of *Bacillus* isolates was IAA producer. Solubilization of phosphate was commonly detected in the isolates of *Bacillus* (80%) followed by *Azotobacter* (74.47%), *Pseudomonas*(55.56%) and *Mesorhizobium* (16.67%). All test isolates could produce ammonia but none of the isolates hydrolysed chitin. Siderophore production and antifungal activity of these isolates except *Mesorhizobium* were exhibited by 10-12.77% isolates. HCN production was more common trait of *Pseudomonas* (88.89(%) and *Bacillus* (50%). On the basis of multiple plant growth promoting activities, eleven bacterial isolates (seven *Azotobacter,* three *Pseudomonas* and one *Bacillus*) were evaluated for their quantitative IAA production, and broad –spectrum (active against three test fungi) antifungal activity. Almost at all concentration of tryptophan (50-500µg/ml), IAA production was higest in the *Pseudomonas* followed by

Azotobacter and *Bacillus* isolates. *Azotobacter* isolates (AZT_3, AZT_{13} AZT_{23}), *Pseudomonas* (Ps_5) and *Bacillus* (B_1) showed broad–spectrum antifungal activity on Muller-Hinton medium against *Aspergillus*, one or more species of *Fusarium* and *Rhizoctonia bataticola.* Further evaluation of the isolates exhibiting multiple plant growth promoting (PGP) traits on soil- plant system is needed to uncover their efficacy as effective PGPR.

Bacillus megaterium TRS-4 was isolated from tea rhizosphere and tested for its ability to promote growth and disease reduction in tea plants by Chakraborty *et al.* (2006) *In vitro* studies reavealed the ability of this bacterium to promote growth of tea plants very significantly. Brown root rot disease, caused by *F. lamaoensis* was markedly reduced by application of the bacterium to the soil. Population of *F. lamaoensis* in soil before and after application of B. *megaterium* as determined by ELISA and dot-blot using PAb raised against the pathogen, was shown to be greatly reduced in presence of the bacterium. Biochemical changes induced in tea plants were also examined by .Root colonization by *B. megaterium* and subsequent inoculation with *F. lamaoensis* led to an increase in polyphenolics, as well as in defense related enzymes peroxidase chitinase, β-1,3- glucanase and phenylalanine ammonia lyase. Determination of mechanism of action of this bacterium reavealed it to be able to solubilize phosphate, produce IAA, Siderophore and antifungal metabolism. The plant growth promotion and reduction of disease intensity have shown to be due to a combination of several mechanisms.

A total of 30 bacteria were isolated from the rhizoplane of rice *cv.* BR29 cultivated in Mymensingh, Bangladesh and from the seedlings obtained from surface-sterilized seeds of BR29 by Islam *et al.* (2007). Upon screening, 6 isolates showed varying levels of phosphate solubilizing activity in both agar plate and broth assays using National Botanical Research Institute's phosphate medium. The bacterial isolates were identified based on their phenotypic and 16S rRNA genes sequencing data as *Acinetobacter* sp. BR-12, *Klebsiella* sp. BR-15, *Acinetobacter* sp. BR-25, *Enterobacter* sp. BR-26, *Microbacterium* sp. BRS-1 and *Pseudomonas* sp. BRS-2. The BR-25 exhibited highest phosphate solubilizing activity followed by BR-15. They grew rapidly in the liquid medium at pH 5 and 7 but almost no growth occurred at pH 3. The pH value of the culture medium was decreased with bacterial growth suggesting that they might

secrete organic acids to solubilize insoluble phosphorus. Scanning electron microscope analysis of two-week-old rice seedlings germinated from seeds previously inoculated with BR-25 and BR-15 revealed dense colonization at the root surfaces presumably using fimbriae on the bacterial cells.

Sood *et al* (2008) studied Rhizospheric soils of plants for bacterial dominance and antagonism. Representatives of *Bacillus* and *Pseudomonas* genera were found to dominate the rhizosphere of established and abandoned tea bushes, respectively. Amongst the isolated species *Bacillus subtilis* and *Bacillus mycoides* appeared to be closely associated with roots of established tea bushes while the rhizosphere of abandoned tea bushes was dominated by *Pseudomonas putida*. Four isolates of both *B. subtilis* and *P. putida* were selected on the basis of maximum antibacterial activity. The bacteriocin- like activity of *B. subtilis* and *P. putida* strains was detected to be active over a range of temperature 0-50°C and was sensitive to proteolytic enzymes. Incubation of indicator strains with different concentrations of bacteriocin- like substances confirmed their bacterial activity. Various species of *Bacillus* and *Pseudomonas* behaved antagonistically amongst themselves due to the production of bacteriocins under in vitro conditions.

Biodegradation of miticide propargite was carried out *in vitro* by selected *Pseudomonas* strains isolated from tea rhizosphere. A total number of 13 strains were isolated and further screened based on their tolerance level to different concentrations of propargite. Sarkar et al., (2009) selected five best strains were and further tested for their nutritional requirements. Among the different carbon sources tested glucose exhibited the highest growth promoting capacity and among nitrogen sources ammonium nitrate supported the growth to the maximum. The five selected *Pseudomonas* strain exhibited a range of degradation capabilities. Mineral salts medium (MSM) amended with glucose provided better environment for degradation with the highest degradation potential in strain SPR 13 followed by SPR 8 (71.9% and 69.0% respectively).

Chakraborty *et al.* (2009) also evaluated one of the PGPRs, *Ochrobactrum anthropi* TRS-2 isolated from tea rhizosphere and its talc based formulation for growth promotion and management of brown root rot disease of tea caused by *Phellinus noxius*. *O. anthropi* could solubilize phosphate, produce siderophore and IAA *in vitro* and also

exhibited antifungal activity against six test pathogens. Application of an aqueous suspension of *O. anthropi* to the rhizosphere of nursery grown tea seedlings of five varieties of tea (TV-18, T-17, HV-39,S-449 and UP-3) led to enhanced growth of the treated plants, as evidenced by increase in height, in the number of shoots and number of leaves per shoot. Treatment with *O. anthropi* also decreased brown root rot of tea. Multifold increase in activities of chitinase, β-1,3- glucanase, peroxidase and phenylalanine ammonia lyase in tea plants was observed on application of *O. anthropi* to soil followed by inoculation with *P. noxius*. A concomitant increase in accumulation of phenolics was also obtained. Further, talc based formulation of *O. anthropi* was prepared and its survival determined every month up to a period of 12 months. *O. anthropi* could survive in the formulation up to a period of 9 months with a concentration of 7.0 $\log_{10}$ CFU g-1, after which there was a decline. Talc formulation was as effective as aqueous suspensions in both plant growth and disease suppression.

Phosphate solubilizing fungi

Phosphorus is one of major limiting factors for crop production on many tropical and subtropical soils as a result of high phosphorus fixation, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants. The concentration of soluble phosphorus (P) in tropical soil is usually very low. While most mineral nutrients in soil solution are present in millimolar amounts, phosphorus is only available in micromolar quantities or less. The majority of applied phosphorus is rapidly fixed in soil into fractions that are poorly available to plant roots. Inorganic phosphates are predominant form of inorganic phosphates in neutral or calcareous soils (Russel, 1973; Sample *et al.*, 1980; Ozanne, 1980; McLaughlin *et al.*, 1988; Dey, 1988; Sanyal and De Datta, 1991; Goldstein, 1994; Norman *et al.*, 1995; Yadav and Dadarwal, 1997; Gyaneshwar *et al.*, 2002).

Pikovskaya (1948) isolated a bacterium from soil and P bearing rocks which he called P bacterium having the ability to form water soluble P from insoluble calcium phosphate. PSMs solubilize insoluble phosphates into soluble form in soil by secreting formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. These acids lower the pH and bring about solubilization. Glucose, sucrose and galactose are the best carbon

source for phosphate solubilization. Decrease pH in the medium during phosphate solubilization is due to the release of organic acid by isolates. Gluconic acid is the most commonly produced acid during phosphate solubilization other mechanism like co_2 and H_2S production and chelation of other acids are also responsible for phosphate solubilization.

Compared with the other major nutrients, phosphorus is by far the least mobile and available to plants in most soil conditions. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. Phosphorus is added in the form of phosphatidic fertilizers, part of which is utilized by plants and the remainder converted into soluble fixed forms. To circumvent phosphorus deficiency, phosphate-solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner.

AM fungi, on the other hand, encourage the plant roots to rapidly absorb solubilized P. Kucey *et al.* (1989) in their review shown that, accordingly the increase in plant growth may be due to the release of certain plant growth promoting substances by the PS organism or AM development and mycorrhizal formation. As we know that Phosphorus is one of the major nutrients limiting plant growth but Most of the soils throughout the world are P deficient and therefore require P to replenish the P demand by crop plants. To circumvent the P deficiency in soils, P fertilizers are applied. However, after application, a considerable amount of P is rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils reported by Norrish and Roster (1983) or Ca in calcareous soils before plant roots have had a chance to absorb it. Further, the use of rock phosphate as a phosphate fertilizer and its solubilization by microbes through the production of organic acids have become a valid alternative to chemical fertilizers, investigated by Kang et al (2002). Rock phosphate is widely distributed throughout the world, both geographically and geologically, confirmed by Zapata, and Roy (2004), in conjugation with phosphate solubilizing microorganism. Rock phosphate provides a cheap source of P fertilizer for crop production. In this regard, several studies of Zaidi (1999), Gull *et al.* (2004) have conclusively shown that PSM solubilizes the fixed soil P and applied phosphates, resulting in higher crop yields. The alternative

approach is to use these PSM along with other beneficial rhizospheric microflora to enhance crop productivity. in this context, the simultaneous application of Rhizobium and arbuscular mycorrhizal fungi by Zaidi *et al.* (2003) has shown to stimulate plant growth more then inoculation of each microorganism alone in certain situation when the soil is P Deficient.

There are many possible reasons why AM fungi fail to show extensive and continuous growth unless they are part of a symbiotic partnership with a host root. It is postulated that they may have a simple nutritional requirement which due to our lack of knowledge has not yet been fulfilled, or it may be necessary to supply some nutrients continuously at a low concentration or else these fungi may have lost a considerable part of their genetic material and this necessitates their interaction with the host's metabolisms. Becard and Fortin (1988) confirmed that growing root organ culture with an AM fungi under aseptic conditions was developed as an alternative system. Another important step forward was root exudates (quercetin, kaemferol, flavonoid, aglycover, formononetin and biochavin) which stimulated the hyphal growth of *Gigaspora margarita* was reported by Diop *et al.* (1992) and Balaji *et al.* (1995).

Mycorrhizal plants show an enhanced growth rate as a result of improved phosphorus uptake the greater drought resistance of AM plants is probably an indirect benefit of improved phosphorus nutrition. Mycorrhizal plants may survive better under competitive field condition then non-mycorrhizal plants. Mycorrhizal fungi encourage the bacteria which grow on or near the hyphal surface or mycorrhizosphere reported by Nelson (1987). Many of these bacteria are antagonistic to plant pathogens or otherwise beneficial to plant growth. Later on Linderman (1994) showed that this antagonism, or improved phosphorus nutrition, may account for reports of arbuscular mycorrhizal fungi protecting hosts from pathogens. The benefits of these kind of symbiosis is, the colonized plant is better nourished and better adapted to its environments as it obtains increased protection against environmental stresses, including drought, cold, salinity and pollution which is confirmed in works of several authors (Davis and Young, 1985; Sylvia and Williams, 1992; Leyval *et al.*, 1994; Charest *et al.*, 1994, Subramanian *et al.*, 1995; Paradis *et al.* 1995; Shetty *et al.*, 1995). In addition, symbiosis tends to reduce the incidence of root diseases and minimizes the harmful effect of certain pathogenic agents

added by Dehne (1982). Mycorrhizal fungi provide an effective alternative method of disease control especially for those pathogen which affect the below ground plant parts was reported by Mukherji *et al.* (1996).

Chemical fertilizer have played a significant role in the green revolution , but unbalanced use of them, had led to reduction in soil fertility and to environmental degradation, reviewed by Gyaneshwar *et al.* (2002). Phosphate availability in soil is greatly enhanced through microbial production of metabolites leading to lowering of PH and release of phosphate from organic and inorganic complexes. A survey of Indian soil revealed that 98% of soils are deficient in Phosphorous. Although P content in an average soil is 0.05% but only a fraction of this (about 0.1% of the total P present in soil) is available to the plants because of its chemical fixation and low solubility. Chemical phosphate fertilizer and their reaction products are only sparingly soluble under the condition in which they are applied to the soil. However, under such condition microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to plants. PSM include largely bacteria and fungi, which can grow on various phosphorous containing compounds such microbes not only accumulate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement. Rhizosphere microorganisms also have the ability to assimilate different macro- and micro- nutrients and release them to the soils. These nutrients are utilized by the plants in a mutualistic way. Rhizosphere microorganisms have been found to solubilize the low soluble calcium phosphates via the production of organic acids and chelating via the production of organic acid and chelating oxo- acids and make them available to the plants reported by Nwaga *et al.* (2000). In particular, soil micro-organisms are effective in releasing P from inorganic P through solubilization, and form organic pools of total soil P by mineralization.

Brunderett (1991) confirmed that the close contact created between the plant and fungus through the filamentous network allows the exchange of nutrients for the survival and growth of the two partners. First, the wide dispersal of the fungus in the soil through its large filament network gives the fungus access to a much larger volume of soil then the roots system itself. The fungus filaments act more or less as a pump, supplying the root with a supplement of water and mineral salts to which it normally would not have

access. In return, the fungus receives from the plant metabolized nutrients that it is unable to synthesize itself, such as sugars, amino acids and secondary metabolites. EMC are the mycorrhizae of many dominant forest trees. The fungi are basidiomycetes or ascomycetes. EMC fungi may spread from root to root or by spores carried by animals or the wind. It may grow on artificial media, but in nature most fungal growth is in association with a host plant

Role of AM fungi in uptake of phosphorus and nitrogen from soil was studied by George *et al.* (1995). Colonization of plant roots by AM fungi can greatly increase the uptake of phosphorus and nitrogen. The most prominent contribution of AM fungi to plant growth is due to uptake of nutrients by extraradicle mycorrhizal hyphe. Quantification of hyphal nutrient uptake has become possible by the use of soil boxes with separated growing zones for roots and hyphae. Many tested fungal isolates increased phosphorus and nitrogen uptake of the plant by absorbing phosphate, ammonium and nitrate from soil. However, compared with the nutrient demand of the plant growth the contribution of AM fungi to plant phosphorus uptake is usually much larger than the contribution to plant nitrogen uptake.

The utilization of soil nutrients may depend more on efficient uptake of phosphate, nitrate and ammonium from the soil solution even at low supply concentration than on mobilization processes in the rhizosphere. In contrast to ectomycorrhizal fungi, non soluble nutrient sources in soil are used to a limited extent by hyphae of AM fungi. Side effects of mycorrhizal colonization on plant health or root activity may also influence plant nutrient uptake. Mycorrhizal fungi produce siderophore was suggested by Haselwandter (1995). Data on mycorrhizal fungi and their potential to produce siderophores are reviewed. Based on a bioassay with *Aureabacterium flavescens* J G-9 it was shown that a number of ectomycorrhizal fungi produce hydroxamate siderophores. An AM grass species which showed greater iron uptake than non mycorrhizal controls tested positively when bioassayed for hydroxamate type siderophores. Ericoid mycorrhizal fungi can also produce hydroxamate type siderophores. However, only in the case of the ericoid mycorrhizal fungi the main siderophores have been isolated and subsequently identified as ferricrocin and fusigen, respectively.

This resistance was correlated with the accumulation of pathogenesis related (PR) proteins, generally assumed to be markers of defense response reported by Ward *et al.* (1991). The phenomenon of systemic acquired resistance (SAR) suggests that there is a signal that originates at the site of elicitor (biotic abiotic) application and moves throughout the plant. The activation of SAR turns the compatible plant pathogen interaction into incompatible one observed by Uknes *et al.,* (1992) salicylic acid is the one among the plant derived substances that has been demonstrated to be an inducer of SAR. These observations led Davis and Ausubel, (1989] come to the conclusion, that there is a mobile signal that travel from the site of application of elicitor to distal plant part and is involved in lignifications of host cell with increase in phenyl propanoid derivatives, which are considered to be the factors involved in the plant defense against pathogen.

Studies on the survival of inocula in suspension showed the following results: at room temperature, inoculum potential of *Gigaspora sp.* decreased after 2 weeks; that of *Glomus mosseae* after 6 weeks and *G. fasiculatum* after 8 weeks. Under refrigeration, inoculum potential of *Gigaspora* sp.decreased drastically after 8 weeks, that of the 2 Glomus spp. also decreased after 8 weeks. At 40°C, inoculum potential of the 3 VAM fungi decreased after 8 weeks.for mycorrhizal roots reduction of inoculum potential for all 3 VAM fungi was observed only after 18 months of storage at ambient condition. Although the inoculum potential did not change considerably there was a reduction of the estimated number of infective propagules after storing them for 20 months at 4°C and refrigeration temperature reviewed by Luis *et al.* (1992).

Brown *et al.* (1995) conducted experiments to develop a method for the production of VAM starter inoculum using Leonard jar and plastic tumbler. The set up was maintained at growth room conditions provided with light intensity of 10-15 klix, photo period at 14 hours a day and temperature at 25-30°C. Among the three host plant and potting media evaluated, corn and perlite combination was found superior for root inoculum production. The composition of the nutrient solution was found to have a great influence on morphological anatomy of mycorrhizal root inoculum and the number of infective propagules. At high concentration of N and P neither arbuscules nor vesicules formed. Both become prominent with increasing dilution of these 2 elements in nutrient

solution. Increasing amount of F, Ca, and Mg from 20pm to 60ppm had no remarkable effect on VAM colonization.

There are several determinants for mechanisms of growth promotion that include bacterial synthesis of the plant hormones, indole 3 acetic acid (IAA), cytokinin, and gibberellin, breakdown of plant produced ethylene by bacterial production of 1-aminocyclopropane-1-carboxylate (ACC), deaminase, and increased mineral and N availability in the soil Glick (1995). PGPR can directly act as antagonists by productions of antimicrobial compounds such as antibiotics, competition for nutrients, or parasitism in the rhizosphere Bower and Rovuva (1999). Besides the direct interaction with soil borne pathogen, PGPR can also stimulate plant defense systemically against foliar pathogens. Phenomenon is known as induced systemic resistance (ISR) which is reported separately by Kloepper *et al.* (1992), Von Loon *et al.* (1998).For mechanism of ISR, previous works demonstrated that several bacterial determinants such as siderophores, SA, and lipopolysaccharides (LPS) contributed to ISR .Since the early 1990s, research on mechanism of biological control by PGPR revealed that some PGPR systemically induce disease resistance against a variety of pathogens in several crops, including bean, carnation, cucumber, radish, and tobacco confirmed in the work of Zhang *et al.* (2002).

Research in last few decades has established that arbuscular mycorrhizae can improve plant growth through improved mineral uptake, resistance to water stress and root pathogens, and synergistic interaction with beneficial soil microorganisms, reviewed by Bagyaraj and Varma (1995).

Under low soil P concentration, most plant species are dependent on a symbiotic association with arbuscular mycorrhizal fungi for the acquisition of P investigated by Smith and Read (1997). Under low N Fertilizer inputs, soil P availability is usually the major factor limiting the rate of N_2- fixation in legume crops reported by Toro *et al.* (1998) and, in the absence of AMF infection, supplementary of P fertilization is generally necessary for the maintenance of N_2-fixation rates by *Rhizobium* at the levels required for economically viable crop production observed by Andrade *et al.* (1998). In legumes the positive synergistic interactions among the members of the tripartite symbiotic association (rhizobium-AMF-legume) result in improved rates of P uptake N_2-fixation

and crop biomass production under conditions of reduced N and P fertilizer inputs reported by Azcon *et al.*(1991), Xavier and Germida (2002).

It has been demonstrated that changes in the soil environment, particularly in terms of its water and nutrient status, are able to appreciably affect the germination, growth, development and pathogenic behaviour of many soil microorganisms. In pathogenic fungi, quiescence of spores, mycelial lysis, and formation of resistant structures can be induced by microbial competition for nutrient and even for space. And the exogenous addition of nutrients can reverse the impact of such competition. Numerous studies have clearly shown that those mycorrhizal root systems are less susceptible to the attack of soil pathogens then non-mycorrhizal systems. Plant responses to fungal attack involve rapid induction of defense mechanisms including morphological, structural and biochemical changes, such deposition of lignin, callose and phenolic compounds, formation of papillae or cell wall appositions, and synthesis of pathogenesis related proteins (PR). Among PR- proteins, two plant hydrolases, ß-1.3-glucanase and chitinase, these two enzymes are of particular interest because many pathogenic fungi contain ß -1, 3- glucans and chitin as major structural cell wall components. And these two enzymes have the capability to degrade fungal wall components, resulting in growth inhibition of fungi. Phosphorus is an essential component of nucleic acids, ATP and other metabolites which are involved in important processes of cell metabolism. Phosphate solubilizing bacteria solubilize insoluble phosphate and make it available for plant growth, development and reproduction. Suppression of plant pathogens by microorganisms to reduce disease incidence is referred as biocontrol without disturbing ecological balance. With increasing awareness of environmental hazards, bio based technologies for sustainable agriculture and biopesticides can provide long lasting, effective solutions. If some of the phosphates solubilizing bacteria are found antagonistic to plant pathogens, the combinations of the two attributes will be of great advantage in crop production. in this present scenario, eco-friendly alternative strategies such as use of bacteria from phylloplane rhizosphere and endophytic bacteria have been explored by Mondol *et al.* (1999), Saha *et al.* (2001) and Bhowmik *et al.* (2002),

Arbuscular mycorrhizal fungi are well known to bring about biochemical changes in plants by increasing various enzymatic activities. Acid phosphatase and nitrate reductase are important enzymes of phosphorus and nitrogen metabolism, respectively. Peroxidase and polyphenol oxidase are important components of the defense mechanism of plants against pathogens. Phenols are also important in plants disease resistance, reviewed by Panwar and Vyas (2002).

The efficiency of eight AM fungi, *Acaulospora mellea* Spain & Schenck, *Gigaspora margarita* Backer & Hall , *Gigaspora gigantica* (Nicol.& Gerd.) Gerd. &Trappe, *Glomus deserticola* Trappe, Bloss & Menge, *Glomus fasciculatum* (Thaxter sensu Gred.) Gred. &Trappe, *Sclerocystis rubiformis* Gred. & Trappe, *Scutellospora calospora* (Nicol.&Gred.) Walker & Sanders and *Scutellospora niger* (Red head) Walker & Sanders, collected from rhizosphere soils of *Acacia leucophloea,* were evaluated on the same host for nutrient uptake and enhancement of acid phosphatase, NR, PRO and PPO activities. Analysis performed after 180d of inoculation showed maximum beneficial effects with *G. deserticola* reported by Panwar and Vyas (2002)

Beneficial plant – microbe interactions in the rhizosphere are primary determinates of plant health and soil fertility. Jeffries *et al.* (2003) in their work observed that Arbuscular mycorrhizas are the most important microbial symbioses for the majority of plants important microbial symbioses for the majority of plants and under conditions of P- limitation, influence plant community development, nutrient uptake, water relations and above ground productivity. They also act as bioprotectants against pathogens and toxic stresses. The mechanism by which these benefits are conferred through abiotic and biotic interactions in the rhizosphere. Attention is paid to the conservation of biodiversity in arbuscular mycorrhizal fungi (AMF). Examples are provided in which the ecology of AMF has been taken into account and has had an impact in landscape regeneration, horticulture, alleviation of desertification and in the bioremediation of contaminated soils. It is vital for the soil scientists and agriculturalists pay due attention to the management of AMF in any schemes to increase, restore or maintain soil fertility.

AM associations are integral functioning parts of plant roots and are widely recognized as enhancing plant growth on severely disturbed sites, including those contaminate with heavy metals. They are reported to be present on the roots of plants

growing on heavy metal contaminated soils and play an important role in metal tolerance and accumulation. Isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems. This review of Gaur & Adholeya (2004) highlights the potential of AM fungi for enhancing phytoremediation of heavy metal contaminated soils.

Soil microbial populations are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural system and natural ecosystems. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low- input biotechnology, to help sustainable, environmental- friendly, agro technological practices. Much research is addressed at improving understanding of the diversity, dynamics, and significance of rhizosphere microbial populations and their co-operative activities. An analysis of the co-operative microbial activities known to affect plant development was the general aim of the review by Barea *et al.* (2005). In particular, they summarized and discussed significant aspects of this general trophic including (i) the analysis of the key activities carried out by the diverse trophic and functional groups of microorganisams involved in co-operative rhizosphere interactions; (ii) a critical discussions of the direct microbe-microbe interactions which results in processes benefiting sustainable agro- ecosystem development; and (iii) beneficial microbial interactions involving arbuscular mycorrhiza, the omnipresent fungus- plant beneficial symbiosis. The trends of this thematic area will be outlined, from molecular biology and ecophysiological issues to the biotechnological developments for integrated management, to indicate where research is needed in the future.

Morgan *et al.* (2005) discussed briefly at plants and their rhizosphere microbes, the chemical communications that exist and the biological processes they sustain. Primarily it is the loss of carbon compounds from roots that drives the development of enhanced microbial populations in the rhizosphere when compared with the bulk soil, or that sustains specific mycorrhizal or legume associations. The benefits to the plant from this carbon loss were discussed. Overall the general rhozosphere effect could help the plant by maintaining the recycling of nutrients, through the productions of hormones,

helping to provide resistance to microbial diseases and to aid tolerance to toxic compounds. When plants lack essential mineral elements such as P or N, symbiotic relationship can be beneficial and promote plant growth. However, this benefit may be lost in well fertilized agricultural soils where nutrients are readily available to plants and symbionts reduce growth. Since this rhizosphere assoctations are common place and offer key benefits to plants, these interactions would appear to be essential to their overall success.

Tarafdar *et al.* (2003) during the course of their work isolated seven efficient phosphatase producing fungi (PPF) and identified as *Aspergillus rugulosus. A. fumigatus, A. terreus, A. niger, A. parasiticus, Pseudeurotium zonatum and Trichoderma harzianum.* Their efficiency to hydrolyze different compounds of organic phosphorus (mono-and hexa) was examined. The fungi reduced the pH of the medium, which was maximum with *A. niger*. A significant negative correlation of pH with development of fungal mats was observed ($r=0.39$, $n=28$, $p<0.05$). The maximum secretion of acid phosphatase by PPF was at 21 d and alkaline phosphatase at 14d. Acid phosphatase produced by PPF was three times higher than alkaline phosphatase. The intracellular phosphatase activity was significantly higher than extra cellular activity. The efficiency to hydrolyze mono-phosphate by phosphatases released from the PPF was 4-times higher than hexa phosphate. *T. harzianum* was found to be most efficient organic P mobilize as compared to the other fungi, tested. The efficiency per unit of enzyme produced by different fungi was different and that indicated the isoenzymes being of different types.

Twenty three isolates of *Azotobacter* were obtained from pesticide contaminated soils of cotton, sugarcane, brinjal and okra fields of HAU farm by enrichment culture technique by Suneja *et al.* (2004) All the *Azotobacter* isolates belonged to *chroococcum* species on the basis of their biochemical properties. Resistance among these isolates was studied on pesticides like endosulfan, ecalux and confidor. The result indicates that resistance to pesticides is common among *Azotobacter* soil isolates. Decreased resistance with increasing concentration of pesticide and reduced among of carbon observed.

Ecology and diversity of phosphate solubilizing microorganisms in 20 soil samples comprising organic and non-organic farming, virgin and barren soils of Gujarat, India were studied by Haque and Dave (2005). No considerable seasonal variation in

population densities of various phosphate solubilizers was observed. Out of 40 phosphate solubilizing microorganisms, *Pseudomonas* spp., *Bacillus* spp., *Saccharomyces* spp. and *Aspergillus niger* were found to be most prevalent as they were present in more then 50% of the soil samples. The phosphate solubilization index for different cultures varied between 104to 240. When the isolates, which gave good phosphate solubilization on solid medium, were further explored for phosphate solubilization in liquid medium, the phosphate solubilization ability decreased to as low as 8% and increased to as high as 99% by these cultures. Moreover , phosphate solubilization in the range of 18.1 to 166.3% was recorded when microbial consortia in the form of soil suspension were inoculated, inspite of semi arid nature of the ecosystem, considerable phosphate solubilizing microbial activity observed indicate the fertility status of the soil in terms of phosphate mobilization.

Mishra *et al.* (2005) isolated fluorescent *Pseudomonas* strain from soil under tea cultivation and designated as RRLJ 134 exhibited in vitro antibiosis against *Fomes lamoensis*, the causal organism of brown rot disease of tea on three different synthetic media. Dressing of two year old tea cutting with this strain enhanced the shoot height, root length, number of buds, leaves and chlorophyll content of the newly emerged leaves in the nursery condition. Also a statistically significant increase in fresh and dry weight of root, shoot, leaf and a bud was noted against control. The application of this strain also showed significant reduction in the number of infected tea cuttings in soil amended with *F. lamoensis* under nursery condition; the result indicates the possible use of this strain as a biocontrol agent of brown rot disease of tea besides enhancing the crop production.

Trivedi *et al.* (2005) studied the microbial diversity of Indian Himalaya and based on a detailed study conducted to isolate microbes from soil samples collected from various tea gardens located in region, two bacteria namely *Bacillus subtilis* and *Pseudomonas corrugate* have been selected as promising inoculants for field application in tea gardens. Bioassays based on the inoculation of seed raised and tissue culture raised tea plants had earlier indicated the biocontrol and growth promotion properties of selected bacteria. With a view to introduce these bacterial isolates eventually in the gardens, suspension cultures were raised and applied in the rhizosphere region of both seedling and cutting raised young tea plants under net- house conditions. Monthly

enumeration of bacterial fungal and actinomycetes populations, up to a period of one year, indicated excellent rhizosphere colonization by the inoculated bacteria. The presence of introduced bacteria in the rhizosphere was confirmed by the use of antibiotic markers. The inoculated tea plants have been transferred to new plantation sites, near Kausani in District Bageshwar, Uttaranchal for further monitoring of growth and overall performance.

Seventeen rhizobacteria isolated from different ecological regions, i.e. Brazil, Indonesia, Mongolia and Pakistan were studied by Hafeez *et al.* (2006) to develop inoculants for wheat, maize and rice. Almost all the bacterial isolates were Gram-negative, fast growing motile rods and utilized a wide range of carbon sources. These isolates produced indole- 3-acetic acid at concentration ranging from 0.8- 42.1 µg/ml, irrespective of the region. Fifteen isolates fixed N at rates ranging from 20.3-556.8 nmole C_2H_2 reduced/h/vial. Isolate 8n-4 from Mongolia produced the highest amount of indole-3-acetic acid (42.1 µg/ml), produced siderophores (0.3mg/ ml) and was the only isolate that solubilized phosphate (188.7 µgP/ml). Inoculation of the wheat variety Orkhon with 8n-4 isolate resulted in the maximum increase in plant biomass, root length, and total N and P contends in plants. Random amplified polymorphism among the bacterial isolates from different geographic regions and a low level of polymorphism among isolates from the same region. The complete 16S rRNA gene sequence analysis demonstrated that 8N-4 is a *Bacillus pumilus* strain, it was concluded that *Bacillus oumillus* 8N-4 can be used as a bioinoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia.

Chen (2006) advocated that the ability of a few soil microorganisms to convert insoluble forms of phosphorus to an accessible form is an important trait in plant growth-promoting bacteria for increasing plant yields. The use of phosphate solubilizing bacteria as inoculants increases the P uptake by plants. In this study, isolation, screening and characterization of 36 strains of phosphate solubilizing bacteria (PSB) from Central Taiwan were carried out. Mineral phosphate solubilizing (MPS) activities of all isolates were tested on tricalcium phosphate medium by analyzing the soluble-P content after 72 h of incubation at 30 °C. Identification and phylogenetic analysis of 36 isolates were carried out by 16S rDNA sequencing. Ten isolates belonged to genus *Bacillus*, nine to

genus *Rhodococcus*, seven to genus *Arthrobacter*, six to genus *Serratia* and one each to genera *Chryseobacterium*, *Delftia*, *Gordonia* and *Phyllobacterium*. In addition, four strains namely, *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. are being reported for the first time as phosphate solubilizing bacteria (PSB) after confirming their capacity to solubilize considerable amount of tricalcium phosphate in the medium by secreting organic acids. P-solubilizing activity of these strains was associated with the release of organic acids and a drop in the pH of the medium. HPLC analysis detected eight different kinds of organic acids, namely: citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of these isolates. An inverse relationship between pH and P solubilized was apparent from this study. Identification and characterization of soil PSB for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field application.

Kathleen *et al.* (2007) reported that Mycorrhizal fungi can contribute to soil carbon sequestration by immobilizing carbon in living fungal tissue and by producing recalcitrant compounds that remain in the soil following fungal senescence. They hypothesized that nitrogen (N) fertilization would decrease these carbon when N availability is high. They measured the abundance of two major gropes of mycorrhizal fungi, Arbuscular mycorrhizal (AM) and Ectomycrrhizal (ECM) fungi, in the top 10 cm of soil in control and N-fertilized plots within three Alaskan boreal ecosystem that represented different recovery stages following severe fire. Pools of mycorrhizal carbon included root- associated AM and ECM structures: soil associated AM hyphae: and glomalin, a glycoprotein produced by AM fungi. A total mycorrhizal carbon pool decreased by approximately 50gCm-2 in the youngest site under N fertilization, and this reduction was driven mostly by glomalin. Total mycorrhizal carbon did not change significantly in the other sites. Root- associated AM structures were more abundant under N fertilization across all sites, and root associated ECM structures increased marginally significantly. They found no significant N effects on AM hyphae. Carbon sequestered within living mycorrhizal structures (0.051-0.21gm-2) was modest compared with that of glomalin stocks within one of the three study sites. As effects on glomalin were inconsistent among sites, an understanding of the mechanism underlying this variation would improve our ability to predict ecosystem feedback to global change.

In the study carried out by Richa *et al.* (2007) *Aspergillus tubingensis* and *A. niger* were tested for their efficacy to solubilize rock phosphate (RP) and also to improve the growth of maize (*Zea mays*) in rock phosphate amended soils. Both the species was able to grow and solubilize rock phosphate and solubilize rock phosphate and soluble P levels were significantly increased in the culture medium as the concentration of RP increased. The results of nursery experiment showed that the growth of maize plants and shoot were significantly increased by these fungi compared to control soil. Soil analysis results showed that the available P, organic carbon levels were significantly increased when compared to initial soil. The soil pH was also lowered compared to initial pH of the soil. These results suggested that *A. tubingenesis* and *A. niger* serves as excellent phosphate solubilizers in alkaline soils amended with RP.

Iman (2008) isolated *Aspergillus niger* and *Penicillium italicum* from the soil and rhizosphere ofdifferent plants. They were tested for their efficacy to solubilize tri-calcium-phosphate (TCP) *in vitro* as well as their effect *in vivo* to promote the growth of soybean (*Glycine max* L.) plants grown in soil amended with TCP. The results showed high solubilizing index in agar plates. Also, they effectively solubilized TCP in Pikovskaya's liquid medium (PVK) and released considerable amounts of P into medium. The efficacy of *Penicillium italicum* to solubilize and release the inorganic P was 275 µg P ml-1 whereas *Aspergillus niger* showed better efficiency and produced 490 µg P ml-1 after seven days of incubation. Drop in pH during growth was more prominent in absence of TCA in liquid medium. This indicated that absence of soluble P in media induces the acid production. The addition of TCP to the broth media produced an increment in fungal biomass. Pot experiment showed that the dual inoculation of phosphate-solubilizing fungi (*A. niger* and *P. italicum*) significantly increased dry matter and yield of soybean plants compared to the control soil. Significant increment in percentage of protein and oil was also recorded. There was an increase in the percentage of N and P content of the plant. It was significantly resulted with N levels of soybean plants but this increase was non-significant with the percentage of total phosphorus, under the experimental conditions. Soil analysis showed that the available P, organic carbon levels were significantly

increased when compared to the initial soil. The pH was also lowered compared to the initial pH of the soil.

Dual application of beneficial microorganisms

Daft and Okusanya (1973), reported increased lignifications of xylem and more starch grains in the stem of tomato and petunia plants colonized by AM fungi, Krishna et al, (1981) observed an increased in leaf thickness, the size of the midrib vein, the mesophyll cells and number of plastids because of mycorrhizal colonization of the roots of finger millet. Certain bacteria which enhance the activity of AM fungi were described as mycorrhization helper bacteria (MHB) by Caroline and Bagyaraj, (1995). These bacteria detoxify the substrate, making it more ideal for mycorrhizal infection and produce hydrolytic enzymes that dilate the cortical cell, thereby enhancing better colonization by mycorrhizal fungi. Further they are known to produce plant growth promoting substances (Duponnois and Garbye, 1991). Co inoculation of AM fungi with MHB was found to have synergistic interaction with consequential benefit on plant growth confirmed by Jayanthi and Bagyaraj (1998).

Both positive and negative interaction of PSMs and VAM on growth and yield of plants have been reported. Plants inoculated with VAM and PSB together have been reported to mobilize significantly higher amount of P then the plants inoculated with these microorganisms separately in P deficient soils by Azcon *et al.* (1976), Barea *et al.* (1975) and Asea *et al.* (1988). PSMs survive longer around mycorrhizal then non-mycorrhizal roots of maize and lavender and sometimes act synergistically with the mycorrhizal fungus to increase plant growth especially when rock phosphate was added to soil.

Solubilization of dicalcium phosphate and tri-calcium phosphate by thermophilic bacteria, actinomycetes and fungi was investigated by Sujatha *et al.* (2004).In general dicalcium phosphate was solubilized more readily then tricalcium phosphate. *Bacillus stearothermophilus, Thermoactinomyces sacchari, Thermomonospora sp.* And *Malbranchea pulchella var. sulfurea* proved to be good phosphate solubilizer and can be exploited for phosphate solubilization.

Rhizosphere inhabiting microorganisms like Vesicular Arbuscular Mycorrhiza (VAM) and phosphate Solubilizing Fungi (PSF) is known to help in P nutrition of crop plants. Mechanisms of P-uptake differ in VAM and PSF. While VAM mobilizes the P from beyond the P depletion zone around the root. PSF solubilizes the insoluble inorganic P from the soil. VAM helps in uptake and utilization of phosphorus even in the presence of aluminum as reported by Morita and Konishi (1989). This can be useful in acidic conditions where aluminium toxicity is a common problem. As such VAM fungi can be better exploited as a biofertilizer in acidic soils of hills.

In a study by Will and Sylvia (1990), it was observed that two fertilizer-N levels, *Klebsiella pneumoniae* and two *Azospirillum* spp. did not provide the plants (sea oats) with fixed atmospheric N; however, *K. pneumoniae* increased root and shoot growth. When a sparingly soluble P source (CaHPO4) was added to two sands, *K. pneumoniae* increased plant growth in sand with a high P content. The phosphorus content of shoots was not affected by bacterial inoculation, indicating that a mechanism other than bacterially enhanced P availability to plants was responsible for the growth increases. When sea oats were inoculated with either *K. pneumoniae* or *Acaligenes denitrificans* and a mixed *Glomus* inoculum, there was no consistent evidence of a synergistic effect on plant growth. Nonetheless, bacterial inoculation increased root colonization by vesicular arbuscular mycorrhizal fungi when the fungal inoculum consisted of colonized roots but had no effect on colonization when the inoculum consisted of spores alone. *K. pneumoniae* was found to increase spore germination and hyphal growth of *Glomus deserticola* compared with the control. The use of bacterial inoculants to enhance establishment of pioneer dune plants warrants further study.

Requena *et al.* (1997) isolated arbuscular mycorrhizal fungi, rhizobium bacteria and plant growth promoting rhizobacteria from a representative area of a desertified semi-arid ecosystem in the south east of Spain. Microbial isolates were characterized and screened for effectiveness by a single- inoculation trial in soil microorganism. *Anthyllis cytisoides* L., a mycotrophic pioneer legume, dominant in the target Mediterranean ecosystem, was the test plant. Several microbial cultures from existing collections were also included in the screening process. Two AM fungi (*Glomus coronatum,* native, and *Glomus intraradices*, exotic), two rhizobia (NR4 and NR9, both native) and two PGPR

(A2,native,and E, exotic)were selected. A further screening for the appropriate double and triple combination of microbial inoculants was then performed. The parameters evaluated were biomass accumulation and allocation, N and P uptake, N_2 – fixation (13 N) and specific root length. Overall, *G. coronatum,* native in the field site was more effective then the exotic *G. intraradices* in co-inoculation treatments. In general, results support the importance of physiological and genetic adaptation of microbes to the whole environment, thus local isolates must be involved. Many microbial combinations were effective in improving either plant development, nutrient uptake, N2 –fixation or root system quality. Selective and specific functional compatibility relationship in plant response between the microbial inoculums, appropriate microbial combinations can be recommended for a given biotechnological input related to improvement of plant performance. And could be exploited in nursery production of target plant species endowed with optimized rhizosphere mycorrhizosphere system that can be tailored to help plants to establish and survive in nutrient deficient, degraded habitats. The relevance of this microbial- based approach in the context of a reclamation strategy addressed to environmental sustainability purposes was discussed

The role of rock- phosphate solubilizing fungi and VAM in growth of wheat plants fertilized with rock phosphate was studied by Omar (1998). A Total of 36 fungal species isolated from soil was tested for their ability to solubilize rock phosphate in agar plates. Most of these fungi were non-rock phosphate solubilizers, but *Aspergilus niger* and *Penicillum citrinum* had high activity. Liquid culture experiments showed that both fungi caused a marked drop in pH of culture media and solubilized considerable amounts of phosphate. The effects on wheat inoculation with VAM fungi and rock- phosphate solubilizing fungi and fertilization with rock phosphate were studied in sterilized pot soils, non-sterilized pot trials and in field plot soila. Rock phosphate fertilization and inoculation with *Glomus constricum* and rock phosphate solubilizing fungi (*A.niger* and *P.citrinum*) significantly increased dry matter yield of wheat plants under all experimental conditions. However, the effect was more evident in non-sterilized pot soils and in the field than in sterilized pots. Rock phosphate had no significant effect on the total phosphorous content of plants grown under pot conditions but it was significantly increased in field plots; the effects of inoculation with fungi (*G. constrictum, A. niger* and *P. citrinum*) on plant phosphorous was closely related to this in dry matter production.

The greatest positive effect on growth and phosphorous contents of wheat plants was recorded in the treatments that received rock phosphate and were inoculated with a mixed inoculum of the three microorganisms used, followed by dual inoculation treatments of *G. constrictum* plus either *A. niger* or *P. citrinum.*

Bora *et al.* (2003) studied the effects of dual inoculation of vesicular arbuscular mycorrhiza (VAM) and phosphate solubilizing Fungi on growth of young tea seedling and shows that dual inoculation in soil mixed with rock phosphate significantly enhanced the growth of tea seedling in terms of plant height , number of leaves, number of branches and dry weight of shoot and root uptake by seedling, mycorrhizal development in terms of spore density and percent colonization in plant roots was also enhanced by the combined inoculation treatment. The effects were a better in presence of rock phosphate then super phosphate applied to the soil. However, population dynamics of phosphate solubililzing fungi was not affected by dual inoculation treatment.

Greenhouse experiments showed that four mixtures of plant growth promoting rhizobacteria (PGPR) strains all *Bacillus* spp. elicited induced systemic resistance in several plants against different plant pathogens. Based on these finding, Jetyanon *et al.* (2003) sought to determine if systemic resistance induced by these PGPR would lead to broad- spectrum protection against several pathogens under field conditions in Thailand. Experiments were conducted during the rainy season (July to October 2001) and winter season (November 2001 to February 2002) on the campus of Naresuan university, Phitsanulok, Thailand. The specific diseases and hosts tested were southern blight of tomoato (*Lycopersicon esculentum*) caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper (*Capsicum annuum* var. *acuminatum)* caused by *Colletotrichum gloeosporiodes*, and mosaic disease of cucumber (*Cucumis sativus)* caused by Cucumber mosaic virus (CMV). Results showed that some PGPR mixtures suppressed disease more consistently then the individual PGPR strain IN93a. One PGPR mixture, *Bacillus amyloliquefaciens* strain IN937a+ *B, pumilus* strain IN937b, significantly protected (P= 0.05) plants against all tested diseases in both seasons. Further, cumulative marketable yields were positively correlated with some treatments.

Interaction between VAM fungus *Glomus mosseae, Azospirillum brasilense and Azotobacter* in soil and their consequent effect on growth and nutrition of Neem seedling

were studied under glass house conditions by Sumana *et.al.,* (2002). Dual inoculation of *G. mosseae* and *A. chroococcum* resulted in maximum plant biomass, nitrogen and phosphorus uptake; biovolume index and quality of neem seedlings. It also increased the mycorrhizal root colonization and spore number in soil of the root-zoon. Dual inoculation of *G. mosseae* and *A. brasilense* improved plant growth more then the single inoculation and uninoculated control. The VAM fungi and phosphate solubilizing bacteria (PSB) are the two groups of soil microorganisms associated with Phosphorous (P) nutrition of plants. VAM fungi produce obligate symbiotic association with plant roots and increase P availability by mobilizing the P with the help of their extrametrical hyphae, particularly in soil with less available P. On the other hand PSB live freely in soil and solubilise insoluble form of P with the help of different organic acids, secreted by them and thus increase the quantity of available P in soil for absorption by plant roots. However, the magnitude of plant growth may vary depending on the type of VAM,PSB strain, crop, amount of P present in soil and presence of co-inoculant. *Azotobacter* has also been found to increase growth and yield of various plants. In the present experiment, the effects of inoculation of VAM, *Pseudomonas,* PSB and *Azotobacter* on growth and nutrition of wheat genotype C- 306 have been studied by Dwiviedi *et al.* (2003).

Anatomical and histochemical changes in the roots and leaves of *Simarouba glauca* due to inoculation with the arbuscular mycorrhizal fungus, *Glomus mosseae* alone and together with the (MHB) mycorrhizal helper bacterium *Bacillus coagulans* in comparison with the uninoculated plants were studied by Sailo *et al.* (2002). The roots cells of plants inoculated with AM + MHB had thicker cell walls compared to plants inoculated with either AM fungus alone or uninoculated plants. The size and /or number of metaxylem, protoxylem, sclerenchyma cells, stele region and pith cells were also higher in plants inoculated with AM fungus+ MHB compared to either mycorrhiza alone inoculated or uninoculated plants.

Arbuscular mycorrhizal (AM) fungi and bacteria can interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition and inhibition of fungal plant pathogens. These interactions may be of crucial importance within sustainable, low-input agricultural cropping systems that rely on biological processes rather than agrochemicals to maintain soil fertility and plant health.

Although there are many studies concerning interactions between AM fungi and bacteria, the underlying mechanisms behind these associations are in general not very well understood, and their functional properties still require further experimental confirmation. Future mycorrhizal research should therefore strive towards an improved understanding of the functional mechanisms behind such microbial interactions, so that optimized combinations of microorganisms can be applied as effective inoculants within sustainable crop production systems. In this context, Artursson (2006) the present article seeks to review and discuss the current knowledge concerning interactions between AM fungi and plant growth-promoting rhizobacteria, the physical interactions between AM fungi and bacteria, enhancement of phosphorus and nitrogen bioavailability through such interactions, and finally the associations between AM fungi and their bacterial endosymbionts. Overall, this review summarizes what is known to date within the present field, and attempts to identify promising lines of future research.

Experiments were conducted by Zaidi and Khan (2006) to evaluate the effects of nitrogen fixing (*Bradyhizobium* sp.(vigna), phosphate solubilizing bacterium (*Bacillus subtilis*), phosphate solubilizing fungus(*Aspergillus awamori*) and AM fungus (*Glomus fasiculatum*)on the growth, chlorophyll content, seed yield, nodulation, grain protein, and N and P uptake of green gram plants grown in phosphorus- deficient soils. The triple inoculation of AM fungus, *Bradhyhizobium* sp. (vigna) and *B.subtilis* significantly increased dry matter yield, chlorophyll content in foliage and N and P uptake of green gram plants. Seed yield was enhanced by 24% following triple inoculation of *Bradyrhizobium* + *G. fasiculatum* + *B. subtilis*, relative to the control. Nodule occupancy, determined by indirect enzyme linked immunosorbent assay (ELISA), ranged between 77% (*Bradyrhizobium* + *A. awamori*) and 96 %(*Bradyrhizobium* + *G. fasiculatum* + *B. subtilis*) at flowering (45DAS), decreasing at the pod –fill (60 DAS) stage with each treatment. Replica immunoblot assay (RIBA) revealed a greater variation in the rhizobial population within nodules and the correlation between nodule occupancy and measured parameters when *A. awamori* was used alone or added to the combination treatments. The present findings showed that rhizosphere microorganisms can interact positively in promoting plant growth, as well as N and P uptake of green gram plants, leading to improved yield

The synergistic effects of plant growth-promoting rhizobacteria and an arbuscular mycorrhizal (AM) fungus (*Glomus fasciculatum*) on plant growth, yield and nutrient uptake of sorghum plants were determined in field conditions by Mathur *et al.* (2009). The triple inoculation of *Azotobacter chroococcum with Bacillus and Glomus fasciculatum* significantly increased the dry matter by 2.6-folds above the control. Grain yield of plants inoculated with *A. chroococcum* together with *Bacillus* sp. And *G. fasciculatum* was 2 –folds higher then that of un-inoculated plants at 135 days after sowing (DAS). The highest increase in grain protein (255 mg/g) was observed in plants inoculated with *A. chroococcum with Bacillus* sp., *G. fasciculatum.* The higher N levels of (33.6 mg/plant) and P (67.8mg/ plant) in sorghum were observed with the co-inoculation of *A. chroococum* with *Bacillus* sp. and *G. fasciculatum.* The N and P contents of the soil at 135 DAS differed the measured parameters. Populations of *A. chroococcum,* phosphate solubulizing microorganisms, percentage root infection, and spore density of the AM fungus in some treatments increased at 80 DAS. Our results show that the multiple inoculations with plant growth promoting rhizobacteria had consistently increased the growth and yield, N and P concentration and quality of sorghum grains.

Two tea rhizosphere microorganisms, *Bacillus megaterium* and *Ochrobacterium anthropi* inhibited the growth of four tea pathogens (*Fomes lamaoensis, Sphaerostilbe repens, Poria hypobrumea* and *Sclerotium roulfsii*) to a certain degree in both solid and liquid medium. Application of *B. megaterium* and *O. anthropi*, either singly or in combination, to rhizosphere of *Camellia sinensis* promoted growth of seedling significantly, but the dual application was more affective. Besides, the bacteria could also control brown root rot of tea, caused by *F. lamaoensis*, *B. megaterium* was more effective than *O. anthropi.* Root colonization by the bacteria, followed by challenge inoculation with the pathogen, induced activities of defense enzymes β-1, 3-glucanase, chitinase, phenylalanine ammonia lyase, as well as peroxidase in tea leaves (Chakraborty *et al.*, 2007).