Rhizosphere is the harbor for a large number of micro-biota, some are beneficial by their positive effect on the plant and others are parasites or pathogens of the plant host. The group of beneficial microbes is designated as the plant growth promoting rhizobacteria (PGPR) due to their growth promoting influence on the phyto-biota.The PGPR activities must be preceded by rhizospheric establishment, regulated strongly by the rhizospheric competence by the microbes.

Several possible mechanisms for plant growth promotion by the rhizobacteria have been proposed, including production of phytohormones (auxin, gibberellin, ethylene), siderophores, solubilising phosphates, fixation of atmospheric nitrogen or indirect mechanisms either by suppression of diseases caused by pathogens or by reducing deleterious rhizobacteria through forming antibiotics PGPR mediated induced systemic resistance (ISR) is an important mechanism of biological disease control. PGPR can also act as bioremediation agents. They can hold soil aggregates, creating channels through which roots grow, soil fauna move and water percolates. Considering the importance of the role of PGPRs in agriculture and understanding their mechanisms of action, several authors have reviewed this topic exhaustively (Lugtenberg *et al.* 2001; Kloepper *et al.* 2004). The main areas of focus of some of the recent reviews have been discussed below.

Rhizosphere colonization is one of the first steps in the pathenogenesis of soil borne microorganisms. It can also be crucial for the action of microbial inoculants used as biofertilizer, biopesticides, and phytostimulators and bioremediators. *Pseudomonas* one of the best root colonizers is therefore used as a model root colonizer. A review by Lugtenberg *et al.* (2001) focused on (a) the temporal spatial description of root colonizing bacteria as visualized by confocal laser scanning microscopically 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. Finally, they identified several noteworthy areas for future research.

Elicitation of induced systematic resistance (ISR) by plant-associated bacteria was initially demonstrated using *Pseudomonas sp* and other gram-negative bacteria. Several reviews have summarized various aspects of the large volume of literature on *Pseudomonas spp.* as elicitors of ISR. Fewer published accounts of ISR by *Bacillus sp*

are available, and Kloepper et al., (2004) revieweed this literature for the first time. Published results were summarized showing that specific strains of the species *B. amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides* and *B. sphaericus* elicitor significant reductions in the incidence or severity of various diseases on a diversity of hosts. Elicitation of ISR by these strains has been demonstrated in green house or field triala on tomato, bell pepper, muskmelon, watermelon, sugar beet, tobacco, *Arabidopsis* sp., cucumber, loblolly pine, and two tropical crops (long cayenne pepper and green kuang futsoi). Protection resulting from ISR elicited by *Bacillus* sp has been reported against leaf-spotting fungal and bacterial pathogens, systemic viruses, a crown-rooting fungal pathogen, root-knot nematodes, and a stem-blight fungal pathogen as well as damping-off, blue mold, and late blight disease. Reductions in populations of three insect vectors have also been noted in the field: striped and spotted cucumber beetles that transmit cucurbit wilt disease and the silver leaf whitefly that transmits Tomato mottle virus. In most cases, *Bacillus* sp that elicits ISR also elicits plant growth promotion. Studies on mechanisms indicate that elicitation of ISR by *Bacillus* sp is associated with ultra structural changes in plants during pathogen attack and with cytochemical alternations. Investigations into the signal transduction pathways of elicited plants suggest that *Bacillus* sp activate some of the same pathways as *Pseudomonas sp* and some additional pathways. For example, ISR elicited by several strains of *Bacillus* sp is independent of salicylic acid but dependent on jasmonic acid, ethylene, and the regulatory gene NPR1-results that are in agreement with the model for ISR elicited by *Pseudomonas sp.* However, in other cases, ISR elicited by is dependent on salicylic acid and independent of jasmonic acid and NPR1.In addition, while ISR by *Pseudomonas sp* does not lead to accumulation of the defense gene PR1 in plants, in some cases, ISR by *Bacillus* sp does. Based on the strains and results summarized in this review, two products for commercial agriculture have been developed, one aimed mainly at plant growth promotion for transplanted vegetables and one, while has received registration from the U.S. Environmental protection Agency, for disease protection on soybean.

2.1. Plant growth promotion

Co-inoculation of plant growth promoting rhizobacteria (PGPR) with *Bradyrhizobium* has been shown to increase legume nodulation and nitrogen fixation

at optimal soil temperatures. Nine rhizobacteria co-inoculated with *Bradyrhizobium japonicum* 532C were tested by Zhang *et al.* (1996) for their ability to reduce the negative effects of low root zone temperature (RZT) on soybean, *Glycine max*(L.) Merr. nodulation and nitrogen fixation. Three RZTs were tested: 25 (optimal), 17.5 (somewhat inhibitory), and 15^{0}C (very inhibitory). At each temperature some PGPR strains increased the number of nodules formed and the amount of fixed nitrogen when co-inoculated with *B. japonicum*, but the stimulatory strains varied with temperatures. The strains that were most stimulatory varied among temperatures and were as follows: 15^{0}C, *Serratia proteamaculans* 1-102; 17.5^{0}C, *S. proteamaculans*1-102 and *Aeromonas hydrophila* P73; 25^{0}C, *Serratia liquefaciens* 2-68

Cook et al. (1998) reported that rhizobacteria, particularly *Pseudomonas* species were (i) able to colonize and maintain populations in the rhizosphere of wheat 5-10 cm and more below the seed, (ii) able to produce one or more antibiotics inhibitory to the target root pathogens and (iii) tolerant to seed-treatment chemicals, needed for the immediate protection of germinating seeds. Their strains were from the rhizosphere of wheat growing in the soil from fields where wheat had been grown continuously for many years, to help ensure that the strains were rhizosphere competent on the crop intended for protection. Initially, they concentrated on *P.fluorescens* 2-79 and *P.aureofaciens* 30-84 with ability to produce phenazine (PHZ) antibiotics. The second phase of their field work concentrated on *Bacillus* species L324-92 with antibiotic activity against three wheat root diseases and on *P.fluorescens* Q69c-80 with no known ability to produce antibiotics, but widely effective in the field. The authors have reported that they are also now concentrating on *P.fluorescens Q8R1-96* with ability to produce the antibiotic 2, 4-diacetyphloroglucinol (PHL). The evidence is strong that PHL producing starins like Q8R1-96 account for take-all decline. Used as a seed treatment, this strain produced the highest yields of wheat at every location where tested in1997. They now have cultures of Q8R 1-96 trasformed to produce PHZ in addition to PHL.

Cattelan *et al.* (1999) selected 116 isolates from bulk soil and the rhizospere of soybean [*Glycine max* (L.) Merr.] and examined them for a wide array of traits that might increase early soybean growth in nonsterile soil (PGPR traits). A sub sample of 23 isolates, all but one of which tested positive for one or more of these PGPR 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 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 promoted 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 results suggest that rhizobacterial able to produce ACC deaminase and, to a lesser extent, β-1, 3-glucanase or siderophores or those able to solubilze P *in vitro* may increase early soybean growth in nonsterile soil. A Proteus strain inhibited mycelial growth of *Fusarium oxysporum in vitro*. Seed bacterization showed significant plant growth promotion and *Fusarium* wilt suppression activity of *Phaseolus mungo* in a gnotobiotic system. The culture filtrates of this strain exhibited three prominent bands in UV-VIS spectra between 300 and 400nm. The growth promotion assay of the extracted compound against different indicator organisms indicated the production of a compound related to a 2-0xoacid type siderophore. The HPLC of the purified ethyl acetate extract of the strains and standard 4- methyl-oxopentanoate (2-oxoisocaproate) revealed a single peak, similarly as the coinjection of the extract and the standard. The production of siderophore, probably 2-oxoisocaproate, was demonstrated by Barthakur (2000).

Experiments were conducted by Lee (2000) to compare the germinability of pepper seeds primed by bacterial strain and solid matrix priming (SMP). Pepper seeds were soaked in the cell suspension of the bacterial strains for 1 hr and incubated at 28^0C for certain period of time then dried in shade and stored. Seed priming with *Bacillus* strains showed even higher germination rate than SMP of chemical osmotic controllers. In pot experiments, pepper seeds primed by *Bacillus* sp. B2-13 showed more than 80% seedling emergency within 7 days, while SMP treatment was 11 days and untreated control was 13 days. When the bio-primed seeds were planted in pots, significant increase of shoot weight and length as well as root weight and length were noted when compared to other treatments. Bio-primed seedling revealed twofold more root biomass than untreated control.

According to Bai (2002) *Serratia proteamaculans* 1-102 (1-102) promotes soybean-bradyrhizobia nodulation and growth, but the mechanism is unkown. After adding isoflavonoid inducers to 1-102 culture, an active peak with a retention time of about 105 min in the HPLC fractionation was isolated using a bioassay based on the stimulation of soybean seed germination. The plant growth-promoting activity of this material was compared with 1-102 culture (cells) and supernatant under greenhouse conditions. The activator was applied to roots in 83, 830 and 8300 HPLC microvolts (μV) per seedling when plants were inoculated with bradyrhizobia or sprayed onto the leaves in same concentrations at 20 d after inoculation. The root-applied activator, especially at 1 ml of 830 μV per seedling, enhanced soybean nodulation and growth at the same level as 1-102 culture under both optimal and sub-optimal root zone temperatures. Thus, this activator stimulation of soybean seed germination is also responsible for the plant growth-promoting activity of 1-102 culture. However, when sprayed onto the leaves, the activator did not increase growth and in higher leaf area, shoot dry weight and root dry weight at 30 days after transplanting were determined. Overall, the best bacterization methods were either root dipping or Bacterization by root dipping was chosen due to its practicability. The most efficient bacterial strains were C210, ENF 16, RAB9 and ENF10. Increases as high as 163.6 %, 107.7% and 87.0 % respectively for shoot dry weight, root dry weight and leaf area were obtained by applying the strain RAB9 by root dipping. All strains were compatible among them and combinations of ENF 10 plus RAB9, ENF 16 plus C2 10 and RAB9 promoted root dry weight increases of 100.3%, 88.1%, 80.1 %, respectively. Production of either IAA or HCN, and solubilisation of phosphate by the strains were not detected under the experimental conditions used here. Only nitrogen amounts in bacterized plantlets had significantly differed from the controls. This work indicated that mixtures of the strains C210, ENF16, RAB9 and ENF10 applied by root dipping could be used to increase biomass production of micropropagated pineapple plantlets, reducing the acclimation period.

Experiments were conducted in pots to determine the growth effect of different rhizobacteria on maize under *Striga hermonthica* infestation. Babalola *et al.* (2002) selected three bacteria based on their plant growth promoting effects. Whole bacterial cells of the rhizobacteria were used to amplify 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase gene by polymerase chain reaction (PCR). Each

bacterial inoculation increased agronomic characteristics of maize although not always to a statistically significant extent. The extent of growth enhancement differed between the isolates, *Enterobacter sakazakii* 8MR5 had the ability to stimulate plant growth; however in the PCR study, ACC deaminase was not amplified from this isolate, indicating that not all plant growth-promoting rhizobacteria contain the ACC deaminase. In contrast, an ACC deaminase specific product was amplified from *Pseudomonas sp.* 4MKS8 and *Klebsiella oxytoca* 10MKR7. This was the first report of ACC deaminase in *K.oxytoca*

Three strains of plant growth promoting fluorescent Pseudomonads (HPR6, RRLJ008 and RRLJ134) were studied for their effect on growth and yield of French bean (*Phaseolus vulgaris* L.) under field conditions (Baruah *et al*, 2003). They examined the effect of these strains on nature of root development 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 increases in specific leaf weight (SLW), net assimilation rate (NAR) and relative growth rate (RGR) by these strains were 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.

Experiments were conducted during 2000 and 2001 to determine the effects of floral and foliar application of the bacterial 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 60 days after full bloom. 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 than on the uncontrolled. The results of this study by Esitken *et al.* (2003) suggest that OSU 142 has the potential to increase the yield of apricot trees.

In onder to select potential Plant growth promoting rhizobacteria (PGPRs), a selection of strains 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), four *Cellulomonas* (Cell), two *Arthrobacter* (Arth), two *Pseudomonas* (Ps), and six *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 Aur 11 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 the screening, and considering the entire variable studied, authors suggested that Aur 6 can be considered a plant growth promoting rhizobacterium suitable for further field trials in other plants and in different production systems.

A bacterial screening was carried out by Mancro *et al.* (2003) in the rhizosphere of two *Digitalis* species, *D. thapsi* and *D. parviflora*, both at the vegetative stage and at flowering. A total of 480 isolates were characterized at genus level, *Bacillus* being the dominant genera in all cases. Fifty percent of the *Bacillus* strains isolated from each species were analyzed by PCR-RAPDs. At 85% similarity, 12 groups were selected for *D. thapsi* and 18 for *D. parviflora*. One strain of each group was selected for biological assay on *D.lanata*, growth promotion and cardenolide content in leaves after inoculation performed in the root system were noted. The plant parameters evaluated were leaf surface area, shoot and root dry weight and leaf number. Lanatoside C content was evaluated by HPLC. Only 17 strains caused significant increases in at least one of the parameters evaluated. The most striking result was that some strains promoted growth and increased cardenolide content at the same time. This effect was detected on leaves while inoculation was carried out on roots. Interestingly, these two parameters are not enhanced simultaneously under regular conditions in pot or in tissue cultures.

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 decrease of 1- aminocyclopropane-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 plant seeds or roots with these bacteria reduced the amount of ACC on plants, thereby lowering the concentration of ethylene.

Ramos *et al.* (2003) either inoculated alder seedling with a suspension of *Bacillus licheniformis*, or left non-inoculated (controls) which were grown in two different soils under controlled conditions. For 8 weeks after inoculation, plant shoot and root systems were measured; nodules counted, and shoot and root length and surface area determined. In addition to plant growth, changes in the bacterial rhizosphere composition and inoculums levels were determined using the phospholipids fatty acid (PLFA) profile from the rhizosphere soil and from culturable bacteria from the rhizosphere (culturable PLFAs), respectively. They showed the differential effect of *B. licheniformis* on alder growth depending on the soil used. Increases in leaf surface area were significant only when grown in soil A, while root growth increased in both soils. Effect was more pronounced in soil A. Changes in the rhizosphere community after inoculation with *B. licheniformis* disappeared within a short period in both soils 6 weeks in soil A and only 2 in soil B. *B. licheniformis* apparently survived at least 8 weeks in the rhizosphere, as revealed by culturable PLFA profiles. Thus, increases in plant growth could be attributed to changes in the rhizosphere microbial communities, especially in the culturable fraction, due to the presence of the inoculated bacteria in soil. Given the different composition of soils, availability of nutrients must also be considered.

Rhizobia form root nodules that fix nitrogen (N_2) in symbiotic legumes. Extending the ability of these bacteria to fix N_2 in non-legumes such as cereals would be a useful technology for increased crop yield among resource-poor farmers. Although some inoculation attempts have resulted in nodule formation in cereal plants, there was no evidence of N_2 fixation. However, because rhizobia naturally produce molecules (auxin, cytokinins, abscicic acids,lumichrome, rhiboflavin, lipo-chito-oligasaccharides and vitamins) that promote plant growth, their colonization and infection of cereal roots would be expected to increase plant development, and grain

yield. Matiru and Dakora (2003) have used light, scanning and transmission electron microscopy to show that roots of sorghum and millet landraces from Africa were easily infected by rhizobial isolates from five unrelated legume genera. With sorghum, in particular, plant growth and phosphorus (P) uptake were significantly increased by rhizobial inoculation, suggesting that field selection of suitable rhizobia/cereal combinations could increase yields and produce fodder for livestock production.

Two strains of *Azospirillum brasilense*, Sp245 and Sp7, were examined by Rothballer *et al.* (2003) for their endophytic potential on German, Brazilian and Israeli wheat cultivars. Plate count and Most Probable Number MPN) methods were applied for quantification, as well as the fluorescent in situ hybridization FISH) technique in combination with confocal laser scanning microscopy for the species specific detection and localization of the two *Azospirillum* strains in roots. Additionally, a plasmid bearing a constitutively expressed gfp gene was transformed into both strains which enable visualization of the bacteria omitting the fixation process during the FISH protocol. The microscopic techniques showed that the potential of strain Sp 245 to grow in the roots of all analyzed wheat varieties as an endophyte was greater than of Sp7, but overall cell densities were rather low under the applied experimental conditions. A plant growth promoting effect was clearly visible in all examined inoculated plants, irrespective of the *A. brasilense* strain used as inocula.

Activities associated with *Paenibacillus polymyxa* treatment of plants in some experiments include nitrogen fixation, soil phosphorous solubilisation, and production of antibiotics, auxin, chitinase, and hydrolytic enzymes, as well as promotion of increased soil porosity. Timmusk (2003) showed that, in stationary phase, *P.polymyxa* released the plant hormone cytokinin isopentenyladenine, in concentrations of about 1.5 nM. In a gnotobiotic system with *Arabidopsis thaliana* as a model plant, it was shown that *P.polymyxa* inoculation protects plants; challenge by either the pathogen *Erwinia carotovora* (biotic stress) or induction of drought (abiotic stress) showed that that pre-inoculated plants were significantly more resistant than control plants. By RNA differential display on RNA from *P.polymyxa* treated or control plants, changes in gene expression were tested. One mRNA, encoding ERD15 (drought stress-

responsive gene) showed a strong inoculation-dependent increase in abundance. In addition, several biotic stress genes were also activated by *P.polymyxa*.Antagonism towards the fungal pathogens *Phytophthora palmivora* and *Pythium aphanidermatum* was studied. *P.polymyxa* counteracted the colonization of zoospores of both oomycetes on *A.thaliana* roots, and survival rates of plants treated with *P.polymyxa* were much higher when challenged *P. aphanidermatum*. Using a green fluorescent protein-tagged isolate of *P.polymyxa*, colonization of *A.thaliana* roots was investigated. Authors drew two main conclusions. Firstly, the bacterium enters the root tissue (but not leaves) and is abundantly present in intercellular spaces. Secondly, the root becomes severely damaged, indicating that- under some conditions- *P.polymyxa* is a "deleterious bacterium", and in others it promotes growth. Based on his work, Timmusk proved that a balance between the activities of a PGPR, the genetic background and physiological state of a plant, and the environmental conditions employed in test system, ultimately determines the resulting effect. (Timmusk, 2003)

Timmusk *et al.* (2005) also showed that this bacterium protected Arabidopsis thaliana against pathogens and abiotic stress. They studied colonization of plant root by a natural isolate of *P.polymyxa* which had been tagged with a plasmid-borne *gfp* gene. Fluorescence microscopy and electro scanning microscopy indicated that the bacteria colonized predominantly the root tip, where they formed biofilms. Accumulation of bacteria was observed in the intercellular spaces outside the vascular cylinder. Systemic spreading did not occur, as indicated by the absence of bacteria in aerial tissues. Studies were performed in both a gnotobiotic system and soil system. The fact that similar observations were made in both systems suggests that colonization by this bacterium can be studied in a more defined system. They discussed the problems associated with green fluorescent protein tagging of natural isolates and deleterious effects of the plant growth-promoting bacteria.

Studies were undertaken by Yan *et al.* (2003) to compare root colonization and persistence of rifampicin-resistant mutants of PGPR strains *Bacillus pumilus* SE34 and *Pseudomonas fluorescens* 89B61, SE34r and 89B61r, on tomato as a function of application method. When the bacteria were incorporated into PromixTM soilless medium at log 6, 7, and 8 colony-formimg units g^{-1}, populations of strain

SE34r per gram of medium maintained the initial inoculum densities, while populations of 89B61r decreased approximately one to two orders of magnitude by 4 weeks after planting. The populations of each PGPR strain colonizing roots after application into the soilless medium showed a similar pattern at 6 weeks as that at 4 weeks after planting, with higher populations on the whole roots and lateral roots than on the taproots. Strain SE34r but not 89B61r moved upwards and colonized the phyllosphere when incorporated into the soilless medium. Following application as seed treatment, populations of SE34r were significantly higher on upper roots and on the taproot than were populations following application through the soilless medium. Conversely, populations were higher on lower roots and lateral roots following application through the soilless medium than were population following application as seed treatment. While strain SE34 enhanced plant growth with application both to the medium and as seed treatment, the level of growth promotion was significantly greater with application in the soilless medium. The results indicate that PGPRs can be successfully incorporated into soilless media in vegetable transplant production systems.

According to Jaizme-Vega *et al.* (2004) soil microbiota communities have demonstrated their crucial role in maintaining the soil ecological balance and therefore the sustainability of either natural ecosystems or agro ecosystems. 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 pathogen stress conditions. 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. The authors evaluated the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micropropagated bananas.

A study by Khalid *et al.* (2004) focused on the screening of effective PGPR strains on the basis of their potential for *in vitro* auxin production and plant growth promoting activity under gnotobiotic conditions. A large number of bacteria were isolated from the rhizosphere soil of wheat plants grown at different sites. Thirty isolates showing prolific growth on agar medium were selected and evaluated for their

potential to produce auxins *in vitro*. Colorimetric analysis showed variable amount of auxins (ranging from 1.1 to 12.1 mg L^{-1}) produced by the rhizobacteria *in vitro* and amendment of the culture media with l-tryptophan (l-TRP), further stimulated auxin biosynthesis (ranging froml.8 to 24.8 mg L-1). HPLC analysis confirmed the presence of indole acetic acid (IAA) and indole acetamide (IAM) as the major auxins in the culture filtrates of these rhizobacteria. A series of laboratory experiments conducted on two cv. of wheat under gnotobiotic (axenic) conditions demonstrated increases in root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 37.7 %) and shoot dry weight (up to 36.3%) of inoculated wheat seedlings. Linear positive correlation (r = 0.99) between *in vitro* auxin production and increase in growth parameters of inoculated seeds was found. Based upon auxin biosynthesis and growth-promoting activity, four isolates were selected and designated as plant growth promoting rhizobacteria (PGPR). Auxin biosynthesis in sterilized vs. nonsterilized soil inoculated with selected PGPR was also monitored that revealed superiority of the selected PGPR over indigenous microflora. Peat-based seed inoculation with selected. PGPR isolates exhibited stimulatory effects on grain yields of tested wheat cv. in pots (up to 14.7% increase over control) and field experiments (up to 27.5% increase over control); however, the response varied with cultivars and PGPR strains. It was concluded that the strain, which produced the highest amount of auxins in nonsterilized soil, also caused maximum increase in growth and yield of both the wheat cultivars. Their study suggested that potential for auxin biosynthesis by rhizobacteria could be used as a tool for the screening of effective PGPR strains.

In order to examine naturally occurring variation in the ability of *Triticum aestivum* L. (hexaploid wheat) to support certain strains of *P.fluorescens*., Okubara *et al.*(2004) have surveyed 27 Pacific Northwest (PNW) cultivars for the ability to undergo root colonization with the aggressive colonizer *P.fluorescens* strain Q8r1-96, and *P.fluorescens* strain Q2-87, a less effective colonizer. In seed inoculation experiments, Q8r1-86 colonized roots of all of the cultivars equally or more effectively than did Q2-87 in a non-pasteurized, non-agricultural soil. Seven cultivars supported significantly ($P< 0.05$) higher rhizosphere population of Q8r1-96 than Q2-87 within 14 days post-inoculation (dpi), two cultivars supported relatively high population densities of the strains. Population densities normalized to root weight

were seen as early as 7 dpi. In pairwise comparisons, the bacterial treatments differentially affected the root morphology of some of the cultivars at 14 dpi. However, principal components (factor) and correlation analysis showed that preferential colonization by Q8r1-96 was independent of root fresh weight, total length, surface area, volume and average diameter, and that differential colonization was not correlated with changes in any specific root morphometric variable. Variation in root colonization of specific cultivars suggests useful genetic stocks for mapping and identifying host genes involved in wheat-rhizosphere interactions.

Rhizobacteria with properties related to plant growth-promotion were isolated from the rhizosphere of the perennial legume *Chamaecytisus proliferus* ssp. *Proliferus* var. *palmensis* (tagasaste) growing in the field conditions by Donate-Correa *et al.* (2005). They collected samples in two localities of the Tenerife Island: La Laguna and EI Tanque, NE and NW at 600 and 1000 meters above sea level, respectively, and in two seasons, winter and summer. The strains were isolated by using culture dependent procedures, and identified by phenotypic (culturable and biochemical) and genotypic (ERIC-PCR fingerprinting) features. The rhizosphere isolates formed a diverse community of mainly Gram-negative bacteria, with members of genera *Pseudomonas*, *Burkholderia* and *Sphingomonas* being predominant. A high level of selectivity was found in the rhizosphere environment as compared to the non-rhizosphere soil where Gram-positive were more abundant. Species richness (number of species) and species abundance were related to the sampling season and the locality, thus, samples obtained in winter at both sites had larger counts than samples obtained in summer, and the higher species riches was found in La Laguna. The species *Pseudomonas fluorescens* showed the highest number of properties related to plant growth promotion (PGP): 1-aminocyclopropane-1carboxylate (ACC) deaminase activity, phytohormone production, nitrogen fixation, fungal growth inhibition and cyanogenesis; thus it seems to be the most suitable microorganism to be tested in PGP-field experiments.

In search of efficient PGPR strains with multiple activities, a total of 72 bacterial isolates to *Azotobacter*, fluorescent *Pseudomonas*, *Mesorhizobium* and *Bacillus* were isolated from diffent rhizospheric soil and plant root nodules in the vicinity of Aligarh by Ahmad *et al.* (2006). These test isolates were biochemically

characterized. These isolates 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 than 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 hydrolyzed 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 highest in the *Pseudomonas* followed by *Azotobacter* and *Bacillus* isolates. *Azotobacter* isolates (AZT_3, AZT_{13}, and 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 plant system is needed to uncover their efficacy as effective PGPR.

A study was conducted by Cakmakei *et al.* (2006) with sugar beet in greenhouse and field at two soil type with different organic matter (containing 2.4 and 15.9 % OM, reffered as the low- and high-OM soil) conditions in order to investigate seed inoculation of sugar beet, with five N_2-fixing and two phosphate solubilzing bacteria in comparison to control and mineral fertilizers (N and P) application. Three bacterial strains dissolved P; all bacterial strains fixed N_2 and significantly increased growth of sugar beet. In the green house, inoculations with PGPR increased sugar beet root weight by 2.8-46.7% depending on the species. Leaf, root and sugar yield were increased by the bacterial inocula by 15.5-20.8, 12.3-16.1 and 9.8-14.7% respectively, in the experiment of low- and high-OM soil. Plant growth responses were variable and depending on the inoculant strains, soil organic matter content, growing stage, harvest date and growth parameter evaluated. The effect of PGPR was

greater at early growth stages than at the later. Effective *Bacillus* species, such as OSU-142, RC07 and M-13, *Paenibacillus polymyxa* RC05, *Pseudomonas putida* and *Rhodobacter capsulatus* RC04 may be used in organic and sustainable agriculture.

In a study by Chen *et al.* (2006), 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 the genus *Bacillus*, nine to genus *Rhodococcus*, seven to genus *Arthrobacter*, six to genus *Serratia* and one each to genera *Chryseobacterium*, *Delftia*, *Gordonia* and *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. were 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 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.

Hafeez *et al.* (2006) isolated seventeen rhizobacteria from different ecological regions, i.e. Brazil, Indonesia, Mongolia and Pakistan 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 concentrations 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 (42.1µg/mL), produced siderophores (0.3 mg/mL) and was the only isolate that solubilized phosphate (188.7 µg P/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 contents in plants. Random amplified polymorphic deoxyribonucleic

acid (RAPD) analysis, conducted with 60 decamer primers, revealed a high level of 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 (Accession number AY548949). It was concluded that *Bacillus pumilus* 8N-4 can be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety *Orkhon* in Mongolia

Hameeda *et al.* (2007) carried out studies to re-cycle crop residues and prepare composts. Their work included isolation and characterization of bacteria for different plant growth promoting traits and antagonistic activity. Two hundred and seven bacteria were isolated from farm waste compost (FWC), rice straw compost (RSC), *Gliricidia* vermicompost (GVC) and macrofauna present in FRC. Percentage of isolates having plant growth promoting traits were 54% from FWC, 56% from RSC, 64% from GVC and 41% from macrofauna. Antagonistic bacteria were 19% from FWC, 38%fromRSC, 39%from GVC and 23% from macrofauna. Twenty-three of 2007 isolates showed plant growth- promoting traits and antagonistic activity against test fungi. These were tested for their plant growth promoting ability on sorghum and pearl millet. Twelve strains significantly increased the plant growth of sorghum and pearl millet. Five of the twelve strains were phosphate- solubilizing bacteria(PSB) and two strains were *Serratia marcescens* EB 67 and *Pseudomonas sp.* CDB 35 showed highest gluconic acid production (67 and 27 mM) and P-solubilization (1036 and 560 uM). Evalution of these potential bacteria in glasshouse condition revealed that there was significant increase in plant growth parameters of sorghum and pearl millet.

Das *et al.* (2007) reported the potentiality of native bacteria of sorghum rhizosphere for early growth stimulation in rabi sorghum. Around 10-15 per cent isolates of the native bacteria in rabi sorghum rhizosphere showed positive effect on early growth in sorghum seedlings. Selected isolates increased seed broad spectrum inhibitory effect on the growth of charcoal rot pathogen (*M. phaseolina*). Two isolates SRB26 and SRB28 had advanced the flowering time of the rabi cultivar M25-1 by 3-5 days under due to production of plant growth hormone, indole acetic acid (IAA), and pathogen suppressing siderophore by rhizobacteria. SRB28 colonized on sorghum

root and developed micro – colonies on the root epidermis which might have improved its survival and bio-efficacy in the rhizosphere. It was concluded that sorghum rhizosphere, harbored plant growth promoting as well plant growth deleterious bacteria and seed treatment with selected strains of native rhizobacteria could enhance seedling growth in sorghum, a property which might be advantageous to rainfed sorghum.

2.2. Biological Control

2.2.1 Rhizobacteria

Pseudomonas fluorescens strains which effectively inhibited mycelial growth of *Fusarium udum*, the pigeon pea (*Cajanus cajan)* pathogen, were isolated from the rhizoplane of different crops (Vidhyasekaran, 1997). Various powder formulations of two efficient *P. fluorescens* strains were effective in controlling the disease, but their efficacies varied depending upon the length of storage. Talc formulations were effective even after 6 months of storage, while peat formulations were effective up to 60 days of storage. The shelf life of vermiculite, lignite, and kaolinite formulations was short. Unformulated bacterial suspensions could not be stored even for 10 days, at which time their efficacy was completely lost. The bacterial strains survived in pigeon pea rhizophere throughout the crop-growth period. The talc-based powder formulations effectively controlled pigeon pea will and increased yield in two field trials. According to the authors, development of powder formulation of P. *fluorescens* will aid large-scale application of biological control in farmers' fields.

Plant growth-promoting rhizobacteria (PGPR) strains INR7 (*Bacillus pumilus*), GB03 (*Bacillus subtilis*), and ME1 (*Curtobacterium flaccumfaciens*) were tested singly and in combinations for biological control against multiple cucumber pathogens by Raupach and Kloepper (1998). Investigations under greenhouse conditions were conducted with three cucumber pathogens-*Colletotrichum orbiculare* (causing antracnose), *Pseudomonas syringae pv. Lachrymans* (causing angular leaf spot), and *Erwinia tracheiphils* (causing cucurbit wilt disease) inoculated singly and in all possible combinations. There was a general trend across all experiments toward greater suppression and enhanced consistency against multiple cucumber pathogens using strain mixtures. The same three PGPR strains were evaluated as seed treatments in two field trials over two seasons, and two strains, IN26(*Burkholderia gladioli*) and

INR7 also were tested as foliar sprays in one of the trials. In the field trials, the efficacy of induced systemic resistance activity was determined against introduced cucumber pathogens naturally spread within plots through placement of infected plants into the field to provide the pathogen inoculums. PGPR-mediated disease suppression was observed against angular leaf spot in 1996 and against a mixed infection of angular leaf spot and anthracnose in 1997. The three way mixture of PGPR strains (INR7 plus ME1 plus GB03) as seed treatment showed that intensive plant growth promotion and disease reduction and disease reduction to a level statistically equivalent to the synthetic elicitor Actigard applied as a spray.

According to Braun-Kiewnick *et al.* (2000) strains of *Pantoea agglomerans* (synanamorph *Erwinia herbicola*) suppressed the development of basal kernel blight of barley, caused by *Pseudomonas syringae pv. syringae* when applied to heads prior to the *Pseudomonas syringae pv. syringae* infection at the soft dough stage of kernel development. Field experiments in 1994 and 1995 revealed 45 to 74% kernel blight disease reduction, whereas glasshouse studies resulted in 50 to 100% disease control depending on the isolate used and barley cultivar screened. The efficacy of biocontrol strains was affected by time and rate of application. Percentage of kernels infected decreased significantly when *P. agglomerans* was applied before pathogen inoculation, but not when coinnoculated. A single *P. agglomerans* application 3 days prior to the pathogen inoculation was sufficient to provide control since population of about 10^7 CFU per kernel were established consistency, while *Pseudomonas syringae pv. Syringae.* Population dropped 100-fold to 2.0×10^4CFU per kernel. An application to the flag leaf at EC 49 (before heading) also reduced kernel infection percentages significantly. Basal blight decreased with increasing concentration 10^3 to10^7 CFU ml^{-1} of *P.agglomerans,* with 10^7 CFU/ml providing the best control. For long-term preservation and marketability, the survival of bacterial antagonist in several wettable powder formulations was tested. Over all formulations tested, the survival declined between 10-to >100-fold over a period of 1.5 years (r = -0.7; P = 0.000). Although not significant, storage of most formulation at 4°C was better for viability (90 to 93% survival) than was storage at 22°C (73 to 79%). However, long-term preservation had no adverse effect on bio control efficacy.

Antibiotic resistant mutants of strains of fluorescent pseudodomonads were isolated by Yeole and Dube (2001) from the rhizosphere of chili, cotton, groundnut and soybean. Isolates produced siderophores and showed plant growth promoting activity with parent crops and showed varied response in their root colonizing capability. The groundnut isolates had highest rhizosphere competence followed by soybean, cotton and chili.

The efficacy of various *P. fluorescens* isolated was tested for the management of fruit rot of chilli caused by *Colletotrichum capsici.* Among the various isolates tested *P. fluorescens isolates viz. Pfl and ATR* increased the plant growth and produced the maximum amount of indole acetic acid. P. *fluorescens* Pfl effectively inhibited the mycelial growth of the pathogen under *in vitro* conditions and decreased the fruit rot incidence under greenhouse condition. Seed treatment plus soil application of talc based formulation of *P. fluorescens* isolate Pf1 effectively reduced the disease incidence. Expression of various defense related enzymes and chemicals was found, involved in the induction of systemic resistance against pathogen infection. Induction of various defense related genes has been discussed for the suppression of pathogen infection by Ramamoorthy and Samiyappan (2001).

Efficacy of seven strains of *Pseudomonas fluorescens (Pfs17),* plant growth-promoting rhizobacteria (PGPR), were tested by Sarma *et al.* (2002) under field conditions for their ability to protect *Cicer arietinum* against *Sclerotium rolfsii* infection. Best protection was observed in strain *Pfs3* where 23% seedling mortality was recorded in comparison to 44% in non-treated control. To correlate the induction of phenolic compounds by the PGPRs with disease resistence, qualitative and quantitative alterations of phenolic compounds in different parts of *C. arietinum* were estimated following PGPR application as seed treatment. High performance liquid chromatographic (HPLC) analysis of the leaves, collars and roots of the PGPR-treated and non-treated (control) plants showed the presence of gallic, ferulic, chlorogenic and cinnamic acids with varied amounts in the PGPR-treated as well as non-treated (control) plants. Maximum accumulation of cinnamic acid was observed in plants treated with *Pfs3* strain (1660 ng g^{1}fresh wt) which was almost 19.5 times higher than untreated control plants and also significantly high when compared to other PGPR treatments. It also caused maximum accumulation of total phenolics and Gallic acid in

all chickpea plant parts as compared to other treatment and untreated control. A direct relationship between the level of total phenolics and seedling survivability was observed. PGPR-mediated induction of phenolics compounds as a biochemical barrier in *C. arietinum* against *S. rolfsii* infection was envisaged by the authors.

Bansal *et al.* (2003) tested the efficacy of *Azotobacter chroococcum* against tomato wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) during rabi 2000-01 and 2001-02 in net house under artificial inoculum conditions. Tomato seedlings var. local treated with *A. chroococcum* before transplanting along with soil application of nitrogen @ 60, 80 and 100kg ha^{-1} showed complete inhibition of plant mortality (7.36%) was also observed when seedlings were treated with *A. chroococcum* only as compared to the seedling without any treatment (17.35%). It may be attributed to the production of antifungal substances by *A. chroococcum.*

Bhatia *et al.*(2003) observed maximum colony growth inhibition due to *Pseudomonas* PS 2 (74%) as compared to PS 1(71 %) on trypticase soy agar (TSM) plates after 5 days incubation. Light and scanning electron microscope examination showed hypal coiling, vacuolation and granulation of cytoplasm resulting in lysis of hyphae of *Macrophomina phaseolina* by pseudomonads. Cell free culture filtrates of strains PS1 and PS 2 restricted the growth of mycelium of *M. phaseolina,* PS 1 and PS 2 caused maximum colony growth inhibition by 57 and 61% respectively at 20% conc. of culture filtrate after 4 days of incubation. Volatile substances produced by PS 1and PS 2 also inhibited the colony growth of *M. phaseolina* by 25 and 32% respectively. Inhibitory effect of volatile substances, however, decreased with advancing incubation period. Colony growth of *M. phaseolina* was significantly decreased by PS 1 and PS 2 as compared to control both in iron sufficient and iron deficent condition. PS 2 showed higher antagogonistic activity than PS1, as evidenced by pronounced colony growth inhibition.

Fourteen plant growth promoting rhiobacteria (PGPR) isolated from rhizoticzones of field-grown green gream (*Vigna radiata* (L.) Wilczek) plants were examined by Gupta *et al.* (2003) for their growth-promoting attributes and ability to affect the growth *in vitro* of 10 strains of *Bradyrhizobium* sp. (*Vigna*). None of the rhizophere bacteria was found to repress or simulate the growth of any of the *Bradyrhizobium* strains tested. However, they produced antibiotic and siderophores

and plant growth promoting substances. Nitrogen fixation and phosphate solubilization was not detected by any of the isolates. Under *in-vitro* condition, nine isolates inhibited growth of soil-borne fungal pathogens; one of them identified as *Bacillus* sp. antagonized all the fungi tested on two different media. All PGPR isolates were tested both in sterile and unsterile soil for their ability to promote nodulation, nitrogen fixation, growth and yield of green gram in the presence of two *Bradyrhizobium* sp. *(Vigna)* strains S 24 and Cog 15. In sterile soil, all PGPR isolates had a positive effect on shoot biomass development, acetylene reduction assay (ARA), and N content when co-inoculated with *Bradyrhizobium* strain Cog 15, but could influence only shoot biomass development in the presence of strain S 24. In unsterile soil, PGPR isolates had a nodule-stimulatory effect on strain Cog 15 and a plant growth promoting effect on strain S 24, after 50 and 90 days of plant growth. Five isolates EG-RS-3, EG-RS-4, and NG-er-7 (*Bacillus* spp), and KG-ER-1 and EG-ER-2 (*Enterobacter* spp) significantly increased yield of green gram in unsterile soil.

A series of laboratory, greenhouse and field experiments were conducted by Niranjan *et al.* (2003) on strains of plant growth promoting rhizobacteria (PGPR). The PGPR were tested as suspensions of fresh culture 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 suspensions 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 *subtilis* strain IN937b (64 and 38% higher than the untreated control, respectively). With powdered formulation, treatment with strain INR7 also resulted in the highest germination and vigor indexes, which were 10 and 62%, respectively, over the untreated control. Under experimental plot condition, prominent enhancement in growth also was observed in the disease tests. Yield was enhanced 40 and 375 over the untreated control by seed treatment with powdered formulation 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 (57%), followed by the PGPR both under greenhouse and field condition. With fresh suspensions, treatment with INR7 resulted in the highest protection (57%), followed by *B. pumilus*

strain SE34 and B. subtilis GB03, which resulted in 50 and 43% protection, respectively, compared with the untreated control. With powdered formulation, PGPR strain INR7 suppresed downy mildew effectively, resulting in 67% protection, while SE34 resulted in 58%, followed by GB03 with 56% protection. Treatment with Apron (Metalaxy) resulted in the highest protection against downy mildew under both greenhouse and field condition. Thus, the present study suggest that the tested PGPR, both as powdered formulation and fresh suspensions, can be used within pearl millet downy mildew management strategies and for plant growth promotion.

Five plant growth promoting rhizobacterial formulations, each consisting of two Bacilli strains with chitson as a carrier were tested for their capacity to promote growth and induce resistance against downy mildew in pearl millet under both greenhouse and field conditions. Three modes of application were tested: seed treatment, soil amendment, and seed treatment+soil amendment. In general, irrespective of application method, most of formulations, in comparison with the control, increased growth and vigor as measured by seed germination, seedling vigor, plant height, fresh and dry weight, leaf area, tillering capacity, number of ear heads, length and girth of ear head, 1000 seed weight and yield. The time of flowering was also advanced by 4-5 days over the control. Likewise all the formulations significantly reduced downy mildew incidence relative to the nontreated control. However, the rate of growth enhancement and disease suppression varied considerably with formulation. Formulation LS 256 and LS 257 besides being the best growth promoters were also the most efficient resistance inducers. None of the formulation matched the level of the fungicide metalaxyl in offering protection against downy mildew. Among the application methods tested, soil amendment was found to be the most suitable and desirable way of delivering the formulation. Combination of seed treatment and soil amendment produced the same effect that was produced by soil amendment alone. This study by Raj *et al.* (2003) demonstrates a potential role for plant growth promoting rhizobacterial formulation in downy mildew management.

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 findings, Jetiyanon *et*

al. (2003) sought to determine if systemic resistance induced by these PGPRs would lead to broad-spectrum protection against several pathogens under field condition 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 disease and hosts tested were southern blight of tomato (*Lycopersicon esculentum)* caused by *Sclerotium rolsii,* anthracnose of long cayenne pepper (*capsicum annuum* var. *acuminatum)* caused by *colletotrichum gloeosporisdies,* and mosaic disease of cucumber (*cucumis sativus)* caused by cucumber mosaic virus (CMV). Results showed that some PGPR strain IN937a+*B. pumilus* strain IN937b, significantly protected (p=0.05) plants against all tested disease in both seasons. Further, cumulative marketable yields were positively correlated with some treatments.

Root colonization by certain non-pathogenic bacteria can induce systemic resistance to pathogen infection in plants. In a split-root assay with tomato plants, Siddiqui and Shaukat (2003) investigated which determinants of the rhizobacterium *Pseudomonas aeruginosa* IE-6S+ were important for induction of resistance to the root-knot nematode *Meloidogyne javanica. P. aeruginosa* IE-6S+ produced 3.9+1.1μg ml^{-1}salicylic acid (SA) in a liquid casamino acid medium under laboratory condition. The bacterial inoculants induced resistantce equivalent to the application of 10 mM synthetic SA. However, SA at this concentration did not produce significant mortality of *M. javanica juveniles in vitro.* Soil iron (2.4 mM $FeCl_3, 6H_2O$) did not markedly alter the resistance that *P. aeruginosa* IE-6S+ induced in tomato roots, which suggested that *P. aeruginosa* IE-6S+ activity was not iron-regulated. However, the resistance reaction was greatly enhanced when IE-6S+ and SA were co-inoculated with 0.5% Tween-20. While IE-6S+ colonized the tomato rhizosphere at 6.38 log cfu g^{-1} fresh weight of root during the first 3 days after inoculation, the bacterial populations declined steadily, reaching a mean population density of 4.73 log cfu g^{-1} fresh weight of root at 21 days. The bacterium was not isolated from the unbacterized half of the split root system.

Two plant growth-promoting rhizobacteria (PGPR), viz., *Pseudomonas fluorescens* strain Pf4 and *P. aeruginosa* strain Pag protected chickpea (*Cicer arietinum)* plants from *Sclerotium rolfsii* infection when applied singly or in

combination as seed treatment (Singh *et al., 2003).* Pag gave the best protection to the seedlings, applied either singly (mortality 16%) or in combination with Pf4 (mortality 17%) compared with 44% and 24% mortality in control and Pf4 treatment. respectively. The two PGPR strains induced the synthesis of specific phenolic acids. Salicylic acid (SA), as well as total phenolics at different growth stages of chickpea seedling with varied amount. The maximum amount of total phenolic was recorded in all the aerial parts of 4-week-old plants. Gallic, ferulic, chlorogenic, and cinnamic acids were the major phenolic acids detected in high-performance liquid chromatography (HPLC) analysis. Induction of such phenolic acids in the seedlings was observed up to 6 weeks in comparison with control. Salicylic acid (SA) was induced frequently during the first 3 weeks of growth only. Between the two strains, Pag was more effective in inducing phenolic acid synthesis applied either singly or in combination with strain Pf4 during the entire 6 weeks growth of chickpea. In the presence of culture filtrate of *S. rolfsii*, the two *Pseudomonas* strain induced more phenolic acids in treated than in non-treated and control plants. The occurrence of salicylic acid was frequent in the first 24 h, but infrequent at 48 and 96 h. foliar spray of *Pseudomonas* strains also enhanced the phenolic acid content as well as total phenolic within 24 h of application. Gallic, chlorogenic, and cinnamic acids were consistently discerned in the treated leaves, whereas SA was absent even up to 96 h of application. Resistance in chickpea plants by *Pseudomonas* strains trough induction of phenolic compounds as well as induced systemic resistance via SA-dependent pathway was evident.

Aflatoxin contamination of groundnut, caused by *Aspergillus flavus* (AF) group of fungi, is a major problem in the rain fed agriculture in the seni arid tropics. Biological control could be one of the components of integrated management to reduce pre harvest kernel investment in the field. Thakur *et al.* (2003) evaluated six *Trichoderma* and three *Pseudomonas* strains that were identified as highly antagonist to AF 11-4 (highly toxigenic strain) *in vitro*, in field to determine their biocontrol potential. The antagonists were applied as seed dressing and soil application in flowering in Af-sick pots. All the antagonists significantly reduced as seed infection in all three field experiments. Two *T. viridae* (Tv 17 and Tv 23), one *T. harzianum* (Th 23) and one *Pseudomonas* (pf 2) isolates provided greater protection to seed

infection by AF 11-4 than others. The reduce seed contamination occurred due to significant reduction in AF population in the rhizosphere of groundnut.

Sclerotinia stem rot of mustard incited by *Sclerotinia sclerotiorum* Lib. De Bary is one of the most important seed as well as soil borne diseases of mustard. Native rhizobacterial isolates of maize, wheat and chili were evaluated by Samanta and Dutta (2004) *in vitro* through dual culture method for their antagonistic activities against four impotant soil borne plant pathogens viz., *Rhizoctonia solani, Macrophomina phaselina, Sclerotium rolfsi* and *Sclerotinia sclerotiorum.* MPf-1 was found to be most effective isolate and suppressed the mycelial growth of all the four soil borne plant pathogenic fungi. Based on the culture, morphological and biochemical characteristics, five rhizobacterial isolates, MPf-1, MPf-2, ChP-1, ChP-2 and PfW1 were tentatively identified as *Pseudomonas sp.* belonging to fluorescent group and another P-2 was tentatively identified as Bacillus sp. Maximum seed germination was observed in MPf-1 and PfW1 treated seeds. Isolates from maize were found to be superior in respect to vigour index of mustard plant. Maximum phosphate solubilization and IAA production (10µg/ml) were observed in MPf-1 isolate. Four metabolites produced by MPf-1 isolate were identified and purified using TLC and HPLC. One metabolite Mp-III was identified as phenolic compound. Crude extract of MPf-1 inhibited the growth *Sclerotinia sclerotiorum* (84% at 2000 ppm). MPf-1 isolate as seed treatment and foliar spray showed better bio-protectant by causing a reduction in incidence of *sclerotinia* stem rot of mustard.

Greenhouse experiments were conducted by Anith *et al.* (2004) to the study the effect of plant growth promoting rhizobacteria (PGPR; *Bacillus pumilus* SE 34, *Pseudomonas putida* 89B61, Bio Yield, and Equity), acibenzolar-S-methyl (Actigard), and a soil amedement with S-H mixture (contains agriculture and industrial wastes such as bagasse, rice husk, oyster shell powder, urea , potassium nitrate, calcium super phosphate, and mineral ash) on bacterial wilt incidence caused by *Ralstonia Solana-cearum* (race 1, biovar 1) in susceptible tomato (*Lycopersicon esculentum* cv. Solar Set). In experiments with PGPR, *Pseudomonas putida* 89B61 significantly reduced bacterial wilt incidence when applied to the transplant at the time of seeding and 1 week prior to inoculation with *Ralstonia solanacearum.* Bio Yield, formulated PGPRs that contained two *Bacillus* strains, decreased disease

significantly in three experiments. Equity, a formulation containing more than 40 different microbial strains, did not reduce wilt incidence compared with the untreated control. With inoculums at low pathogen densities of 1 x 10^5 and 1 x 10^6 CFU m^{-1}, disease incidence of Actigard-treated plants was significantly less than with nontreated plants. This is the first report of actiguard-mediated reduction of bacterial wilt incidence in a susceptible tomato cultivar. When PGPR and Actigard application were combined, Actigard plus *P. putida* 89B61 or Bio Yield reduced bacterial wilt incidence compared with the untreated control. Incorporation of S-H mixture into infested soil 2 weeks before transplanting reduced the bacterial wilt incidence in one experiment. Combination of Actigurd with the S-H mixture significantly reduced bacterial wilt incidence in tomato in two experiments.

A pool of 11 randomly selected, uncharacterized *Bacillus pumilus* isolates from sugar beet were evaluated by Bargabus *et al.* (2004) using a high-throughput screened that utilized labotary-based tests for 2 pathogenesis-related proteins, chitinase and β-1,3glucanse, and biphasic hydrogen peroxide production. The screen was followed by a glasshouse test for induction of systemic acquired resistance for control of *Cercospora* leaf spot in sugar beet. These isolates were compared to the known biological control agent, *Bacillus mycoides* isolate Bac J. and a chemical inducer of resistance, acibenzolar-S-methyl. All laborty-based screens identified *B. pumilus* isolates 203-6 and 203-7, which reduced *Cercospora* leaf spot symptoms by approximately 70% even when spatially separated from the casual agent, *Cercospora beticola.* This level of control was similar to *B. mycoides* isolate Bac J and acibenzolar-S-methyl. In all cases, systemic resistance elicitation was marked by an increase in 2 pathogenesis-related proteins, chitinase and β-1,3glucanse, and was preceded by biphasic hydrogen peroxide production, also found in incompatible plant-pathogen interaction in which systemic resistance is induced. A combination of glycol chitin and aniline blue plate assays correctly identified all in plant inducers of systemic acquired resistance without the inclusion of false positive identification, reducing the workload in subsequent disease challenge assays by nearly 70%.

Jeun et *al.* (2004) compared the expression of induced resistance between cucumber plants induced with either plant growth-promoting rhizobacteria (PGPR) or chemicals. Inoculation with PGPR strains *Serratia marcescens* (90-166) and

Pseudomonas fluorescens (89B61) induced systemic protection in the aerial part of cucumber plants against the anthracnose pathogen *Colletotrichum orbicutaure.* Disease development was significantly reduced in these plants compared to control plants that were not inoculated with the PGPR strains. Inoculation with the PGPR strains caused no visible toxicity, necrosis, or other morphological changes. Induction with DL-3-aminobutric acid (BABA) or amino salicylic acid (ASA) also significantly reduced disease development. Soil drenched with 10mM BABA and 1.0mM ASA-induced resistance in cucumber leaves without any toxicity to the plants. Higher concentrations of ASA (up to 10mM) were phytotoxic, resulting in plant stunting and blighted appearance of leaves. Cytological studies using fluorescent microscopy revealed a higher frequency of autoflourescent epidermal cells, which are related to accumulation of phenolic compounds, at the sites of fungal penetration in plants induced with PGPR and challenged by the pathogen. Neither spore germination rate nor formation of appressoria was affected by PGPR treatments. In contrast, both BABA and ASA significantly reduced spore-germination rate and appressoria formation, while there were no differences from controls in the frequency of autoflourescent epidermal cells at the sites of fungal penetration. Their findings suggest that PGPR and chemical inducers cause different plant response during induced resistance.

Talc based bioformulation containing cells of *Pseudomonas fluorescens, Bacillus subtilis* and *Saccharomyces cerevisiae* were evaluated for their potential to attack the mango (*Mangifera indica* L.) anthracnose pathogen C*olletotrichum gloeosporioides* Penz. under endemic conditions by Vivekananthan *et al.* (2004). The pre harvest 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 minimum induction of flowering, a yield attribute in the pre harvest stage, consequently reduced latent symptoms were recorded at the 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. The enhanced expression of defense-mediating enzymes may collectively contribute to suppress the anthracnose pathogen, leading to improved yield attributes.

Khabbaz *et al.* (2005) isolated five bacteria from different ecosystems viz., phylloplane of paddy leaves, water pond and industry effluent were screened against the major fungal diseases of rice viz., blast, sheath blight, brown spot and foot rot. Among the collected isolate, isolate no. S12 from phylloplane of rice leaves showed promising results against *Pyricularia oryzae, Rhizoctonia solani, Helminthosporium oryzae* and *Fusarium moniliforme* by producing strong inhibition in dual culture technique. Percent inhibition of mycelial growth was recorded based on the inhibition zone. The isolate S12 was identified as *Bacillus* sp. based on the biochemical tests. The same isolate can be formulated and used effectively under field conditions.

Pure culture of *P. aeruginosa* isolated from soil and charecterised according to Bergey's manual of determinative bacteriology were studied by Sharma *et al.* (2005) for siderophore production as well as antifungal activity. Siderophore production was determined by CAS reagent using top layer method. Antifungal activity of this strain against *Fusarium moniliformae*, *Alternaria* and *Helminthosporium halbdes* was assayed by seeding the bacterial lawn with fungal dics and incubating the plates at 37^0 C. Antifungal activity of cell free filtrate of *P. aeruginosa* was also studied. Eight-millimeter diameter wells were made in an agar plate seeded with fungal discs and filled with cell free filtrate of 5 days old culture of *P. aeruginosa*. The plates were inoculated at 37^0 C Inhibition of growth of all three fungi by as well as cell free filtrate was observed. Inhibition of fungal pathogens is due to production of antifungal secondary metabolites by *P. aeruginosa*. Use of cell free filtrate of as a bio control agent can thus provide an eco-friendly option and hence suggested.

Bhatia *et al.* (2005) studied ten isolates of fluorescent pseudomonads from rhizosphera of sunflower, potato, maize and groundnut. All the isolates produced fluorescent pigment in succinate broth displayed siderophore production. Production of hydrocyanic acid (HCN) and indole acetic (IAA) by all the isolates was reduced besides phosphate solubilisation. Out of the ten strains, *Pseudomonas* PS I and PS II was found most potential. Bacterisation of sunflower seeds with fluorescent *Pseudomonas* PS I & PS II resulted in increased seed germination, root length, shoot length, fresh and dry weight of roots ands shoots, and yield of sunflower. Seed bacterisation with strains of fluorescent *Pseudomonas* PS I & PS II reduced incidence

of collar rot by 69.8% and 56.9% respectively, in *Sclerotium rolfsii*-infested soil, making the organism a potential bio control against collar rot of the sunflower.

A number bacterial strains isolated by from rhizophere of different crop plants including tea (*Camellia sinensia* L. (O) kuntze) were screened for their ability to suppress two root disease-brown rot and charcoal rot of tea under gnotobiotic and nurseray condition. The strains were initially selected based on their *in vitro* antibiotic against *Fomes lamaoensis* and *Ustulina zonata*, the causative organism of brown and charcoal rot disease of tea. Three fluorescent *Pseudomonas* strain designated as RRLJ, B4, RRLJ 04, RRLJ 706 and a non fluorescent *Pseudomonas* strain AMJ showed significant suppression of both the disease under nursery condition. Seed dressing of the stem cutting with these strain also enhanced the percentage of survival of cutting in the nursery condition besides enhanced the number of leaves with high chlorophyll content. The application of these strain reduced the disease incidence of charcoal rot in field condition. The bio active metabolites isolated from these strain also showed plant growth protein and disease suppression properties. RRLJ 134 produced six different bioactive metabolites of which three have been identified as phenazine analogues. (Dileep Kumar *et al.*2005)

The *Pseudomonas flouresens* isolate 1 (Pf1) was found to protect the ragi [*Eleusine coracana* (L.) Gaertner] blast fungus *Pyricularia grisea.* Induction of defense proteins *viz.* chitinase, β-1, 3 glucanase, peroxidase (PO) and poly phenol oxidase (PPO) by the Pfl isolate was studied against *P. grisea* by Radjacommare (2005). Analysis of chitinase in a resistant, susceptible and commonly used cultivar with and without challenge inoculation of *P. grisea,* revealed changes in the isoform pattern by UV illumination after staining the gel with fluorescent brightner 28. Native PAGE (polyacrylamide gel electrophoresis) of PO showed the single isoform in all the treatments including the control and a significant increase in the intensity of the band in the inoculated control and Pfl treatment in all the varieties. Isoform analysis of PPO showed the induction of PPO in *P. fluorescens* treated plants challenged with *P. grisea.* Application of Serenade, a commercial biofungicide formulation containing the bacterium *Bacillus subtilis,* to the cinematic surface of open blue berry flowers suppresses floral infection by the mummy berry fungus *Monolinia vacciniicorymbosi.*

Out of 500 rhizobacteria isolated from soil, rhizosphere and rhizoplane of healthy tomato plants one isolate was previously selected by Romeiro *et al.* (2005) in laboratory, green house and field tests as a good inducer of systemic resistance. This plant growth-promoting rhizobacterium (PGPR) was identified as *Bacillus cereus* by fatty-acid analysis. *Bacillus cereus* bacterial cells were removed from liquid culture by centrifugation and the supernatant repeatedly dialyzed (cut-off =12 000 daltons) against distilled water. Dialysates applied to roots protected tomato plants against leaf fungal and bacterial pathogens gives evidence that macromolecules synthesized by the PGPR and released into the environment act as elicitors of systemic resistance.

The aim of a study by Demoz and Korsten (2006) to determine the Avocado flower inoculated with a liquid commercial formulation of *Bacillus subtilis* B246 were observed at different time intervals under the scanning electron microscope (SEM). Population dynamic of the antagonistic on the flower were determined by means of total viable counts using reference culture and background counts from the control. Flowers were also inoculated with antagonistic-pathogen (*Dothiorella aromatica* and *Phomopsis perseae*) in combination to determine *in vivo* interactions. The SEM observation and population dynamics study confirmed that the antagonistic could effectively attach, colonize, and survive on avocado flowers. It could also attach to conidia and hyphae of the pathogens and cause cell degradation. These modes of action can give new insights into the control of pathogens by *Bacillus subtilis.*

Different formulations of *Bacillus licheniformis* were evaluated on their own and in combination with prochloraz and stroburilin for their ability to reduced mango post-harvest fruit diseases [anthracnose and stem-end rot (SR)] when applied as a dip treatment in a mango pack house. Untreated fruit and fruit treated with either prochloraz or stroburilin alone served as controls. In these trials treatments integrating chemical pesticides with *B. licheniformis* controlled antharacnose and SR as effectively as the chemical control of post-harvest disease when fruit were kept in cold storage to simulate export conditions. In two of three trials, results obtained when fruit was treated with the antagonist in combination with the commercial chemical were comparable to that obtained with the commercial chemical control. In this study by Govender and Korsten (2006), it was found that the antagonist when

used in mango pack house treatments could provide an effective alternative which can be successfully incorporated into the existing pack line.

Chakraborty *et al.* (2006) isolated *Bacillus megaterium* De Bary TRS- 4 from tea rhizosphere and tested for its ability to promote growth and cause reduction in tea plants. *In vivo* studies revealed the ability of this bacterium to promote growth of tea plants very significantly. Brown root rot disease, caused by *Fomes lamaoensis* was markedly reduced by application of the bacterium to the soil. Population of *F. lamaoensis* in soil before and after supplication 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. Root colonization by *B. megaterium* and subsequent inoculation with *F. lamaoensis* also led to an increase in polyphenolics, as well as in defense related enzyme- peroxidase, chitinase, □-1,3-glucanase and phenyl alanine ammonia lyase. Determination of mechanism of action of this bacterium revealed it to be able to solubilize phosphate, produce IAA, siderophore and antifungal metabolite. The plant growth promotion and reduction of disease intensity have been shown to be due to a combination of several mechanisms.

In greenhouse experiments, plant growth promoting rhizobacteria (PGPR) *Serratia marcescens* NBR11213 was evaluated for plant growth promotion and biological control of foot and root rot of betelvine caused by *Phytophthora nicotiane* (Lavania *et al.*,2006) . Bacterization of betelvine (*Piper betle* L.) cuttings with *S. marcescens* NBR11213 induced phenylalanine ammonia-lyase, perxodase, and polyphenoloxidase activities in leaf and root. Qualitative and quantitave estimation of phenolic compounds was done through high-performance liquid chromatography (HPLC) in leaf and root of betelvine after treatment with *S. marcescens* NBR1213 and infection by *P. nicitianae.* Major phenolics detected were gallic, protocatechuic, chlorogenic, caffeic, ferulic, and ellgic 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 phenolic was increased in *S. marcescens* NBR11213-treated plants infected with *P. nicotianae.* In a greenhouse test, bacrerization using *S. marcescens* NBR11213 decreased the number of diseased plants compared with 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 than untreated controls. This is the first report of PGPR-mediated induction of phenolics for biological control and their probable role in protecting betelvine against *P. nicotianae, an important soil-borne phytopathogens*fungus.

Saika *et al.* (2006) attempted to control an algal pathogen of tea, *Cephaleuros parasitica* by biological means. The pathogenic alga was isolated from the leaves and twigs of red rust effected tea plant from Gotonga Tea Estate, Jorhat, Assam during July, 2002. Algal colony on the culture medium was detected after 48 hours of inoculation. The organisms (RR3) used for preparation of algaecide is a heterotrophic bacterium isolated from tea garden soil of Assam which grows well in a specific medium at pH 6.9. *In vitro* algaecides activity of the bacterium was studied by using live culture, culture filtrate and bacterial metabolite. Results showed complete inhibition of growth of the algal pathogen at a concentration of 20:100 when live culture and culture filtrate was used. Bacterial metabolite at a concentration 0.6 g/ 100ml was effective against the algal pathogen. Besides the algaecidal activity, the bacteria (RR3) and its metabolite enhance plant growth. The growth was determined by measuring the biomass and number of new off-shoot emergence in the treated tea plants.

Pieterse *et al.* (2006) developed an *Arabidopsis*-based model system using *Fusarium oxysporum f sp raphani* and *Pseudomonas syrigae pv tomato* as challenging pathogens, in order to study the molecular basics underlying the systemic resistance. Colonization of the rhizosphere by the biological control strain WCS417r of *P. flourescens* resulted in a plant-mediated resistance response that significantly reduced symptoms elicited by both challenging pathogens. Moreover, growth of *P. syringae* infected leaves was strongly inhibited in *P. flourescens* WCS417 treated plants. Transgenic *Arabidopsis* NahG plants, unable to accumulate SA, and wild-type plants were equally responsive to *P. flourescens* WCS417r-mediated induction of resistance. Furthermore, *P. flourescens* WCS417r-mediated systemic resistance did not coincide with the accumulation of PR mRNAs before challenge inoculation. The result indicated that *P. flourescens* WCS417r induces a pathway different from the

one that controls slassic systemic acquired resistance and this pathway leads to a form of systemic resistance independent of SA accumulation and PR gene expression.

Plant growth-promoting rhizobacteria (PGPR) bioformulations (*Pseudomonas* and *Bacillus)* were tested for their efficacy against blister blight (*Exobasidium vexans)* disease in tea (*Camellia sinensis)* under field conditions for two seasons. Among the bioformulations tested, foliar application of *P. flourescens* Pfl at 7-d intervals consistently reduced the disease incidence of blister for two seasons, almost comparable with that of chemical fungicide. In addition to disease control, it also increased tea yield significantly compared to the untreated control. Induction of defense enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase, β-1, 3-glucanase and phenolics were studied. The enzyme accumulation was greater in *P. flourescens* Pfl-treated plants compared to control. The study revealed the probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance in tea plants against blister disease by PGPR bio formulation (Saravanakumar *et al.* 2006).

Pseudomonas corrugate, a soil bacterium originally isolated from a temperate site of Indian Himalayan Region (IHR) was examined by Trivedi *et al.,*(2006) for its antagonistic activities against two phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum.* Although the bacterium did not show inhibition zones due to production of diffusible antifungal metabolites, a reduction in growth between 58 % and 49 % in both test fungi, *A. alternata* and *F. oxyporum,* was observed in sealed Petri plates after 120 h of incubation due to production of volatile antifungal metabolites. Reduction in biomass of *A. alternata* (93.8) and *F. oxyporum* (76.9) in Kings BSNL broth was recorded after 48 h of incubation in dual culture. The antagonism was observed to be affected by growth medium, pH and temperature. The reduction in fungal biomass due to antagonism of bacteria was recorded maximum in the middle of the stationary phase after 21 h of inoculation. The production of siderphore, ammonia, lipase and chitase in growth medium by *P. corrugata* were considered contributing to the antagonistic activites of the bacterium.

Two tea rhizosphere microorganisms, *Bacillus megaterium* and *Ochrobactrum anthropi* inhibited the growth of four tea pathogens, *Fomes lamaoensis, Sphaerostilbe repens, Poria hypobrunnea* and *Sclerotium rolfsii,* to a

certain degree, in both solid and liquid medium. Chakraborty *et al.* (2007) showed the application of B. *megaterium* and *O.anthropi,* either singly, or in combination, to rhizosphere of *Camellia sinensis* promoted growth seedling significantly, but the dual application was more effective. Besides, the bacteria could also control brown root rot of tea, caused by *Fomes lamaoensis*. *B. megaterium* was more effective than 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.

Peanut are frequently invaded by toxigenic *Aspergillus fiavus* prior to harvest, especially in hot, dry condition. Strategies for reducing aflatoxin contamination of peanut have concentrated on minimizing postharvest infestation of seed with toxigenic strains of *A.flavus* and *A. parasiticus.* Historically, these strategies have emphasized cultural practices, since chemical control and prevention of preharvest fungal infestation have generally been ineffective.Biological control offers one potential strategy for preventing seed invasion by *Aspergillus flavus* or preventing production of aflatoxin *in situ.* One approach for biocontrol which is currently being investigated is the incorporation of nontoxigenic strains of *A. parasiticus* into peanut field soil which results in displacement of toxgenic *Aspergillus* spp. The date, there has been no extensive evaluations of bacteria as potential biological control agents against aflatoxigenic fungi on peanuts. In assay optimization experiment, two *in vivo* assays (seed and root radical) were developed and optimized for screening 119 strains of geocarposphere bacteria as candidate biological control against *A. flavus*,. Seven bacterial strains viz. *Bacillus megaterium, B. laterosporus, Cellulomonas cartae, Flavobacterium odoratum, Phyllobacterium rubiacearum, Pseudomonas aurofaciens* and *Xanthomonas* maltophila prevented colonization of seeds and root radicales by the fungus. Root growth promotion, noted with some strains, may indicate that bacteria alter the host physiology, which in turn, could be advantageous for control of aflatoxin production. In green house experiment, inoculation of root regions of 1 to 2 – week old peanut plants with toxigenic *Aspergillus flavus* and geocarposphere bacterial resulted in lower synthesis of aflatoxin B_1 in the peanut kernels at maturity, than those in plants inoculated with the toxigenic strains alone. Of seven bacterial strains tested, four strains showed reduction in aflatoxin production in varying extents. Pre-inoculation of bacterial strains (1-day earlier) resulted in greater

inhibition of aflatoxin accumulation. However, toxin level was not much reduced when the bacterial strains were introduced 1-day after inoculation of toxigenic *A. flavus* strain. *Bacillus megaterium* showed maximum inhibition of aflatoxin biosynthesis as compared to remaining three potential bacterial strains. The morphological interactions among A.flavus and other bacteria were also examined on peanut extract agar medium. The results suggest the potential of bacteria as biological control agents against pre- harvest aflatoxin contamination of developing peanuts. (Chourasia, 2007)

Growth promotion and pathogen suppression are the two well known mechanisms exhibited by PGPR. Bio–innovation research with PGPR and biocontrol research has led to the development of bacterial inoculants that consistently promise higher crop productivity. Gnanamanickam *et al.* (2007) conducted research programmed in India and US during 2006 and 2007 for three of their products whose active ingredients are *Bacillus* or *Pseudomonas* strains. EcoGuard GN is a EPA-registered biofungicide whose active ingredient is *Bacillus licheniformis* 3086. In a field test conducted during 2006 at Coimbatore, weekly applications of Eco Guard GN at 260 l/ha led to yields of 30.5 t/ha while the untreated control crop produced 12.6 t/ha (58% increase). The biofungicide used at this concentration also reduced the incidence of down mildew (caused by *Plasmopara viticola*). The percent disease index (PDI) in treated plots was reduced by 38.2% compared to untreated control. TAEGRO, also an EPA- registered biofungicide formulated in cornstarch contains 24.5% of freeze-dried spores of *Bacillus subtilis* var *amyloliquefaciens* FZB24. In US trials carried out in 2007, TAEGRO afforded substantial control of *Xanthomonas campestris* pv. *vesicatoria* (bacterial speck) and Phytophthora root rot of tomato better than. Kocide 2000. In addition to its well known fungicidal properties against *Rhizoctonia*, *Fusarium*, and Phytophthora (in particular the ridomilresistant isolates of pink-rot of potato pathogen, *Phytophthora erythroseptica*), TAEGRO has also shown consistent enhancement of crop yields in crops such as tomato, potato, cucumber and several ornamental crops. In recent greenhouse tests, the incorporation of TAEGRO in potting mix at 0.613 g/gallon pot suppressed *Ralstonia solanacearum* (bacterial wilt of tomato) by 75% over that of the untreated control. The third product under investigation is stain 3621 of *Pseudomonas congelans*. It suppresses the growth of *Ralstonia solanacearum*, X.c. pv. *vesicatoria* and *Erwinia amylovora* in laboratory

and greenhouse assays. This Gram-negative strain with proven PGPR and biocontrol traits now available as a wheat bran product and also in alginate beads is yet to be field tested. At present their believe that TAEGRO has the potential of a biobactericide that can control important diseases such as bacterial with and fire blight and a mixture of TAEGRO and *P. congelans* would be a much superior microbial product for disease control and growth enhancement/crop productivity in the organic and global agricultural market segments worldwide.

Liquid formulations of PGPR (*Rhizobium, Azotobacter, Azospirillum*, PSM, etc.) have become a preferred method for inoculating plants for improved availability of nutrients in soil and thereby promote growth of plants. A seed survival study was conducted by Acharya *et al.* (2007) during May-2007 of *Azotobacter chroococcum* (ABA-1), Anand isolate in liquid and carrier formulations and for their ability to support growth and promote survival of *Azotobacter* during storage on *Pennisetum glaucum* cv. GHB-558. In a polyethylene bag, 100g seeds were treated with 0.5 ml of liquid or 2.5 g carrier based formulation and approximately 2-5 ml sterile distilled water was added for uniform coating of inoculants on seeds. *Azotobacter* count was done on Ashby's mannitol agar medium by serial dilution method, various additives like glycerol and polyvinyl phrolidone (PVP) @ 2% to liquid inoculants promote higher cell of all the formulation depended on the *Azotobacter* strain and additives, when stored at room temperature. Cell count up to 6.8×10^5 x, 8.0×10^5, 6.6×10^5 CFU/g seed respectively after 48 hours at 30^0C, whereas Charcoal and Lignite based formulation maintained 6.4×10^4, 6.0×10^4 CFU/g seed respectively. After 48 hours survival of bacteria on seed was reduced drastically. Seed germination effect of all inoculants formulation was observed on seed agar keeping an uninoculated check. After 48 hours of incubation in dark increased root and shoot length with secondary root formulations gave better germination compared to untreated check. The present investigation indicated that liquid inoculants with additives promote survival of bacteria on seed and also increase germination.

Effect of different plant growth promoting rhizobacteria (PGPR) and the method of application of PGPR were investigated by Hariprasad and Umesha (2007) to determine whether biocotrol of bacterial spot disease of tomato caused by *Xanthomonas vesicatoria* (Doidge) Dye could be improved. The PGPR strains

(*Bacillus subtilis* strain GB03, *Bacillus amyloliquefaciens* strain IN937a, and *Brevibacillus brevis* strain IPC11) were selected based on the reported capacity to induce resistance against various bacteria pathogens of tomato. PGPR applications were made by seed, root and fohar spray treatment separately and in combinations in field. Among them, GBB03 was the most effective in providing significant suppression of bacterial spot and was well correlated with increased activity of defense related enzymes viz, peroxides and phenylalanine ammonia lyase. Combination treatments proved to be the best in reducing the bacterial spot incidence. Plant growth promoting rhizobacteria that were effective in green house were also able to induce resistance in under field conditions against bacterial spot of tomato.

Maiti *et al.* (2008) made an attempt to isolate and utilize a potential biocontrol agent against two fungal diseases of *Stevia rebaudiana* caused by *Alernaria alternata* and *A. steviae*. *Pseudomonas aeruginosa* WS-1, a rhizosphere isolate among 134 isolates, showed in vitro antagonistic activity against both the phytopathogens. Microscopic examination after antagonism showed hyphal shriveling, swelling, vaculation, short branching and granulation of cytoplasm resulting in lysis of hyphae of the pathogens. Correlation of antifungal activity activity of this isolate has been found to be linked with the production of siderophore, proteases and chitinases. Furthermore, Talc based formulation of the antagonist @ 4 gm/ 1 (containing 10^6 cells/ ml) showed 84% and 71% protection against leaf spot disease caused by *A. alternata* and leaf blight disease caused *A. steviae* respectively when applied at an interval of 15 days in field condition.

Vermicompost based bioformulation of *Pseudomonas aeruginosa* was effective in suppression of bacterial wilt (Ralstonia solanacearum) incidence of chilli (*Capsicum annum*) in field. Quantitative assays of the population dynamics of *P.aeruginosa* revealed that the shelf-life of this bioagent was maintained upto 200 days of storage at room temperature (26 ± 2^0C). Bioformulation of vermicompost with *P.aeruginosa* carboxy methyl cellulose and mannitol was the best to maintain shelf-life and high population recovery of *P.aeruginosa* during storage (10^6 x 10^7 cfu/ml). Application of this bioformulation as seed treatment followed by root and soil application at transplanting and soil application at 30 days after transplanting of chilli, showed minimum wilt incidence(8.7%), maximum yield (87.39/ha) and high

recovery (58.93 x10^7 cfu/g) of *P.aeruginosa* from the crop rhizosphere (Bora and Deka, 2008)

2.2.2. Fungi

Madi and Katan (1998) reported that infiltration of *Penicillium janczewskii* conidia or its culture filtrate into melon and cotton leaves induced systemic resistance and protected the lower part of the stem of melon and cotton plants against *Rhizoctonia solani*, leading to up to a 100% reduction in the incidence of damping-off. Hypersensitive reaction like responses was observed in melon and tobacco, but not in cotton. Peroxidase activity, associated with induced systemic resistance, increased in treated plants of both species compared to the control plants. Gel elctrophoresis of peroxidases from melon plants treated with culture filtrate exhibited enhanced activity of all three isozymes present in untreated plants, but mostly of the slowest migrating isozyme. Phenylalanine ammonia-lyase activity in stems of melon plants treated with *P. janczewskii* or its culture filtrate increased two-fold compared to the untreated plants. Western blot analyses revealed induction of β-1, 3-glucanase, a pathegenesis-related protein, and HSP 70, a member of the heat shock protein family in melon. Altered root development was observed in cotton plants infiltrated with *P. janczewskii* conidia or its metabolites. Our results suggest that treating leaves with *P. janczewskii* or its culture filtrate triggers the signal transduction cascade, activating different defense genes in melon and cotton, thus protecting the lower parts of the stem.

Assam and Tamil Nadu isolates of *Trichoderma harzianum*, *T. viride* and *T. virens* were tested by Hazarika and Das (1998) for their potential to suppress *Rhizoctonia solani*, the French bean root rot pathogen under *in vitro* conditions. All isolates inhibited growth of *R. solani*. Culture filtrates of *T. harzianum* and *T. viride* inhibited mycelial growth and sclerotial germination. Wheat bran substrate supported maximum growth of all isolates followed by farm yard manure and tea waste. Both *T. harzianum* and *T. viride* effectively controlled the bean root rot disease when they were applied as seed and soil treatment.

Singh *et al.* (1998) *Trichoderma viride* and *T. harzianum* when applied as soil inoculation plus seed treatment proved most effective in reducing incidence of dry root rot and increase phenolic compound and carbohydrate contents of chickpea

followed by *Pseudomonas fluorescens, Bacillus subtilis* and *Aspergillus flavus.* Soil inoculation plus seed treatment was the best method of application followed by soil inoculation and seed treatment alone.

Seven *Trichoderma* spp., seven isolates of *Pseudomonas fluorescens,* two isolates of fluorescent pseudomonad, *Bacillus subtilis* and one yeast, namely, *Saccharomyces cerevisiae* were screened against *Colletotricum capsici* (Syd) Butler and Bisby, both in *in vitro* and on the plant. Jeyalakshmi *et al.* (1998) found, among the fungal antagonists, *S. cerevisiae* exhibited maximum reduction of the mycelia growth followed by *T.viride* Among the 10 bacterial antagonists, *B. subtilis* showed the maximum growth reduction followed by *P.fluorescens* isolate 27. In pot culture experiment *S. cerevisiae* recorded the maximum reduction of fruit rot intensity and incidence and die-back incidence followed by *B. subtilis,* when sprayed on 105 and 120 days after sowing. However, it was next only to carbendazim (0.1%)

Potential of seven promising biocontrol agents (BCAs) *Chaetomium globosum, Coniothyrium minitans, Gliocladium virens, Laetisaria arvalis, Trichoderma harzianum, T.hamatum* and *T. viride* against *Rhizoctonia solani* Kuhn, causing root rot of French bean (*Phaseolus vulgaris* L.) was studied by Mathew and Gupta (1998) under *in vitro* and glasshouse conditions. *In-vitro* evaluation of BCAs by dual-culture method revealed that *T. harzianum* caused maximum inhibition, followed by *T.hamatum,T. viride* and *G. virens.* In pot experiments, *G. virens* and *T. harzianum* proved superior to other antagonists in reducing pre-emergence root rot to 6.7 and 13.3%, respectively, as compared to 36.7% in control. *T. harzianum* was also effective to reduce post-emergence root rot. Pre-inoculation of antagonists proved to be a superior method to check post-emergence root rot.

Rhizome rot of ginger is caused by either *Pythium* or *Fusarium* spp. or both (mainly *P. myriotylum* and *F. solani*). Resident biocontrol agent (BCA) *Trichoderma harzianum* isolated from rhizome rot suppressive soils reduced the disease and increased plant stand and yield. In order to further enhance the efficiency of disease suppression, Ram *et al.* (1999) used a bacterial BCA *Pseudomonas* sp individually, in combination with and also with fungicidal rhizome treatment. Combination of both BCAs resulted in better germination and plant stand, reduced disease, and increased yield. Soil application of BCA was more effective compared to their seed treatment.

Integration of soil application of BCA with fungicidal rhizome treatment (bavistin + ridomi MZ) increased the efficiency of disease control as compared to their individual treatments. Soil application of and *T. harzianum* rhizome treatment with *Pseudomonas* sp and fungicides was the most effective among all the tested treatments.

Bunker and Mathur (2001) evaluated three biocontrol agents(BCAs) individually and in combinations, and in integration with Bavistin seed treatment in pathogen infested soil in pots, for suppression of dry root rot pathogen *Rhizoctonia solani* in bell pepper (*Capsicum frutescens* cv. *california* Wonder. Seed treatment with the biocontrol agents was as effective and bavistin seed treatment. Integration of seed and soil application of individual BCA resulted in higher germination and reduced mortality due to disease. Combination of two biocontrol agents, particularly of *Trichoderma harzianum* and *T.aureoviride* was better than the individual ones. Population of BCAs in chilli rhizoshere and soil was directly related to suppression of *R.solani*. Application of mixture of *T.harzianum* and *T.aureoviride* as seed and soil treatment was the most promising in increasing the germination and suppression of chilli root rot pathogen and the disease.

Wilt caused by *Fusarium oxysporum f.sp. cumini* (Foc) is the most important and destructive disease of cumin (*Cuminum cyminum*).To reduce the population of this pathogen and the incidence of wilt on cumin in field, efforts were made to evolve environmentally sound method of management by utilizing native bio-control agents. *Aspergillus versicolor* highly antagonistic to *F .oxysporum* was isolated from arid soils. Dual culture tests were performed to confirm its antagonistic activities and were also compared with that of *Trichoderma harzianum*, a known biocontrol agent. In Foc and *A.versicolor* infested soil, an initial population of 4.2×10^4 CFU g^{-1} soil of Foc drastically declined to 2.8×10^3 CFU g^{-1} soil after 15 days of incubation causing 93.3% reduction compared to 73.8% reduction in Foc prop gules in the presence of *T.harzianum*. In liquid culture tests, cell-free filtrates even at 0.5 ml concentration of both the bio-control agents could inhibit mycelial growth of Foc. Reduction in mycelial growth of Foc in the the cell-free filtrates of *A. versicolor* and increase in reduction with increased concentration of cell-free filtrate is a clear evidence that *A. versicolor* has released certain antibiotics. Studies related to thermal resistance to

A .versicolor showed that it was able to survive and multiply even at 65^0C. An initial count of 6.3 x 10^4 CFU g^{-1} of *A. versicolor* increased many fold with the increase in the time interval at a temperature range of 50-55^0C under moist conditions. Studies on integration of soil amendments and bio-control agents revealed that amending soil with *A. versicolor* alone or in combination with *T.harzianum* or residues was significantly better in reducing Foc propagules compared to non-amended control. Better survival and multiplication of *A .versicolor* at low soil moisture content and at high soil temperature are of beneficial consequences of utilizing its potentials against control of *Fusarium* in dry sandy soils, where temperatures often reaches in the ranges of 50-60^0C during hot summer months. (Isreal *et al.* 2002)

Fungal isolates antagonistic to *Phomopsis vexans* were isolated from plant rhizosphere and phylloplane. An isolate Bb-III of *Beauveria bassiana* was found very effective during preliminary investigations. Efficacy of this isolate was tested *in vivo* in pots using soil infested with *P.vexans* virulent isolate. This soil was amended by Chani *et al.* (2002) with different concentrations viz. $1x10^5$, $1x10^6$, $5x10^6$ and $1x10^7$ cfu/100 g and seeds of brinjal variety Pb. Barsati were sown in each pot. The biocontrol isolate reduced seed rot by 70% and seedling blight by 57%, the damping off incidence was reduced by 65.6% and final plant stand was 87.3% compared to 51.4% in control. Highest concentration $1x10^7$ cfu/100 g soils was the most effective. Biocontrol agent performed better than seed dressing with captan. Further studies on its formulation and application technology could lead to its development as a biofungicide

Collar rot disease caused by *Phytophthora cactorum* (Leb. and Cohn) Schrot results in 12-15 percent mortality of apple plants in the nurseries and also causes extensive economic losses in established orchards by out rightly killing the grown up plants. Since it is difficult to manage this disease by chemical treatments, therefore two non-chemical methods.viz.employment of biocontrol agents (BCAs) and addition of amendments (plant leaves, cakes) in the soil were evaluated in controlling this disease under pot culture conditions. Out of seventy three fungal BCAs and thirty two bacterial BCAs isolated from the soil samples collected from apple orchards and nurseries, twenty three (fungal) and twelve (bacteria) were found effective against the target pathogen under laboratory conditions. Pot culture evaluation of *in vitro*

effective BCAs of fungal origin revealed that five namely, *Trichoderma longibrachiatum, T.harzianum,T. viride,T virens* and *Penicillium funiculosum* were found highly effective and gave 89.96 percent disease control. Similarly, out of twelve bacterial BCAs four namely, *Bacillus subtilis, Enterobacter aerogenes, Pseudomonas putida* and *Bacillus spp.* were found effective to c heck the seedling mortality up to an extend of 78.6-89.2 percent. Further, in evaluation of BCAs of mycorrhiza origin (8 No.) separate addition of two viz. *Glomus mosseae* and *G.macrocarpus* checked the disease development up to the extent of 71.3 and 68.7 percent, respectively under pot conditions. In another experiment, screening of fifteen different soil amendments (plant leaves, cakes) against the target pathogen indicated that addition of dried leaves of *Lantana camara, Vitex negunda, Melia azadirachta, Murraya koeningii* and Mustard cake were found effective in controlling the disease up to 66.3-78.4 percent. (Sharma *et al.* 2002)

Indiscriminate use of synthetic pesticide for controlling plant diseases not only causes environmental pollution, species disappearance, pressure on natural resources but their toxic residues enter into animals and humans and causes several health hazards. Alternatively biological management of plant diseases using soil borne antagonistic fungi are ecofriendly and reduces use of pesticides and may be employed as a component of sustainable agriculture. The chickpea wilt complex is caused by pathogens viz., *Fusarium oxysporum f. sp. Ciceri* (Padwik), *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn. Use of various antagonistic fungi such as *Gliocladium virens* /in combination with fungicide (vitavax. 0.1%) not only controlled chickpea wilt reduce environment pollution. Preparation of powdered base *G. virens* / *T. harzianum* using different carriers were made which costs about Rs. 25 per kg, other preparations that are available in the market costs about Rs. 125 per kg hence, it may be suggested that use of along with (vitavax. 0.1%) in the form of powdered preparation not only controls chickpea wilt complex effectively but also shows growth promoting activities. (Singh *et al.* 2004)

Yamada (2004) isolated *Sporothrix* sp. from tea phylloplane and screened for their antagonistic activity against tea anthracnose fungus *Colletotricum theae-sinensis.* Screening was done by pathogen- antagonist dual culture test. Culture filtrate of *Sporothrix* sp. inhibited elongation of germ tube of conidia of *C. theae-sinensis.*

Lesion size of anthracnose was decreased when conidia suspension of *C. theae-sinensis* was inoculated together with *Sporothrix* sp. by wound inoculation method using detached mature leaves

Singh and Singh (2005) reported the case of a formulation of *Trichoderma harzianum* as vine treatment to control collar rot of betel vine (*Piper betle* L.) caused by *Sclerotium rolfsii*. The strains were isolated from disease suppressive soil in the conservatories located at Mahoba, UP, India.The per cent mortality in the vines treated with *T. harzianum* formulation was 9.39% as compared to 76.91% in inoculated control. In addition to the disease control, the yield of betel vine in terms of number of leaves was also increased in *Trichoderma* treated vines in comparison to both inoculated and uninoculated control.

Chili (*Capsicum frutescens* L.) is an important spice cum vegetable crop. Leaf spot and fruit rot caused by *Alternaria alternata* (Fr.) Keissler is severe and common disease in entire chilli growing area in the country. It infects all the aerial parts of the plant causing severe losses. Eight known antagonists viz., *Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *Aspergillus flavus*, *A. niger*, *Chaetomium globosum*, *Gliocladium virens* and *Bacillus subtilis* were evaluated *in vitro* by dual culture, pathogen at periphery and pathogen at centre method. Gohel *et.al* (2005) reported that all the antagonists were significantly superior in checking the growth of the pathogen. In case of dual culture method, *T. longibrachiatum* produced maximum inhibition (73.21 %) of the pathogen.Whereas *T. harzianum* (49.46%) proved best in pathogen at periphery method. While in case of pathogen at centre method.*A.flavus* (77.00%) produced maximum inhibition. Overall *Trichoderma* spp., A.*flavus* and *B.subtilis* were found better against *A.alternata.*

In a study by Jha and Jalali (2005) the culture filtrates of different fungal antagonists' viz., *Trichoderma viride, Aspergillus niger, A. flavus, A. terreus, A. sydowi* and *Spicaria sylvatica* were tested at 5%, 10% and 20% concentration in relation to their effect on radial growth of *Fusarium solani f sp.pisi* by dual culture method. They found that the culture filtrates of all the tested antagonists showed significant inhibition of radial growth of the pathogen *F. solani f.sp.pisi* and an increase in the concentration of culture filtrates resulted in greater inhibition of the growth of pathogen.The culture filtrate of *T. viride* recorded the maximum inhibition

of radial growth of pathogen by 38.8,54.12 and 60.11% at 5,10 and 20% concentration, respectively, followed by that of *A. niger,* where the radial growth of the pathogen was inhibited by 21.32,42.27 and 52.38% at 5,10, and 20% concentration of culture filtrate,respectively.Like-wise the culture filtrates of *S. sylvatica and A. terreus* also showed marked inhibition of radial growth of the pathogen which was 24.40 and 19.30%,respectively, at 10% concentration and 29.41 and 25.73% at 20% concentration of filtrates. On the other hand the culture filtrate of *A.flavus* recorded relatively lesser inhibition while that of *A. sydowi* exhibited the least inhibition of radial growth of the pathogen.The inhibition of radial growth of *F. solani f.sp.pisi* by culture filtrates of various antagonists might be due to production of antifungal metabolite by the respective antagonists.

Collar rot of brinjal (*Solanum melongena* L.) caused by *Sclerotium rolfsii* Sacc, was also reduced by different antagonists viz., *Trichoderma viride, T. harzianum, T. longibrachiatum, Aspergillus flavus, A. niger, Gliocladium virens* and *Bacillus subtilis* which were evaluated by Patel *et al.* (2005). The antagonists were applied @ 50/ Kg soil in previously inoculated (60g inoculun/Kg soil) pots. *T.harzianum* and *T. viride* were found superior in reducing pre and post-emergence seedling mortality, enhanced germination and resulted in better final plant stand.The next best in order of efficacy were *A. niger* and *T. longibrachiatum.*

Multilocational field studies were conducted (1999-2000 to 2002-2003) by Gaur *et al.* (2005) to evolve the best application technique of *Trichoderma harzianum* for controlling dry root rot (*Rhizoctonia solani*) of chickpea. The studies (rain fed and irrigated) conducted on the basis of preliminary green house results showed that dry root rot of chickpea can be effectively and economically managed either by the soil application of 10-15 days pre-inoculate . *T.harzianum* (TG-1) @ 10 Kg ha-^{1} in 200 kg 0f FYM or by the practice of seed treatment with talc-based formulation of *T.harzianum* containing 2x10^{6} c.f.u./g @ 10 g Kg-^{1} seed in combination with soil application of $ZnSO_4$ @ 25 Kg ha^{-1}.Under rainfed situation, seed treatment with talc based formulation of *T.harzianum* @10 g Kg-^{1} seed proved economically better. Therefore, it may serve as an ecofriendly substitute to carbendazim.Wheat bran-based formulation of *Trichoderma* gave better control of disease than talc-based formulation for soil application. Similarly soil application of *Trichoderma* was found significantly

superior over seed treatment. *Trichoderma* @ 10g Kg^{-1} seed gave better control than the lower dose of 4 g Kg^{-1} seed. Technique of seed dip and solid matrix priming did not prove effective.

Chandel *et al.* (2005) isolated different microorganisms from the rhizosphere of carnation plants which were tested under *in vitro* in pot and field conditions to ascertain their antagonistic property. Different microorganisms namely, *Aspergillus sp., Fusarium sp., Penicillium sp., Rhizopus* sp., *Trichoderma, T. viride, T. hamatum,* bacteria *Bacillus* sp. *Pseudomonas* sp. and actinomycetes were isolated from the carnation rhizosphere. Maximum inhibition of the mycelial growth of the fungus (*Fusarium oxysporum f. sp. Dianthi)* was shown by *T. harzianum* (76.54%) with minimum radial growth (9mm) that was superior over the treatment and was closely followed by *T. viride*(70.06%). However, minimum inhibition was recorded in *Rhizopus* sp. (28.70%) that was statistically on par with the In pot culture, all the antagonists were able to reduce the wilt disease incidence over control, maximum being recorded in *T. harzianum* followed by *T. viride, Aspergillus sp.,* and *Bacillus* sp. while in field conditions *T. harzianum* followed by *T. viride* gave more than 80% disease control. However, *Penicillium sp.,* were found least effective in disease control. Both the root dip and soil drench method were found equally good with no significant difference in the disease incidence. The present disease incidence was comparatively less after 35 days of planting as compared to 60 days.

Aspergillus sp., followed *Trichoderma* was found to be dominant in the turmeric rhizosphere (Dutta *et al.* 2005b). On the basis of microscopic observation and growth characterisatics native Tri-Pun and Tri-Pun 2 isolates were tentatively identified as *T. virens* and *T. hamatum* respectively. Tri-Pun2 followed by Tri-p-Pun inhibited growth of soil-borne plant pathogens during dual culture method. Potato dextrose agar and Oat meal agar media were found to be most suitable for growth and sporulation of native *Trichoderma* isolates. Though the competitive saprophytic ability (CSA) of Thdl (*T. hamatum* Delhi isolate) was best as compared to native isolates, Tri-Pun and Tri-Pun2 also belong to higher group. Paddy grain and paddy husk were found to be the best locally available substrates for mass multiplication of native Tri-Pun 2 isolate.Eighty one percent spore viability was observed after 137

days at normal temperature in paddy grain substrate. Tri-Pun 2 was found to be most effective after Bavistin for management of web blight disease of black gram.

Betel Vine (*Piper betle* L.) is an important commercial crop of Karnataka, mainly cultivated for its leaves, known as 'Pan'. Raghavebdra *et al.* (2005) isolated *Pythium vexans*, a soil-borne fungal pathogen of betel vine in the Tarikere taluk of Southern transition agroclimatic zone of Karnataka, from diseased plant parts by baiting technique. The pathogencity tests revealed that the isolated fungus *P. vexans* was pathogenic. The *in vitro* control of *P. vexans* was carried out by following 'Poison food technique'. The cold-water extracts of *Allium sativum* (Garlic) and *Azadirachta indica* (Neem) showed significant inhibition of colony growth. *A. sativum* showed maximum inhibition of *P. vexans* colony at 80% and 100% concentrations by 49.57% and 52.59% respectively. *A. indica* at 80% and 100% concentration showed maximum inhibition of P. vexans colony by 18.53% and 48.71% respectively. The dual culture studies revealed that the native isolates of *Trichoderma harzianum* was effective against *P. vaxans*. The average inhibition percentage of *P. vexans* by *T. harzianum* was 22.29%. Experiment on *in vivo* control of the disease was carried out under green house conditions. The test plants (*P. betle*) were raised in the pots, for the treated plants, the soil was amended with 100g of *T. harzianum* that was mass multiplied in the substrate 'paddy straw-sorghum' mixture, for the untreated plants (control) only substrate was added to the soil. For both treated and control plants four mycelial discs were placed around the root zone. After ten days the roots of the treated host plants were observed for the disease symptoms. The treated host plants containing the inoculums of *T. harzianum*, did not develop any disease symptoms and remained healthy and the disease was expressed in untreated host plants

Devi and Paul (2005) collected soil and wilt/root rot infected plant samples from pea growing areas. Five pathogens viz., *Fusarium oxysporum f. sp. pisi, F. solani f. sp. pisi, Rhizoctonia solani, Phoma medicaginis var. pinodella* and *Sclerotionia sclerotiorum* were found to be associated with the disease. Thirteen antagonists were isolated from the soil and screened against the pathogens. Out of these, JMA-4(*Trichoderma harzianum*), SMA-5 (*T. harzianum)*, DMA-8 (*T. koningi)* and JMA-11 (*T. koningi*) were found to be more promising on the basis of their broad

spectrum activity and growth characteristics. Hyphal interaction studies revealed several hyphal abnormalities in the pathogens such as swollen hyphae, excessive branching, hyphal sliming, hyphal apex deformation, excessive vacuolation and lysis. Soil factors like 30% soil moisture, 25^0C soil temperature and 6.6 pH were found to be most suitable for the growth of JMA-4. Among ten plant extracts evaluated, *Ranunculus muricatus* showed strong antifungal activity against pathogens and biomass. *Eupatorium adenophorum* did not affect the growth of bioagents but was inhibitory to pathogens. Spictaf (0.1%) was ineffective to biomass whereas, it caused more than 50% inhibition of pathogens. Out of three fungicides tested, Bavistin was found to be highly toxic to pathogens as well as the bioagents. Different delivery systems evaluated against test pathogens revealed that wheat bran based formulation was most effective against *R. solani* and *F. solani*. Application of bioagent in the form of sodium alginate pellets resulted in lowest disease caused by *F. oxysporum* and *P. medicaginis*. Seed treatment was found to be effective against wilt/root rot complex disease in pot experiments. Under field conditions use of sodium alginate pellets was found to be highly effective delivery system. Integration of seed treatment with bioagent + soil application with wheat bran based formulation plus mulch followed by seed treatment with Spictaf (0.5%) + soil application with wheat bran plus mulch were found to be most effective in managing the disease.

Thakur *et al.* (2006) used different bioagents against *Rhizoctonia bataticola in vitro* and *in vivo*. *In vivo* studies revealed that seed treated with spore suspension of *Trichoderma harzianum* increases significantly. *Trichoderma harzianum* was found superior over *T. viride*, EM solution of *Bacillus subtilis*. Seed treatment with *T. harzianum* influenced the seed germination and there was considerable increase in plant height also. *In vitro* experiment revealed that *T. harzianum* was superior in arresting the growth of *Rhizoctonia bataticola* followed by *T. viride* over control. EM solution was found to be effective even at lower concentration of 2 percent and *Bacillus subtilis* was also found effective in restricting *Rhizoctonia bataticola*. *T. harzianum T. viride* significantly reduced the root rot incidence to 23.18% and 28.35% respectively compared to 83.6% incidence in control. Thus, it may be inferred that seed treatment with *T. harzianum* could bring down the menace of root rot caused *Rhizoctonia bataticola* (Taub.) Butler in Sesamum.

Chakraborty *et al.* (2006) reported that two tea rhizospheric bacteria- *Bacillus pumilus* TRS3 and *Bacillus megaterium* TR S4, as well as native strains *Trichoderma harzianum* and *T. viride* were found to be highly antagonistic *in vitro* against *Poria hypobrunnea*, *Fomes lamoensis* and *Spherostilbe repens*, causing root rot, brown root rot and violet root rot, respectively. *In vivo* applications of the bacteria and fungi led to enhancement in growth of tea plants over respective untreated control. Significant control of root rot diseases was also achieved due to treatment with the bacteria and *Trichoderma sp.* Species of *Glomus*, along with other mycorrhizal fungi were isolated from tea rhizosphere. *In vivo* application of *Glomus* to the rhizosphere also significantly increased plant growth and reduced root rot intensity. Dual inoculation of VAM and PGPR markedly enhanced the growth of the tea seedlings in terms of plant height, number of leaves, number of branches and dry weight of root and shoot. Phosphorous uptake was also enhanced by tea seedlings by combined inoculation with VAM and PGPR. Significant increases were obtained in mojor biochemical components- proteins, total phenols and O-dihydroxy phenols following various treatments. Defense related enzymes- phenyl alanine ammonia lyase, peroxidase, β-1, 3 glucanse and chitinase also showed increased activity when treated with VAM and PGPR, either singly or in combination. Catechins, which are major flavonoid flavour components of tea leaves, were analyzed by HPLC to determine changes in the different isoforms. Several new peaks were observed following bacterial treatments. Antifungal phenolics were extracted from treated tea roots, analyzed by HPLC and bioassay was performed using spore germination of *S. repens* and radial growth assay of *F. lamoensis*. Treatment with *Trichoderma* did not enhance activity of defense enzymes or phenolics. Population of pathogens in soil and root tissues, as determined by immunodiagnostic tests, were greatly reduced in soil after application of *Trichoderma* sp. Results of present study indicate while *Bacillus megaterium* and *Bacillus pumilus* act by inducing systemic resistance in tea plants, *Trichoderma sp.* act by their direct antibiosis against the pathogens.

"Som" (*Persea bombycina* Kost) the primary food plant of muga silk worm is prone to various diseases that affect the quality and quantity of leaves and cocoon production. Among them Grey blight is one of of the major disease of *P.bombycina* caused by *Pestalotiopsis disseminate* (Thum) Stey. The leaf loss due to grey blight disease is estimated at 1273 kg per hectare per annum. Indiscriminate use of

chemicals to control plant diseases is a threat to environment; therefore the alternative approach is necessary to adopt integrated disease management using eco-friendly methods by biological controlling agents.With this view, an *in vitro* experiment was carried out to study the effect of three fungal antagonists of *Trichoderma spp viz. T.harzianum, T. viride,T.hamatum* and four bacterial antagonists isolates of *Pseudomonas fluorescens* against mycelial growth and conidial development of *Pestalotiopsis disseminate* the causal organism of grey blight disease of "Som" by dual culture technique.The antagonists' treatment on the mycelial growth showed that the maximum growth inhibition of the pathogen was exerted by *T. viride*(51.7%) followed by *T.hamatum*(45.9%)The plates amended with *Trichoderma spp* and isolates *Pseudomonas fluorescens* did not show any reduction in sporulation of the pathogen and was almost at par with check.(Das *et al.* 2006)

Potentiality of *Trichoderma harzianum* Rifai as seed treatment agent on reduction of white mold incidence of French bean caused by *Sclerotinia sclerotiorum* (Lib.) de Bary was assessed in FYM amended soil under field condition. Further, the effect of FYM amended soil on the distribution of *Trichoderma spp* and associated microflora in root rhizosphere was also studied by Gohain and Das (2006). In the present investigation, efficacy of four different concentrations of water extract of FYM amended soil viz., 0.1%.0.5%, 1.0% and 1.5% at three different period of incubation. Viz., 1, 2 and 3 weeks were tested for stimulation of growth of *T.harzianum* and inhibition of *S sclerotiorum.* The highest radial growth of *T.harzianum* was recorded in 1.5 percent water extract of FYM after two weeks of incubation *in vitro*, which was statistically at par with 1.0 percent after same period of incubation. On the other hand, minimum radial growth of was observed in 1.5 per cent of water extract of FYM amended soil after 2 weeks of incubation which was statistically at par with 1.0 per cent after same period of incubation. In field condition, seed treatment with *T.harzianum* along with 0.05% carbendazim in 1.0% FYM amended soil was found effective in reducing the white mold incidence of French bean. Increased dry weight of roots shoots and yield of crop was also observed in the field due to the same treatment as compared to inoculated control. The population density of *Trichoderma spp* in root rhizosphere of French bean was found highest in amended soil irrespective of different concentrations than unamended soil in field condition. Maximum population of *Trichoderma spp* in rhizosphere soil (both surface

and soil of 6 cm depth) was found when seeds were treated with along with 0.5% of carbendazim. At the end of the experiment, microflora associated with rhizosphere of French bean were identified as *Aspergullus sp.*-1, *Aspergullus sp-2, Rhizopus sp., Penicillium sp., Bacillus sp-1, Bacillus sp-2* and *Bacillus sp-3.* The sclerotial productions of *S sclerotiorum* were arrested significantly when seeds were treated along with 0.05% carbendazim in 1.0% FYM amended soil under field condition. From the analysis of soil before and after amendment with FYM, decreased soil pH with increased organic carbon was observed in amended soil as compared to unamended soil.

Anwer and Khan (2006) carried out an investigation to examine effectiveness of selected soil isolates of *Aspergillus niger* against *Rhizoctonia solani in vitro* and *in vivo.* Numerous isolates of *A. niger* were collected from different crop fields and pure culture on potato dextrose agar. The isolates were characterized for production of ammonia, hydrogen cyanide, hydrogen sulphide, indole acetic acid, siderophores, phosphate solubilization and antagonism against *R. solani in vitro.* Four soil isolates of *R. solani* viz. Anc_2, AnR_3, $AnPP_2$ and AnS_2 were selected on the basis of above characters to evaluate their effectiveness against root rot caused by *R. solani* on egg plant, *Solanum melongena* cv. Pusa Kranti in 25 cm clay pots filled with 2 kg sterilized soil (field soil+ compost, 3:1). The isolates were applied in soil (1 g/kg soil) and on roots (bare root dip treatment).Before seedling planting the soil in pots was inoculated with 2 g sorghum seed colonized by *R. solani* (2g/kg soil). Inoculation with *R. solani* resulted to 75% decrease in the seed germination. The cultivar Pusa Kranti was found highly susceptible to the infection by *R. solani* and exhibited significantly decrease in the plant growth (16%) and flowering (21%). Application of *A. niger* isolates increased the seed germination, decreased the root rot and improved the growth variables ($P< 0.05$). Soil application with the isolate AnC_2 checked the root rot (21%) and improved the dry matter production (11%) and flowering (17%).

Reddy and Raghavender (2006) used arbuscular mycorrhizal fungus, *Glomus fasciculatum* in biological suppression of charcoal rot fungus *Macrophomina phaselina.* It is clear from their study that charcoal rot disease was controlled (physical and physiological process) to a large extend when the sorghum plants are inoculated with the mycorrhzal fungi. No disease was observed in the varities of RS-29 and E-36-1 when inoculated with mycorrhizae and pathogen. The disease

incidence was reduced upto 80% even in the variety CSV-8R which was considered as highly susceptible to charcoal rot. The reduction in disease severity may be due to competition of sites, through the main reason is by strengthening the host plant so that damage caused by the pathogen was offset by improved plant growth.

Fungal biocontrol agents (BCA) viz, *Trichoderma viride, T.harzianum, T.hamatum, T.virens* and bacterial isolates B1 and B2 of *Bacillus* sp. were tested by Verma and Sharma (2007) *in vitro* and *in vivo* against *Fusarium solani* causing mango seedling wilt. Fungal BCA were more effective than bacterial BCA under *in vitro* as well as in pot studies. *T.harzianum* caused a maximum (72%) *in vitro* inhibition, followed by *T. virens*(71%), *T. viride* (67%), *T.harzianum* (58%), *Bacillus* isolates B1 and B2 were the least effective. In pre-as well as simultaneous applications, *T.harzianum* recorded the least population of *F.solani* in pot soil and *T. virens, T. viride* and *T.hamatum* were the next best treatments. *Bacillus* isolates were the least effective. The pre-applications of biocontrol agents *T. virens* and *T. viride* were more effective. Maximum disease control was obtained when the pathogen was inoculated 10 days after the application of biocontrol agents. The least level of disease control was in post-application of biocontrol agents *T.harzianum* and *T. virens* recorded the highest disease control and *T. viride* and *T.hamatum* were the next best in pre- application treatments. Mango seedlings were healthier and recorded maximum increases in shoot and root lengths as well as weights.

Trichoderma harzianum and *Pseudomonas fluorescens* the compatible antagonists to the pathogen *Phytophthora capsici* were used in combination to control root rot of chillies by More and Baig (2007). *T.harzianum* inhibited mycelial growth of *P. capsici in vitro* after 48 h by dual culture method with a drastic growth reduction by 89%. *T.harzianum* arrested the spread of the pathogen by covering the complete surface of the pathogen colony and sporulated as evident of microscopic studies where as *P.fluorescens* produced a zone of inhibition. The formulation prepared on a mixture of soil and vermiculite was effective against the pathogen up to 2 years when stored at room temperature in the laboratory. The optimal dose of the antagonists in the compound formulation was $3.5x10^{-8}$ spores/ml of *T.harzianum* $1.0x10^{-9}$ cfu/ml of *P.fluorescens.* The two antagonists in the formulation were effective at pH from 3.5 to 5.6 at 20-40^{0}C.