Application of Fungi as a Biocontrol Agent and their Biofertilizer Potential in Agriculture

Pandya, U* and Saraf, M
Department of Microbiology, School of Sciences, Gujarat University, Ahmedabad-09

Abstract

The need for new and useful compounds to provide assistance and relief in all aspects of the human life is ever growing. There are enormous difficulties in raising enough food on certain areas of the earth to support local human populations. Environmental degradation, loss of biodiversity and spoilage of land and water also add to the problems faced by mankind. In the continual search by both pharmaceutical and agricultural industries for new products, natural selection has been found superior to the combinatorial chemistry for discovering novel substances that have the potential to be developed into new industrial products. The plant is an extraordinarily common source of organic energy. It is thus likely that a huge array of microbes like bacteria and fungi reside inside the plant tissues and interact with them. Of these, a range can be isolated from apparently healthy tissues, many of which have never been documented to be associated with disease; others may cause disease when environmental conditions change. The intent of this review is to provide insights into the presence of fungi in nature and their diversity, their interaction with the host plant and their role in plant growth promotion.

Key words: Plant growth promoting fungi, phosphate solubilization, siderophore, cell wall degrading enzymes.

Introduction

Microorganisms are important component of soil. Soil bacteria and fungi mediate soil processes such as decomposition, nutrient mobilization and mineralization, storage and release of nutrients and water, nitrogen fixation and denitrification. In the frame of agriculture, the micro flora is of great significance because it has both beneficial and detrimental influence upon man's ability to feed himself[1]. All the environmental factors which influence the distribution of bacteria and actinomycetes also influence the fungal flora of soil. The quality and quantity of organic matter present in soil have a direct bearing on fungal numbers in soil since most fungi are heterotrophic in nutrition. Fungi are dominant in acids soils because acidic environment is not conducive for existence of either bacteria or actinomycetes, resulting in the monopoly of fungi for utilization of native substrates in soil. Isolation of fungi from different horizons of soil profiles shows that these organisms exhibit selective preference for various depths of soil. Those fungi which are common in lower depths are rarely encountered on the surface of soil which may be explained on the basis of the availability of organic matter and the ratio between oxygen and carbon dioxide in the soil atmosphere at varying depths. Seasonal fluctuations in fungal numbers are not uncommon. Farm practices including crop rotation and fertilizer or pesticide applications influence the nature and dominance of fungal species[2].

Diversity of Fungi

The number of fungi recorded in India exceeds 27,000 species, the largest biotic community after insects. The true fungi belong to kingdom Eukaryota which has 4 phyla, 103 orders, 484 families and 4970 genera. The eighth edition of '*Dictionary of the Fungi*' has recognized eleven phyla. The Deutromycotina is not accepted as a formal taxonomic category. The number of fungal genera reported from the world and that from India between 1905 and 1995, are shown in Table 1.

Table 1 Fungal genus[3]

Phyla	World	India
Myxomycotina	450	380
Mastigomycotina	308	205
Zygomycotina	55	50
Ascomycotina	2000	745
Basidiomycotina	357	232
Deuteromycotina	4100	468
Total	7270	2080

Fungi interact with their hosts, and also with abiotic variables in the environment. They occur on rocks, in soil, in sea and fresh water in extreme habitats, experiencing high and low temperature, on

*Corresponding Author : Email- urjapandya1@yahoo.co.in

dry substrates and in concentrated nutrients. Members of mucorales are considered as ruderals since they survive in soil as long as nutrients are available although they are not capable of degrading cellulose or lignin. Fungi like *Fusarium*, *Gliocladium*, *Penicillium* and *Trichoderma* are stress tolerant. Majority of fungi are mesophilic with maximum growth between 25 and 30ºC (*Mucor mucedo*, *Mortierella*, *Penicillium chrysogenium*) however *Cylindrocarpon* sp., *Candida scotti* are cold tolerant (Psychrotolerant) and can grow near 0ºC; others are thermo tolerant and grow above 40ºC (*Rhizomucor*,*Thermomyces*,*Talaromyces*). Xerotolerant fungi can grow on dry material (*Aspergillus*, *Penicillium*) with low matric potential (a_w) while Osmotolerants grow at very low osmotic potential (*Pichia* sp.). Dung of herbivorous mammals harbors a large number of fungi, termed coprophiles, of which *Pilobolous*, *Ascobolus* and *Basidiobolous* are famous for their special shot gun dispersal mechanism. Fungal endophytes are micro fungi that colonize living tissues of plants without producing any apparent symptoms or obvious negative effects. Fungi that are biotrophic mutualists, benign commensals or latent pathogens are included under the broad term 'endophytes'[3].

Role of Fungi in Agriculture

Similar to PGPR (plant growth promoting rhizobacteria), some rhizosphere fungi able to promote plant growth upon root colonization are functionally designated as 'plant-growth-promoting-fungi' (PGPF)[4] .PGPF belong to genera *Penicillium*, *Trichoderma*, *Fusarium* and *Phoma*. Several species of PGPF have been shown to trigger systemic resistance against various pathogens in cucumber plants[5]. Plant growth promoting fungi (PGPF), which are non-pathogenic soil inhabiting saprophytes, have been reported to be beneficial to several crop plants not only by promoting their growth but also by protecting them from diseases[6,7]. Among these PGPF, some isolates of *Phoma* sp. and *Penicillium simplicissimum* GP17-2 were highly effective in inducing systemic resistance against cucumber anthracnose caused by *Colletotrichum orbiculare*[8,9].

One of the primary functions of filamentous fungi in soil is to degrade organic matter and help in soil aggregation. Besides this property, certain species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Dematium*, *Gliocladium*, *Helminthosporium*, *Humicola* and *Metarhizium* produce substances similar to humic substances in soil and hence may be important in the maintenance of soil organic matter. Some of the fungi capable of forming ectotrophic associations on the root system of forest tree such as pine belonging to the genera *Boletus* and *Lactarius* help in the mobilization of soil phosphorus and nitrogen into plants. In many instances establishment of new forest becomes difficult unless mycorrhizal fungi are artificially introduced into soil by inoculation[2].

The fungus *Trichoderma harzianum* has long been investigated as a biological control organism against several soil borne pathogens and shown to be capable of increasing plant growth and yields. These increases usually were attributed to the reduction of plant diseases. This fungus could also have the potential to stimulate plant growth independent of any plant disease. Applications of *T. harzianum* in plant production, therefore, can reduce the use of fungicides, growth regulators and labor which eventually will lower the production costs and environmental impact[10]. For example, *Trichoderma harzianum* was shown to solubilize phosphate and micronutrients that could be made available to plant[11]. *Piriformospora indica* (hymenomycetes, Basidiomycoya) was described as a cultivable endophyte plant growth promoting fungi[12]. Differential inducible defense mechanism against bacterial speck pathogen in *Arabidopsis thalina* by plant growth promoting fungus *Penicillium* sp.GP 16-2 and its cell free filtrate is reported[13]. Interactions between plant growth promoting fungi (*Phoma* sp.GS8-2 & GS8-3; *Penicillium simplicissimum* GP17-2) and *Glomus mosseae* and interaction of systemic resistance to anthracnose disease in cucumber is studied[14]. It is reported that gene hal I from *S. cerevisiae* protein is involved in regulation of K^+ transport and showed higher level of salt tolerance and transgenic which were able to retain more K^+ than controls under salt stress[15]. Arbuscular mycorrhizal fungi (AMF) are the most widespread root fungal symbiont and are associated with the vast majority of higher plants. AMF have been shown to improve soil structure and have great importance due to their great capability to increase the plant growth and yield through efficient nutrient uptake. AMF might alleviate some nutrient deficiencies, improve drought tolerance, overcome the detrimental effects of salinity and enhance tolerance to pollution[16].

Role of Fungi as PGP (Plant Growth Promoter)

Plant roots support the growth and activities of an array of microorganisms that may impart profound effects on growth and health of plants. Diversity of such microorganisms is studied for certain culturable bacteria, fungi, actinomycetes and other eukaryotic microorganisms. Plant growth mechanisms can be grouped as follows: direct- like asymbiotic fixation of atmospheric nitrogen, solubilization of minerals such as phosphates and production of plant growth regulators like auxins, gibberellins, cytokinin and ethylene; and indirect- like HCN production, antibiotics, siderophores, synthesis of cell wall lysing enzymes and competitions with detrimental microorganisms for sites on plant roots[17].

Phosphate solubilization

Phosphorus (P) is one of the major essential macronutrients for biological growth and development[18]. Filamentous fungi are widely used as producers of organic acids[19] particularly black *Aspergilli* and some species of *Penicillium*. These species have been tested for solubilization of rock phosphates[20,21]. Inoculation of phosphate solubilizing fungi and mycorrhizal fungi improve the physico-chemical, biochemical and biological properties of rock phosphate amended soil. Higher available P and aggregate stability levels, higher soil carbon levels, enzyme activities and lower soil pH were also reported due to inoculation of these fungi[22]. Among phosphate solubilizing microorganisms, fungi perform better in acidic soil conditions[23]. Species of *Aspergillus*, *Penicillium* and Yeast have been widely reported solubilizing various forms of inorganic phosphates[1,24]. Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria[25]. *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., had the more solubilizing ability of inorganic insoluble phosphate than bacteria, viz., *B.subtilis* and *B. megatarium*[26]. They concluded that application of biofertilizer prepared by above mentioned fungi should be helpful to reduce the salinity of soil by neutralization phenomenon. Increased P uptake by plants is reported due to inoculation of *Aspergillus niger*[27]. The phosphate solubilization and antifungal activity of *Aspergillus niger*, *Curvularia lunata*, *Rhizoctonia solani* and *Fusarium oxysporium* is reported[28] and the beneficial prospects of these organisms for better crop productivity is also described.

Siderophore production

The acquisition of iron by microorganisms in organic environments[29] presents a difficult problem since the solubility products constant for ferric hydroxide is about 10^{-38}. Thus at pH 7, the freely available iron is at a concentration of no more than 10^{-17} M, which is far below that required for microbial and plant growth. Iron in an aerated environment exists in the ferric form and hence is highly insoluble in neutral or alkaline soil[30]. To solve this problem, microorganisms are genetically observed to utilize a high affinity iron transport system. The synthesis and secretion of a low molecular weight ferric-specific chelation agent to solubilize iron is termed as Siderophore[31,32]. Microbial siderophore may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots or indirectly by competitively inhibiting the growth of plant pathogens with less efficient iron uptake system[33]. In soil, plant roots normally coexist with bacteria and fungi which may produce siderophores capable of sequestering the available soluble iron and hence interfere with plant growth and function. Siderophores are produced during extreme iron-depleted conditions for the solubilization of extracellular ferric iron by most bacteria and fungi. The most significant feature of siderophores is their extremely high affinity for ferric ion, which can be so high (iron binding constant of $>10^{30}$ for three bidentate ligands) as to even permit microbial extraction of iron from stainless steel. The majority of fungal siderophores are hydroxamates, apart from the carboxylate -type siderophore rhizoferrin produced by Zygomycetes. Siderophores are generally named based on their iron charged forms and the prefix deferri- or desferri- is used to denote the iron-free (deferrated) form of the ligand. Hydroxamate siderophores are derived from the non-proteinogenic amino acid ornithine and can be classified into four structural families; fusarinines, coprogens, ferrichromes and rhodotorulic acid[34]. Most species of the genus *Aspergillus* are known to produce several hydroxamate type siderophores and many reports on the isolation and characterization of siderophores have been publishe[35].

Phytohormones production

Plant growth promoting fungi (PGPF) are well known for the production of useful secondary metabolites. The plant growth promoting capacity of fungal endophytes is partly due to the endophytes' production of phytohormones such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances and/or partly owing to the fact that endophytes could have enhanced the hosts' uptake of nutritional elements such as nitrogen and phosphorous. PGPF are associated with plant roots, and they secrete a number of secondary metabolites including gibberellins in the rhizosphere. Gibberellin (GA) secretion by PGPF was reported by several researchers which showed the importance of GA producing fungi in plant growth and development, especially under nutrient-deficient conditions[36]. Phytohormones have been found to be synthesized not only by plants but also by microorganism including bacteria and fungi. Both indole-3-acetic acid and gibberellins were found in microorganisms long before their recovery in flowering plants. Fungi may affect the growth and development of plants in many different ways. Mutualistic associations between mycorrhizae and plants often result in increased biomass production, better health and better resistance of the host plant to attack by pathogens. The major plant auxin, indole-3-acetic acid (IAA), was first detected in culture filtrates of the fungus *Rhizopus suinus*, even before IAA was identified in plants[37]. The effects of tryptophan, IAA and IAM on IAA biosynthesis in plant pathogenic fungus *Colletotrichum gloeosporioides* f.sp. *aeschynomene* axenic cultures and on in planta IAA production is studied[38]. Biosynthesis of indole-3-acetic acid by the gall inducing fungus *Ustilago esculenta* is reported[39]. The production of IAA in culture by fungi was solely dependent on the presence of tryptophan .The addition of thiamine (Vitamin B_1) to medium greatly enhanced fungal growth whereas IAA production was completely inhibited. Maximum amount of IAA (ca.1µg mL^{-1}) was obtained after 8 days of incubation. The production of IAA was highly correlated with the amount of tryptophan. Absciscic acid (ABA) and indole-3-acetic acid (IAA) concentration in *Funalia trogii* (Berk) *Bondartsev* and *Singer* and *Phenerochaete chrysosporium* Burds.ME446 under salt stress is studied[40]. The levels of ABA and IAA were determined by gas chromatography (GC) and UV-VIS spectrophotometry. These results showed that, in fungi, ABA concentrations are positively and IAA concentrations negatively correlated with salt stress. IAA is reported as one of the metabolites that have been isolated from *Pythium aphanidermatum* showed the same symptoms of *Phythium* red blight on bent grass at concentration of 1000mg L^{-1} that was 200 times higher[41]. These results suggested that IAA produced by this fungus was the causal substance of *Phythium* red blight on bentgrass. *Phenerochaete chrysosporium* ME 446 synthesized the growth substances IAA, GA_3, ABA and zeatin as primary and secondary metabolites[42]. Recovery of IAA, GA_3, ABA and zeatin were respectively 55.50%, 74.6±8%, 51.6±10% and 56.63±6%.

Fungi as biocontrol

Currently, the role of BCAs (Biological Control Agents) is a well established fact and has become increasingly crucial, and in several cases, complementary or even replacing the chemical counterparts where antagonistic fungi play an important part. Fungal based BCAs have gained wide acceptance next to bacteria (mainly, *Bacillus thuringiensis*), primarily because of their broader spectrum in terms of disease control and yield. In this context, *Trichoderma* spp. has been the cynosure of many researchers who have been contributing to biological control pursuit through use of fungi[43]. Fungi of the genus *Trichoderma* are important biocontrol agents (BCAs) of several soil borne phytopathogens[44]. The molecular characterization of several wild isolates has shown a certain degree of polymorphism and the presence of three different ITS lengths[45] and the secondary metabolites involvement in biocontrol has been recently reviewed[46]. *Trichoderma* use different mechanisms for the control of phytopathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes[47]. In addition, *Trichoderma* could have a stimulatory effect on plant growth[48] as a result of modification of soil conditions. *Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soil born pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds[49].

Arbuscular Mycorrhizal Fungi (AMF) is the major components of the rhizosphere of most plants and plays an important role in decreasing plant disease incidence. Several AMF species have been found to control soil borne pathogens such as species of *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Pythium*, *Rhizoctonia*, *Sclerotinium* and *Verticillium*. Under green house conditions *Glomus fascicuatum* and *Gigaspora margarita* were shown to decrease root rot diseases caused by *Fusarium oxysporum* f.sp.*asparagi* in asparagus and *Glomus clarum* was shown to decrease root necroses due to *Rhictonia solani* in cowpea. The AM fungus *Glomus mosseae* was shown to systemically reduce take-all disease infection caused by *Gaeumannomyces graminis* var. *tritici* in barley[50].

Filamentous fungi of the genus *Trichoderma* have long been recognized as agents for the biocontrol of plant diseases. *Trichoderma* spp. can directly affect mycelia or survival propagules of other fungi through production of toxic secondary metabolites, formation of specialized structures, and secretion of cell wall-degrading enzymes[51]. This mycoparasitic activity of *Trichoderma* spp. against phytopathogenic fungi and oomycetes due to lytic activity of cell wall-degrading enzymes has been widely studied. In addition to mycoparasitism, other mechanisms have been proposed to account for biocontrol of plant disease by *Trichoderma* spp., including the induction of resistance in the host plant and competition for nutrients and potential infection sites[47]. All of these mechanisms have been shown to be employed effectively by *Trichoderma virens*. This biocontrol agent has been recognized as an aggressive mycoparasite capable of competing ecologically when colonizing potential sites of infection. Different strains have been shown to induce phytoalexin production and systemic resistance. *T. virens* produces secondary metabolites, including gliotoxin, gliovirin, and peptaibols with known antimicrobial activities that have been shown to act synergistically with lytic enzymes to enhance the destruction of host cell walls[52].

The performance of endophyte *Piriformospora indica* in different substrata under greenhouse and practical field conditions is studied[53]. Roots of winter wheat were colonized efficiently, and biomass was particularly increased on poor substrata. In greenhouse experiments, symptoms of severity of a typical leaf (*Blumeria graminis* f. sp. *tritici*), stem base (*Pseudocercosporella herpotrichoides*), and root (*Fusarium culmorum*) pathogen was reduced significantly. However, in field experiments, symptoms caused by the leaf pathogen did not differ in *Piriformospora indica*-colonized compared with control plants. In the field, severity of *Pseudocercosporella herpotrichoides* disease was significantly reduced in plants colonized by the endophyte. Increased numbers of sheath layers and hydrogen peroxide concentrations after *B. graminis* attack were detected in *Piriformospora indica*-colonized plants, suggesting that root colonization causes induction of systemic resistance or priming of the host plant. Although the endophyte is not well suited for growth at Central European temperature conditions, it remains to be shown whether *P. indica* is more suitable for tropical or subtropical farming.

Fungi as Biofertilizer

Biofertilizers are live formulations of agriculturally beneficial microorganisms, which upon application to seed, root or soil can mobilize the availability of nutrients by their biological activity and help to improve the soil health. Microbes involved in these formulations not only mobilize N and P but also secrete various growth promoting and health promoting substances. In broad sense, the term 'biofertilizer' may be used to include all organic resources for plant growth, which are rendered in an available form for absorption through microorganisms or plant association or interactions. The effects of different Arbuscular Mycorrhizal Fungi (AMF) species on the growth and nutrient contents of pepper seedlings (cv.Demre) grown under moderate salt stress is studied[16]. Two different mycorrhizas (*Glomus intraradices* and *Gigaspora margarita*) were tested on a growing media containing moderate salt stress (75 ppm NaCl). Some nutrients such as P, K, Ca and Na and plant growth parameters such as shoot height, stem diameter, root length and dry and fresh weights of species had positive effects on salt tolerance based on the plant growth parameters and nutrient contents. *G. intraradices* caused better response in seedling development compared to *G. margarita,* though insignificantly. The influence of VAM on nutrient uptake and growth of Custard-apple is studied[54]. The result indicates that symbiotic association of mycorrhizal fungus amounts to greater uptake of phosphorus and increased chlorophyll content in VAM treated plants than non mycorrhizal plants. Different growth parameters like height of the plant, fresh and dry weight of the roots and shoots were observed to be significantly high in *G. fasciculatum* treated plants compared to the respective controls. The quantum of herbage in VAM treated plants may possibly be due to enhanced uptake of essential mineral nutrients.

The influence of Vesicular Arbuscular Mycorrhizal fungi on growth and nutrient uptake in chickpea is studied[55]. Chickpea plants were inoculated with five VA-mycorrhizal fungi (*Glomus mossese*, *G.fascictum*, *Aculospora levis* and *Gigaspora gilmorei*). Inoculation with all the mycorrhizal fungi resulted in increased plant height, dry weight, nutrient content, mycorrhizal colonization, sporocarp numbers in soil and number of pods per plants as compared to the control. Among these mycorrhizal fungi *G. mossese* exhibited most pronounced effects. The ability of cellulolytic fungi and wheat straw incorporation to improve the nodulation, growth and nitrogen status of fenugreek grown in saline soils is studied[32]. Three of these fungi, *Aspergillus niger*, *Chaetomium globosum* and *Trichoderma harzinum* strongly decreased (up to 1%) the enzymatic activity of the ten tested moulds. Application of wheat straw with cellulolytic fungi significantly enhanced growth, nodulation and nodule efficiency at 0.5 and 1 % salinity. The greatest values of nodulation and growth parameters were obtained with a straw-*Trichoderma harzianum* combination. Cellulolytic fungi and wheat straw increased the concentration of Ca, Mg and K in the shoots and roots of plants. The increase in dry matter production and N content was mainly due to improved N_2 fixation reflected by enhanced formation and growth of nodules as well as nitrogenase activity. Results of pot experiment with dual inoculation of phosphate -solubilizing fungi (*A. niger* and *P. italicum*) showed significantly increased dry matter production and yield of soybean plants compared to the control soil[56]. Significant increment in percentage of protein and oil was also recorded. There was an increase in the percentage of N and P content of the plant. It was significantly resulted with N levels of soybean plants but this increase was non-significant with the percentage of total phosphorus, under the experimental conditions. Soil analysis showed that the available P and organic carbon levels significantly increased compared to the initial soil. The pH was also lowered compared to the initial pH of the soil.

The stimulatory effect of phosphate -solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L.cv.GPF2) is studied[57].The effect of six phosphate -solubilizing fungi (PSF, two strains of *Aspergillus awamori* and four of *Penicillium citrinum*) on the growth and seed production of chickpea plants (*Cicer arietinum* L.cv.GPF2) was studied in pot experiments. Maximum stimulatory effect on chickpea plants growth was observed by inoculation of two *A.awmori* strains. This treatment resulted in 7-12% increase in shoot height, nearly three-fold increase in seed number and two-fold increase in seeds weight as compared to the control (un-inoculated) plants. Inoculation of four strains of *P.citrium* exhibited lesser stimulatory effect. It showed 7% increase in consortium of all the six fungal isolates resulted in no stimulatory effect on chickpea plants growth.

The efficacy of *Aspergillus tubingensis* and *Aspergillus niger* to solubilize rock phosphate (RP) and to improve the growth of maize (*Zea mays*) in rock phosphate amended soils was studied[58]. The results of nursery experiment showed that the growth of maize plants and shoot P levels were significantly increased by inoculation of these fungi compared to control soil. Soil analysis showed that the available P, organic carbon levels were significantly increased compared to initial soil. The soil pH was also lowered compared to initial pH of the soil. The positive effect of *Trichoderma* spp. inoculation on chickpea variety *Annegeri-1* in terms of plant height, number of branches, biomass and P/N-uptake by shoots/roots is observed[59].

Conclusion

Fungi are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds. Although work on the utilization of this vast resource of microorganisms has just begun, it has already become obvious that an enormous potential for organism, product and utilization discovery in this field holds exciting promise. This is witnessed by the discovery of a wide range of products and microorganisms that already hold inkling for future prospects. The mechanisms through which fungi exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek study and spend time isolating micro flora components. This may facilitate the product discovery processes. Direct antiherbivore properties of fungi may be exploited, for instance, in biocontrol through developing natural pesticides or improvement of herbivore resistant cultivars by introducing biologically active (high mycotoxin production, etc.) fungal strains into cultivars. Alternatively, fungal strains, which do not produce mycotoxins but increase plant growth, seed production, seed germination rate and/or stress tolerance, can be used to increase forage productivity when introduced to the cultivars used as forage. Similarly, many fungi are able to control insect pests. This property can be exploited to transmit the capacity to resist the host against certain pests by their artificial inoculation in endophyte free plants.

References

1. Whitelaw, MA. 2000. *Growth promotion of plants inoculated with phosphate solubilizing fungi*. In: Advances in Agronomy (Donald L. Sparks, ed.), Academic Press. 69: 99-151.
2. Subba Rao, NS.1999. *Soil Microbiology*. Oxford and IBH publication.39-42.
3. Manoharachary, C, Sridhar, K, Singh, R, Adholeya, A, Sauryanarayanan, TS, Rawat, S and Johri, BN. 2005. Fungal biodiversity: distribution, conservation and prospecting of fungi from India. *Current Science*, 89(1): 58-71.
4. Hyakumachi, M. 1994. Plant growth promoting fungi from Turfgrass rhizosphere with potential for disease suppression. *Soil Microorganisms*, 44: 53-68.
5. Shoresh, M, Yedida, I and Chat, I. 2005. Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology*, 95:76-84.
6. Shivanna, MB, Meera, MS and Hyakumachi, M. 1994. Sterile fungi from Zoysiagrass rhizosphere as plant growth promoters in spring wheat. *Canadian Journal of Microbiology*, 40: 637-644.
7. Shivanna, MB, Meera, MS and Hyakumachi, M. 1996. Role of root colonization ability of plant growth promoting fungi in the suppression of take-all and common root rot of wheat. *Crop Protection*, 15: 497-504.
8. Meera, MS, Shivanna, MB, Kageyama, K and Hyakumachi, M. 1994. Plant growth promoting fungi from Zoysiagrass rhizosphere as potential inducers of systemic resistance in cucumbers. *Phytopathology*, 84: 1399-1406.
9. Koike, N, Hyakumachi, M, Kageyama, K, Tsuyumu, S and Doke, N. 2001. Induction of systemic resistance in cucumber against several diseases by plant growth promoting fungi: lignification and superoxide generation. *European Journal of Plant Pathology*, 107: 523-533.
10. Phuwiawat, W and Soytong, K.1999. Growth and yield response of Chinese radish to application of *Trichoderma harziznum*. Thammasat *International Journal of Science and Technology*, 4 (1):68-71.
11. Altomore, C, Norvell, WA, Bjorkman, T, Harman, GE.1999. Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma*

harzianum Rifai 1295-22. *Applied and Environmental Microbiology*, 65:2926-2933.

12. Verma, A, Verma, S, Sudha, Sahay, N, Butehorn, B and Franken, P.1999. *Piriformospora indica*, a cultivable plant growth promoting root endophyte. *Applied and Environmental Microbiology*, 65(6):2741-2744.
13. Hossain, MM, Sultana, F, Kubota, M and Hyakumachi, M. 2008. Differential inducible defense mechanisms against bacterial speck pathogen in *Arabidopsis thalina* by plant growth promoting fungus *Penicillium* sp. GP16-2 and its cell free filtrate. *Plant Soil*, 304:227-239.
14. Chandanie, WA, Kubota, M and Hyakumachi, M. 2006. Interaction between plant growth promoting fungi and arbuscular mycorrhizal fungus *Glomus mosseeae* and induction of systemic resistance to anthracnose disease in cucumber. *Plant Soil*, 286:209-217.
15. Gisbert, C, Rus, AM, Bolarin, C, Lopez CM, Arrillaga, I, Montesinos, C, Caro, M. Serrano, R and Moreno, V. 2000. The yeast HAL1 gene improves salt tolerance of transgenic tomato. *Plant Physiology*, 123:393-402.
16. Turkmen, O, Sensoy, S, Demir, S and Erdinc, C. 2008. Effects of two different AMF species on growth and nutrient uptake of Pepper seedlings grown under moderate salt stress. *African Journal of Biotechnology*, 7(4):392-396.
17. Ahmad, F, Ahmad, I, Aquil, F, Khan, MS and Hayat, S. 2008. *Diversity and potential of non-symbiotic diazotrophic bacteria in promoting plant growth*. In: Plant- Bacteria Interactions (Ahmad, I., Pichtel, J. and Hayat, S. ed.): 81-82.
18. Ehrlich, HL.1990. *Mikrobiologische and biochemische verfahren stechnik*. In:, Geomicrobiology, second ed. (Einsele, A, Finn, RK, Samhaber, W. eds.) VCH Verlagsgesellschaft, Weinheim.
19. Matty, M. 1992. The production of organic acids. *Critical Review in Biotechnology*, 12:87-132
20. Goenadi, DH, Siswanto and Sugiarto,Y. 2000. Bioactivation of poorly soluble phosphate rocks with a phosphate solubilizing fungus. *Soil Science Society of America Journal*, 64:927-932.
21. Narsain, V and Patel, HH. 2000. *Aspergillus aculeatus* as a rock phosphate solubilizer. *Soil Biology and Biochemistry*, 32: 559-565.
22. Caravaca, F, Alguacil, MM, Azcon, R, Diaz, G and Roldan, A. 2004.Comparing the effectiveness of mycorrhizal inoculation and amendment with sugar beet, rock-phosphate and *Aspergillus niger* to enhance field performance of the leguminous shrub *Dorycnium pentaphyllum*. L. *Applied Soil Ecology*, 25: 169-180.
23. Ahmed, N and Jha, KK. 1968. Solubilization of rock phosphate by microorganism isolated from Bihar soil. *Journal of Applied Microbiology*, 14: 89-95.
24. Asea, PEA, Kucey, RMN and Stewart, JWB. 1998. Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. *Soil Biology and Biochemistry*, 20: 459-464.
25. Nahas, E. 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World Journal of Microbiology and Biotechnology*, 12: 567-572.
26. Rajankar, PN, Tambekar, DH and Wate, SR. 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of purna river basin. *Research Journal of Agriculture and Biological Sciences*, 3(6): 701-703.
27. Medina, A, Jakobsen, I, Vasiilev, N, Azcon, K and Larsen, J. 2007. Fermentation of sugar beet waste by *Aspergillus niger* facilitate growth and P uptake of external mycelium of mixed populations of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 39: 485-492.
28. Srivastav, S, Yadav, KS, Kundu, BS. 2004. Prospects of using phosphate solubilizing *Pseudomonas* as biofungicide. *Indian Journal of Microbiology*, 44:91-94.
29. Lindsay, WL and Schwab, AP. 1982. The chemistry of iron in soils and its availability to plants. *Journal of Plant Nutrition*, 5: 821-840.
30. Shenker, M, Ghirlando, R, Oliver, I, Helmann, M, Hadur, Y and Chen, Y. 1995. Chemical structure and biological activities of a

siderophore produced by *Rhizopus arrhizus*. *Soil Science Society of America Journal*, 59:837-843.

31. Neilands, JB. 1981.Microbial iron compounds. *Annual Reviews in Biochemistry*, 50:715-731.
32. Abd-alla, MH and Omar, SA.1998. Wheat straw and cellulolytic fungi application increases nodulation, nodule efficiency and growth of fenugreek (*Trigonella foenum-graceum* L.) grown in saline soil. *Biology and Fertility of Soils*, 25:58-65.
33. Marek-Kozaczuk,M, Deryto, M and Skorupska, A. 1996. The insertion mutants of *Pseudomonas* sp.267 defective in siderophore production and their effect on clover (*Trofolium pretense*) nodulated with *Rhizobium legminosarum* bv. *Trifolli*. *Plant Soil*, 179:269-274.
34. Johnson, L. 2008. Iron and siderophores in fungal-host interactions. *Mycorrhiza Research*, 112:170-183.
35. Dube, HC, Vala, AK and Vaidya, SY. 2000. Chemical nature and ligand binding properties of siderophores produced by certain *Aspergillus* from marine habitats. *Natural Academy of Science Letters*, 23:98-100.
36. Muhammad, H, Khan, SA, Iqbal, I, Ahmad, B and Lee, IJ. 2010. Isolation of a gibberellin producing fungus (*Penicillium* sp. MH7) and growth promotion of crown daisy (*Chrysanthemum coronarium*). *Journal of Microbiology and Biotechnology*, 20 (1):202-207.
37. Tudzymski, B and Sharon, A. 1994. *Biosynthesis, biological role and application of fungal phytohormones*. In: The Mycota: A comprehensive treatise on fungi as experimental systems for basic and applied research: 183-185.
38. Maor, R, Haskin, S, Levi-Kedmi, H and Sharon, A. 2004. In planta production of Indole-3-acetic acid by *Colletotrichum gloeosporioides* f. sp. *Aeschynomene*. *Applied and Environmental Microbiology*, 70(3): 1853-1854.
39. Chung, KR and Tzeng, DD. 2004. Biosynthesis of Indole-3-acetic acid by the gall-inducing fungus *Ustilago esculenta*. *Journal of Biological Sciences*, 4(6): 744-750.
40. Unyayar, S. 2001. Changes in Abscisic acid and Indole acetic acid concentrations in *Funalia trogii* (Berk.) Bondartsec & Singer and *Phanerochaete chrysosporium* Burds ME446 subjected to salt stress. *Turkish Journal of Botany*, 26: 1-4.
41. Shimada, A, Takeuchi, S, Nakajima, A, Tanaka, S, Kawano, T and Kimura, Y. 2000. Phytotoxicity of Indole-3-acetic acid produced by the fungus *Phythium aphanidermatum*. *Bioscience, Biotechnology and Biochemistry*, 64(1): 187-189.
42. Unyayar, S, Topcuoglu, SF and Unyayar, A. 1996. A modified method for extraction and identification of Indole-3-acetic acid and Zeatin produced by *Phanerochaete chrysosporium* ME446. *Bulgarian Journal of Plant Physiology*, 22(3-4): 105-110.
43. Verma, M, Brar, SK, Tyagi, RD, Surampalli, RY and Valero, JR. 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control *Biochemical Engineering Journal*. 37:1-20.
44. Benitez, T, Rincon, AM, Limon, MC and Codon, A. 2004. Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology*, 7(4):249-260.
45. Hermosa, MR, Grondona, I, Iturriaga, EA, Diaz-Minguez, JM, Castro, C, Monte, E and Garcia-acha, I. 2000. Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. *Applied and Environmental Microbiology*, 66 (5): 1890-1898.
46. Reino, JL, Guerrero, RF, Hernandezalan, R and Collado, IG. 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochemistry Reviews*, 7(1):89-123.
47. Harman, GE, Howell, CR, Viterbo, A, Chet, I and Lorito, M. 2004.*Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2:43-56.
48. Naseby, DC, Pascual, JA and Lynch, JM. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil

microbiol communities and soil enzyme activities. *Journal of Applied Microbiology*, 88(1):161-169.

49. Ghahfarokhi, RM and Goltapeh, ME. 2010. Potential of the root endophytic fungus *Piriformospora indica*; *Sebacina vermifera* and *Trichoderma* species in biocontrol of take-all disease of wheat *Gaeumannomyces graminis* var. *tritici in vitro*. *Journal of Agricultural Technology*, 6(1): 11-18.
50. Al-Askar, AA and Rashad, YM. 2010. Arbuscular mycorrhizal fungi: A biocontrol agent against common bean *Fusarium* root rots disease. *Plant Pathology Journal*, 9(1):31-38
51. Sarrocco, S, Mikkelsen, L, Vergara, M, Jensen, DF, Lubeck, M and Vannacci, G. 2006. Histopathological studies of sclerotia of phytopathogenic fungi parasitized by a GFP transformed *Trichoderma virens* antagonistic strain. *Mycological Research*, 110:179-187.
52. Djonovic S, Pozo, MJ and Kenerley, CM. 2006. Tvbgn3, a ß1, 6-Glucanase from the biocontrol fungus *Trichoderma virens*, is involved in Mycoparasitism and control of P*ythium ultimum*. *Applied and Environmental Microbiology*, 72(12): 7661-7670
53. Serfling,A, Wirsel, SGR, Volker Lind, V, and Deising, HB. 2007. Performance of the biocontrol fungus *Piriformospora indica* on Wheat under greenhouse and field conditions. *Biological Control*, 97(4):523-531.
54. Ojha, S, Chakaraborty, MR, Dutta, S and Chatterjee, NC. 2008. Influence of VAM on nutrient uptake and growth of custard -apple. *Asian Journal of Experimental Sciences*, 22 (3):221-224.
55. Kumar, R, Jalali, BL and Chand, H. 2002. Influence of vesicular arbuscular mycorrhizal fungi on growth and nutrient uptake in chickpea. *Journal of Mycology and Plant Pathology*, 32(1):11-15.
56. El-Azouni, IM. 2008. Effect of phosphate solubilizing fungi on growth and nutrient uptake of soybean (*Glycine max* L.) plants. *Journal of Applied Sciences Research*, 4(6): 592-598.
57. Mittal, V, Singh, O, Nayyar, H, Kaur, J and Tewari, R. 2008. Stimulatory effect of phosphate - solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). *Soil Biology and Biochemistry*, 40: 718-727.
58. Richa, G, Khosla, B and Reddy, SM. 2007. Improvement of maize plant with by phosphate solubilizing fungi in rock phosphate amended soils. *World Journal of Agricultural Sciences*, 3(4):481-484.
59. Rudresh, DL, Shivrakash, MA and Prashad, RD. 2005. Effect of combined application of rhizobium, phosphate solibilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). *Applied Soil Ecology*, 28:139-146.