CHAPTER 2

REVIEW OF LITERATURE

Review of relevant literature pertaining in the investigation is an important aspect. Before starting the research work numbers of publications in the form of books, journals, news articles research papers etc. were briefly reviewed under the following subheads.

2.1 General Introduction:

Reviews on beneficial aspects of mycorrhizal fungi were done elaborately by different workers in the recent times covering the reviews of earlier pioneers in this field. The reviews cover the evolutionary trend, occurrence of AM fungi in angiosperms, gymnosperms, bryophytes and pteridophytes, nutrient uptake, soil, water and mycorrhizal plant relationship, work on plantation crops etc. (Bolan, 1991; Jariwala and Rai., 1998; Misra, 2001; Brundrett, 2002; Auge, 2004; Andrade et al., 2009). Wang and Qiu, (2006) done a detailed survey of 659 papers mostly published since 1987 and compiled a checklist of mycorrhizal occurrence among 3,617 species (263 families) of land plants.

Several workers (Rodríguez and Fraga, 1999; Vassilev et al., 2001; Bhattacharyya and Jha, 2011) reviewed the interaction of diazotrophs,
phosphate solubilizing microorganisms and vesicular mycorrhizal fungi (Bonfante and Anca, 2009) and their role in nutrient uptake as well as the influence of shoot to root ratio (Wilson, 1988) in plant growth.

BassiriRad (2000) reviewed an importance of root to shoot interaction that might determine the overall response of plants and the ability of the root system to adjust nutrient acquisition capacity to meet variations in shoot demand caused by environmental changes. Plant roots can alter their nutrient acquisition capacity by adjusting their physiological, morphological and/or architectural characteristics to meet changes in shoot nutrient demand.

Osorio Vega (2007) reviewed the beneficial aspects of rhizosphere bacteria on plant nutrition. The interaction between plant and phosphate-solubilizing bacteria was explained in more detail and used as model to illustrate the role that rhizosphere bacteria i.e Azotobacter (Aquilanti et al., 2004; Singh, 2006) Azospirillum (Singh et al., 2001; Saharan and Nehra, 2011). Plant growth promoting rhizobacteria (Bashan, 1999; Bhattacharyya and Jha, 2011; Saharan and Nehra, 2011) play important role in soil nutrient availability.

The role of mycorrhizal symbiosis was long related to its impact on the mineral nutrition of the host plant and consequently on the development of the plant species (Jha et al., 1991; Sreevani and Reddy,
2004). However, it is proved that this symbiotic process significantly interacts with other biological components of the ecosystem for eg. microflora, microfauna and mesofauna etc. to optimize the implication of these microorganisms in the operation of the major biochemical cycles.

The success of mycorrhizal evolution has been attributed to the role that mycorrhizal fungi play in the capture of nutrients from the soil of all ecosystems (Hayman, 1974, Hayman, 1982a; Bonfante and Perotto 2000; Bonfante and Anca, 2009). The symbiosis is characterized by the exchange of nutrients where carbon in the form of Hexose sugars flows to the fungus and inorganic nutrients are passed to the plant, thereby providing a linkage between the plant root and the soil (Sylvia et al., 1998). Mycorrhizal fungi differ from other plant fungus associations because of their ability to create an interface for nutrient exchange which occurs within living cells of the plant (Brundrett 2002; Brundrett 2004).

Some nonmycorrhizal plants belonging to the families Amaranthaceae, Brassicaceae, Cyperaceae, Chenopodiaceae and Caryophyllaceae are less attractive to mycorrhizal fungi (Gerdemann, 1968; Hayman, 1974; Sharma, 1997) but at times attempts were made to colonize their roots (Kruckelmann, 1975; Ocampo et al., 1980; Brundrett, 2002). The inability of these plants to support mycorrhizal colonization may be due to the accumulation of chemicals in the roots which fail to elicit differential hyphal branching (Giovannetti and Sbrana, 1998; Brundrett 2002).
Mycorrhizal fungi interact with plants at different levels and can be grouped into obligate mycorrhizal, facultative mycorrhizal and non-mycorrhizal plants (Brundrett, 2004). Classically a mycorrhiza is defined as an interaction from which both partners benefit. Generally it is claimed that mycorrhizal fungi improve the nutrient uptake of the host plant through their hyphae, which in return receive plant carbohydrates that are essential for completion of the fungal life cycle. This retains the concept of mutualism, i.e., an interaction of net benefit to both parties (Bonfante and Anca, 2009).

Now all mycorrhizal fungi are grouped in single domain Glomeromycota (Schüßler et al., 2001; Redecker and Phillip, 2006; Hibbett et al., 2007). Vesicles are generally found in *Glomus*, *Acaulospora* and *Entrophospora* (Issac, 1992). However, their formation depends on environmental conditions such as high or low P levels that affect vesicle development (Smith and Read, 1997).

The role of soil microorganisms in sustainable development of agriculture was well documented (Lee and Pankhurst, 1992; Wani and Lee, 1992). Soil microflora plays an important role in the maintenance of soil fertility because of their ability to carry out biochemical transformation and also due to their importance as a source and sink for mineral nutrients (Jenkinson and Ladd, 1981). Soil structure and porosity are much influenced by the activities of soil organisms (Juma, 1993).
Application of fertilizer containing macro and micronutrient has contributed significantly to the huge increase in world food production. As the world’s population continues to expand almost exponentially, there is an urgent need to consider novel ways of increasing food production that are compatible with sustainability and the retention of environmental quality. After photosynthesis, nitrogen fixation and assimilation is perhaps the second most important biological progress currently limiting crop yield (Sindhu and Dadarwal 2001). So, for efficient nutrient uptake, most land plants need to be associated with mycorrhizal fungi that supply minerals, increasing their productivity and conferring resistance to stress. The exploitation of AM fungi symbioses in natural and agronomic environments is of high environmental and economic value at present (Bonfante and Anca, 2009).

The excessive use of chemical fertilizers in developed countries has led to series of problems of nutrient, pollution, leaching of toxic nitrate into ground water and volatilization of nitrogen oxides into the environment (Bohool et al., 1992). Therefore time has come to develop biological fertilizers for retention or remediation of soil and environmental quality.

Inoculation of plants with naturally occurring plant growth promoting Rhizobacteria (PGPR) is an inexpensive and environmental
friendly technique for increasing plant productivity (Kloepper and Schroth, 1981; Chanway, 1995; Bhattacharyya and Jha, 2011).

The knowledge about the survival of microorganisms in the rhizosphere of different crops is inadequate for better understanding of soil and plant health. Survival experiments were mostly described for single bacterial strains of the genera *Pseudomonas* (Glandorf *et al.*, 1992; de Freitas and Germida, 1992 a, b; Kemp *et al.*, 1992), *Azospirillum* (Bashan and Levanony, 1989a,b) or *Rhizobium* (Theis *et al.*, 1992; Baldiani and Weaver, 1992) and often for only one single plant species. So, Wiehe and Hoflich (1995) have done a field study of the survival of selected associative *Rhizobium* and *Pseudomonas* strains on loamy sand soil under temperate climatic conditions. It is known that these two strains are able to colonize the rhizosphere of different crops under greenhouse conditions (Hoflich *et al.*, 1995). Plant and specific bacterial colonization, influence of soil humidity, colonization of deeper root parts as well as migration of the introduced bacteria to non-inoculated crops and weeds are taken into consideration (Wiehe and Hoflich, 1995).

The rhizosphere of established tea bushes have some specific characteristics, which are associated with long lived nature of tea plants viz negative rhizosphere effect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species (Lynch 1987; Barua, 1989; Pandey and Palni, 1996; Sood *et al.*, 2007a)
The overall interactions amongst tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seems to favour the growth of microbes, which are known to produce strong antibiotics with potential biocontrol agents (Sood et al., 2007; Dutta et al., 2007).

Sood et al. (2007) also studied whether or not in tea rhizosphere *Bacillus* and *Pseudomonas* species produce bacteriocins. They stated that dominant isolates of *Azotobacter* and phosphate solubilizers etc. are indicative of microbial diversity in tea rhizosphere. The occurrence and distribution of *Azotobacter*, *Azospirillum*, Actinomycetes, *Rhizobium* and phosphate solubilizing microbes in tea plantation was studied in the recent times (Baby et al., 2002; Dutta et al., 2007).

The most important fact in tea soil is the presence of Aluminium, iron and clay that render the applied phosphorus unavailable to the plants by forming insoluble complexes which is a limiting factor in the growth and production of tea (Barua, 1989; Verma and Palani, 1997; Vyas et al., 2007). Hence, isolation and identification of native microorganisms resistant to acidic soil condition and also promoting tea growth is in demand.
2.2 Arbuscular Mycorrhizal Fungi (AMF):

2.2.1 Taxonomy of Endomycorrhiza:

Beniamino Peyronel in 1923 was the first to recognize that the vesicular arbuscular mycorrhizal (VAM) fungi are members of Endogonales (Trappe and Schenck, 1984) now called as AM fungi, since not all genera produce vesicles, revised as AM fungi by Friberg, (2001). Endogonaceae are among the more common and widely distributed of the soil–borne fungi. According to latest taxonomy, AM fungi belong to the phylum Glomeromycota, including 4 families and 12 genera (Schüßler et al., 2001; Redecker and Phillip, 2006). Gerdemann and Nicolson, (1963) developed a procedure for collecting spores from soil and described new species found by their technique. Mycorrhizas are found in many environments and their ecological success reflects a high degree of diversity in the genetic and physiological abilities of the fungal endophytes (Bonfante and Anca, 2009).

AM fungi are classified based on morphology and germination characteristic of asexual spores under the Division of Eumycota, Class Zygomycetes, Order Endogonales, Family Endogonaceae. The genera of AM fungi so far recognized are *Glomus, Gigaspora, Scutellospora, Acaulospora* and *Entrophospora* form mutualistic symbiotic relationship with many plant families (Schüßler and Walker, 2010).
It is a well established fact that many plants cannot grow adequately without AM fungi, especially in phosphate deficient soils (Mosse, 1973b; Gerdemann, 1975; Hayman, 1978).

Mostly mycorrhizal infections occur solely in the epidermis or esoderm and the cortical parenchyma of roots which present a primary structure. The infection does not penetrate the endodermis and is therefore not present in the central vascular cylinder, nor is it present in the meristematic regions of angiosperms. As observed by the pioneers in this field, the infection develops in stages: Firstly an extrametrical phase with extrametrical hyphae and external vesicles or spores scattered in the surrounding soil and secondly an intraradical phase with intracellular unbranched hyphae (vesicles), branched intracellular (arbuscules) hyphae (Bonfante, 1984).

The presence of vesicles and arbuscules is the diagnostic criterion for identifying a vesicular arbuscular mycorrhizal fungus in root. Vesicles is nearly borne terminally or on short lateral branches (Hayman, 1982b). The external vesicles largely vary from 20 to 150µm in diameter and are thick walled, with a dense cytoplasmic content rich in oil globules. Vesicles are usually oval, sometimes round and occasionally irregularly lobed (eg. Acaulospora laevis). They are believed to function as storage organs (Bonfante, 1984). In older roots they can develop thick walls and sometimes function as resting spores when the root decay
(Hayman, 1982b). With age they become vacuolated (Nicolson, 1959; Mosse, 1959). Not all AM fungi eg *Gigaspora margarita* form vesicles within roots (Gerdemann and Trappe, 1974; Bonfante, 1984).

The arbuscules are considered to be the primary structures involved in the bidirectional transfer of nutrients between the fungal symbiont and host plant. They are present in the inner layers of the cortical parenchyma (Harley, 1969). The arbuscule is the most significant structure in the AM fungi complex as it is considered to be the functional unit in the whole association and scientists agree that the arbuscule is the preferential site for fungus-plant metabolite exchanges (Cox *et al.*, 1975).

All endophyte fungi, recognized by Gerdemann and Trappe (1974) belonging to the genera *Glomus*, *Gigaspora* and *Acaulospora* form arbuscules. Roots collected from the field often show large arbuscular clumps, formed by the aggregation of smaller ones, filling the host cell. In older plants this situation of senescent arbuscules is more frequent than that of the active arbuscules easily observed within young mycorrhizal roots obtained under control conditions (Bonfante, 1984).

### 2.2.2 Components of AM fungi association:

Arbuscular Mycorrhizal fungi (AM fungi) is a balanced mutualistic symbiosis in which both partners can be benefited. AM fungi have three major components, the root itself which provides carbon in the form of
sugars to the fungus, fungal structures within cortical cells of plant root that provide contact between the fungus and the plant cytoplasm and the extraradical hyphae that aid in uptake of nutrients and water (Smith and Read 1997).

Soil fertility also plays a key role in governing the extent to which a plant can be benefited from mycorrhiza. On the basis of soil analysis, especially estimates of plant, available phosphate (Olsen et al., 1954) for soluble P, the growth response to AM fungi can be predicted. Any AM fungal species can infect to some extent the AM mycorrhizal plant species depending upon the suitability of the soil. *Acaulospora lactis* and *Glomus fasciculatus* are best in acid soils, *Glomus mosseae* in alkaline-neutral soils. At the same time, population changes when virgin land is brought into cultivation (Hayman, 1978).

Percentage root colonization has been reported to be one of the major parameters used to measure biological productivity and efficiency of plants inoculated with AM fungi (Kurle and Pfleger, 1994). This parameter also reflects the viability of AM fungal propagules in soil. The viability of AM fungal spores has always been questioned in terms of root colonisation because spores are not the only propagules capable of colonising host plant roots. Both the spores of AM fungi and colonised root pieces can be effective propagules, initiating typical AM fungi colonisation in host plants although the ability of AM fungi to persist in
soil may depend partly on the type of propagules formed (Sparling and Tinker, 1975; Powell, 1976; Hetrick, 1984).

2.2.3 Occurrence of AM fungi:

The occurrence of AM fungi are ubiquitous in geographic distribution and except waterlogged conditions, they are distributed worldwide in various kinds of environment i.e marine, saline patches, mangrove vegetation, desert area, ridges (Jariwala and Rai, 1998), in heavy metal contaminated soils (Gaur and Adholeya, 2004) and acidic soils (Hayman, 1978). They have also been reported from forest tree species (Thapar and Khan 1973; Mishra and Sharma, 1981), in aquatic plants (Chaubal et al., 1982), in epiphytic orchids (Katiyar et al., 1986). AM fungi association are also formed in gymnosperms (Harley 1969), in Bryophytes (Parke and Lindermann, 1980; Bonfante, 1984) and in pteridophytes (Rothwell, 1996).

AM fungi are formed by nearly all cultivated plants whether they are agricultural, horticultural or fruit crops (Jariwala and Rai, 1998). AM fungi populations of cultivated lands are affected by the various soil, plant and environmental factors that affect them in natural ecosystem plus various agricultural and horticultural practices (Hayman, 1982a).

Misra (2001) in his review paper stated that the important crops where benefits from mycorrhiza have been drawn which include forage
legumes, cereals like upland rice, maize, wheat, barley, sorghum etc. pulses, oilseeds, vegetables and commercial crops (tobacco, cotton, sugarcane) and plantation crops (tea, coffee, rubber, olive, oil palm).

Venkataramanan et al., (1990) reported the presence of Gigaspora nigra, Glomus mosseae and Sclerocystis rubiformis in soils of North East India. Baruah (1994) reported 14 species of AM fungi belonging to four genera i.e Glomus, Gigaspora, Scuttelospora and Sclerocystis from green gram rhizosphere samples of Jorhat district of Assam.

2.2.4 AM fungi and tea:

Spore number in the root region of Assam lemon soil remained more or less constant throughout the year with slight decline in the month of July (Rahman, 1996). Chakravarty and Talukdar (1997) also reported that AM fungi spores gradually decreased from June onward and reached minimum in the month of February in four micro-agro ecosystem of Assam. They also reported that more spore were prevalent in tea soils for long period of time as compared to native grasses, maize or legume where spore number decreased much before onset of autumn. They speculated that white coloured tea roots remained active until late autumn, which might support AM spore production.

The occurrence of four genera of AM fungi viz Glomus, Gigaspora, Acaulospora and Sclerocystis in association with tea crop was reported from Assam (Anonymous 1982). Barthakur et al.(1987) in their
preliminary experiment with few Tocklai clones found that the extent of AM fungi colonization in roots ranged between 32 to 64 percent and this variation was affected by the pattern of weed management. Occurrence of AM fungi in some predominant weeds of tea plantation was also observed (Barthakur et al., 1989).

Thseg and Chen (1984) found AM fungi association in Taiwan tea plantation. Its association has also been reported from China (Zhi 1993), Bangladesh (Mridha et al., 1995). Sieverding and Toro (1987) observed the impact of different isolates of AM fungi on tea and found a three–fold increase in growth parameters compared to non- mycorrhizal plants. Study by Roy et al. (2002) reported that a good correlation could be established between AM fungi population, root colonization, and plant growth for the tea varieties.

2.2.5 Factors affecting colonization and sporulation:

Root colonization, subsequent spore production, its survival and performance of AM fungi are influenced by a wide range of host plant, environment, soil fertility and cropping practices. In general, most of the AM fungi spores and infection of roots occur in the top 20 cm of the soil. But there is an exponential decline in both infection and spore number with depth of soil (Harris et al., 1987). In many cases, the factors, which stimulate or inhibit colonization probably also stimulate or inhibit
sporulation as both these phenomenon are often closely related (Hayman 1970; Daft and Nicolson, 1972).

Maximum root colonization and sporulation occur in low fertile soils (Hayman 1970). However, increase in barley yield grown in soil with 40 ppm available P (NaHCO$_3$ extractable) was observed due to AM fungi inoculation (Clark and Mosse, 1981). Hazarika, (2000) also observed variation in spore population of AM fungi in tea plantation. He studied the spore load and also confirms the presence of four species of chlamydospores in tea soil and its variability in different tea gardens. Bouamri, (2006) done a survey of arbuscular mycorrhizal fungi (AMF) diversity in date palm rhizosphere and found that there was a significant difference in spore density between season. The effect of high soil fertility on root colonization depends on the host plant. Menge et al.(1978) has shown that much of the influence of soil fertility on root colonization is plant mediated.

Both phosphorus (Daft and Nicolson, 1969; Hayman 1970; khan 1972) and nitrogen (Porter and Beute, 1972; Redhead, 1975) may significantly reduce colonization if present at high levels. AM fungi enter into inactive phase due to application of high dose of phosphorus in later stage of tea cultivation (Barthakur et al., 1987).
Both temperature and light have a significant influence on colonization and sporulation of AM fungi under greenhouse conditions. Higher temperature generally result in greater root colonization in temperate zone, however the reverse may be true in the tropics (Hayman, 1974). Longer day lengths and increased light intensity generally increases percentage root colonization (Hayman, 1974).

Certain species of AM fungi are reported to be adversely affected by low pH of the soil (Noordwijk and Hairiah, 1986). However, Robson and Abbott (1989) stated that there was no correlation between AM propagules and soil acidity. Some AM fungi are restricted to either acidic or alkaline soils whereas, other occur both in alkaline or acidic soils. The optimum pH for spore germination differs with each AM species and the environment to which each is indigenous (Hetrick, 1984).

Application of farmyard manure (FYM) stimulated AM fungi (HariniKumar and Bagyaraj, 1988) while long fallow period reduced mycorrhizal colonization of crops grown latter (Thompson, 1987).

AM fungi are remarkably non host specific. Some AM fungi species may be more efficient in stimulating the growth of certain plant species, but each AM fungus is able to colonize every AM host species (Mosse, 1973b). Cereals in rotation with legume showed higher root colonization and more number of AM propagules than cereals grown in
monocropping (HariniKumar and Bagyaraj, 1988; Wani et al., 1991). In mixed cropping infection in the host plant wheat was reduced by the non-host mustard (Iqbal and Qureshi, 1976). After many years of deleterious cropping schedule, however, AM population may be markedly reduced (Kruckelmann, 1975).

Seasons variations with AM fungal populations have been demonstrated in many surveys based usually on the spore numbers isolated (Hayman, 1970; Sutton and Barron, 1972). Abundance of AM fungi is generally higher in sandy than clay soils and different area may have different dominant AM species. Moreover, the AM fungi population level was not correlated with any of the soil physical or chemical characteristics examined nor with avocado cultivar or age (Hass and Menge, 1990). Abundance of AM fungi also depends on the type of soil and other characteristics of soil (Baruah, 1994; Das and Barthakur, 1999). *Camellia sinensis* can also differentially alter fertility and other physical and chemical characteristics of soils (Singh et al., 2008) which in turn can affect the AM community structure.

Lambert et al. (1980) stated that indigenous fungi are often more efficient in increasing plant growth in soils to which they have become adapted. The spore dynamic in soils may vary widely depending upon the agro-ecosystem, geographical location etc. (Buwalda et al., 1985). Root colonization and numbers of resting spores in rhizosphere were higher in
summer and lowest in winter (Ietswaart et al., 1992; Chakravarty and Talukdar, 1997). Kausal and Srivastavava (1995) also reported lowest spores in summer and maximum during rainy season and decreased slowly towards winter in arid and semi-arid region of Gujarat.

The water relations of arbuscular mycorrhizal (AM) plants have been compared often. Auge (2004) summarized the findings that support the assertion that colonization of soil may play as important a role as colonization of roots regarding how AM symbiosis affects the water relations of host plants.

2.2.6 Symbiotic benefits:

The role of mycorrhizal symbiosis was long related to its impact on the mineral nutrition of the host plant and consequently on the development of the plant species. Arbuscular mycorrhiza (AM) confer several benefits on host plants. (Udaiyan et al., 1997) reviewed that the AM fungi help plants not only in the better utilization of soil phosphorus (Hayman, 1982b; Koide, 1991) through increased uptake but also of other elements such as N, K, Zn, Mg, Cu and S (Lambert et al., 1979; Abbott and Robson, 1984; Stribley, 1987; Barea, 1991). Arbuscular mycorrhizas are also known to increase resistance of plants to conditions such as drought (Safir et al., 1972) and extreme soil acidity (Mosse, 1973a). The improvement in the water relations of plants as a result of mycorrhizal
infection has been reviewed by Cooper (1983) and Harley and Smith (1983). But it is difficult to distinguish direct mycorrhizal effects from those that could be mediated via improved mineral nutrition (Nye and Tinker, 1977; Nelson and Safir, 1982). Increased root length and development of external hyphae may influence water relations of mycorrhizal plants (Kothari et al., 1990; Auge, 2004).

Most research on effects AM fungi on plant nutrition has been concerned with phosphate because it is a major plant nutrient and AM fungi can produce dramatic benefits to plants growing in P-deficient soils. Hence, it is widely accepted that AM fungi plays a recognized role in nutrient cycling in the ecosystem (Harley and Smith 1983; Rodríguez and Fraga, 1999). It is a well established fact that AM fungi increased productivity in AM plants compared to non mycorrhizal plants. Interest in AM fungal symbiosis arose in agriculture, forestry, rehabilitation and in different environments (Friberg, 2001). The major benefits of AM fungi to symbiosis includes enhanced nutrient uptake ,increased tolerance to root pathogens, drought resistance, tolerance to toxic heavy metals and improved soil aggregation and structure (Bolan, 1991; Jariwala and Rai,1998; Misra, 2001).
2.2.6.1 Nutrient uptake:

Ion uptake by plant roots from soil is governed by two major factors; transfer of ions through the soil and the absorbing power of the root (Nye and Tinker, 1977). It has been experimentally proved that inoculation with mycorrhizal fungi in forest and fruit plants at nursery stage and field crops at sowing time increases the absorption of almost all the nutrients required by them for their growth, particularly those which are relatively immobile in soil such as P, S, Cu, Zn and others (Abbott and Robson, 1984; Misra, 2001).

Apart from the well known effect of AM fungal association on P-uptake (Gray and Gerdemann, 1969; Hatting et al., 1973) there are also reports to show that they increase Zn uptake in pea, Red clover and maize (Gilmore, 1971) and Sulphur uptake (Grey and Gerdemann, 1973). The fungal hyphae associated with mycorrhizal plants can ramify in a greater soil volume and produce a greater absorptive surface area, than root hairs alone on a non mycorrhizal plants and as a result of which there is increased accumulation and absorption of plant nutrients mainly P, Zn, Cu, S (Abbott and Robson, 1984) and other elements like K, Mg, Mn, Fe etc. (Krishna and Bagyaraj, 1991) through greater soil exploitation.

Nutrients are believed to be released from the fungus to the plant by secretion or leakage from hyphae and intact arbuscules and during
arbuscule breakdown. Electron microscope studies indicate transfer of material in oil globules, exocytotic vesicles from intact arbuscules and pieces of fungal membrane from disintegrating arbuscules (Crush, 1973; Cox and Sanders, 1974)

2.2.6.1: Nitrogen uptake by Mycorrhizal plants:

Nitrogen is of great importance for plant growth. It is obtained by the extra-radical hyphae of AM fungi in different forms ranging from amino acids, peptides to recalcitrant organic nitrogen forms (Ames et al., 1983; Tobar et al., 1994; Lipson et al., 1999; Hawkins et al., 2000).

Lanowska (1996) showed that *Pisum sativum* plants infected with AM fungi generally have a lower tissue nitrogen concentration as percent dry weight than those without Mycorrhizae. While coffee seedlings inoculated with *Glomus fasciculatum*, *Gigaspora mariagata* and *Acaulospora levis* (Andrade et al., 2009) and tea seedlings with *G.fasciculatum* registered increased nitrogen uptake over non mycorrhizal plants (Hazarika, 2000)

A number of mechanisms were suggested to explain the effect of nitrogen uptake by AM fungi. This include direct uptake of nitrogen from soil (Johansen et al., 1992) improved biological nitrogen fixation (Barea et al., 1983a) and nitrogen transfer between host plants (Hamel et al., 1991).
2.2.6.2: Phosphorus uptake by Mycorrhizal plants:

Mycorrhizal infection can enhance the uptake of P by plants roots is well established fact. The improved phosphorus uptake has been reported in some plantation and orchard crops due to inoculation of AM fungi. Mycorrhization of tea with AM fungi helps in increased uptake and utilization of phosphorus even in presence of aluminum and iron in acid soils (Morita and Konishi, 1989). AM fungi synthesize polyphosphate vacuolar granules from soil phosphate and the granules are broken down in the arbuscules to inorganic phosphate for release to the host (Callow et al., 1978). The uptake of P is being attributed to the solubilization of soil phosphate by the production of acids and phosphatase enzymes by phosphate solubilizers as well as AM fungi.

Barthakur et al. (1992) reported that addition of single super phosphate (SSP) and AM fungi propagules resulted in very good growth and fairly high rate of success of tea cuttings. But, with the same dose of SSP without AM fungi resulted in high mortality of cuttings with moderate plant growth. Similarly, the uptake of P, K, Cu and Fe in tea plant was significantly improved due to inoculation of Glomus fasciculatum (Zhi, 1993). It was reported that root infection rate decreased with increasing application of soluble phosphorus. However, the infection rate and P uptake were promoted by the application of rock phosphate (Hazarika, 2000).
There was also report of enhanced leaf harvest and uptake of phosphorus by tea shoot due to inoculation of *G.fasciculatum* (Barthakur *et al.*, 1994). They also reported that the effect was more pronounced when phosphorus was omitted from the fertilizer mixture. While Rajagopal and Ramarithinam, (1997) reported that phosphorus uptake was significantly increased in tea seedlings inoculated with *Glomus fasciculatum* in presence of phosphorus. In tea about 60% of P absorbed by the bushes may be removed from the field and 40% either returned to the soil or tied up in perennial growth (Ranganathan, 1980).

AM fungi have been reported to increase the efficacy of P uptake (Goswami and Kamath, 1984). Effect of AM fungi on the growth yield and P –uptake of several crops has also been reported, both in the presence and absence of fertilizer P. These include sorghum (Krishna and Bagyaraj, 1981) pearl millet (Krishna and Dart, 1984) cotton (Bagyaraj and Manjunath, 1980) and chillies (Bagyaraj and Sreeramulu, 1982).

**2.3 Association of AM fungi in weed species of tea plantation:**

Arbuscular Mycorrhizal (AM) fungi form symbiotic associations with the roots of many plants including important weeds.

The soil factors which influence weed persistence are soil water aeration, temperature, pH and fertility level and also cropping system. Weeds are found in soils differing quite widely in physical characters,
soil-moisture-holding capacities and soil reaction. The fact that they are weeds indicates that they have adaptability to a wide range of soil environment. Weed species like *Cynodon dactylon*, *Digitaria sanguinalis*, *Pteridium* spp and *Borreria hispida* inhibit only acidic soils (Barua, 1989).

Bilalis *et al.* (2011) studied the AM root colonization and N% of weeds and compared it with two olive production systems (organic and conventional). They observed that AM root colonisation of weeds influences the density and biomass of competitive weeds. They also indicate that organic cultural practices significantly increased weed biomass and AM root colonization. Weeds are an important variable in organic crop production, both economically and ecologically. Weeds may serve to maintain diversity and agronomically beneficial taxa of AM fungi (Vatovec *et al.*, 2005).

Chen *et al.* (2004) observed that the number of AM fungal spores increased significantly with increasing weed species number. Barthakur *et al.* (1994) studied the association of AM fungi in tea field weeds and found that weeds specially *Ageratum conizoydes*, *Borreria hispida*, *Mimosa pudida* and *Mimosa invisa* were highly colonized by AM fungi. Further studies were initiated after that to utilize it as a nutrient pool in the recent year (Dutta *et al.*, 2007). Moreover, weeds like *Mimosa invisa* and *Mimosa pudica* are commonly grown in tea plantation of North East India in rehabilited areas for N$_2$ fixation.
2.4 Rhizosphere Microflora:

The rhizosphere is the thin cylinder of soil that immediately surrounds the roots. Its pH and other characteristic are considerably different from that of the whole soil (Hiltner, 1904). Normally, the pH of the rhizosphere will be more than one unit less than the soil as a whole, the result of released H ions from the respiration process. There is considerable biological activity in the rhizosphere as carbonaceous materials are released or sloughed off from the extending and /or expanding root as it moves through and into the soil, providing readily usable food for bacteria and fungi .This is one of the reasons why plants can survive on relatively poor soils i.e soils whose pH and level of available essential elements may be less than ideal. Plants roots, their function, and extent of soil contact will significantly influence the growth and development of the whole plant. Fluctuation in rhizosphere activities occur in response to changes in plant, microbial community, edaphic, and environmental factors (Rao and Johri, 1999). The rhizosphere microflora play important role in plant growth and development.

2.4.1 Free living - Nitrogen Fixers and its role in plant growth:

Plant growth in soils is influenced by many abiotic and biotic factors. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most
abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates (Lynch, 1990) creating a very selective environment where diversity is low. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Saharan and Nehra, 2011).

Among the nitrogen fixing microorganisms non-symbiotic including associative symbiotic group viz. *Azotobacter* and *Azospirillum* are generally the dominant forms in the rhizosphere. Non-symbiotic nitrogen fixation has a great agronomic significance. One main limitation that it faces is the availability of carbon and energy source for the energy intensive nitrogen fixation process. However, this limitation can be compensated by moving closer to or inside the plants, viz. in diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. Some important non-symbiotic nitrogen-fixing bacteria like *Azotobacter* sp, *Azospirillum sp*. *Bacillus* sp, *Beijerinckia* sp, *Pseudomonas* sp were reported in close association with crop plants. (Saharan and Nehra, 2011).

Free living nitrogen fixing bacteria and the associative nitrogen fixing bacteria, colonize rhizosphere and endorhizosphere, respectively and fix atmospheric nitrogen. They have significant potential for commercial applications as biofertilizers (Bashan and Holguin, 1997).
inoculation of *Azospirillum* as seed bacteriazation, root dipping and through soil application is a common practice (David, 1992). Seed inoculation is the most successful technology of bioinoculant inoculation, which provides high population of desirable strain in the close vicinity of the young growing roots. But, this method appears to be difficult to treat the small sized seeds especially for transplanted crops, *viz.* tomato, chilli, brinjal, tobacco *etc.* There are few reports of inoculating *Azospirillum* directly to the nursery in case of transplanted crop like tea which might have the potential of improving the proliferation of the endosymbioant (Baby *et al.*, 2002).

*Azcon et al.* (1978) have reported that formation of phytohormones may help in synergistic interactions between the soil microorganisms and establishing dual symbiosis with plants. But, little work was carried out on the utility of single or dual inoculation on tea.

**2.4.1.1 *Azospirillum* as N₂ - fixer and Plant growth promoter:**

*Azospirillum* is a free-living, plant-growth-promoting bacterium (PGPB), capable of affecting growth and yield of numerous plant species, many of agronomic and ecological significance.

Dobereiner and Baldani, (1981) reported that possession of C4 – dicarboxylic acid pathway of photosynthesis by tropical grasses favours the establishment of nitrogen fixation in the roots because of the ability of
these plants to use the intense radiation in tropics efficiently. The growth in NFM (nitrogen free medium) is always accompanied with alkali production and high rates of acetylene reduction (Dobereiner et al., 1976). They were isolated from the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates (Dobereiner et al., 1976).

Inoculation with *Azospirillum* sp. mainly changes growth or morphology of roots by increasing the number of lateral roots and root hairs; the enlargement of the root surface results in better nutrient uptake and improved water status that may be the main factor enhancing plant growth (Lin et al., 1983; Bottini et al., 2004).

Among the free living nitrogen fixing bacteria *Azospirillum* is considered to be more efficient with nitrogenase properties comparatively better than the other nitrogen fixers. Nitrogen fixation is the first major mechanism for the enhancement of plant growth by *Azospirillum* (Prasad and Govindarajan, 2001).

Acetylene reduction activity was detected in all *Azospirillum* strains isolated from rice roots in Zanzibar, Tanzania by Yasmin et al. (2004) which ranged from 5.9 – 76.4 nmole C$_2$H$_2$ reduced/h/mg protein.

In India extensive work on *Azospirillum* have been done by Laxmikumari et al., 1976, Subba Rao, 1981. They reported the
association of *Azospirillum* with many crop plants. The performance of *Azospirillum* in pot experiments (Subba Rao, 1982) and field trials (Subba Rao, 1981) was found to be highly significant in increasing crop growth and yield.

In tea, Baby *et al.* (2002) studied the effect of artificial inoculation of *Azospirillum*. Incorporation of *Azospirillum* bioformulations resulted in higher growth response of the tea plant, increase in soil and plant total nitrogen and leaf nitrate reductase activity.

Both in greenhouse and in field trials, *Azospirillum* was shown to exert beneficial effects on plant growth and crop yields (Okon and Labandera- Gonzalez, 1994). Inoculation of *Azospirillum* spp to wetland rice under acidic conditions improved shoot growth, straw yield and N-uptake (Govindan and Bagyaraj, 1995). Mohanty *et al.* (2001) reported that rhizosphere and rhizoplane samples revealed the presence of *Azopirillum* in soils of Orrisa in sugarcane plantation. They also recorded phosphatase activity of the four *Azopirillum* isolates. The most interesting and notable feature of their study was the excellent performance of the *Azospirillum* strains in a soil having pH from 4.5 -5.8. Singh *et al.* (2001) studied that total *Azospirillum* population was found to be increased by more than 100 fold irrespective of the treatments till 90 to 120 days of *Azospirillum* application.
The differences between plant species in relation to *Azospirillum* occurrence and its nitrogenase activity have been observed by Lakshmi *et al.* (1977). The occurrence of this bacterium in soil is dependent on pH. But the nitrogenase activity could be detected in the roots of *P. maximum* even in acid pH up to 5.2 probably due to the proliferation of *Azospirillum* within the roots (Subba Rao, 1981).

In connection with numerous manifestations of a beneficial action of *Azospirillum* bacteria on plants, since the beginning of studies on bacterial species of this genus attempts have been made to use them practically in agriculture through inoculation of crops with these bacteria. (Swedrzynska and Sawicka, 2001).

Thakuria *et al.* (2004) studied three isolates of *Azospirilla* group and identified them as *Azospirillum brasilense* and the fourth isolate was identified as *Azospirillum amazonense* in the acidic soil of Assam. *Azospirillum* strains have no preferences for crop plants or weeds or for annual or perennial plants and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots. It appears that *Azospirillum* is not a plant specific bacterium and is a general root colonizer (Saharan and Nehra, 2011).
2.4.1.2 *Azobacter* spp. and its role in plant growth:

*Azobacter* spp. are Gram negative, aerobic, asymbiotic free living nitrogen fixing bacterium belonging to family *Azobacteriaceae*, section VI of *Bergey’s Manual of Determinative Bacteriology* that play an important role in improving plant growth and yield by producing plant hormones and antimicrobial substances (Subba Rao, 1981; Sandeep *et al.*, 2011).

Subba Rao, (1981) stated that lack of organic matter in soil is a limiting factor in the proliferation of *Azobacter* in soil. He studied in detailed the aspects of *Azobacter* inoculum. Singh, (2001) in his review stated that nitrogen fixing capacity of *Azobacter* found to vary considerably on condition of cultivation of the strain, composition of the nutrient medium and its acidity, temperature, carbon and nitrogen source.

The artificial inoculations of seeds of crop plants with *A. chroococcum* were first introduced by Gerlach and Vogel in 1902 (Singh *et al.*, 2001).

The phenomenon of increasing the yield of agricultural crops is attributed to the multiple action of *Azobacter*. It can act not only by fixing atmospheric nitrogen but also by altering the microbial balance and by producing metabolites that stimulate plant development thus giving the plants advantage over the uninoculated controls. *Azobacter* is also
reported to affect the plant growth indirectly by changing the microflora of rhizosphere and by maintaining the balance between harmful and beneficial organisms in soils (Singh et al., 2001).

Panosyan et al. (1967) reported for the first time that various strains of *Azotobacter* were having a high phosphatase activity.

Singh, (2006) reviewed the plant growth parameters in cereals inoculated with *Azotobacter* and stated that nitrogen requirement of paddy could be reduced by 20 to 40 kg/ha by *Azotobacter* inoculation.

An increase in rice grain yield was observed when rice seedlings were inoculated with a mixed inoculum comprising *Azotobacter chroococcum, Pseudomonas striata* and *Aspergillus awamorrii* and with and without application of N and P fertilizers than single species inoculation (Kundu and Gaur, 1984).

Sharma et al. (2002) indicated that the treatment combinations of N levels with and without FYM and *Azotobacter* levels exerted a highly significant influence on the mean yield of tea ranging from 0.68 to 26.09 percent over control.

2.4.2 Phosphate solubilizing microorganisms and its role in plant growth:

Plants acquire P from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through
precipitation with cations such as Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{3+}\) and Al\(^{3+}\), depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. Hence, phosphate solubilizing microorganisms initiate this process by different microbial mechanisms.

Species of *Aspergillus* and *Penicillium* are among fungal isolates identified to have phosphate solubilizing capabilities. Among the bacterial genera with this capability are *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Arthrobacter*, *Alcaligenes*, *Serratia*, *Enterobacter*, *Acinetobacter*, *Flavobacterium* and *Erwinia* (Rodriguez and Fraga, 1999).

Vassilev *et al.* (2001) reviewed that involved immobilized microorganisms related to Rock Phosphate solubilization and Phosphorus plant nutrition, and pointed out possible future trends in this field of research. Rodríguez and Fraga, (1999) reviewed the principal mechanism for mineral phosphate solubilization, the production of organic acids, and acid phosphatases activity of phosphate solubilizing microorganisms. Mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. The presence of a significant amount of phosphatase activity in soil has been reported (Lynch, 1990; Feller *et al.*, 1994). The major source of phosphatase activity in soil is considered to be of microbial origin (Garcia *et al.*, 1992; Xu and Johnson, 1995). Phosphatase
activity is substantially increased in the rhizosphere (Tarafdar and Junk, 1987).

Burns,(1983) studied the activity of various phosphatases in the rhizosphere of maize, barley, and wheat, showing that phosphatase activity was considerable in the inner rhizosphere at acidic and neutral soil pH. Rodríguez and Fraga,(1999) stated that although several phosphate solubilizing bacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the property of phosphate solubilization for plant yield enhancement.

An alternative approach for the use of phosphate-solubilizing bacteria as microbial inoculants is the use of mixed cultures or co-inoculation with other microorganisms. Several studies demonstrate the beneficial influence of combined inoculation of phosphate-solubilizing bacteria and \textit{Azotobacter} on yield, as well as on nitrogen (N) and P accumulation in different crops (Kundu and Gaur, 1984). They concluded that mixed inoculants provided more balanced nutrition for the plants, and that the improvement in N and P uptake was the major mechanism involved. This evidence points to the advantage of the mixed inoculations of PGPR strains comprising phosphate-solubilizing bacteria. AM fungi
along with phosphate solubilizer causes a synergistic interaction that allows for better exploitation of poorly soluble P sources (Ray *et al.*, 1981).

*Aspergillus niger* solubilized insoluble phosphate well in a liquid medium supplemented with tricalcium phosphate (Vazquez *et al.*, 2000) and caused a remarkable drop in pH of culture media and solubilized considerable amounts of phosphate (Omar, 1998). The absence of soluble phosphate in media induces the acid production (Pradhan and Sukla, 2006). Patgiri and Bezbaruah, (1990) estimated that only 10.86% phosphate solubilizing bacteria (PSB) were found among the 46 heterotrophic bacteria isolated from the tea soils of Assam. Thus the abundance of PSB in soils in terms of in terms of percentage varied widely and ranged between 6 to 42% of the total microflora.

Nopparat *et al.* (2007) studied ten fungal species of Thailand, in Pikovskaya’s medium supplemented with tri-calcium phosphate and found that Alkaline phosphatase (pH 11) liberated maximum 0.042 units/ml in 3rd day of experiment and in case of Acid phosphatase (pH 6.5) the maximum was recorded 0.041 units/ml on the 3rd day. The results showed variability in liberation of both acid and alkaline enzyme at different growth periods from 3rd to 7th day culture.

Recently, Balamurugan *et al.* (2010) studied phosphatase enzyme in phosphate solubilizing bacteria (PSB) in Pikovskaya’s broth where
TCP was replaced with organic source (p-glycerophosphate) following Eivazi and Tabatabai, (1977) method and found effective PSB strains in the tea soil of South India.

2.5 Interaction between Rhizosphere microflora and AM fungi:

Kumari and Balasubramanian, (1993) studied the effect of combined inoculation of AM fungi (Glomus fasciculatum, Gigaspora margarita and Acaulospora laevis) with Azospirillum brasilese on growth and nutrient uptake of coffee seedlings grown in the nursery. Dual inoculation of maize plants with Azospirillum brasilese and Glomus mosseae, stimulated the development of mycorrhizal fungi and produced plants of a similar size, N content and a higher P content than those supplied with N and P from the nutrient solution (Barea et al., 1983).

Interactions of AM fungi with other soil microorganisms were also reviewed by many workers. Field trials had shown gains of 21 kg N/ha by inoculating grass seed with Azospirillum and increased yields in sorghum, maize and barley as observed by Tilak, (1985). Azospirillum brasilese also stimulated the AM infection of maize and ryegrass by Glomus mosseae as stated by Barea et al. (1975). Bagyaraj and Menge, (1978) studied the interactions between Azotobacter chroococcum and the AM fungus Glomus fasciculatum in tomato and found a synergistic effect on plant growth.
Manjunath et al. (1981) conducted a triple interaction study between the free living nitrogen fixing bacterium *Beijerinckia mobilis*, phosphate solubilizing fungus *Aspergillus niger* and the mycorrhizal fungus *G. fasciculatum* and found a synergistic beneficial effect on the growth of onions with all three organisms.

Raj et al. (1981) studied the effect of *Glomus fasciculatum* and a non phytohormone producing strain of the phosphate dissolving bacterium *Bacillus circulans* on phosphate solubilization of finger millet and found that AM fungi do not solubilize unavailable forms of P but still enhanced the P-uptake, which was attributed to a better exploration of soil.

Root-associated diazotrophs fix atmospheric nitrogen but translocate only little of this fixed nitrogen from cells to the host plant (Christiansen-Weniger et al. 1992) Perhaps this might be the reason that some of the isolates could uptake least N and P in their tissues. In few cases, however, there was strong evidence of nitrogen-fixing bacteria contributing to the nitrogen accumulation in the plants (Boddey and Dobereiner, 1988).

### 2.6 Biofertilizer:

Biofertilizers form an integral part of Integrated Plant Nutrient Supply (IPNS) system and organic farming which constitutes the present as well as future mandate of Indian agriculture. *Azospirillum* is one of the
important biofertilizer, which was found to fix nitrogen in association with crops like rice, maize, sorghum, wheat and millets (Mubeen et al., 2006; Stella and Sivasakthivelan, 2009).

Mahdi et al. (2010) in the review discussed the importance of biofertilizer in the present scenario and showed the problems of indiscriminate use of synthetic fertilizers that had led to the pollution and contamination of the soil. The role and importance of biofertilizers in sustainable crop production have been reviewed by several authors (Biswas et al., 1985; Katyal et al., 1994). It may be noted, only 30% of India’s total cultivable area is covered with fertilizers where irrigation facilities are available and the remaining 70% of the arable land, which was mainly rain fed, very negligible amount of fertilizers are being used. Farmers in these areas often use organic manures as a source of nutrients that are readily available either in their own farm or in their locality (Mahdi et al., 2010).

The North-Eastern (NE) region of India provides considerable opportunity for organic farming due to least utilization of chemical inputs. It was estimated that 18 million hectare of such land was available in the NE that can be exploited for organic production. With the sizable acreage under naturally organic/default organic cultivation, India have tremendous potential to grow crops organically and emerge as a major supplier of organic products in world’s organic market. The incorporation
of bio-fertilizers (N$_2$-fixers) plays major role in improving soil fertility, yield attributing characters and thereby final yield has been reported by many workers (Kachroo and Razdan, 2006; Sabashini et al. 2007). In addition, their application in soil improves soil biota and minimizes the sole use of chemical fertilizers (Sabashini et al., 2007; Mahdi et al., 2010).

Bio-fertilizers are carrier based preparations containing beneficial microorganisms in available state intended for seed or soil application and designed to improve soil fertility. Further, the potential of phosphate solubilizers in solubilising P and mycorrhizae in mobilizing P made agricultural scientists to think over the possibility of exploiting these organisms in integrated nutrient management programme. Though Balamurugan et al.(2010) successfully isolated some phosphate solubilizers from tea soil samples and exploit it very well by tea plantation for its higher productivity in South India only at the recent times, exploitation of biofertilizers in plantation crop like tea have not yet been well explored (Balamurugan et al., 2010).