Review of Literature
Several microbes such as bacteria, fungi and actinomycetes are widely distributed in soil and they work in a tandem to fix atmospheric nitrogen, solubilize phosphorus to be absorbed by the plants, increase nutrient absorbing surfaces (endomycorrhiza), have non specific hormonal effects on the plant growth and many even control pathogens. They are collectively known as “Biofertilizers” and at times some of them are also known as plant growth promoting rhizobacteria (PGPR). According to Singh et al. (2005) the biofertilizers or microbial inoculants are the preparations of live microorganisms used to improve plant nourishment and soil fertility achieving there by more and sustainable production. Biofertilizers offer a cheap, low capital intensive, non bulk and eco-friendly source to boost productivity. Having their dependency on renewable energy the biofertilizers offer an attractive source of nutrients for sustaining crop productivity.

Among these the free-living nitrogen fixers like *Azotobacter* and *Azospirillum*, Phosphorus solubilizers like *Aspergillus awamori*, *Bacillus megaterium*, *Bacillus polymyxa* etc. and Vesicular Arbuscular Mycorrhizal fungi (VAM) are important in mulberry cultivation and other non-leguminous crop plants due to absence of symbiotic microbial associations in their systems. An account of such economically important and beneficial microorganisms about their distribution, utility in mulberry and other crop plants is presented here.

### 2.1 *Azotobacter* diazotroph and its characteristics

*Azotobacter*, a free living heterotrophic nitrogen fixing bacterium, belongs to the family *Azotobacteriaceae*. *Azotobacter* species are found in soil, water, rhizosphere etc. It is a gram-negative motile soil organism and can be isolated and cultured ex-situ conveniently. It is larger compared to other prokaryotes (4-7 μm in diameter) and has
yeast like in appearance. *Azotobacter* grows best in neutral to alkaline soil, but does not grow when the pH is below 6 and hence not present in acidic soil. It forms cysts that serve as resting bodies. Colonies are moderately slimy turning blackish brown on ageing (Becking, 1992).

*Azotobacter* is a highly aerobic organism, which fixes atmospheric nitrogen asymbiotically. The important species comprise of *A. chroococcum, A.vinelandii, A.paspali, A.beijerinckii* etc. *A.chroococcum* strain was isolated by Subramani and Abraham (1962) from red loamy soils in India. Studies on the distribution of *Azotobacter* species indicated that they are widely spread in top layers of soil and their population decreases with depth (Tilak, 1991).

*A.chroococcum* produces characteristics black pigment, melanin especially in older cultures. This pigmentation is due to the oxidation of tyrosine by tyrosinase an enzyme which has copper. *A.beijerinckii* and *A.vinelandii* produce green yellow pigment whereas *A. insignis* produces yellow brown colour. The related species like *Derxia gummosa* which fix nitrogen are yellow to mahogany brown whereas *Beijerinckia indica* is rust brown (Mishustin and Shilnikova, 1971).

### 2.2 Mode of Action of *Azotobacter* on plant growth

#### 2.2.1 Nitrogen fixation

*Azotobacter* can utilize a variety of carbon sources to fix atmospheric nitrogen in the soil (Mishustin and Shilnikova, 1969; 1971). Iswaran and Sen (1960) reported that the presence of Calcium at optimum levels is essential for better growth of *Azotobacter* and its nitrogen fixation. Similarly, many reports (Iswaran and Sundara Rao, 1960; Iswaran and Subbarao, 1966) indicate that the presence of combined nitrogen, trace elements and sodium chloride in the medium could influence nitrogen fixation by
Azotobacter. It is found in the rhizosphere of many plants, fixes nitrogen and produce growth promoting hormones (Shende and Apte, 1982).

2.2.2 Growth promoting and other substances produced by *Azotobacter*

Different workers like (Mishustin and Shilnikova, 1971; Subbarao, 1989) have reported that in addition to nitrogen fixation, *A.chroococcum* synthesizes and secretes thiamine, riboflavin, pyridoxine, cyanocobalamine, nicotinic acid, panthothenic acid, IAA, gibberellins or gibberellin like substances. Mallikarjunaiah and Bhide (1981; 1982) reported that *A.chroococcum* inhibited some of the fungi like Alternaria, Fusarium, Helminthosporium, Rhizoctonia, etc. due to the production of antibiotic and fungistatic substances. *A.chroococcum* cultured with sodium benzoate as the sole carbon source produced humic acid like substances (Hardisson and Robertgero, 1966). These substances are known to stimulate the salt uptake by plants. Robertgero *et al.* (1967) reported that *A. chroococcum* cultures produced quinines which are known to give protection to plants against plant pathogens and also affect the oxidative phosphorylation of plants (Brown, 1974) and he also reported that the root exudates of *Azotobacter* inoculated plants contained tryptophan or related compounds that could act as precursors for IAA synthesis. *A.chroococcum* of rhizosphere is the most active producer of thiamine, biotin, inositol, pyridoxine, panthothenic acid and nicotinic acid (Umetov *et al.*, 1968).

El-ruan *et al.* (1973) reported the beneficial effect of *A.chroococcum* on plant by virtue of its ability to synthesize IAA, GA3, some vitamins, amino acids and nitrogen fixation. *A.chroococcum* also produces antifungal and antibiotic substances which inhibit a variety of soil fungi. In fact, these twin attributes of *Azotobacter* can explain the beneficial effects of the bacteria on the germination of seeds (Shende *et al.*, 1977).

In addition to the nitrogen, the ability of species of *Azotobacter* to synthesis and secrete thiamine, riboflavin, pyridoxine, cyanocobalamine, nicotinic acid, indole acetic
acid, gibberellin and gibberllin like substances is well documented. The production of auxin, gibberellin and cytokinin by Azotobacter have been confirmed by many workers (Gonzalez-Lopez et al., 1986; Martinez Eoledo et al., 1988 and Salmeron et al., 1990).

2.3 Effect of Azotobacter on plant health and productivity

2.3.1 Response of various crops to Azotobacter

Application of Azotobacter resulted in beneficial effects on several crops such as rice, wheat, sorghum, maize, pearl millet and cotton. The study in India indicated increased yield of cereals by 10% and 13-39% in vegetables and available data also indicated that Azotobacter was more effective on manured soil (Bagyaraj and Patil, 1975).

Reddi and Reddi (1981) reported that Azotobacter inoculation significantly increased the grain yield by 10.7-12.1% in pearl millet.

Anita et al. (1981) observed that there was an improvement in plant root, boll number/plant, boll width, seed cotton yield, oil content in seeds and quality of cotton (Gossypium hirsutum) due to inoculation of C2 and M4 strains of Azotobacter.

Wani (1988) reported increased yield in gram, pearl millet from 0-27% by Azotobacter application. Palpawar (1983) reported an improvement in germination of seeds by 30% and 16-30% yield increase in wheat due to the inoculation of Azotobacter. It also improved the plant height, tillers, ear length and grain yield of wheat over non inoculated control (Sharma et al., 1987).

Yadav et al. (1990) curtailed 50% of the recommended dose of nitrogen fertilizer on pearl millet cultivation by application of A.chroococcum. The Azotobacterization of wheat had given significant increase in grain and straw yield in poor fertile soil as reported by Kolte et al. (1990).
Seed inoculation of wheat varieties with P solubilizing and phytohormones producing *A. chroococcum* showed better response and increase in grain (12.6%) and straw (11.4%) yield (Kumar, 2001).

### 2.3.2 Response of mulberry to *Azotobacter*

Introduction of *Azotobacter* to soil could increase the yield and growth in several crop plants including mulberry (Kasiviswanathan *et al.*, 1981; Shabaev *et al.*, 1991).

Gangwar and Thangavelu (1992) observed that the sprouting, rooting, survivability, height of the plant, number of leaves, branches per plant and protein content of leaves increased in mulberry variety Kanva-2 inoculated with *A. chroococcum* by using FYM as adherent and bulking medium.

Siddiqui *et al.* (1993) reported that *Azotobacter* inoculation in mulberry significantly increased plant height, number of leaves, number of branches and leaf nitrogen content significantly.

Bongale and Dandin (1993) studied the efficient utilization of nitrogen fixing bacterial systems in mulberry cultivation and reported that the soil organic matter content has to be maintained for regulating the efficacy of biofertilizers. Among different groups of nitrogen fixing bacteria, the species of *Azotobacter* and *Azospirillum* were found to be effective in supplementing the nitrogen requirement substantially in mulberry cultivation.

Das *et al.* (1994) found that it is possible to curtail 50% of the recommended dose of nitrogen (300 kg N/ha/yr) supplied through *Azotobacter* biofertilizer without any adverse effect on leaf yield and quality as assessed from plant growth, leaf yield, chemical analysis of leaf and silkworm rearing.

Similarly Yadav and Kumar (1994) and Balasubramaniam (1995) reported positive response of mulberry to the application of *Azotobacter* inoculants.
A field experiment conducted to study the response of six mulberry varieties (S54, S41, S36, S30, Kanva-2 and Local) to the application of *A. chroococcum* biofertilizer under irrigated condition revealed that all the varieties responded positively to *Azotobacter* biofertilizer application along with half of the nitrogen i.e., 150 kg N/ha/yr. However, the response of the variety S-54 to *Azotobacter* biofertilizer was significantly superior over all other varieties. The local variety showed the least response (Das *et al.*, 1995).

Datta (1995) reported the use of *Azotobacter* biofertilizer under irrigated mulberry cultivation along with foliar application of growth promoter n-triacontanol could save the chemical nitrogen fertilizer application up to 50% and increase the leaf yield by 28% over control.

Satpathy *et al.* (1995) found that inoculation of *Azotobacter* with application of 150 kg nitrogen, yielded 29,697 kg of leaf/ha/yr which was at par with control where 300 kg of inorganic nitrogen was applied. Basavaiah *et al.* (1995) recorded leaf yield in the treatments having 50 N plus *Azotobacter* biofertilizer which was on par with the treatment of 100% N without biofertilizer. Significant improvement in leaf moisture content and leaf nitrogen contents were also observed.

Sreenivasulu *et al.* (1995) opined that inoculation of *Azotobacter* increases the crop yield by 5-15% and a nitrogen contribution of about 25 kg/ha. Apart from nitrogen fixing ability, this microorganism produces growth promoting substances which favor better growth of plants. Das *et al.* (1996) also studied differential response of mulberry cultivars to the inoculation of *Azotobacter* biofertilizer under irrigated condition and observed that all the recommended varieties responded positively to the inoculation and 50% nitrogen application could be saved.

The application of *Azotobacter* not only fixes the atmospheric nitrogen, but also reduces the disease incidence in the plants. The *Azotobacter* produces soluble fungistatic
substance which inhibits the growth of fungi like *Alternaria, Helminthosporium, Fusarium* etc., when tested under laboratory conditions on Agar media (Lakshmi *et al.*, 1975; Singh, 1997).

Sharma *et al.* (1996) reported that the incidence of major foliar disease of mulberry caused by *Cercospora moricola, Phyllactinia corylea, Cerotelium fici, Fusarium pallidoroseum, Alternaria alternata* and *Pseudomonas syringe* was found to be effectively reduced when *A. chroococcum* biofertilizer was applied.

Mishra *et al.* (1996) observed that the organic farming is possible with the use of bacterial biofertilizer which was found to reduce the input of nitrogenous fertilizer by 50%. The yield of mulberry was also maintained on par with that obtained through application of full N dose.

Bongale *et al.* (2000) elucidated the efficiency of new strains (Prakrathi, R.F.C) of *A. chroococcum* in improvement of mulberry crop. Philip (2000) proved the use of biofertilizer and triacontanol to be quite promising in increasing the mulberry leaf yield in Kerala substantially besides saving 50 percent cost on urea.

Sinha *et al.* (2000) studied the effect of *Azotobacter* on nitrogen fixation in mulberry and reported that the application of *Azotobacter* @ 10 kg/ha/year in two splits per crop recorded an increase of 9.23% in leaf yield than untreated ones indicating that *Azotobacter* can alone significantly supplement the nitrogen requirement to the mulberry plants. They also reported that *Azotobacter* not only helps in curtailing the dependence on chemical fertilizers but is also economical.

Venkataramana *et al.* (2001) studied the effects of triacontanol and *Azotobacter* application in mulberry and reported 16.04% increase in leaf yield and 7.3 kg increase in cocoon yield per 100 DFLs compared to control.

The work of Baqual and Qayoom (2004) reveal that inoculation of mulberry with *Azotobacter/ Azospirillum* resulted in better plant response and saving of nitrogenous
fertilizers. The rate of sprouting, rooting and other quantitative parameters were found to be increased in K2 mulberry variety with the use of *Azotobacter* biofertilizers.

While comparing the effects of chemical fertilizers with that of biofertilizers in mulberry Sannappa *et al.* (2005) found that the leaf area, leaf area index and leaf yield did not differ significantly.

Susheelamma *et al.* (2005) reported that due to scarcity and non availability of farmyard manure under dry farming areas, the soil fertility cannot be maintained at required level and this problem could be overcome by the use of biofertilizers which saves nitrogen up to 50% in addition to improving the soil health and leaf quality.

Vijayan *et al.* (2007) observed that foliar application of *A.chroococcum* to mulberry grown under saline soil conditions showed significant level of improvement in biochemical and morphological parameters of leaf.

It is reported (Susheelamma *et al.*, 2008) that, high requirement of nitrogenous and phosphatic fertilizers in mulberry escalates the cost of cultivation. They found that the application of 500 kg neem leaves + 25 kg *Tridax sinensis* + 5 MT FYM + 4 kg biofertilizers /ac/crop effectively reduced the weed and pest population besides improving the soil health cutting down the expenditure on nitrogenous fertilizer.

2.4 *Azospirillum* Diazotrophs

Dobereiner and Day (1976) were the first to report the wide distribution of *Azospirillum* in the rhizosphere of several tropical grasses. Since then *Azospirillum* has been isolated from the roots of numerous wild and cultivated grasses, cereals and other crops from tropical, subtropical and temperate soils (Ladha *et al*., 1987; Sundaram *et al*., 1988; Wong *et al*., 1980).

Tarrand *et al.* (1978) proposed *Azospirillum* as the genus and distinguished two species *A-brasilense* and *A.lipoferum* based on physiological differences and on
homology experiments. Reinhold et al. (1987) isolated highly salt tolerant species namely *A. haploferans* from the roots of kallar grass.

### 2.4.1 Characteristics of *Azospirillum* spp

The genus *Azospirillum* comprises of bacteria which are gram negative, spiral shaped and 1.5μ in diameter (Dobereiner and Day, 1976). When grown in N-free medium it behaves as a micro- aerophilic and fixes nitrogen and when supplemented with fixed nitrogen it grows as an aerobe. It prefers salts of organic acids such as maleate, succinate and pyruvate as carbon and energy sources.

### 2.5 Mode of Action of *Azospirillum* on plant growth

#### 2.5.1 Nitrogen fixation by *Azospirillum*

Nitrogen fixation was the first major mechanism of action suggested for the enhancement of plant growth by *Azospirillum* (Hurek et al., 1988; Helwin et al., 1989). The nitrogen fixing activity of *Azospirillum* cultures is governed by the fluctuations in soil redox potential, pH and organic matter content (Charyulu and Rao, 1980). The studies of Okon et al. (1983) suggested that only 5% of the fixed nitrogen is incorporated in to plant tissue. Kucey (1989) found that 5-10% of the plant N was derived from bacterial N\textsubscript{2} fixation in maize and wheat. In general the nitrogen fixation activity of *Azospirillum* culture is governed by the fluctuation in soil factors. Soil pH is one of the important factors deciding the nitrogenase activity of *Azospirillum* (Dobereiner et al., 1976). An optimum pH of 7.1 to 7.4 was recorded by Okon et al. (1977). Gupta and Tauro (1992) using mutants of *A.brasilense* under defined cultural conditions showed the importance of hydrogenase uptake in the process of N\textsubscript{2} fixation.

#### 2.5.2 Growth promoting substances produced by *Azospirillum*

Tien et al. (1979) and Horemans et al. (1985) reported production of IAA, gibberellins, cytokinins by *A.brasilense*. Horemans and Valassak (1985) studied the
IAA production by *A. brasilense* in the presence of NH$_4$ @ 40 mg/ml and found that IAA was produced into the medium without the addition of tryptophan.

Prabhu and Balasubramanian (1989) reported the production of IAA by *A. lipoferum* which varied from 4 to 42 mg/ml in various strains. Applications of hormones either synthetic or purified from bacterial culture to seedlings completely reproduced the effects of *Azospirillum* on root development and morphology (Harri *et al.*, 1988). Karthikeyan (1994) investigated the effect of pH on IAA production by *Azospirillum* isolates. Under tryptophan supplement condition as well as in the absence of tryptophan, the isolate AC$_8$ produced the maximum quantity i.e., 23.40, 24.55 and 29.48 mg/ml of IAA at pH levels of 4.5, 6.0 and 7.5 tested respectively.

### 2.6 Effect of *Azospirillum* on plant health and productivity

#### 2.6.1 Response of various crops to *Azospirillum* inoculation.

The *Azospirillum* inoculation increased total yield of field grown plants which generally ranged from 10-30% (Kapulnik *et al.*, 1981; Rao *et al.*, 1983; Watanabe and Lin, 1984). Kolb and Martin (1985) found that the root proliferation and root to shoot ratio improved favorably in wheat plants due to inoculation with *Azospirillum*. In the case of field grown wheat, winter legume and non legume a significant increase in the growth parameters, dry matter accumulation, grain yield and protein content was recorded due to *Azospirillum* inoculation (Boddy *et al.*, 1986; Dart, 1986).

The effect of inoculation with *A. brasilense* on growth and yield of *Sorghum bicolor* in hydroponic system was significant with enhancement of dry matter content, leaf area development and grain yield. At later stages of growth, leaf senescence was delayed in inoculated plants thus favoring dry matter accumulation and grain filling (Sarig *et al.*, 1990). Paramaguru and Natarajan (1993) reported that local and CA-42 chilli cvs. showed highest plant height (56.13 cm and 57.86 cm) and higher fruit yield.
92.25 mt/ha and 2.24 mt/ha when inoculated with *A. brasilense* along with 75% of recommended nitrogen. Sundaravelu and Muthukrishnan (1993) observed enhanced leaf number, leaf length and yield in radish due to *A. brasilense* inoculation and GA treatment. Jeevajothi *et al.* (1993) recorded increased yield of cabbage when *Azospirillum* inoculated was in combination with NPK.

Fallik *et al.* (1994) observed changes in the concentrations of free indole 3-acetic acid and indole 3- butyric acid and specific activities of enzymes in TCA cycle and glycolysis pathway in roots of maize and other plants inoculated with *Azospirillum*. Lee *et al.* (1994) found a good response of pearl millet and sorghum to the inoculation of N$_2$ fixing bacteria in unsterilized soil.

Subbiah (1994) recorded highest pod yield in chillies and bulb yield in bellary onion due to the application of *Azospirillum* along with 75 % N and full doses of P & K.

Kalyani *et al.* (1996) recorded increased plant height, more number of leaves, dry matter content of plant and yield in cauliflower when *A. brasilense* was inoculated along with 120 Kg N per hectare. Hameeduennisabegum (1998) observed a significant increase in plant height and fruit yield of tomato when inoculated (seed treatment) with *Azospirillum* along with 75% recommended nitrogen at 30, 45 and 60 days after planting.

Durai and Ravichandran (1996) studied the use of *Azospirillum* sp as supplemental source of N in sugarcane crop cv CO-6304. Application of 7 kg of *Azospirillum* sp. along with 225 kg of N/ha recorded same cane yield as that of 300 kg N/ha resulting in saving of 75 kg N/ha.

Inoculation of *A. brasilense* along with normal recommended dose of nitrogen fertilizers recorded increased growth and yield parameters and grain yield in coriander (Subramanian and Vijayakumar, 2000). It has been found that the inoculation of maize crops with an active strain of *A. brasilense* has a beneficial effect on maize vigor and yield (Swedrzynska and Sawicka, 2000).
Nanthakumar and Veeraghavathatham (2001) reported highest yield per hectare (36.94 mt/ha) as also maximum carbohydrate (29.26%), crude protein (13.65%) and higher ascorbic acid (14.69 mg / 100 g) contents in fruits of brinjal when inoculated with *A. brasilense* along with 100% NPK and FYM.

### 2.6.2 Response of mulberry to *Azospirillum*

Santhanakrishnan and Obilisamy (1980) conducted pot culture experiments to study the influence of *Azospirillum* and *Azotobacter* individually and in combination with growth regulators on the growth of mulberry and reported better rooting due to *Azospirillum* inoculation.

Nagarajan *et al.* (1986) and Das *et al.* (1989) observed the increase in plant height, shoot biomass, root biomass and leaf weight due to *Azospirillum* inoculation in mulberry. The influence of *Azospirillum* was studied on mulberry by Yadav and Kumar (1991) and improvements in growth and uptake have been reported.

Yadav and Kumar (1992) have reported that the application of *A. brasilense* (SL-33) to roots of mulberry (*Morus alba* L) sapling and an additional dose of FYM as soil inoculum mixture at 10 kg/ha after 30 days after planting as second dose increased root volume and nitrogen uptake significantly.

Balasubramanian *et al.* (1992) concluded that the individual and combined inoculation of *Azospirillum* and phosphobacteria enhanced the mulberry leaf yield by 7.59% and 14.36% respectively. They also isolated 49 *Azospirillum* cultures from rhizosphere of mulberry from different locations in Tamil Nadu and screened for their efficiency of N2 fixation and IAA production. Among these isolates A2P2, A2P7 performed well.

Kumutha *et al.* (1992) stated that the combined inoculation of *A. brasilense* and VAM had a significant influence in mulberry and increased the number of leaves and leaf fresh weight.
Sharma et al. (1993) observed that incidence of major mulberry diseases was minimum due to the combined inoculation of *Azospirillum* and *Azotobacter* over the control.

Association of *Azospirillum* with mulberry is reported to exist in nature (Sudhakar et al., 1993) and further inoculations by way of soil application and foliar spray may increase the population of this nitrogen fixing bacterium in different spheres and planes of mulberry plant which stimulates plant growth and leaf yield.

Das et al. (1994) reported best results with *A. brasilense* in mulberry which reduced urea fertilizer requirement and its costs.

*Azospirillum* inoculation along with 225 kg N recorded mulberry leaf yield of 31,315 kg/ha/yr registering a gain of 4.5% over control indicating the possibility of substituting 25-50% inorganic nitrogen input in mulberry through the use of these microbes as biofertilizers (Satpathy et al., 1995).

Inoculation of *Azospirillum* stimulates plant growth in mulberry with an increase in the number, length and weight of roots, number of leaves and leaf weight besides bud development and also improves the economic characters of silkworm and silk characters (Anilkumar and John, 2000).

Nagarajaiah et al. (2004) reported that 75% of recommended fertilizers in combination with *Azospirillum* bio-fertilizer performed equally better to that of 100% in enhancing the growth, leaf yield and quality in mulberry, thus saving of 25% of fertilizer if supplemented with biofertilizer.

The addition of organic manure (20 MT/ha), mulching with organics for 5 cm height immediately after planting cuttings for weed management, incorporation of *T.viride* and *P.fluorescens* (each 2.5 kg/ha) and dipping the cuttings in *Azospirillum* solution for 30 minutes before planting resulted in enhancement of sprouting (57%),
shoot dry weight (11.08g), leaf dry weight (6.04g), root no. (7.77) and root length (18.17 cm) compared to conventional technology (Krishnan, 2008).

2.7 Phosphate solubilizing microorganisms (Phosphobacteria)

The occurrence of phosphate solubilizing bacteria in the rhizosphere of wheat plants was reported by Urova (1956). Nowotony and Golctriowska (1956) found that lupin in the rhizosphere stimulated the growth of microorganisms, solubilizing tricalcium phosphate. Sperber (1958) observed dissolution of hydroxypatite by several species of bacteria and fungi isolated from rhizosphere soils of leguminous plants. Paul and Rao (1971) isolated phosphate solubilizing bacteria, *Bacillus megaterium*, *B.subtilis*, *B.brevis*, *B.palvifaciens*, *B.pumilus* and others from the rhizosphere of berseem and green gram.

The occurrence of phosphate solubilizing microorganisms in rhizosphere soil of fertilized maize was noticed by Ghazvinizadeh et al. (1980). The most active ones were *B.megaterium* and *B.cereus* whereas *Streptomyces* showed mild activity.

2.7.1 Effect of Phosphate solubilizing microorganism on plant health and productivity

Phosphorus a major element is an essential plant nutrient required for plant growth. It accelerates tillering, flower formation, good pod and seed setting besides early maturation in several economically important and cultivated crops (Salisbury and Ross, 1986). Under tropical conditions approximately 70-75% of phosphorus gets converted into a form, which is directly not available to the plant roots and hence the deficiency of phosphorus may also occur in crops grown in soil having adequate phosphorus (Hayman, 1975). These are rendered available to plant roots by soil microorganisms through the secretion of organic acids. Thus the P solubilizing microflora plays an important role in correcting the deficiency of phosphorus (Natarajan, 1999).
The action of organic acid has been established for their ability to form suitable complexes with metallic elements. In addition to phosphate solubilization, they can mineralize organic phosphates into soluble form. This reaction takes place in the rhizosphere because the microorganism makes more phosphorus available in solution than is required for their own growth and metabolism and this surplus is available for plant absorption (Vyas and Modi, 1998). The phosphate solubilizers also produce growth promoting substances which influence the plant growth (Ani and Lee, 1995).

2.7.2 Response of various crops to Phosphobacteria

Rao (1968) conducted multilocational phosphobacterial inoculation trials with different crops and reported yield improvement by 20-30% in maize, 16-37% in wheat, 16-19% in pulses and 19-31% in paddy.

Similar trials conducted by Gaur and Gaind, (1984) in different crops also showed increased yield ranging from 0-50% due to inoculation with culture of phosphate solubilizing bacteria with or without rock phosphate addition. Potato yields were increased dramatically (50-60%) due to the inoculation of *Aspergillus awamori*. The residual effect of inoculation was also observed on the succeeding crop.

The application of Phosphobacteria to plant organs, seeds and roots has resulted surprisingly consistent increase in plant growth and yield even well over 100% (Schroth and Hancock, 1982). This provides an indication of the potential of this group of microorganisms improving plant growth as well as yield.

The best results were obtained with radish by Kloeper and Schorth (1978) where a 30 day crop was treated with various strains of *Pseudomonas sp* which recorded an increase in root weight ranging from 60-144% in different field trials.

In the experiments conducted by Savithry and Gnanamanickam (1987) *Pseudomonas fluorescens* as seed inoculant caused significant increase in plant height,
number of pods per plant and fresh weight of pods in groundnut. The increase in plant height was 25.74% and yield was 59% over the controls.

The bacterization of rice with *P. fluorescens* strains had reduced the incidence of sheath rot disease and had enhanced the yield. Phosphobacteria treatment of 8 commercial rice cultivars showed enhanced grain yield which ranged from 3-160%. Treated plants also showed increase in plant height and number of tillers per plant (Sakthivel and Gnanamanickam, 1987). In another experiment, rice plants showed a 56% increase in number of tillers per plant and over 69% increase in number of grains per ear head (Anuradha, 1986) when treated with phosphate solubilizing bacteria.

Tilak (1991) reported that inoculation of phosphate solubilizing bacteria (PSB) with low grade rock phosphates can add about 30-35 kg P$_2$O$_5$/ha to the soil. Field trials with PSB showed significant increase in the yield in case of rice, wheat, chickpea, pigeon pea, soybean, groundnut and berseem (Chhonkr, 1994). In various agricultural crops similarly, significant improvement of yield were recorded due to the application of Phosphate solubilizing microflora (Tarafdar and Marshner, 1995; Rao and Rao, 1996).

Balamurugan and Gunasekaran (1996) reported the effect of combined inoculation of *Rhizobium* strain Tt9 with a phosphobacterium, *B. megaterium* var. *Phosphaticum* (PB1) at different levels of phosphorus in groundnut (CO-1) wherein maximum crop growth, nodulation, plant dry weight and yield were obtained in treatments receiving 100% chemical fertilizer alone which was at par with the combined inoculation of *Rhizobium* and phosphobacteria at 50% chemically sourced phosphorus indicating a saving of 50% chemical fertilizer.

The ability of phosphorus solubilizing rhizobacteria to enhance the growth and phosphorus uptake in canola (*Brassica napus* l.cv.*Legend*) was studied in potted soil experiments. Out of eleven isolated bacteria, nine plant growth promoting rhizobacteria were screened for P-solubilization *in-vitro*. The best P solubilizing activity was recorded...
in isolates namely *B. brevis, B. megaterium, B. polymyxa, B. sphaerucus, B. thuringiensis* and *Xanthomonas maltophilia* (PGPR strain R 55). The results indicated increased pod yield, seed yield and plant weight demonstrating potential use of phosphate solubilizing rhizobacteria as inoculants for Canola (Freitas et al., 1997).

The results of field experiments carried out using some of the culture suspensions with and without super phosphate or rock phosphate on the yield of wheat and rice (Gaur and Gaur, 1998), demonstrated significant increase in grain yield when wheat was inoculated with *P. striata* in the presence of rock phosphate @ 100 kg P₂O₅ per hectare. Similarly, grain yield was significantly increased when rice (paddy) was inoculated with *B. polymyxa* in the presence of rock phosphate.

A field experiment was conducted at IARI, New Delhi during 2002-2004 in maize (*Zea mays* L)-wheat (*Triticum aestivum* L) crop sequence. The treatments Nitrogen 120 kg/ha, Single Super phosphate 30 kg/ha, VAM and Nitrogen 120 kg/ha, Rock phosphate 30 kg/ha, phosphate solubilizing bacteria proved its superiority in respect of grain yield of maize- wheat crop sequence and also resulted in better fertility build-up of soil organic carbon, soil available N and P (Banerjee et al., 2005).

2.7.3 **Response of mulberry to Phosphate solubilizing microorganisms**

Gowda (1996) observed the positive effect of P solubilizing microorganism in mulberry production. Recently two efficient strains (MPM1 and MPM2) of phosphate solubilizing microorganisms have been developed for mulberry and marketed as Seriphos (Kumar et al., 2000).

The highest fresh yield of mulberry was observed by Krishnababu (1994) due to the application of *Aspergillus awamori* with P fertilizers including rock phosphate. The application of *A. awamori* with low cost rock phosphate is very effective in decreasing the cost of mulberry cultivation.
In the field trials conducted to study the influence of phosphate solubilizing microorganisms isolated from mulberry rhizosphere viz., *A. awamori, A. niger, Bacillus sp.* in mulberry nursery, all the inoculants increased the plant height, shoot weight and root biomass significantly as compared to uninoculated saplings. Among different isolates *A. awamori* was found more efficient. However, significant differences were not observed with reference to the survival of sapling (Nagendrakumar and Sukumar, 2001).

**2.8 Effect of Vesicular Arbuscular Mycorrhiza on plant health and productivity**

Other important microorganisms that are very effective in mobilizing phosphatic nutrient uptake in plant are mycorrhizae. The term mycorrhiza literally means “fungus roots”. Mycorrhizae have been classified into many types of which Vesicular Arbuscular Mycorrhiza (VAM) have a wide host range. They are represented by species viz., *Glomus mosseae, G.fasciculatum* associated with most of the agricultural crops and 90% of them are vesicular plants (Sylvia, 1994).

VA mycorrhiza is generally non-specific and non-culturable outside the plant host. Thus with VAM infection the plant roots get increased surface area available for contact with P containing minerals. In other words the absorption area is enhanced by making the hyphal network around the host root which increase the uptake of micronutrients such as Zn, Fe, Cu, Ca, S, K, Mg etc. (Dandin *et al.*, 2005).

The role of VA mycorrhiza in crop productivity has been studied intensively throughout the world. The results of various studies revealed that the mycorrhizal plants possess a greater ability to absorb phosphorus from the soil than the control plants (Tilak, 1987; Vyas, 1993). Moreover one of the current topics of mycorrhizal research is to manipulate the symbiosis to improve plant growth under a variety of conditions. Under the tropical condition the use of VAM fungi can be especially beneficial to perennial
crops which are produced in nurseries and planted in the field (Feldmann and Iderezak, 1992).

The uptake of different nutrient from soil by roots is governed by two major factors, transfer of ions through soil and absorbing power of roots (Nye and Tinker, 1977). The transformation of ions to the roots occurs primarily by mass flow or by diffusion. The mass flow and diffusion depends on the mobility of the particular ion in to the soil. Ions like H$_2$PO$_4$, NH$_4$, Zn, Cu are having poor mobility. For that reason the limiting step in the uptake of these ions by plants is the movement of ions in the soil solution to the root surface. Under such circumstances the plant uptake would be increased if ions could be moved rapidly through the fungal hyphae rather than diffusing slowly through the soil (Bieleski, 1976).

Bolan (1991) demonstrated that the beneficial effect of mycorrhiza on plant growth is due to increase in the uptake of immobile nutrients especially phosphorus. He also opined that increase in the uptake of phosphorus by mycorrhizal plants include exploration of larger soil volume with an increase in the efficiency of phosphate ions and decreased distance that phosphate ions must diffuse to plant roots better by increasing the root surface area for absorption and faster movement of phosphorus in to mycorrhizal hyphae.

2.8.1 Response of various crop plants to VA mycorrhiza

VAM root colonization has been reported by several scientists in different crop plants (Faber et al., 1990; Dhillon and Antoran, 1992; Zhao and Li, 1994 and Bhardwaj and Dadeja, 1998). It has been well documented that VA mycorrhiza play an important role in uptake of different nutrients in different crop plants (Bowen and Smith, 1981; Cooper, 1984).

Saif (1987) reported significant increase in the total uptake of phosphorus, potassium, calcium and magnesium in 24 tropical forage legumes and grasses after VAM
inoculation. According to Kucey and Janzen (1987) VAM increased the plant dry matter production of field bean by increasing the uptake of P, Zn and Fe.

Bagyaraj and Verma (1988) reported that VAM fungi could save the phosphatic fertilizer to the tune of 25-30% besides improving plant yield. The role of VAM in enhancing the growth and yield of many crop plants has been well established (Raju et al., 1990). There are several reports on biomass increase due to VAM inoculation (Reddy and Bagyaraj, 1990; Singh and Tilak, 1990; Sulochana and Manoharachary, 1990). Nemec and Vu (1990) found that VAM inoculation in citrus improved photosynthetic CO₂ fixation and growth of the plant particularly in the areas where soil P is a limiting factor.

Michelson and Rosendhal (1990) reported increased shoot dry weight in Acacia nilotica and Leucaena leucocephala due to VAM inoculation. VAM fungi are known to play an important role in the utilization of less or unavailable sources of phosphorus for plant growth (Diederichs, 1990) and application of rock-phosphate along with VAM fungi have shown increased plant growth and other parameters (Chhabra and Jalali, 1991).

Increased fresh and dry weight, plant height, root length, number of lateral branches and stem diameter of Hevea brasiliensis inoculated with VAM fungi have been reported by Ikram et al. (1992). About 70% increase in plant dry weight was also recorded by them due to VAM inoculation.

Hooker et al. (1992) studied root colonization by VAM in poplar plants and observed a significant changes in root morphology in terms of root length and root branching. However the study of Baon et al. (1993) showed that mycorrhizal infection alters the allocation of P in inefficient cultivars and improves the efficiency of P utilization with respect to shoot growth.
Rachel et al. (1993) recorded increased biomass of sunflower due to VAM inoculation amended with phosphorus. Champawat and Pathak (1993) have suggested that VAM fungi could be extremely useful in pearl millet to get higher plant growth and nutrient uptake.

Konde and Bhosale (1994) recorded higher green chilli yield and nutrient uptake due to VAM inoculation amended with rock phosphate in a P deficient soil having pH of 8.2. Similarly, inoculation of bamboo seedlings with Glomus fasciculatum was found beneficial by Verma and Jamaluddin (1995).

Raverkar and Bhandari (1995) have observed improved health of tomato seedlings in terms of dry matter accumulation in shoot, root and P accumulation in shoots due to VAM inoculation in nursery.

Sundaram and Arangarasan (1995) have also observed higher fruit yield and improved quality attributes like vitamin C and total soluble sugars in tomato inoculated with VAM. Kehri and Sudhir Chandra (1995) have found improvement in spore count in rhizosphere soil, mycorrhizal colonization in roots, higher nodulation, shoot biomass and P content in shoots of chickpea inoculated with VAM. Similarly higher root proliferation and higher micronutrients (Cu, Zn and Mo) resulting in increased biomass of soybean treated with VAM was reported by Bhandari and Rawat (1995).

Kamalprasad and Bilgrami (1995) have recorded maximum increase in biomass when VAM inoculated sugarcane plants were grown in soil amended with rock phosphate followed by diammonium phosphate and single super phosphate. Devi et al. (1995) have reported that VAM along with rock phosphate could also be successfully used in alkaline soils with appreciable results.

Sulochana et al. (1995) have observed better growth and yield of cassava inoculated with VAM with full dose of nitrogen and potash and 50% of recommended
dose of phosphorus application while Subashini and Krishnamurthy (1995) have reported the possibility of saving 40 kg \( P_2O_5/ha \) in tobacco by application of VAM.

Vejsadova et al. (1992) also reported the positive effect of the VAM on foliar concentration of P, Zn and Cu in crop plants. Similarly Ho (1993) found higher concentration of Cu, Mg, Mn, Fe and Zn in mycorrhizal maize plants, whereas increased concentration of P and Cu in both shoot and grains of mycorrhizal maize has been reported by Sylvia et al. (1993).

The inoculation of VA mycorrhiza to increase the uptake of P, K, Mg, Mn, Cu, Ca, Zn and Mo has been well documented by many workers (Ikram et al., 1992; Samina khalil et al., 1994; Bhandari and Rawat, 1995). Besides these nutrients, it was also observed that mycorrhizal plants absorb \( NH_4 \) nitrogen more efficiently than non-mycorrhizal plant (Smith et al., 1985) and it is probable that \( H^+ \) extrusion which is an inevitable consequence of ammonium assimilation in cell (Raven and Smith, 1976; Bolan et al., 1989) would occur from the hyphae as well as from the roots (Raven et al., 1978). This could reduce the pH around an infected root and thereby affect the availability of slowly soluble source of phosphorus such as rock phosphate (Bolan, 1991).

Champawat (1992) reported increased plant dry weight and total phosphorus uptake in chickpea inoculated with \( G.fasciculatum, G.constrictum \) and \( Gigaspora calospora \) in sterilized soil in pots over uninoculated control.

Rathore and Singh (1995) have also recorded increased concentration of phosphorus in maize shoot inoculated with VAM and reported a saving of phosphatic fertilizer equivalent to 30 kg of \( P_2O_5/ha \).

Amaranthus and fenugreek plants responded significantly high to VA mycorrhizal inoculation in terms of improved plant growth, yield and increased P concentration of the inoculated plants (Sreeramulu et al., 1996).
Hemavathi et al. (2006) reported that inoculation of *Ocimum basilicum* with *G. fasciculatum* and plant growth promoting rhizobacteria namely *B. megaterium* and *P. fluorescens* enhanced the plant height, number of branches, herbage yield, phosphorus content, mycorrhizal colonization and spore numbers in the root zone soil.

Sandeepa et al. (2008) observed that the inoculation of four selected plant species namely *Cajanus cajan*, *Ocimum tenuiflorum*, *Phyllanthus amarus* and *Arachis hypogaea* with *G. mosseae* significantly increased shoot height, shoot number, root length, root number and size of leaves, number of flowers and mycorrhizal colonization compared to control in all the plant species studied except *P. amarus*.

### 2.8.2 Response of VA mycorrhiza to mulberry

Muthukrishnan et al. (1981) noticed the occurrence of VA mycorrhiza in the fine feeder roots of mulberry and that more number of colonization and spore number were found in light textured soil compared to heavy textured soil. They further reported that of the six main species of VA mycorrhizal fungi, *G. mosseae* and *G. fasciculatum* were found to be beneficial for mulberry cultivation.

Katiyar et al. (1989) observed the endomycorrhizal fungi ie., Vesicular Arbuscular Mycorrhiza (VAM) to be associated with mulberry roots which can mobilize and improve the uptake of available phosphorus from soil through mycorrhizal network in the soil through plant root hairs by colonizing the roots with elongated filaments.

Katiyar et al. (1989) also observed VAM root colonization on six different mulberry varieties during different season. The variety Mysore local had maximum % of VAM colonization in roots followed by S-41 during summer season. Similarly Ambika et al. (1994) observed the occurrence of five genera of VAM in the rhizosphere of different genotypes of mulberry.
Mycorrhizal associations in mulberry play a significant role in the control of certain root diseases like root rot which are known to reduce the quality and quantity of available plant biomass (Teotia, 1991).

The VAM inoculation also plays an important role as biocontrol agents against the plant pathogens in different crops (Bagyaraj, 1989; Singh et al., 1990; Sreenivasa et al., 1992; Sharma et al., 1993). Sharma et al. (1995) reported inoculation of VAM effectively reduced the major foliar disease of mulberry.

Gowda and Rao (1992) have also reported the beneficial effect of inoculating mulberry with VA-mycorrhizal fungi. Kumutha et al. (1993) have reported improved mulberry growth and positive increase of phosphorus in leaf due to mycorrhizal inoculation.

Gowda and Rao (1993) also reported that mulberry var. Kanva-2 inoculated with *G. intraradices* showed increased percent root colonization and biomass improvement.

The inoculation of nursery beds with *G. mosseaee* increased growth, development and survival of mulberry saplings in comparison with the uninoculated control. The inoculated saplings raised in the nursery beds were much healthier and contained more nitrogen, phosphorus and potash in leaves and stems compared to uninoculated saplings (Das et al., 1995a).

Studies conducted by Katiyar et al. (1995) revealed that the inoculation of mulberry with *G. mosseaee* in combination with 30 kg P/ ha/ yr had similar effect on plant growth, leaf yield and leaf chemical constituents when compared to control, which received the full dose of phosphatic fertilizer (120 kg P/ ha/ yr) without inoculation. Silkworm rearing (moulting test) also did not reveal any significant difference in the leaf quality even after reducing phosphorus application by 75% in mulberry. The root colonization was significantly higher in VAM inoculation at the lower levels of phosphorus compared to uninoculated control receiving the full dose of phosphate.
fertilizer (120 kg P/ ha / yr) suggesting that low phosphorus levels in soil promote better VA-mycorrhizal symbiosis in mulberry.

The inoculation of VAM in established mulberry garden through intercropping of maize as host plant successfully with curtailment of P fertilizer has also been reported (Katiyar et al., 1995).

Setua et al. (2000b) found three strains of VAM (Glomus spp.) to be very effective even at very low doses of phosphate @ 30 kg/ ha/ yr which yielded 6077.0, 6026.7 and 6082.4 kg/ ha/ crop of leaf yield respectively which were at par with the control (5973.1 kg / ha / crop).

Fathima et al. (1996) reported significant increase in cocoon yield due to the inoculation of mulberry with G.mosseae and G.fasciculatum and application of only 30 kg P/ ha/ yr.

Chandrashekar et al. (1997) reported VAM fungal inoculation in mulberry (Morus alba L.) reduced the root-knot nematode besides promoting the growth characters like height of the plant, shoot and root fresh and dry weight, percentage of colonization, root-knot index in comparison to the control. The presence of the nematode also did not affect the VAM colonization in mulberry.

VAM inoculated S-1 mulberry saplings in the nursery stage revealed that except leaf moisture, leaf and root dry matter and other parameters exhibited significant increase in survival (10.40%), plant height (5.07%), leaf area (11.80%), root length (9.80%), no. of lateral roots per plant (56.10%) and plant dry biomass (27.40%) over control, besides enhanced phosphorus uptake by 13.5% as also reduced tukra infestation compared to non-mycorrhizal saplings. Thus, the mycorrhizal saplings performed better in respect of important qualitative and quantitative characters of mulberry (Setua et al., 1995; 1997).
Different strains of *Glomus* sp with reduced phosphate doses showed promising result in respect to plant growth characters and leaf yield in mulberry and enabled a saving of Rs 2125/ ha/ yr (Setua *et al.*, 1999).

Katiyar *et al.* (2000) and Reddy *et al.* (2001) suggested that the established mulberry garden can also be effectively inoculated with VAM by growing maize as carrier host for saving phosphatic fertilizer to economize mulberry cultivation.

The quality and quantity of mulberry variety S1 associated with three strains of VA mycorrhiza separately with graded levels of phosphorus (180, 90, 60 and 30 kg P/ha/yr) were studied by Setua *et al.* (2000a) and found an overall improvement in leaf yield, leaf moisture percentage and P uptake by leaves in all the VAM-treated mulberry plants.

VAM is a multi-beneficial organism (Padma *et al.*, 2000) which plays an important role in plant growth and health, helps in the mobilization of phosphorus and also other elements such as N, K, Zn, Mg, Ca and S in soil.

Kumar *et al.* (2001) studied the effect of VAM in established mulberry gardens under farmers condition and the results revealed that even though the application of phosphatic fertilizer could be curtailed up to 50% in the VAM treated plot (300:60:120 kg/ha/yr; yield: 11266 kg/ha/crop), the leaf yield was found to be at par with that of control plot, where full dose of recommended fertilizer was applied (300:120:120 kg/ha/yr; yield: 10769.3 kg/ha/crop).

The inoculation of VAM was highly beneficial for the growth of mulberry and even phosphorus application could be reduced by 50% with the use of VAM fungal inoculum (Baqual and Qayoom, 2004; Beevi *et al.*, 2004).

Vijaya *et al.* (2005) opined that the VAM enhances host plant growth by improving the supply of mineral nutrients of low mobility in the soil, phosphorous in particular and also micro elements like Cu and Zn. In addition, VAM increases host
tolerance against many plant pathogens. It is found that the application of *G. fasciculatum* to the clonally propagated mulberry had significantly increased the leaf area, number of leaves, fresh and dry weight of roots, leaves and shoot. The DNA and protein content was also found increased in *G. fasciculatum* treated plants over control.

Beevi and Qadri (2008) reported significant difference in the colonization percentage between treated and untreated plants recording better survival, leaf moisture, fresh weight of roots and root colonization on 60th and 120th day of inoculation compared to non-inoculated saplings.

2.9 Combined inoculation of biofertilizers on plant health and productivity

2.9.1 Response of combined inoculation of biofertilizers in other crops

Combined inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria *Pseudomonas striata* or *Bacillus polymyxa* on field grown sorghum significantly increased grain and dry matter yields and N and P uptake as compared with single inoculation of individual organisms (Alagawadi and Gaur, 1992). Veerasamy *et al.* (1992) reported that mixed inoculation of *A. brasilense* and the VAM fungus *G. intraradices* in sorghum created a synergistic interaction resulting in significant increase in many plant growth parameters including mycorrhizal infection with a concomitant increase in levels of root phosphatases, increase in phosphorus content in plants and enhanced uptake of nitrogen, zinc, copper and iron. This double inoculation could replace the application of N and P fertilizers.

A number of researchers have reported that dual inoculation of VAM and bacterial biofertilizer is more effective in increasing the growth of different crop plants (Panwar, 1993; Thakur and Panwar, 1995; Sansamma *et al.*, 1998; Sreeramulu *et al.*, 2000 and Sumana and Bagyaraj, 2002). Maximum plant growth was noticed in combined
inoculation of rhizobial strains with *Azospirillum* and phosphobacteria in groundnut (Balamurugan and Gunasekaran, 1996).

Seed inoculation of wheat varieties with P-solubilizing and phytohormones producing *Azotobacter* showed better response and increase in grain yield by 12.6% and straw yield by 11.4% (Kumar, 2001).

Greep *et al.* (2005) evaluated the effect of different liquid biofertilizers *Azotobacter*, phosphate solubilizing organisms, VAM (soil based) and potash mobilizing bacteria as individual as well as in combinations along with compost and phosphocompost in chillies and found them superior when compared to the control.

Onion plants inoculated with *Azotobacter + VAM + PSB* receiving 50% of recommended N and P showed significant increase in plant height, number of leaves, diameter of bulb, marketable yield per plot and significant decrease in weight of unmarketable yield per plot, bolting and jointed bulb percentage there by saving cost of 50% nitrogen and phosphorus compared to control (Jadhao *et al.*, 2005).

*Pseudomonas sp*, a plant growth promoting rhizobacteria (PGPR) isolated from peanut rhizosphere when applied to peanut plants enhanced the growth and yield of the crop significantly (Dey *et al.*, 2004).

Luigi *et al.* (1998) reported that inoculation of *Burkholderia cepacia, P.fluorescens* and *Enterobacter sp* in *Sorghum bicolor* enhanced the root colonization and plant growth promotion.

### 2.9.2 Response of combined inoculation of biofertilizers in mulberry

Balasubramanian *et al.* (1992) reported that the combined inoculation of *Azospirillum* (AZP2) and phosphobacteria increased the leaf yield by 7.59% compared to recommended doses of N and P alone in mulberry.

Chandrashekar *et al.* (1994) studied the effect of combined inoculation of *Bacillus megaterium var.phosphaticum, Azospirillum brasilense* and *Acaulospora laevis*
with single super phosphate as well as rock phosphate in mulberry and recorded increased leaf over control. The growth parameters like number of branches, length of branches and number of leaves per plant were also increased when compared with control.

The research works carried out on use of various biofertilizers at CSRTI, Mysore (Das et al., 1994; Katiyar et al., 1995) indicated that *Azotobacter* biofertilizer and VAM were highly beneficial and also cost effective in mulberry production.

Dipping of mulberry cuttings in bacterial inoculants like *Azospirillum*, Phosphobacteria and their combination along with cow dung enhanced the bud development over control (Santhi and Ponnusamy, 1995).

Application of P solubilizing bacteria *B. megaterium* with nitrogen fixer *Azotobacter* and VA-mycorrhizal fungus significantly improved the mulberry leaf yield (Chandrashekar et al., 1996).

Katiyar et al. (1996) reported that the dual inoculation of mulberry with *Azotobacter* and VAM and application of 50% of recommended dose of N and P have yielded mulberry leaf on par with uninoculated control. Further higher VAM colonization in the dual inoculated plants was recorded. This indicates the beneficial interaction of VAM with *Azotobacter* on improvement of mulberry crop.

Mulberry saplings inoculated with VAM and *Azotobacter chroococcum* exhibited considerable increase in plant growth and development (Umakanth and Bagyaraj, 1998).

Mamatha and Bagyaraj (1999) obtained significantly higher yield in mulberry when inoculated with mycorrhiza and associated bacteria.

Reddy et al. (2001) opined that the dual inoculation of VA mycorrhiza and biofertilizers at farmers' level reduced the use of chemical fertilizer without sacrificing the yield and quality of mulberry leaf.
Dual inoculation of mulberry with Azotobacter and VA-mycorrhiza could curtail both N and P fertilizers by 50 percent against control receiving full dose of N and P @ 300 and 120 kg/ha/yr respectively (Das et al., 2000).

Anilkumar and John (2000) reported that the combined application of Azospirillum, VAM and PSB in mulberry had significantly improved total fresh leaf production (30758 kg/ha), total leaf dry matter production (10279 kg/ha), total stem dry matter production (9684 kg/ha) and total root dry matter production (5422 kg/ha) compared to their individual applications registering corresponding figures of 24471 kg, 8154 kg, 7683 kg and 5011 Kg/ha in Azospirillum, 29464 kg, 9860 kg, 9306 kg and 5343 kg/ha in VAM and 22051 kg, 7329 kg, 7032 kg and 4684 kg/ha in PSB and 16574 kg, 5535 kg, 5438 kg and 4527 kg/ha in control respectively.

Foliar application of three nitrogen fixing bacteria (NFBs) namely Azotobacter, Azospirillum and Beijerinckia in mulberry (Morus spp) along with half of the recommended dose of N as a basal application of chemical fertilizer improved the leaf yield., leaf quality, silkworm rearing and cocoon production. (Sudhakar et al., 2000b).

Sukumar et al. (2000) investigated the effect of combined inoculation of phosphate solubilizing B. megaterium and the diazotroph Azospirillum brasilense on mulberry leaf yield and uptake of nutrients at graded levels of N and P (75 and 100 per cent) and observed significant increase in leaf yield, N and P uptake with dual inoculations as compared to the uninoculated control and individual single inoculations of either organisms.

Application of VAM and biofertilizer under dry farming mulberry cultivation with an objective of curtailing 50 per cent of chemical nitrogenous and phosphatic fertilizers for productivity has been proved successful besides improving the soil fertility in an eco-friendly way (Susheelamma et al., 2000 and Sundar Raj et al., 2001).
Field studies conducted by Sukumar et al. (2001) to assess the response of M-5 mulberry (*Morus indica* L.) to co-inoculation with the diazotrophic *A. chroococcum* isolate RFB and the P solubilizing *B. megaterium* at graded levels of N and P revealed an increase in the total chlorophyll content and total population of *A. chroococcum* and *B. megaterium* in the rhizosphere respectively.

Response of mulberry (*Morus indica* L.) to combined inoculation with vesicular arbuscular mycorrhiza *G. mosseae* and phosphate solubilizing bacteria *B. megaterium* was studied under field conditions by Padma et al. (2001). Significant increase in growth, yield and nutrient uptake was recorded in the inoculated treatments as compared to un inoculated control. Significant increase in the population of *B. megaterium* in the rhizosphere of VAM inoculated plants was also recorded (Sukumar et al., 2000; 2001).

A study conducted by Sudhakar et al. (2001) on the effect of foliar application of nitrogen fixing bacteria (NFBs) viz., *A. chroococcum, A. brasilense* and *B. indica* on plant growth and leaf yield of mulberry individually and in combination under reduced nitrogen input (150 kg/ha/yr) level indicated the possibility of substituting 50 percent N input in mulberry through foliar application of efficient NFBs like *A. chroococcum* and *B. indica*.

Srikantaswamy et al. (2001) inferred that, application of VAM (*G. mosseae*) along with *Azotobacter* has potential in saving on costly fertilizers like Nitrogen and Phosphorus thereby improving leaf yield and quality besides enriching the soil fertility.

Reddy et al. (2003) opined that VAM and *Azotobacter* inoculation (alone and in combination) in saplings of V1 and S13 mulberry varieties improved the survival percentage, growth characters, leaf moisture, total biomass nitrogen, phosphorus, potash, chlorophyll and carbohydrate status in the inoculated saplings. The increase was more pronounced with integrated application, in both the mulberry varieties which may be due
to the increase in microbial population and root colonization in the rhizosphere (Sudhakar et al., 2003).

Yadav and Kumar (2004) studied the response of mulberry to inoculation with N\textsubscript{2} fixing bacteria – \textit{A. brasilense} (SL33), \textit{A. lipoferum} (ICM-1001) and \textit{A. chroococcum} (ICM2001) in pots containing unsterilized soil and different nitrogen levels and found significant increase in yield and nitrogen uptake due to the combined inoculation of \textit{A. brasilense} (SL33) and \textit{A. chroococcum} (ICM-2001) over inoculation of individual organisms.

Baqual (2003) has clearly indicated that it is possible to curtail the application of nitrogeneous and phosphatic fertilizers in mulberry cultivation to an extent of 50% without any adverse effect on leaf yields and quality by supplementing nitrogen and phosphorus through the use of nitrogen fixing bacteria (\textit{Azotobacter}) and phosphate solubilizing bacteria (\textit{B. megaterium}) and VAM. The study also indicated that an expenditure of up to Rs.4500 per hectare per year could be saved on the input cost of nitrogen and phosphorus in mulberry cultivation.

Kashyap et al. (2004) reported that the use of VAM and bacterial biofertilizers effectively curtailed the recommended dose of chemical fertilizers. Philomena et al. (2005) opined that the application of eco-friendly organic manures like \textit{Azotobacter} 20-23 kg/ha/yr (2 splits/yr) + VAM 1000 kg (once)+ Seriphos 25 kg/ha/yr (2 splits/yr)+ Vermicompost 20 MT/ha/yr (2 splits/yr) + Green manure 20-25 kg \textit{Sesbania aculeata} /ha/crop enhanced the nutritional value of mulberry leaf, yield and soil fertility.

Sreeramulu et al. (2005) reported that the combined inoculation of \textit{A. brasilense} and \textit{B. megaterium} improved the plant height, number of branches and leaves and leaf yield in mulberry.

Rashmi et al. (2005) reported significant enhancement of soil fertility status and microbial population increase in S36 mulberry garden when biofertilizers namely \textit{A.}
*brasilense* and *Aspergillus awamori* @ 10 kg each + 20% each recommended N through compost, green manure, castor cake, vermicompost and chemical fertilizers + remaining P and K through fertilizers were applied.

The biofertilizers like *A. brasilense* and *A. awamori* helped in enriching the soil with the major nutrients like N and P which are mainly essential for luxuriant growth of mulberry crop (Praburaj *et al.*, 2005).

The importance of treating mulberry leaves with liquid and carrier based biofertilizer combinations and its significance on the growth and development, cocoon production and silk productivity in silkworm was assessed by Ramarethinam *et al.* (2005). Significant increase in the nutritional factors, larval and cocoon characteristics were observed in the treatment groups as compared to the untreated control group.

The biofertilizers enriched with bacteria and fungi have proven to be of great importance in improving the yield and quality of various agricultural crops and in mulberry which is the sole food for silkworm (Baqual *et al.*, 2005).

Swathi and Sujathamma (2005) observed that application of *Azotobacter* and Phosphobacteria improved the qualitative and quantitative parameters in mulberry.

Jaishankar *et al.* (2005) reported that the inoculation of mulberry with the biofertilizers viz., *Rhizobium* a symbiotic bacteria, *Azotobacter* a free living nitrogen fixing bacteria, phosphate solubilizing bacteria and an obligate symbiotic VAM-fungi along with curtailment of 50% and 30% of inorganic N and P revealed 24.60% improvement in leaf yield and 34.63% in cocoon yield over the control. The soil fertility also showed improvement by maintaining the pH and increase in the available phosphorus content. There was also significant improvement in the microflora population viz., bacteria, fungi and actinomycetes.

Baqual and Das (2006) reported that the co-inoculation of mulberry with phosphate solubilizing microorganisms, nitrogen fixing bacteria and VAM increased the
uptake of macronutrients like N (484.12 kg/ha), P (59.83 kg/ha) and K (244.61 kg/ha). Significantly higher effective rate of rearing, single cocoon weight and single shell weight were recorded by feeding the silkworm larvae with the leaves harvested from the inoculated plants. The influence of VAM fungi and bacterial biofertilizer (BBF) with 50% reduction in the recommended dose of (N and P) chemical fertilizers on leaf quality traits of mulberry and its impact on silkworm growth and cocoon characters revealed that reduction in chemical fertilizers did not affect the leaf quality or cocoon traits (Ramarao et al., 2007).

Experiments conducted by Rashmi et al. (2008b) to assess the leaf quality, soil fertility and microbial status of soil in the case of mulberry variety M-5 as influenced by application of different organic manures and inorganic fertilizers revealed that application of 10 kg each of Azospirillum + Aspergillus awamori + 20% recommended N through each of compost, green manure (Glyricidia maculata), oil cake (Castor cake) and vermicompost indicated significantly higher plant height, number of shoots per plant, leaves per plant, fresh leaf yield/ha besides increasing total chlorophyll, total soluble sugar, crude protein, macro and micronutrients in the leaf.

Sudhakara et al. (2008) observed maximum pupal weight, rate of pupation, rate of moth emergence and less melting percentage by feeding the leaves harvested through inoculation of 10 kg of Azospirillum + 10 kg of Aspergillus awamori/ha/yr + 20% recommended N through each of compost, green manure, castor oil cake, vermicompost and fertilizer + remaining P, K through fertilizer.

Venugopal et al. (2008) inferred that raising of green manure like Sesbania aculeata or Crotalaria juncea with Rhizobium seed treatment along with application of biofertilizer (Azospirillum and PSB) mixed with FYM and applied near rhizosphere of mulberry enhanced the soil microflora viz., bacteria, fungi and actinomycetes population. The alkalinity of the soil was reduced and organic carbon content, phosphorus (kg/ha)
and potash (kg/ha) were increased considerably with resultant increase in mulberry leaf yield by 13.29%.

Investigations carried out by Waktole and Bhaskar (2008) revealed that the use of bio inoculants viz., *Azotobacter sp* @ 20 kg/ha/yr, *Aspergillus awamori* @ 25 kg/ha/yr and *Trichoderma harzianum* @ 20 kg/ha/yr had positive effect on growth, yield and quality of M-5 mulberry under rainfed condition and its subsequent effect on cocoon parameters of silkworm hybrid PMxCSR2.

### 2.10 Role of certain effective microorganisms on the management of soilborne diseases

Sithambaram and Parker (1978) used a mixture of fluorescent strains from wheat rhizosphere to reduce the incidence of wheat diseases. They also showed that there was significant increase in the yield of wheat in the sterile sand assay. In vitro antagonistic potential of *Pseudomonas fluorescens* against *Fusarium solani* was assessed by dual culture technique (Upadhyay and Rai, 1987) by measuring the radial growth of the pathogen as well as that of *P. fluorescens*. Different mechanisms by which *P. fluorescens* act as biocontrol agent have been proposed by various workers such as competition for nutrition and space (Dube, 1995), antibiosis by production of various antibiotics (James Gutterson, 1986), production of lytic enzyme and siderophore (Elad and Baker, 1985 and Yeole and Dube, 1997), production of hydrogen cyanide (Defago *et al.*, 1990) and degradation of patho-toxins (Borowitz *et al.*, 1992). In addition to direct antagonism against pathogen this bacteria also induces systemic resistance in host plants (Ramamoorthy *et al.*, 2001).

The contribution of VAM fungi in the biological control of root rot pathogens is well documented. The disease suppression may be attributed to enhanced nutrient uptake and production of certain hormones (Davis, 1980; Sampangi and Bagyaraj, 1989). The interaction between *G. fasciculatum* and root pathogen *Sclerotium rolfsii* in peanut
showed reduced number of sclerotial bodies in VAM inoculated plants (Krishna and Bagyaraj, 1983). Davis and Menge (1980) showed that citrus roots colonized by *G. fasciculatum* and *Phytophthora parasitica* were more healthier and weighed more than that of roots infected with pathogen alone. Meyer and Linderman (1986) demonstrated that microbial suppression of *Phytophthora sporangium* production in mycorrhizosphere soil, compared to non mycorrhizal soil.

Caron et al. (1986) reported reduction in population of *Fusarium* in tomato mycorrhizosphere. Chakravarthy and Mishra (1986) observed similar results where in the VAM fungi controlled *Fusarium* wilt in the tree species *Albizzia procera* and *Dalbergia sissoo*. Pre inoculation with the VAM fungus, *G. fasciculatum* suppressed the damage caused by root rot pathogen *Aphanomyces eutriches* in pea roots (Rosendahl, 1985). Bagyaraj and Padmavathi (1993) attributed the mechanism of suppression of root pathogens by mycorrhizal fungi to modification of cell wall, production of antimicrobial compounds and altered rhizosphere microflora.

Prashanthi et al. (1997) reported that among different VAM species *G. fasciculatum* suppressed *Rhizoctonia bataticola* in safflower with increased seedling survival of 65.2 percent on inoculation with *G. fasciculatum*. Harlapur (1990) noticed a reduction in disease incidence by *Sclerotium rolfsi* in wheat by inoculation with fungi, of the four mycorrhizal fungi tested *G. fasciculatum* provided higher disease reduction.

Rabie (1998) observed induction of fungal disease resistance in *Vicia faba* by dual inoculation with *Rhizobium leguminosarum* and fungi. Champawat (1991) studied the interaction between VAM and *F. oxysporum* f.sp. cumini and reported enhanced nutrient uptake and reduced disease severity. Datnoff et al. (1995) obtained significant decrease in *Fusarium* crown and root rot of tomato by *G. intradices* and *Trichoderma harzianum*.  

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Seed treatment with *T. viride* and soil application of *T. viride* and *T. harzianum* in mustard was found to reduce damping off disease by 63.7, 71.3 and 64.4 percent respectively compared to control (Ebenezar *et al.*, 1996). Effective control of rhizome rot of ginger caused by *Phytophthora aphanidermatum*, *P. myriotylum* and *F. solani* complex was observed when *Trichoderma* and *Gliocladium* were used. (Usman *et al.*, 1996). When *Trichoderma* strains were used against French bean root rot under green house conditions, *T. harzianum* was found to be the most effective and the bioagent as pre-inoculation antagonist proved useful for controlling the root rot (Mathew and Gupta, 1998). Kumar (1993) reported that pre and post emergence damping off of *Pinus roxburghi* caused by *F. solani* was effectively controlled by *T. viride* incorporation to potting mixtures before sowing the seeds.

Ram *et al.* (1997) reported that two biocontrol agents, *T. harzianum* and *P. fluorescens* when introduced to soil for control of rhizome rot of ginger caused by *F. solani* and *Phytophthora myriotylum* inhibited the growth of the pathogens. Manka *et al.* (1997) reported that *T. harzianum* and *T. viride* applied during rooting of carnation cuttings strongly promoted the growth of plants and gave a good control of *F. oxysporum* f.sp. *dianthi*.

Antagonistic fungi and bacteria like *T. harzianum*, *T. viride* and *Bacillus subtilis* are extensively used in mulberry for control of wilt disease caused by *Fusarium* fungus which is very serious in nurseries and old plantations (Govindaiah and Pradipkumar, 1992). Wilt disease caused by a fungus *S. rolfsi* forms a white mycelial mat on the collar portion of the newly sprouted mulberry cuttings resulting in death of the saplings to a considerable extent. The spread of the fungus can be prevented by spraying *T. harzianum* which bears antagonistic properties specific to *S. rolfsi* (Das, 1992).

Dileepkumar and Dube (1992) reported that seed bacterization reduced the incidence of chickpea wilt by *F. oxysporum* f.sp. *ciceris* in the wilt sick soil by 52
percent. Leeman et al. (1995) reported suppression of *Fusarium* wilt of radish by seed treatment with *P. fluorescens* WCS 374 in commercial green house trials. There was a reduction of wilt disease by 42.6% and increase in yield by 44.7%. Shamin et al. (1998) reported seed dressing with *P. aeruginosa*, *Paecilomyces lilacinus* and *T. koningii* significantly reduced the infection of *M. phaseolina*, *R. solani* and *F. solani* on cotton roots in pot experiments and in the field. Combined use of *P. aeruginosa* strain CMG63 with *T. koningi* produced greater plant height and fresh weight of shoots in the field.

Sheela et al. (1995) observed that *T. hamatum* (isolate 1) when multiplied in FYM and applied to soil recorded the least incidence (33.34%) of wilt disease caused by *F. solani* followed by *T. viride* (isolate 1) (38.89%) as compared to control (73.89) in egg plant. The antagonist *T. hamatum* (isolate 1) when multiplied in coirpith compost recorded the least incidence of wilt (37.78%) followed by *T. viride* (isolate 1) (45.56%) as compared to control (73.89%).

*T. viride* isolated from cowpea phylloplane hyper parasitised the mycelium of *Colletotrichum truncatum*, causal agent of brown blotch disease of cowpea in vitro. Whereas its spore suspension application in the form of a seed dip and soil drench (10-8 conidia/ml) was very effective in reducing infection from brown blotch infected seeds (Bankole and Adebanjo, 1996).

Pythium blight and root rots caused by *Pythium aphanidermatum* and *P. graminicola*, brown patch caused by *Rhizoctonia solani*, and collar spot caused by *Sclerotinia homoeocarpa* are most common diseases on golf course turf and home lawns and are effectively controlled by the application of different strains of *T. harzianum* (Lo, 1997).

Lo et al. (1997) observed *T. harzianum* strain 1295-22 as an effective biocontrol agent for several fungal diseases. Spray applications of conidial suspensions of strain
1295-22 significantly reduced all the diseases of creeping bentgrass turf in both greenhouse and field experiments.

Gupta (1998) reported a biofungicide formulation namely Nursery guard (T. pseudokoningii) to control the nursery diseases like stem canker and die-back (Botryodiplodia theobromae), stem cuttings rot (F. solani) and collar rot (Phoma sorghina and P. mororum) in mulberry.

Sharma (1998) opined that the application of a bionematicide Verticillium chlamydosporium and neem cake reduced the mulberry root knot disease severity by about 85-90% and increased the leaf yield by 23% compared to the recommended cultural or chemical method (use of neem oil cake or Furadan) where the severity of disease was reduced by 63-73% and leaf yield increased by 16-18%.

Plant growth-promoting rhizobacteria (PGPR) strains INR7 (Bacillus pumilus), GBO3 (B. subtilis), and ME1 (Curtobacterium flaccumfaciens) were tested singly and in combinations for biological control against multiple cucumber pathogens. The combined inoculation of the above PGPR treatment showed intensive plant growth promotion with considerable disease reduction (Raupach George and Kloeper Joseph, 1998).

Sheela et al. (1998) observed that soil application of P. fluorescens along with peat soil as carrier lowered the incidence of collar rot disease (23.33%) in groundnut against the control (85%).

Efficacy of four species of VAM fungi viz., G. mosseae, G. fasciculatum, G. etinicatum and G. margarita against the root rot disease of Casuarina equisetifolia Forst was tested by Rajeswari et al. (1998) and it was found that all the four fungi significantly reduced the root rot incidence in the nursery. However G. fasciculatum was superior over others with the least disease incidence and maximum dry weight.
Buchenauer (1998) reported the utilization of antagonistically active rhizosphere microorganisms collectively as an effective alternative method to control soil-borne diseases.

Treatment of pearl millet seeds with pure culture of *P. fluorescens* followed by foliar spray increased seedling vigor and inhibited sporulation of downy mildew pathogen (Umesha *et al.*, 1998).

Jubina and Girija (1998) reported that the rhizobacteria were superior to *T. harzianum* and *P. fluorescens*. All the plants treated with *T. harzianum* succumbed to infection after 30 days and those exposed to *P. fluorescens* were killed in 90 days. Isolate B-13 rhizo-bacteria was identified as the most promising one in reducing plant mortality, and providing prolonged foliar blight protection whereas isolate B-7 had the dual function of disease suppression as well as growth promotion. The antagonistic bacteria were identified as endospore forming *Bacillus*. Woeng *et al.* (1998) studied antagonistic activity of *Pseudomonas chlororaphis* PCL 1391 against tomato root rot caused by *F. oxysporum f.sp radices lycopersici*. This particular isolate was found to be an excellent biocontrol agent producing a broad spectrum of antifungal factors like phenazine 1-carboxamide (PCN), hydrogen cyanide, chitinases and proteases.

Philip and Sharma (1999) screened seven isolates of *B. subtilis* against root rot pathogens of mulberry *F. solani* by agar plug and pot culture methods and found up to 68% control of disease by these isolates.

Sridar *et al.* (2000) reported that among the different antagonistic microorganisms *B. subtilis* showed the highest inhibitory effect on the mulberry root rot pathogen *Macrophomina phaseolina* under laboratory conditions. *B. subtilis* was found to reduce the pathogen load in the mulberry rhizosphere also.

VAM plays an important role in the biological control of root pathogens and also helps in reducing the nematode infection of plant roots due to the production of
chemicals and physiological changes in host plants. It offers resistance in plants to fight against leaf and shoot pathogens. It provides greater strength to fight against vascular pathogens by providing a strong vascular system enabling an increase in the flow of nutrients (Padma et al., 2000).

Sudhakar et al. (2000a) investigated the possibility of checking mulberry leaf diseases through use of nitrogen fixing bacteria as foliar spray. Foliar application of *B. indica*, *A. chroococcum* and *A. brasilense* under a reduced nitrogen input level of 150 kg/ha/yr reduced powdery mildew by 34.3, 39.5 and 16.3 %, black leaf spot by 27.3, 23.24 and 1.74 %, leaf rust by 42.8, 35.6 and 19.2 % and bacterial leaf blight by 43.2, 20.4 and 40.8 %, respectively over control (300 kg N/ha/yr without NFB). *B. indica* and *A. chroococcum* were found to be more effective than *A. brasilense* in controlling fungal diseases, while *B. indica* and *A. brasilense* excelled over *A. chroococcum* in controlling bacterial blight.

The application of *T. harzianum* along with root dipping with 0.1% Indofil-M-45 has showed higher survival of mulberry plants (63.70%) in the identified hot spot for root rot compared to a survival of 12.6% in control (Beevi et al., 2000).

The efficacy of a talc based bioformulation of *T. harzianum* helped to reduce the population of *F. solani* and *F. oxysporum* by about 85 percent and controlling the disease by about 75%. There was no significant reduction in the efficiency of the bioformulation even after 90 days of storage at room temperature (Philip et al., 2000).

The interaction of thirteen biocontrol agents, including nine *Trichoderma* isolates at primary and secondary metabolite level against mulberry root rot pathogens, *F. solani* and *F. oxysporum* was investigated (Philip et al., 2001). Except *T. viride* (TV-1) all other biocontrol agents inhibited the radial growth of either or both of the pathogens significantly.
The incidence of root rot in Black gram caused by *Macrophomina phaseolina* was effectively reduced (50%) by the combined application of *T.harzianum* and *T.viride* along with the rhizobium biofertilizers under glass house and field conditions (Indra and Gayathri Subbiah, 2003).

Kazempour (2004) reported that the *P. fluorescens* isolates inhibited the growth of *Rhizoctonia solani* Kuhn, the rice sheath blight pathogen and suggested that the *P. fluorescens* isolates have an excellent potential to be used as biocontrol agent against *Rhizoctonia solani* in rice under field conditions.

*P. fluorescens* is reported to have a great potential to be used as biofertilizer and biocontrol agent for enhanced growth and in controlling crop diseases (Gehlot *et al.*, 2005).

Roy *et al.* (2006) reported that the microbial flora of plant leaf surface commonly known as phyllosphere microflora consists of various microorganisms which grow and multiply on the leaf and are capable of inhibiting the growth of other microbes. This unique property of surface microflora is successfully exploited in various agricultural crops for biocontrol of important diseases.

Treatment with vermicompost in combination with plant growth promoting rhizobacteria (PGPR) *P.syringae* reduced the seedling mortality in chickpea (*Cicer arietinum*) caused by *S. rolfsii*. The PGPR not only reduced the mortality but also increased the availability and uptake of minerals like P, Mn and Fe in chickpea seedlings resulting in an increased plant growth (Sahni *et al.*, 2007).

Yogesh *et al.* (2008) reported incongruent performance of *Pseudomonas fluorescens* (Pf) strains with respect to plant growth promotion and biocontrol activities when tested under controlled and field conditions against *Fusarium solani* f. sp. *pisi*. In vitro studies on the efficacy of *Trichoderma* spp. against soil borne pathogens revealed
maximum inhibition of mycelial growth by \textit{T.harzianum} against \textit{Rhizoctonia solani}, \textit{Pythium debaryanum}, \textit{Sclerotina minor} and \textit{Fusarium oxysporum} f.sp.\textit{pisi}.

Murugesh and Mahalingam (2008) reported that application of neem cake (800 kg/acre) with the repeated application of antagonistic bacteria (\textit{P.fluorescens}) and fungi (\textit{T.viride} and \textit{T.harzianum}) along with FYM in the ratio of 1:1:1:20 at an interval of 45 days for three times brought down the root disease incidence from 31.53\% to 2.63\% in mulberry.

Rashmi \textit{et al.} (2008a) reported that application of 50\% N through \textit{Azotobacter} and 50\% through inorganic fertilizers resulted in higher leaf yield (715 g), total soluble sugars (51.64 mg/ml) and total soluble protein (29.21\%). Plating for microbes also revealed more population of bacterial (145.66 CFU/g) counts.

Bioagent \textit{T.viride} showed maximum inhibition of mycelial growth of \textit{S.rolfsii}. Similarly, the culture filtrates of \textit{T.harzianum} showed maximum inhibition of mycelial growth of \textit{R.solani}, \textit{P.debaryanum}, \textit{S.minor}, \textit{F.oxysporum} f.sp.\textit{pisi} and \textit{S.rolfsii}. \textit{T.harzianum} inhibited significantly the conidial germination of \textit{F.oxysporum} f.sp.\textit{pisi} and sclerotial germination of \textit{S.rolfsii} as compared to other bioagents (Kapoor, 2008).

Rani \textit{et al.} (2009) evaluated the performance of six \textit{Trichoderma} and four \textit{Pseudomonas} isolates for their ability to induce systemic resistance against \textit{F.solani} causing wilt of chilli and found that maximum inhibition was noticed in \textit{T.viride} (indigenous) followed by \textit{T.viride-16} and \textit{T.harzianum-10}. Among four bacterial bioagents an indigenous isolate of \textit{P.fluorescens} (Pf-1) was most efficient with 74.26\% inhibition followed by \textit{P.fluorescens}-PGPR isolate.

The pre-inoculation spray of \textit{T.koningii} 5201+ \textit{Chaetomium cochliodes} 3319 against rice sheath blight caused by \textit{Rhizoctonia solani} showed maximum reduction (55.1\%) of the disease incidence, disease severity (70.8\%), relative lesion height
(49.1%), maximum 1000 grain weight, grain yield and straw yield (Tamilvanan and Kandhari, 2009). Chawla and Gangopadhyay (2009) studied the antagonistic potentiality of *T. harzianum, T. viride, P. fluorescens* and *B. subtilis* along with organic amendments viz., FYM, vermicompost and mustard cake against *Fusarium oxysporum* f.sp.*cumini* and reported maximum inhibition of mycelial growth of *F. oxysporum* f.sp.*cumini* in presence of *P. fluorescens* followed by *T. harzianum*. These bioagents suppressed the pathogen population in soil and also enhanced the shoot and root lengths and dry weight of cumin plants.

The effect of recommended doses of oil cakes like *Azadirachta indica* L. and *Guizotia abyssinica* and pesticides like Dithane M-45 and Furadan used for the control of soil borne diseases in mulberry on rhizosphere and rhizoplane microflora indicated that the bacterial population was maximum in terms of colony forming units (CFU/g) followed by fungi and actinomycetes in both the habitats. This microflora was significantly higher in rhizosphere as compared to rhizoplane (Sharma *et al.*, 2009).

Various isolates of *P. fluorescens* collected from healthy rhizosphere of mulberry and screened under in vitro conditions against pathogen complexity (*F. solani, F. oxysporum, Botryodiplodia theobromae* and *Macrophomina phaseolina*) causing root rot in mulberry indicated the efficacy of *P. fluorescens* (Psf-7) against all pathogens (Chowdary *et al.*, 2009).

### 2.11 Constraints in the use of Biofertilizers

Although biofertilizers are known for their low cost and eco-friendly nature their adoption has been limited in our country. In most cases the rate of adoption has been stagnating around 10-15% of the area for many years. The inconsistent performance of biofertilizers in the field remains a concern due to various reasons which include i) high population of native organisms which are not always effective ii) poor survival and
multiplication of the crop inoculant in the soil iii) nutrient deficiencies in the soil iv) inadequate crop management besides this high temperatures and pesticides residues in crop often affect the performance of the inoculant (Venkateswarulu, 2005). Soil temperature plays a pivotal role in the survival of the organism (Joshi, 1994). The optimum temperature lies between 25° - 30° C and India being a tropical country the major constraint is the use of biofertilizer particularly in summer months when soil temperature rises very high (45°- 50°C) at 5 cm depth. The number of bacteria (Azotobacter, Azospirillum and Rhizobium) in soils decline drastically as the soil dries. Water logging during rainy season creates a barrier for better functioning of biofertilizers as living cells contained in them face lack of oxygen. This becomes a major problem for root respiration and can rapidly result in loss of nitrogenase activity and hence N fixation ability (Motsara et al., 2000). Nitrogen fixing organisms are adversely affected by low soil pH (below 5.0) which is often associated with Al toxicity and Ca deficiency. This retards the growth of the organisms. The growth of Azotobacter is suppressed below pH 5.8. Salinity is common in coastal arid and irrigated soils. Salts of Na and Ca are known to be toxic to biofertilizers. Salt concentration (generally sodium chloride) of more than 1% inhibit the growth of biofertilizers. Although some strains of Azotobacter and Azospirillum can tolerate high salt concentration, increase in salinity sharply decreases their proliferation (Motsara et al., 1995).

In rainfed areas low fertile soils and frequent droughts limit the biomass production in crops, which leads to lowered N/P requirement and consequent poor response to inoculation under field condition. For the best results soil fertility management and moisture conservation practices have to be integrated with biofertilizer use which is not possible always (Bisoyi, 2000).

Presence of native ineffective strains which cannot be replaced by inoculated strains, if they are not competitive and effective. It has been often stated that poor
organic matter and low soil moisture status lead to lower levels of establishment of inoculated strains of microorganisms. Presence of antagonistic organisms in rhizosphere also minimizes the number of beneficial microorganisms in rhizosphere. Non availability of soil, crop and region wise specific strains, poor shelf life of biofertilizers besides rainfall, soil type, soil moisture and temperature and methods of inoculation also affect the survival of biofertilizers in the crop ecosystem (Saleema et al., 1982). These factors and all others discussed under the para 2.11 above collectively contribute to the poor response to inoculation of biofertilizers in field condition, whereas the same is not true in the case of effective microorganisms (EM) as they do not require any specific conditions for their multiplication and survival. Therefore the present study has been taken up to study the effect of different biofertilizers along with effective microorganisms in mulberry.

2.12 Role of Effective Microorganisms (EMs) in agro-eco system

EM creates a balance and harmony of microorganisms, when inoculated in the soil. The EM consists of a combination of aerobic and anaerobic organisms. Microorganisms already present in the soil, combined with those in the EM inoculant, create a living soil which results in the release of nutrients for plant growth. EM creates a fermentative culture which increases the microfloral environment for the digesting the crop residues into nutrients utilized by plant life. EM also can increase the efficiency of applied fertilizer while promoting the growth of beneficial microorganisms in the soil.

The use of microbes in the form of animal manure and slurries has a long history in traditional agriculture. The use of rhizobial and mycorrhizal inoculation added a new dimension to the technology of microorganisms in agriculture. In recent times research has clearly shown the benefits of using inoculation of naturally occurring microbes in
increasing productivity of both conventional and organic farming systems (Tisdal, 1994; Zarb et al., 2001). However, the use of microbial inoculation containing many species obtained from the respective ecosystems to develop the benefits has not received much attention. EMs basically comprise of a liquid concentrate containing a consortium of beneficial microbes that act as microbial inoculants and antioxidants in the soil creating a conducive environment for the crops to grow. Contrary to the traditional use of fertilizers, the purpose of EMs is to increase the number of beneficial microorganisms in the soil thus improving the soil health and promoting a healthy environment for plants (Nair, 2006).

Today Effective microorganisms (EMs) are used in many systems pertaining to agriculture and environmental management which range from crop and animal production systems to livestock and aquaculture units. Effective microorganisms are used widely in environmental management for decomposition and more importantly for recycling the wastes, both solids and liquids. More recently researchers from Japan and USA have reported the ability of Effective Microorganisms (EMs) based products to reduce dioxin contents (Yadav, 2000). Research has shown that the inoculation of EM cultures to the soil/plant ecosystem can improve soil quality, soil health and growth yield and quality of agricultural crops (Higa, 1991; Higa and Wididana, 1991a). The Effective Microorganism consists of selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms. All these are mutually compatible with one another and can coexist in liquid culture. (Higa and Parr, 1994). The beneficial role of this microbial solution lies in its ability to break down organic matter, thereby providing plant nutrients and enhancing physical and chemical properties. EM is not a substitute for other management practices. It is however an added dimension for optimizing soil and crop management practices such as crop rotations, use of organic
amendments, conservation of tillage, crop residue recycling and biocontrol of pests. If used properly, EM can significantly enhance the beneficial effects of these practices (Higa and Wididana, 1991b).

2.12.1 Efficacy of effective microorganisms in growth and productivity of different crops

Higa (1991) and Parr et al. (1994) reported that the microorganisms are useful in eliminating problems associated with the use of chemical fertilizers and pesticides, which are now widely applied in nature farming and organic farming systems. Beneficial and effective microorganisms applied as soil, plant and environmental inoculants appears to hold the greatest promise for technological advances in crop production, crop protection and natural resource conservation (Higa, 1995).

Higa and Wididana (1991a) and Higa (1994;1995) have noted the use of beneficial microorganisms as soil plant inoculants to shift the microbiological equilibrium in a way that enhance soil quality, yield and quality of crops. EM improves soil health and growth, yield and quality of crops over a wide range of agro-ecological conditions (Higa and Parr, 1994). It is also reported that the foliar application of EM results in a large number of beneficial microorganisms at the leaf surface or phyllosphere (Pati and Chandra, 1981). Certain microorganisms in EM culture including photosynthetic bacteria and N-fixing bacteria enhance the plants photosynthetic rate and efficiency and N-fixing capacity as well.

Treatment of seeds having poor viability with EM and biofertilizers effectively improved the germination (Santos, 1992). Siqueira et al. (1992) observed that the seed inoculation with effective microorganisms (0.1% and 1.0%) for 30 minutes and a biofertilizer Vairo (biofertilizer produced by anaerobic fermentation of cow manure) for 10 minutes showed increased germination and vigor in carrot, cucumber, pea, beet and tomato.
EMs are currently used in many countries as a beneficial microbial inoculate for processing organic materials so that they can be recycled back into agricultural systems (Afzal et al., 1994). The products made out of wastes from the banana industry can be used as beneficial microbial inoculates for soil regeneration and fertilization (Higa, 1996). Daly and Stewart (1999) stated that the EMs plus molasses increased the onion yield by 29% and the proportion of highest grade onion by 76%, pea yield by 31% and sweet corn cob weights by 23%.

Yadav (2000) reported no visible difference in the vegetative growth of radish but the yield of radish roots (the edible parts) were significantly higher in the plots treated with EMs compared to the control. More important observation was the delayed hardening quality of radish roots in effective microorganism applied plots. He also reported increased cabbage yield (91.58%) and better compactness of cabbage heads due to the foliar application of effective microorganism.

Zarb et al. (2001) opined that the use of microbes singly or in mixtures of free living and naturally occurring species could enhance the productivity of most farming systems significantly.

The reports on the success of EMs in crop production are many. Research on papaya in Brazil (Chagas et al., 2001), herbage grasses in Holland and Austria (Bruggenwert, 2001; Harder, 2001), vegetables in New Zealand and Sri Lanka (Daly and Stewart, 1999; Sangakkara and Higa, 2000) and apples in Japan (Fujita, 2000) illustrate success phenomenon very clearly. All these studies also highlight that the use of Effective Microorganisms or EM based products increase crop yields in traditional organic systems over a period of time.

The beneficial effects of EM have been attributed to many factors, which include greater release of nutrients from organic matter (Sangakkara and Weersekera, 2001), enhanced photosynthesis (Xu et al., 2001) and protein activity (Konoplya and Higa,
2001), greater resistance to water stress (Xu, 2000), greater mineralization of carbon (Daly and Stewart, 1999) and increased soil properties (Hussein et al., 2000). Better penetration of roots with the use of effective microorganism has also been reported (In Ho and Ji Hwan, 2000).

The impact of EM in promoting plant growth by controlling or suppressing pests and diseases has also been reported from many countries (Sangakkara and Higa, 2000). Kremer et al. (2001) reported the control of Sclerotinia in turf grass with EM. Guest (1999) and Wang et al. (2000) highlighted the control of Phytophthora with EM derivatives in China and Australia. Wood et al. (1999) reported the control of pickleworm in cucumber with EM. Elango (1999) and Tabora et al. (1997) reported the control of black Sigatoka leaf spot in Costa Rica using EMs.

Crop residues and animal wastes were effectively composted to produce biofertilizers using EM (Van Bruchem et al., 1999). The compost prepared out of animal or crop residue using EM increased the yields of crops applied with this compost in the traditional organic systems (Shintani, 2006).

The EM acts as a microbial inoculant as well as a soil conditioner and used as a decomposing agent which helps in minimising the composting time (Juyal et al., 2002). A series of weed and fertility management trials associated with the commercial production process of peas, onions, beans and sweetcorn were established on organic farms in Canterbury. The results demonstrated a consistent positive response with the use of EM in crop production and indicated the potential of this technology to reduce fertilizer use and increase the yield and quality of crops (Szymanski and Patterson, 2003).

The control of pepper fruit anthracnose under green house condition using EMs indicated that EM reduced the number of fungus colony recovered from senescent flowers and the number of young commercial diseased fruits. The shelf life of harvested
fruits was longer with EM treatment (http://en.wikipedia.org/wiki/Effective microorganisms).

A field experiment conducted in Brazil in citrus garden with EM indicated that the EM treated plot recorded significantly higher soil organic matter content, levels of major nutrients and cation exchange capacity (CEC). Yield of oranges was increased by 17% besides increase in total soluble sugars, juice contents and average weight of the fruit in plots where EM solution was sprayed both to the soil and foliage (http://en.wikipedia.org/wiki/Effective microorganisms).

Singh (2007) reported that the EM treatment increased seed protein, crude fat and seed yield in soybeans, increased N uptake by cowpea from crop residues, increased yields in banana, oranges, peanuts, papayas and mangoes besides controlling the disease caused by Sclerotinia sp in turf grass. EM application also increased the efficiency of compost production from three months to three weeks (www.cityfarmer.org/bokashi.html).

Arshad (2006) found that the foliar application of EM in combination with proper soil amendment enhanced the nodulation, nodule number, nodule biomass and grain yield in pea. The application of EM improved the root growth, total nitrogen, total chlorophyll contents and yield in paddy and Chinese cabbage. It also increased the beneficial microorganisms populations in soil and nutrient uptake by the plants (O-Chol-Min et al., 2008).

2.12.2 Efficacy of effective microorganisms in growth and productivity of mulberry crop

Ganeshkeremane et al. (2004) opined that EM application as foliar spray in three mulberry varieties exhibited positive effect on the moisture retention capacity of mulberry leaves which was encouraging in terms of silkworm rearing. Further studies to elucidate effect on crop productivity indicated that there was an increase in the
chlorophyll content, plant growth parameters and leaf yield by the foliar spray of EM (0.1%) in mulberry compared to the control plots (Gnanaselvi, 2007). Vinoj (2008) reported that treatment of mulberry seed cuttings with EM along with vermicompost and biofertilizers viz., *Azospirillum*, PSB and *Rhizobium* resulted in higher sprouting, survival, total soluble sugars, total soluble protein, chlorophyll content, total phenol and other growth parameters in the nursery. Plating for microbes also revealed more population of bacterial and fungal colonies.

The exhaustive review of literature speaks of voluminous works which have been carried out with a number of microbial inoculants on various agricultural and horticultural crops, but the reports on microbial inoculation along with EM on mulberry are either very scanty or not available at all. Moreover the inoculation of microbial complexes and their impact on mulberry plant health, productivity and cocoon yield have not been studied. This has necessitated generating more useful information on use of EMs in mulberry to help the sericulture industry to achieve sustainable progress.