Discussion
5. DISCUSSION

5.1 Effect of certain effective microorganisms (EM) along with reduced levels of N and P on mulberry under nursery condition

5.1.1 Effect on sprouting and survival

The treatment of mulberry cuttings with microorganisms like species of *Azotobacter*, *Azospirillum*, and phosphobacteria with other EM formulations showed significant increase in sprouting and survival percentage irrespective of mulberry varieties. The nursery inoculation with biofertilizers is known to increase the growth, sprouting and survivability of many horticultural crops. Inoculation of nursery with different VA mycorrhizal fungi has been known to enhance the survival of citrus (Menger et al., 1977), white ash seedlings (Furlan et al., 1985) and other horticultural crops (Gianinazzi et al., 1989). Presence of increased microbial populations in the treated soil accelerates the process of decomposition of organics thereby improving the availability of nutrients particularly "N" to the sprouting cuttings. The treatment T10 of the present study has, therefore, resulted in enhanced sprouting and survival at 30, 60, 90 and 150 DAP of cuttings. Increased sprouting and survival of cuttings due to the application of *Azotobacter chroococcum* and *Azospirillum brasilense* is also reported to be due to the higher production of IAA and gibberellin like substances closely resembling GA3 (Rao et al., 1976; Gangwar and Thangavelu, 1992).

5.1.2 Effect on growth parameters

The selected microorganisms treatment in nursery showed significant increase in plant height. The treatment T10 exhibited enhanced plant height compared to other treatments at 90 and 150 DAP (87 cm, 116 cm and 66 cm, 91 cm) respectively in mulberry varieties V1 and MR2. Increased plant height may be attributed to the effective function of biofertilizers and EM which produced bioactive substances with probiotic effect. These results are in agreement with the findings of many research workers who
reported increased plant height due to microbial application on various crops like chilli (Amirthalingam and Balakrishnan, 1988), bhendi (Parvatham et al., 1995), tomato (Hameedunnisa Begum, 1998), coriander (Subramanian and Vijayakumar, 2000), onion (Nagarajan et al., 2001) and mulberry (Nagarajan et al, 1986; Gangwar and Thangavelu, 1992).

Biofertilizers and EM applied to nursery recorded significantly higher shoot weight per plant at 90 and 150 DAP in the treatment T9 in V1 (76.23 g, 120.30 g) and T5 in MR2 (89.67, 100.83 g) respectively, which also recorded significantly higher root colonization by VAM fungi in both the treatment combination. This could be attributed to the enhanced mobilization of nutrient N and P₂O₅ as reported by earlier workers (Badr eldin and Moawad, 1988; Chhabra and Jalali, 1995). Similar effect has been observed on the number of leaves and plant growth due to the treatment T10, Azospirillum+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM at both the stages of plant growth viz., 90 and 150 DAP recording significant improvement in both V1 and MR2 varieties. The microorganisms are also reported to produce growth promoting substances like auxins, gibberellins and cytokinins and mobilizations of various micronutrients besides supplying nitrogen and phosphorus (El-ruan et al., 1973) and hence increased growth of plants observed in the present study. Higher number of leaves in this treatment could be attributed to efficient growth regulating activity which is in accordance with the findings of Kumaraswamy and Madalageri (1990) who also recorded maximum number of leaves in tomato due to Azospirillum brasilense inoculation along with 30 Kg N per ha. Like wise more number of leaves were recorded due to Azospirillum inoculation in cauliflower (Kalyani et al., 1996) and VAM inoculation in onion (Nagarajan et al., 2001).

Biofertilizer and EM application also recorded significant increase in leaf weight. The combination of Azospirillum+PSB+VAM+ (75% N+25% P+ full dose of K /ha/yr)
+EM resulted in maximum leaf weight compared to other treatments at 90 and 150 DAP (67.13 g, 93.08 g and 45.95 g, 92.56 g) respectively in both the varieties. Increased leaf weight could be attributed to the improved photosynthetic activity as could be ascertained from significantly high chlorophyll a, b and also total chlorophyll observed in plants receiving biofertilizers and effective microorganisms. These results are in accordance with the findings of Nagarajan et al. (1986) and Gangwar and Thangavelu (1992). El-ruan et al. (1973) reported increased supply of nitrogen and phosphorus to plants due to inoculation of EM which contributed for better growth of leaves.

The root biomass is the important character recorded in this experiment. Root length and root biomass was enhanced due to the treatment T10 (Azospirillum+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM), though individual inoculation with reduced fertilizer nutrient was not exhibiting much influence on root length, root fresh and dry biomass. This was in accordance with the findings of Kuberanarayanan (1995) who reported the combination of Azospirillum with phosphobacteria resulting into 44% and 46% increase in root length and root weight respectively. The response on root biomass is explained by the hormonal effect of microbial population colonized on and around roots (Das et al., 1992). In the present study mycorrhizae inoculated saplings in nursery were also found superior recording maximum root length and root biomass in comparison with untreated control. This could be corroborated with the studies of Ianson and Linderman (1989); Danielhylton and Ahmed (1994) who have also recorded better plant response and more symbiotic association of VAM with the mycorrhizal plants and that increased colonization of roots resulted in to increased volume of root system to absorb and translocate elements mainly P through external hyphae. The phosphate solubilizing microorganisms have been shown to increase the root proliferation in mulberry (Nagendrakumar, 1998) which in turn helps in efficient absorption of nutrients from a larger volume of soil. Santhanakrishnan and
Obilisami (1980) and Balasubramanian (1995) have reported better growth and root development of mulberry cuttings due to inoculation with *Azospirillum* and *Azotobacter*. The combined inoculation has significantly improved the dry root biomass of the saplings in both the mulberry varieties over uninoculated control studied and reported herein. This is in line with the studies of Danielhylton and Ahmed (1994) who have also recorded increased growth in kidney beans (*Phaseolus vulgaris* L.) due to co-inoculation of VA mycorrhizal fungi and rhizobia.

5.1.3. Effect on biomass production

It was observed that there is significant improvement in total above ground fresh biomass and total above ground dry biomass of saplings in the treatment T11-Azotobacter+PSB+VAM + EM receiving lesser dose of nitrogen and phosphorus (50% N+25% P+ full dose of K/ha/yr) than the uninoculated control treatment (T0) or treatment receiving full dose of fertilizers (T1). The results of Young (1990) demonstrated similar significant beneficial effect of co-inoculation in increasing growth of *Leucaena leucocephala* using PSB and VA mycorrhiza. Similar results were also obtained in mulberry Chandrashekar *et al.* (1996). This is attributed to the enhanced microbial interaction due to inoculation of different beneficial microorganisms in the rhizosphere of mulberry where they play a pivotal role in various physiological processes including solubilization and transportation of minerals to the leaves. This accentuates the availability of plant nutrients in the rhizosphere thereby improving the growth and yield of plants. This point out beneficial effect of PSB, nitrogen fixing bacteria and VA mycorrhiza in effectively supplementing nitrogen and phosphorus supply which in turn improves the growth and biomass production in mulberry even after curtailing chemical fertilizers to a great extent (Satpathy *et al.*, 1995).
5.1.4. Effect on qualitative parameters

Biofertilizers and EM treatment of mulberry cuttings showed significant increase in chlorophyll a, b and total chlorophyll content with the treatment *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K ha/yr) +EM (T10) exhibiting highest chlorophyll content compared to other treatments at 90 and 150 DAP in both the varieties. These results are in agreement with the findings of Kuberanarayanan (1995); Baqual (2003) and Balasubramanian and Kumutha (1994). The synergistic action of introduced microorganisms increased the vigor and vitality of the mulberry plant which resulted in enhanced metabolic activities of the plant thus contributing for the increase in chlorophyll content. The increased trend in chlorophyll content was observed at 25, 40 and 60 DAP of mulberry cuttings in nursery due to the combined application of *Azospirillum*, PSB, Rhizobium and EM in soil (Vinoj, 2008) and as foliar spray of EM alone in mulberry (Gnanaselvi, 2007) which might be due to synergistic interaction of biofertilizers and EM. The increased amount of chlorophyll content in leaves indicated the photosynthetic efficiency and it can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin, 2000). Higher levels of chlorophyll are indicative of higher photosynthetic efficiency of plants (Patil et al., 1998; Watson, 1952; Raj and Tripathy, 1999). The present study is also in tune with the report of Singh et al. (1991); Das et al. (1994) and Ramarethinam et al. (2005) who reported that chlorophyll content significantly increased due to the application of urea, triacontanol and biofertilizers respectively in mulberry.

Biofertilizers and EM applied to the cuttings in the present study showed significant increase in total carbohydrates, total soluble protein and total phenolic content in leaf. Such types of increase in the nutritional status of the mulberry leaves in terms of biochemical contents through the application of fertilizers and biofertilizers have also been recorded by earlier workers (Loganath and Sivashankar, 1986; Madhubabu et al.,
The synergistic action of introduced organisms increased the vigor and vitality of plant which resulted in enhanced metabolic activities of the plant. All these things may contribute for the increase in chlorophyll and carbohydrate contents of the leaves. The biochemical analysis of leaf samples of mulberry from various treatments revealed a significant increase in parameters such as total soluble proteins, total carbohydrate, total chlorophyll content, total amino acids etc., in the biofertilizer treatment groups as compared to the control group (Ramarethinam et al., 2005). The N2 fixing property of Azospirillum and Azotobacter increased the N availability which in turn might have resulted in increased protein content.

Similar to other qualitative parameters higher crude protein content (18.75% and 18.13%) was obtained in the treatment T10 (Azospirillum+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM) on 150 DAP in V1 and MR2 varieties respectively. Higher crude protein content in leaves could be attributed to the efficiency of biofertilizers and EM used in rendering nutrients mainly N available additionally. These results are in accordance with the findings of Gangwar and Thangavelu, 1992 and Ramarethinam et al. (2005) who also reported presence of higher crude protein in leaves of mulberry treated with Azotobacter and Azospirillum.

The mycorrhizal colonization in the roots of mulberry saplings was higher in the plants treated with VAM and other biofertilizers + EM compared to others with maximum in the treatment T9 (Azotobacter + VAM + (50% N+50% P+ full dose of K /ha/yr) +EM in V1 (56.33%, 66.53%) and MR2 (50.23%, 57.73%) varieties respectively on 90 and 150 DAP. In some other crop plants like onion (Allium cepa) such increased root colonization due to mycorrhizal inoculation has been reported by Manjunatha et al. (1981) where as Fathima et al. (2000) had also reported that increased root colonization is an indication of better association of VAM fungi in the rhizosphere of mulberry which
in turn increase the mobilization of phosphorus and micronutrients thereby enhancing growth and yield. Physiological and anatomical studies have brought out that mycorrhizal plants have increased rates of respiration, photosynthesis and increase amounts of sugar, amino acids, RNA etc., and larger and or more number of chloroplasts, mitochondria, xylem vessels, motor cells (Hayman, 1983; Krishnan et al., 1981; Dyaladoss et al., 1988). Colonization of roots by VAM fungi may result into changes in the root exudation and also altered rhizosphere organisms in crop plants (Secilia and Bagyaraj, 1987; Mada and Bagyaraj, 1993). The increase in the uptake of immobile nutrients especially phosphorus by mycorrhizal plants is explained on account of exploration of larger soil volume and decreasing the distance that phosphate ions must diffuse to plant roots and by increasing the root surface area for absorption and faster movement of phosphorus in to mycorrhizal hyphae by increasing the affinity for phosphate ions (Bolan, 1991).

Significant reduction in the root colonization percentage was also observed in the present study in the treatment which received full doses of phosphatic fertilizers. The efficacy of the VAM fungi in soil is also known to decrease as the level of available phosphorus of the soil increases (Daft and Nicolson, 1969; Abbot and Robson, 1977 and Tinker, 1978). Moreover decreased magnitude of mycorrhizal response with the increase in P levels have also been demonstrated by Ames and Linderman (1978); Maronek et al. (1980), Krishna and Bagyaraj (1983); Lim and Cole (1984) and Umadevi and Sitaramaiah (1990).

5.1.5. Effect on nutrient contents in mulberry leaf

Other than biochemical constituents, increase in major nutrient elements like N, P and K content were recorded in biofertilizer and EM treated plants. Highest N (3.0%, 2.9%), P (0.61%, 0.55%), K (1.99%, 1.88%) was recorded in T10 (Azospirillum+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM) and lowest
in uninoculated control (T0) plants N (2.0%, 1.88%), P (0.33%, 0.29) and K (1.69%, 1.54%) respectively in varieties V1 and MR2. These results are in accordance with the findings of Murumkur and Patil (1996). Dhillon et al. (1980) and Shabaev et al. (1991) also have observed supplementation of nitrogen in soil and more nitrogen fixation by *Azotobacter* inoculation in the cases of wheat and oat respectively. The P and K content of leaf in some varieties of mulberry increased significantly due to biofertilizer application with 50% cut in nitrogen fertilizer. This is in confirmation with the results of Das et al. (1994). The increase in leaf N and P content was registered by co-inoculation of biofertilizers and EM treatment which may be due to the synergistic action of the introduced organisms and their efficiency. Similar increase in N and P content in mulberry leaves was reported earlier by Yadav and Kumar (1991) and Das et al. (1992).

### 5.2 Effect of certain effective microorganisms along with reduced levels of N and P on plant health and productivity of mulberry in main field condition

The role of microbial bio-inoculants in maintaining the soil nutrient status and fulfilling the nutrient demand of crops cannot be over ruled. Among various contributors of beneficial soil microbes, *Azospirillum*, *Azotobacter*, Phosphate solubilizing bacteria (PSB) and VA mycorrhiza play significant role as eco-friendly plant growth promoting microorganisms. To improve soil health and fertility *Azospirillum* and *Azotobacter* which are free living non symbiotic bacteria can be employed to fix atmospheric nitrogen, while PSB as phosphate solubilizers can convert unavailable form of P to available form in soil. On the other hand VAM is a phosphate mobilizer which mobilizes P and also improves the uptake of other nutrients like K, Ca, S, Mg, Zn, Cu, Fe etc., through proper association with the roots of plants (Dandin *et al.*, 2005). Therefore the application of
biofertilizers along with EM for effective crop nutrient management has emerged as a blooming thought to be enunciated.

5.2.1 Effect on quantitative plant growth parameters

Biofertilizers as soil application and EM as foliar spray in both the varieties V1 and MR2 showed significant increase in plant height in the first and second year. The treatment T10 resulted in enhanced plant height compared to others. Increased plant height may be attributed to the effective function of different microbes which produced bioactive substances with increased nitrogen availability (Chandrashekar et al., 2005). Similar results were reported in maize and wheat plants where the inoculation with *Azospirillum* along with reduced doses of synthetic fertilizers mostly increased the height of shoots and root (Rousta et al., 1998). Better growth components in *Azospirillum*+EM are attributed to N2 fixation by these free living bacteria as stated by Sahu et al. (1997). In addition to fixing atmospheric nitrogen *Azospirillum* is also known to produce growth promoting substances such as GA3, IAA, cytokinins and beta vitamins (Rao, 1981) which stimulate germination, root growth and enhance mineral uptake (Lin et al., 1983). The increased root growth might have resulted in better utilization of soil moisture and nutrients ultimately producing better growth components. The enhanced growth characteristics could also be due to their ability to improve the physical, chemical and biological properties of the soil. Further more the stimulative effect may be related to the good equilibrium of nutrients and water in the root zone (Abdelziz et al., 2007) or to the beneficial effects of bacteria on the vital enzymes and hormonal stimulating effects on plant growth (Bashan et al., 1989).

Similar to plant height longest shoot length of the plant was obtained in the treatment T10 in both the varieties in the first and second year. The increase in longest shoot length may be attributed to the bioactive substances with probiotic effect produced by microorganisms (Praveenkumar, 2003). This is in accordance with the findings of
Karuthamani et al. (1995) who reported longest vine length in cucumber due to inoculation of *A. brasilense* and phosphate solubilizing bacteria along with reduced doses of N and P.

Soil application of biofertilizers and foliar spray of effective microorganisms produced significantly more number of shoots per plant in the variety V-1 during the first and second year in the treatment T10 compared to control. The reason for the highest number of shoots could be attributed due to enhanced release of nitrogen from the growth promoting substances produced by the microbes resulting into induction of more number of shoots. Adequate quantity of enzymes present in cells of microbes may be a reason for the favored growth and higher number of shoots as reported in the case of potato by Singh and Bury (1998). Increased number of shoots in this treatment could be attributed to efficiency of different microorganisms in terms of nitrogen fixation, P solubilization and its mobilization and production of growth promoting substances, whereas in the variety MR2 the treatment T6 and T7 produced more number of shoots per plant during the first and second year. Similar observations were made in different horticultural crops (Sriharibabu et al., 1998; Deka et al., 1996). The pronounced effect of co-inoculation of *Azospirillum* and PSB and *Azospirillum* and VAM is mainly due to the better compatibility of these organisms which cumulatively enhanced nutrient assimilation as well as growth hormone production (Balasubramanian, 1995).

The maximum number of leaves per shoot and leaves per plant was obtained in the treatment T10 in both the varieties during two years of the experimental period. The number of leaves is an important parameter since leaves are structures bearing photosynthetic machinery and an increase in leaf number may promote better translocation of water uptake and deposition of nutrient and yield. Plant growth promoting substances such as IAA, GA3 and cytokinins produced by *Azospirillum* are known to promote plant growth (Tien et al., 1979, Tiwary et al., 1998). Kumarasamy
and Madalageri (1990) reported maximum number of leaves (47.31) with 30 kg N/ha in tomato due to triple inoculation of *A. brasilense*, PSB and *Glomus fasciculatum* made along with FYM. Increase in the number of leaves per plant in other horticultural crops is also reported (Sreeramulu *et al.*, 1996; Kalyani *et al.*, 1996, Nirmala and Vadivel, 1999). Increased amount of phytohormones produced by microorganisms might have resulted in better absorption of nutrients contributing ultimately to increased number of leaves per plant. This is in agreement with the results of Sivakumar (1994) in sunflower. Increased leaf number is also considered a net result of better utilization of nutrients as per El-Hariri *et al.* (1988), Jeganathan *et al.* (1990) and Mala (1995). Of late it is confirmed in rice that increased biomass was due to biological nitrogen fixation by *A. amazonense* as measured by 15N isotope technique (Rodrigues *et al.*, 2008).

Higher leaf area and leaf area index were observed in the treatment T10 in both the varieties during both the year of study. The plants treated with *Azospirillum* along with synthetic fertilizers have recorded maximum leaf area in several crop plants. Corresponding to the increased leaf number, an increment in leaf area and leaf area index could be observed (Varma, 1993). The leaf area index is related to primary productivity which exhibit maximum efficiency in CO₂ assimilation and C₆H₁₂O₆ production. So through the application of microorganisms it is obvious that leaf production and nutritive value of leaves also increase considerably. Watson (1952) reported that the leaf area was highly related to the crop yield since it provided room for the photosynthetic system. Any treatment which influenced leaf area could have a control on economic yield of the plant and leaf area could be manipulated by organic and inorganic fertilization. Further the acceleration in leaf area could be attributed to the level of auxins, carbohydrates and other organic compounds produced as a result of application of organic nutrients as soil and foliar along with inorganic fertilizers (Anamika and Lavanya, 1990). Ambika *et al.* (1994) also reported increased leaf area in mulberry due to VAM inoculation. Mohandas
(1987) reported higher leaf area in tomato plants inoculated with *A. vinelandi* and VAM. Patil (1993) also observed higher leaf area and LAI in chilli plants inoculated with *G. macrocarpum* along with reduced dose of P than plants treated with only synthetic P. Vyas (2003) reported higher leaf area per plant in mustard when inoculated with *Azotobacter* and PSB compared to control.

### 5.2.2 Effect on yield parameters

The total above ground fresh biomass, total above ground dry biomass and leaf yield/ha/crop were significantly higher in the present study due to the combined application of biofertilizers and EM particularly in the case of treatment T10. Similar findings were observed due to inoculation of *Azospirillum* in mulberry at reduced levels of N (Yadav and Kumar, 1993). Not only the diazotroph *Azospirillum* but also the phosphate solubilizing organisms such as phosphobacteria and VA mycorrhiza are of importance in mulberry cultivation. P is one of the essential elements and its availability in soil is meager since it many a time becomes unavailable. Phosphobacteria is one such organism capable of converting unavailable P to available P due to organic acid production and mineralizing the organic phosphorus compounds (Gaur, 1972; Arora and Gaur, 1979). Significant increase in yield as well as phosphorus uptake of several crops have been reported (Gaur, 1985). The combined influence of *Azospirillum*+PSB+VAM is mainly due to the enhanced nutrient assimilation as well as growth hormone production and the compatibility of the organisms among themselves (Balasubramanian, 1995). Similar results were reported by Jha and Mathur (1993) in *Pennisetum glaucum* inoculated with *Azospirillum*. The increase in plant height, shoot biomass, root biomass and leaf weight due to *Azospirillum* + VAM combined inoculation in mulberry was reported earlier by Nagarajan *et al.* (1989). Yadav and Kumar (1991) reported that inoculation was beneficial at low levels of fertilizer nitrogen and that *Azospirillum* inoculation indicated positive response in increasing mulberry leaf yield. Similarly the
enhancement in leaf biomass by VAM has been reported by many workers (Kandasamy et al., 1986; Rajagopal et al., 1989; Katiyar et al., 1989; 1992 and Das et al., 1995b) which may be attributed to higher plant growth and better nutrient uptake specially phosphorus which had resulted in higher fresh and dry biomass production. Chandrashekar et al. (1996) also indicated improvement in growth parameters of mulberry due to synergistic effect of microorganism used for soil inoculation. Earlier a number of researchers like Bose et al. (1973), Bagyaraj and Menger (1978), Gaskin and Hubbel (1979), Berkum and Bohlool (1980), Cohene et al. (1980); Okon (1982); Dart and Wani (1982); Lin et al. (1983) and Alagawadi and Gaur (1988a, 1988b) have also recorded significant beneficial effect of using free living nitrogen fixing bacteria (Azotobacter / Azospirillum), VA mycorrhiza and phosphate solubilizers as bio-inoculants in agricultural crop production. The microorganisms in EM formulations have the ability to break down the organic matter thus releasing beneficial soluble substances such as amino acids, sugar, alcohol, hormones and similar organic compounds which are absorbed by plants and thus growth is enhanced (Higa, 1999). Higa (1994), Myint (1994) and Sangakkara (1994) have also reported that EM could improve crop quality and yield.

Significantly higher stem yield was recorded due to the treatment T10 in both the mulberry varieties studied due to the inoculation with Azospirillum, PSB and VAM with reduced doses of N and P along with EM. Stem yield data revealed that though N and P were applied in reduced dose yet there was a perceptible difference in stem yield and nutrient uptake. This suggests that the activity of PSB in association with VAM enhanced the solubilization of phosphatic fertilizers in soil which in turn could increase the uptake of available phosphorus in to the plants through VA mycorrhizal symbiosis. Further the presence of nitrogen fixing Azospirillum could have increased the available nitrogen content in the soil resulting in to enhanced uptake of nitrogen as observed in the present study. This synergistic effect of various microorganisms contributed to the better
growth and nutrient uptake in mulberry. Since the requirement of nitrogen and phosphorus during the early period of plant growth is very high, the use of PSB, VAM and *Azospirillum* might have helped in meeting this requirement of the plants quicker than the uninoculated plants. Similar results were also obtained by Nagaraju and Nanjundappa (1996) on growth and yield of cowpea plants in alfisols after application of single super phosphate inoculated with PSB.

The data on significant improvement in leaf moisture % in inoculated mulberry plants in spite of using reduced fertilizer doses is due to the increased root biomass which might have contributed for the enhanced moisture content in leaves of mulberry due to more water uptake. This can be justified by the observation made by Bagyaraj and Sreeramulu (1982), Bolan (1991), Katiyar *et al.* (1995) and Setua *et al.* (1999). In mulberry leaf moisture plays vital role in improving nutritive levels of leaves which in turn improve the palatability of leaves for silkworm (Bose and Bindroo, 2001). Hence the moisture content in the leaves may serve as one of the criteria in estimating their quality. Many scientists reported favorable effect of high moisture content of leaves on their palatability and digestibility by silkworm (Parpler, 1968; Waldbauer, 1968; Kasiviswanathan, *et al.*, 1973). Paul *et al.* (1992) reported that higher moisture content in the leaves enhances the feeding efficiency of the larvae which in turn increases the growth rate.

5.2.3 Effect on biochemical parameters of mulberry

The green color pigment chlorophyll plays a vital role in photosynthesis. The higher amount of chlorophyll helps in higher photosynthesis and there by increasing the total dry matter production. An increased trend in the chlorophyll content in different treatments during each stage of growth was observed. Though all the treatments had influenced the chlorophyll content, much effect was recorded in T10 and T2. The increased amount of chlorophyll content in leaves indicated the photosynthetic efficiency
and thus it can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin, 2000). The improvement in chlorophyll content might be due to synergistic effects of both biofertilizers and EM. The organisms in EM formulation increase the photosynthetic rate and efficiency and there by more photosynthesis and N-fixing capacity in EM treated plants (Pati and Chandra, 1981).

Regarding total carbohydrate content, a similar trend was observed as that of chlorophyll content. Higher amount of carbohydrates accumulated in the treatment T10 followed by T7. Application of biofertilizers and EM stimulate the plant growth and development through improvement in the photosynthetic efficiency due to increased chlorophyll content in leaves which in turn help the plants to accumulate more amounts of food materials (Shabaev et al., 1991). In mulberry leaves carbohydrates are available in plenty and it was reported to be the chief sources of energy for silkworm (Hori, 1978). Significant increase in total soluble protein content in leaves denotes an increase in the nutritional status of mulberry leaves in terms of biochemical contents through application of microorganisms (Madhubabu et al., 1992). Further the nitrogen fixing activity of microorganisms increased the nitrogen availability which in turn might have increased the protein content in the leaves. The increase in N and P content in leaf due to co-inoculation of biofertilizers and EM application recorded in this study may be due to the synergistic action of the introduced microbes and their efficiency.

5.2.4 Effect on soil and mulberry leaf microbial population

The population of the microorganisms such as bacteria, fungi and VA-mycorrhiza in soil depends up on certain factors like soil moisture, soil fertility, soil organic matter content and soil environment. The growth, survivability and population of these microbes are directly co-related with the soil factors. Application of high amount of chemical fertilizers decreases the population of microorganisms. High phosphorus levels in soil have invariably proved to reduce mycorrhizal intensity (Graham and Sylvester,
1981 and Cade et al., 1990) by reducing root colonization by VA-mycorrhiza. This also further confirms the results of an earlier study (Mondal, 2002) in which reduction in the population of bacteria and fungi in rhizosphere was observed in the treatments receiving higher amount of chemical fertilizers and at the same time increase in microbial population at a faster rate in the treatments receiving reduced doses of inorganic fertilizers. Bagyaraj and Manjunath (1980) observed an increase in population of bacteria in the rhizosphere due to the dual inoculation of *A. chroococcum* and VAM fungi which is also in confirmation with the findings of the present study. Srihari and Sreenivas (1995) also recorded higher microbial population in the rhizosphere of chilli when inoculated with *G. macrocarpum* and or *B. polymixa* as compared to uninoculated plants.

Bacterial populations in the soil particularly species of *Bacillus* are known to improve soil aggregation and water retention property. Fungi are useful for *in situ* composting of the crop residue enriching the soil and the actinomycetes induce disease resistance in crop plants to pest and diseases besides improving photosynthetic activity (Jayaraj et al., 2005 and Sannappa et al., 2005). The microbiological analysis of mulberry leaves also indicated enhanced bacterial and fungal population due to the application of effective microorganisms in the present findings. This can be clearly supported by the evidence that growth and development of plants is affected through a number of biochemical and physiological mechanisms coupled with the interaction of several intermediates. These intermediary compounds are mainly the precursors of various growth promoting substances actually involved in promoting growth and development in plants (Sudhakar et al., 2000b). Production of growth substances such as auxins, gibberellins and cytokinins in the presence of these growth promoting rhizobacteria have been observed resulting in enhancement of plant growth (Arshad and Frankenberger, 1991). Foliar application of EM results in an increased number of beneficial microorganisms at the leaf surface which suppresses the development of
harmful pathogens at the leaf surface there by providing a measure of plant protection through biocontrol (Wididana and Higa, 1995).

5.2.5 Effect on soil health parameters

The use of biofertilizers and effective microorganisms in different treatment studied here had improved a number of soil properties significantly. The pH and electrical conductivity were decreased whereas organic carbon, available phosphorus and available potassium levels were markedly increased in the treatments receiving EM. These microorganisms have the ability to dissolve insoluble phosphates in soil in to soluble forms by secreting organic acids and once the population of the organisms stabilizes in the soil their activity continues for many years (Subba Rao, 1986). These acids aid in lowering the pH of the soil and bring about the dissolution of bound forms of phosphates to usable forms such as orthophosphate thus increasing the potential availability of phosphorus for plants (Kucey, 1989). Effective microorganisms enhance the degradation and chemical break down of organic materials and stimulate the process of mineralization of organic matter (Higa and Kinjo, 1991; Hussein et al., 1999), releasing more nutrients in to soil plant system (Daly and Stewart, 1999) there by increasing the organic carbon content in the soil. EM enhances the decomposition of organic materials and improves chemical and biological properties of soil (Higa, 1988).

5.3 Effect of certain effective microorganisms on the management of root rot disease in mulberry

Mulberry is a commercially important crop raised for its nutritious leaves which are required for production of commercially important silkworm cocoons. It is currently encountering a serious problem due to wide spread incidence of root rot disease caused mainly by species of Fusarium resulting in to excessive damage as also reduction in reage (Beevi and Qadri, 2008). The conventional chemical control measures are unable to provide total crop protection and also have many other undesirable effects on
biological soil health (Beevi and Qadri, 2005). Indiscriminate use of chemical inputs is already known to cause many deleterious effects in soil as also in crop plants. In this context there is an increasing trend of exploiting antagonistic fungi, bacteria besides effective microorganisms as biocontrol agents in combating the disease.

In the present study, the treatment Trichoderma harzianum + T.viride + EM (T5) recorded significantly higher survival of plants under sick soil condition in both the varieties studied. The antagonistic effect of T.harzianum towards several root pathogens is well established. Volatile and non-volatile antibiotics produced by T.harzianum are responsible for the inhibitor action against root pathogen, Fusarium culmorum (Iqbal et al., 1994) F. oxysporum (Michrina et al., 1995) and R. solani (Kapoor, 2008). The role of Trichoderma in increasing the growth, survival and yield of plants is also reported. Trichoderma was found to induce increased growth of various horticultural crops (Ralph and Chun, 1986) and increased shoot and dry weight of tomatoes and tobacco by producing growth regulating factors (Baker et al., 1986) and increased the weight of fresh shoots, fresh roots and dry roots besides enhancing the root system (Joshi and Harsh, 2009) in Dalbergia sissoo. Robert et al. (1993) reported that seed treatment with T. harzianum and T. viride was more effective in controlling Rhizoctonia solani in Phaseolus vulgaris under both green house and field conditions.

The reduction in root rot and damping off disease by Trichoderma species may be due to higher antagonistic potential that includes antibiosis, parasitism and production of lytic enzymes (Singh et al., 2004). Mukhopadhyay et al. (1992) suggested that production of fungal metabolites by Trichoderma was responsible for biocontrol. Volatile and non-volatile antibiotics produced by T. harzianum inhibited F. culmorum by 42.3% on agar plates (Michrina et al., 1995). Trichoderma spp multiply in the soil and protect plants over the entire growing season by inducing systemic acquired resistance against the microbial pathogens (Harman, 2000 and Howell et al., 2000). The availability
of higher and constant number of colony forming units (CFUs) per gram of soil make the use of these Trichoderma species very attractive for the control of root rot (Sharma et al., 2009). The soil suppressiveness by specific microorganisms is a complex phenomenon involving the interaction of several factors including plant species and cultivars (Weller et al., 2002; Mazola, 2004; Singh et al., 2008).

Effective microorganisms applied in to the soil have the capacity to assert a powerful regenerative effect on soil (Higa, 1994) helping to re-establish a balanced soil ecology and combat oxidative stress in plants (Deiana et al., 2002). The EM when mixed with organic manure like compost or FYM activates and the beneficial microbes which proliferate, fermenting the waste in to nutrient and antioxidant rich compost that competitively excludes pathogenic bacteria and fungi (Higa, 1991). Disease inducing soil can be transformed in to disease-suppressing soil by inoculating the problem soil with EM (Parr et al., 1994). The application of EM to the soil enhances and releases the availability of nutrients to plants and reduce the disease incidence and destructive effects of plant pathogens (Panchaban and Pipatweeravat, 1975).

A combination of microorganisms applied to the soil has much higher chance of establishing itself than that of inoculation of single strain (Higa, 1995) which is in accordance of the present findings. The next best treatment was T8, T.harzianum +VAM + EM recording survival of 70%, 69.3% and 69% on 60, 90 and 150 DAP. The dual inoculation of T.viride + G.mosseae reduced the disease incidence and showed maximum effect on growth characters of Coleus barbatus (Boby, 2000). This type of synergism has also been reported in the case of marigold by T.aureoviride + G.mosseae (Calvet et al., 1993).

Increased production of vegetative spores by G.mosseae in the presence of Trichoderma species has also been observed (Calvet et al., 1990). VAM fungi are known to increase resistance of plants to pathogens by modification of cell wall, production of
antimicrobial compounds and altered rhizosphere microflora (Bagyaraj and Padmavathi, 1993). VAM fungi alleviating the severity of disease caused by root pathogenic fungi has been reported by earlier workers also (Prashanthi et al., 1997; Chakravarthy and Mishra, 1986). Datnoff et al. (1995) reported suppression of Fusarium crown rot and root rot of tomato by the use of G. intraradices + T. harzianum. VAM application resulted in suppressing rhizome rot caused by F. solani besides enhancing the growth. Improved nutritional status of plants with mycorrhiza makes them more resistant to certain root diseases (Sieverding, 1991). Total phenol content in mycorrhizal roots was found to be higher than non mycorrhizal roots and hence higher amount of phenols might be one of the factors responsible for increased resistance against Fusarium sp (Bhatia et al., 1972).

5.3.1 Population of microflora in rhizosphere soil of mulberry

5.3.1.1 Bacteria

Microbial population in soil differed significantly with the treatment. The treatment of the sick plot with T. harzianum + EM (T1) and T. harzianum + T. viride + EM (T5) recorded maximum number of bacterial population at 60, 90 and 150 DAP. These results are in accordance with the observations made by Srihari and Sreenivas (1995) who recorded higher microbial population in the rhizosphere of chilli when inoculated with antagonistic microorganisms as compared to uninoculated control plants. The occurrence of higher bacterial CFUs resulted in substantial decrease in rot incidence and increase in plant dry biomass in chilli and brinjal (Bohra et al., 2006). Due to its specific attributes like production of broad spectrum antibiotics and ability to grow under dry conditions, the use of bacterial species seems to have immense potential for biological control of root rot diseases in crop plants (Mariappan, 1995). Significant improvement in soil microbial population due to application of various biofertilizers and organics were observed by Jaishankar and Dandin (2005).
5.3.1.2 Fungi

Fungal CFUs in rhizosphere differed significantly with the treatment. The higher fungal population was observed in *T.harzianum* + *T.viride* + EM (T5) and *T.harzianum* + *T.viride* + *Pseudomonas fluorescens* + EM (T4) treated plants. This is in accordance with the findings of Bohra *et al.* (2006) who reported high population density of *T.harzianum* and *T.hamatum* in treated brinjal rhizosphere. When biocontrol agents and neem formulation were used for the management of damping off caused by *Pythium aphanidermatum* in brinjal and chilli, the biocontrol agents varied in their ability to establish and proliferate in the rhizosphere. Biological control using the *Trichoderma sp* helps the plant to attain better growth and yield by the destruction of the resting propagules of many soil-borne pathogens without any environmental hazards (Katan *et al.*, 1983 ; Tamilvanan and Kandhari, 2009 and Kapoor, 2008)).

The introduction of antagonists through appropriate food base is suggested for its successful establishment. Wright (1956) emphasised that the production of antibiotics by antagonists was influenced by the food base on which they were grown. FYM has been reported to be an effective substrate for the growth and multiplication of *T. harzianum* and *T.viride* (Kousalya and Jeyarajan, 1988; Chawla and Gangopadhyay, 2009) which has been used as substrate under this study. The antagonistic fungi utilized the cellulose and chitin present in the organic substrates by elaborating enzymes like β-1, 3 glucanases and chitinases which inhibited the growth of *Fusarium* like pathogens. The *Trichoderma* species show antagonistic effects against soil-borne plant pathogens and are good for use in bio-control, since it is easy to isolate and culture them, grow rapidly on many substrates, act as mycoparasite, compete well for food and site, produce antibiotics and have an enzyme system that ultimately destroy a wide range of plant pathogens (Boby, 2000).
5.4 Effect of certain effective microorganisms along with different levels of N and P on silkworm rearing

There was a clear indication of improvement in various cocoon characters of silkworm as a result of feeding mulberry leaves from the biofertilizer and EM inoculated plants. The combined effect of biofertilizers and EM along with reduced doses of N and P and full dose of K had a significant impact on growth parameters of silkworm. There was marked increase in the larval weight of silkworm (53.80 g / 10 larvae) in the treatment T10 receiving *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM followed by the treatment receiving full dose of recommended chemical fertilizer. This is attributed to the increased nitrogen, phosphorus and potassium contents in the treated leaves. This was also evident from the high leaf moisture in treated leaves which in turn has resulted in improved quality of leaf suitable for silkworm rearing (Baqual, 2003). The role of quality mulberry leaf in production and increased quantity of quality cocoons has been well established (Krishnaswami, 1978; Datta, 1992; Ravikumar, 1988). Katiyar *et al.* (1995) reported increased mulberry plant growth and improved moulting percentage of silkworm larvae through VA- mycorrhizal inoculation and reduced ‘P’ dose through single super phosphate application. Das *et al.* (1993) also observed improvement of larval weight due to application of *Azotobacter* along with 150 kg of N/ha/yr.

Other rearing parameters including economic characters were however not affected even after 50% reduction in chemical nitrogen fertilizer. The ERR by number and weight recorded due to the treatment T6-*Azospirillum*+PSB + (75% N&P + full dose of K/ha/yr) +EM and T10- *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM were superior over control. The higher ERR by number and weight is mainly due to the better leaf quality in terms of higher N, P and K contents. This is in accordance with the findings of Fathima *et al.* (1995) and Rama Rao *et al.* (2007) who
observed improvement in cocoon yield when *G. mosseae* treated leaves were fed to the silkworm.

The chemo assay of leaf for N, P and K content revealed higher nutrient content in leaf due to the application of bio-inoculation along with reduced doses of chemical fertilizers. This explains the superior quality leaf production due to microbial treatments in the present study. Improved nutrient content in leaf could be due to the efficiency of microbial inoculants which compensated reduced doses of chemical fertilizers by fixing atmospheric N and mobilization of phosphorus. Additionally it is reported that synthesis of other nutrients, vitamins, amino acids, hormones by nitrogen fixing microorganisms helped to enhance the growth by increasing lateral root formation of the host plants (Yoav Bashan and Gina Holguin, 1997) with the result that leaf quality and yield was improved and due to this reason improvement in silkworm growth and cocoon characters were observed (Sannappa *et al.*, 2005 and Rama Rao *et al.*, 2007).

There was a clear indication of improvement in various cocoon characters like single cocoon weight, single shell weight and silk ratio % of silkworms because of feeding leaf from the inoculants treated plot. This is attributed to the increase in N, P and K content in leaf due to microbial applications. The studies of Baqual *et al.* (2005) have revealed increased NPK and chlorophyll content of mulberry leaf due to co-inoculation with microbial consortium. The increased leaf nutrient content in turn could contribute for better economic characters of silkworms as has been observed in the present study. Similarly the importance of nutritive care for young age silkworm rearing and its influence on cocoon crop production is also reported (Chaluvachari, 1995). Since the young age silkworm needs more amount of sugar and protein in mulberry leaf for their better survivability in late age, the increased protein and other essential nutrients in mulberry leaf due to combined application of microbial consortium further underlines the
importance of the use of eco-friendly and beneficial microorganisms as bio-inoculants for better yields in mulberry (Baqual and Das, 2006).

In mulberry leaf moisture content and leaf moisture retention capacity are the two important factors that maintain the nutritive levels of leaves which in turn improve its palatability for silkworm (Hori, 1978). Leaves possessing higher leaf moisture content and moisture retention content are identified as good quality leaves which are highly essential for young age silkworm rearing (Bongale and Chaluvachari, 1995; Sujathamma and Dandin, 2000). It is also reported that higher leaf moisture content is significantly associated with the growth and nutritional parameters of silkworm (Fathima et al., 1996 and Rahamathulla, et al., 2004). Further it is also observed that the leaf protein as well as the chlorophyll content was significantly enhanced due to the microorganisms in mulberry which explains improvement in the quality of mulberry leaf. The importance of protein, sugars, aminoacids and other biomolecules in the silkworm feed has been well documented by Ito and Arai (1965). Mishra et al. (1993) have clearly indicated beneficial effect of commercial formulation triacontanol with A.chroococcum in improving mulberry leaf quality which in turn significantly improved all the economic and commercial characters of silkworm cocoons and this is in confirmation with the results of present investigation. The results of Das et al. (1994) in improving mulberry leaf quality due to bacterial biofertilizer inoculation also confirm potentiality of using various microbial inoculants in mulberry cultivation for obtaining better silkworm cocoon production. The plant nutrients improvement as reported in this study through the use of beneficial microorganisms in mulberry cultivation can bring a significant contribution in reducing cost of production and improving soil health through an integrated plant nutrient management (Das et al., 2003).