



USE OF BIOFERTILISERS IN VARIOUS AGRICULTURAL CROPS WITH SPECIAL REFERENCE TO MULBERRY

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ABSTRACT

The increased population and growing need of food grains has imposed tremendous pressure on agricultural land and many methods have been employed to increase productivity and cater to the needs of such population either through evolution of more productive varieties as a long term measure or excessive use of fertilisers which earlier underlined the concept of green revolution. Although, these fertilisers and pesticides have played a definite role in elevating the productivity of Indian agriculture but this increase has been at the cost of soil health, fertility status, health hazards and environmental pollution etc. This undesirable role of synthetic fertilisers has grown to be of a major concern and now efforts are on to focus more on the sustainability part of soil which was attached least importance earlier. After much damage to ecosystem, alternative ways of increasing agriculture food production have gained momentum and more importance is given to the use of bio-fertilizer & bio-pesticides, organic farming, biodynamic farming, low input agriculture and sustainable agriculture integrated farming practices etc throughout the world. These alternative sources of building soil health especially use of biofertilisers has been found to be environmentally safe, ecologically friendly and economically viable means of restoring the soil sustainability for prolonged use. Many types of biofertilisers viz., *rhizobia* which are symbiotic bacteria that fix atmospheric nitrogen in plant root nodules and establish mutually helpful relationship with their host plants, nitrogen fixers including *Azotobacter* and *Azospirillum* which are free living non-symbiotic nitrogen fixing organisms producing certain substances good for the growth of plants, *azolla* which is found in abundance in water bodies and is also capable of fulfilling the nitrogen requirements of the plants by the process of nitrogen-fixation, *Arbuscular mycorrhizal* fungi colonising the root system and result in phosphorus mobilization, *phosphate-solubilizing bacteria (PSB)* and *plant growth promoting rhizobacteria (PGPR)* which represent a wide variety of soil bacteria and work with the association of host plant have been found to be useful in agriculture. The impact of different forms of these biofertilisers on various agricultural crops along with the mulberry which is an important commercial crop grown extensively as a food plant for silkworm (*Bombyx mori* L.) is reviewed.

KEY WORDS: - Biofertilisers, Agricultural crops, mulberry.

INTRODUCTION

In view of negative effects posed to soil by the application of chemical fertilisers, the use of alternative source viz., biofertilisers, in various agricultural cropping systems has gained more and more popularity. The concept of biofertilisers revolves upon the observation that they can have a beneficial effect on plant and crop growth (Davidson, 1988). These biofertilisers are essentially the microbial inoculants of living cells which aid in nutrient assimilation through the process of colonization, mobilization or solubilisation of nutrients. They are ready to use live formulations of such beneficial micro-organisms which on application to seed, root or soil mobilize the availability of nutrients by their biological activity and help build up the micro-flora and in turn the soil health. Minerals, organic components and microorganisms are three major solid components of the soil which profoundly affect the physical, chemical, and biological properties and processes of terrestrial systems. Soil infertility continues to be one of the

most important constraints limiting crop yield in developing nations worldwide, and especially among farmers who have marginal land holdings and are resource poor. Therefore, maintaining soil quality through the use of economically viable alternative like biofertiliser can reduce the problems of land degradation, decreasing soil fertility and rapidly declining production levels that occur in large parts of the world needing the basic principles of good farming practice. These biofertilisers through the presence of beneficial microorganisms accelerate and improve plant growth and protect plants from pests and diseases (El-yazeid *et al.*, 2007). Use of such natural products like biofertilizers in crop cultivation will help in safeguarding the soil health and also the quality of crop products for sustainable agriculture. These have been proved to increase crop yield by 20-30%, replace chemical nitrogen and phosphorus by 25% in general, stimulate plant growth, activate the soil biologically, restore natural soil fertility and provide protection against drought and some soil borne diseases. Reduce our dependence on chemical fertilizers and

pesticides that has created problems in agriculture (Chaturvedi, 2006) and also their frequent use for a prolonged period has deteriorated the surface soil characteristics and affected the availability and uptake of nutrients by the plants Subbaswamy *et al.* (1994). In addition to this there has been a realisation that chemical fertilisers have done much damage to ecosystem and this thinking has lead to use of products like bio-fertilizer and some of the practices that were started by the developed as well as the developing countries (Kolanu & Kumar, 2007) to make the agriculture sustainable.

A small dose of biofertiliser is sufficient to produce desirable results because each gram of carrier of biofertilisers contains at least 10 million viable cells of a specific strain (Anandraj and Delapierre, 2010). *Azotobacter* and *Azospirillum* are the two most important non-symbiotic N fixing bacteria in non-leguminous crops. Under appropriate conditions, *Azotobacter* and *Azospirillum* can enhance plant development and promote the yield of several agricultural important crops in different soils and climatic regions (Okon and Labendera-Gonzalez, 1994). The biofertilisers have been proved to enhance crop yield both, if applied singly or in combination with other inoculants. According to Goswami (1997) nitrogen fixing bio-inoculants can add 20 to 400 kg N per hectare where as P solubilizing

bio-inoculants can solubilize 20-30 kg P₂O₅ per hectare under optimum conditions depending up on the crop and agro-ecological conditions. Further, these crop benefiting bio-inoculants are known to secrete plant growth promoting substances and vitamins needed for improved crop growth and are also known to produce certain antibiotic substances, which help in suppressing the incidence of several root borne pathogens as well as foliar diseases. The consortium of biofertilisers has been proved to enhance crop yield in a sustainable way. The available information on the combined use (co-inoculation) of *Vesicular Arbuscular mycorrhiza*, *nitrogen fixing bacteria*, *phosphate solubilizing bacteria* and fungi have resulted in enhancement of soil fertility and thereby improved plant growth through their increased biological activity in the rhizosphere Subba Rao (1998). The role of VAM in the nutrition of agricultural and horticultural crops has received much attention (Tinker, 1978; Menge *et al.* 1977). Further, inoculation of soil with these organisms enriches rhizosphere microflora which can have a vital influence on plant growth through mineral uptake Sukhada (1988). Nutritional quality of any product grown under the influence of biofertiliser has been found to be significantly better (Pascale, S. De *et al.*, 1995). The production figures of chemical vis-a-vis biofertilisers in India from 1992-93 to 2010-2011 are indicated in table 1 below:-

TABLE 1: Production of Chemical and Bio-Fertilizer in India

Year	Production of Chemical Fertilizer (tonnes)	Production of bio fertilizer (tonnes)
1992-1993	12154.5	2005
1993-1994	12366.3	3084
1994-1995	13563.8	5800.5
1995-1996	13876.1	6692.3
1996-1997	14308.1	7406.6
1997-1998	16187.8	7104.6
1998-1999	16797.5	5972.1
1999-2000	18069.7	5716
2000-2001	16702.3	6242.7
2001-2002	17359.7	9019.2
2002-2003	16090	7181.7
2003-2004	16800	8701.4
2004-2005	18398	10479
2005-2006	20340	11752.4
2006-2007	21651	15871
2007-2008	22571	20111
2008-2009	24909	25065
2009-2010	24686	20040
2010-2011	28122	37997.61

Sources: Agriculture at a glance, Directorate of Economics & Statistics, Ministry of Agriculture, Govt. of India, FAI

Use of biofertilisers in Agricultural crops

In view of greater health concerns coupled with the increased cost of synthetic fertilizers, biofertilisers have been found to be cheap and economically viable alternative for sustainable crop production and they have found role in increasing the productivity of almost all the agricultural crops either directly or indirectly. Their role in mitigating the pathogen attack, reducing disease incidence or increasing germination rate of seeds has been proved beyond doubt.

They can be used to minimize the input cost involved in the use of chemical fertilizers and at the same time their use results in maintaining the soil health and fertility for sustainable agriculture which is more important for prolonged sustenance of mankind which are directly or indirectly associated with agricultural produce. The dual benefit of sustainable agriculture coupled with economisation of input cost holds a greater promise for the stakeholders who have less and fractured land holdings. As

such the use of biofertilisers in conjunction with minimal use of fertilizers is advocated.

VAM fungi are found associated with majority of agriculture crops with enhanced accumulation of plant nutrients. It has also been suggested that VAM stimulate plant growth by physiological effects or by reducing the severity of diseases caused by the soil pathogens (Gupta, 2004).

Application of mycorrhizal fertilisers particularly *Glomus fasciculatum* improved resistance of millet plant to drought stress and could compensate some of the effects of the drought stress (Ali Arab *et al.*, 2013). According to Sajedi and Madani (2008) mycorrhizal fertiliser significantly increased grain per row, number of grains per ear, ear weight and grain yield of corn. Tomato (*Lycopersicon esculentum* Mill.) seedlings treated with *Azotobacter*, *Azospirillum*, *Azotobacter* + *Azospirillum* indicated significantly high performance in whole plant dry weight (g/plant), plant height (cm) number of leaves/plant, number of fruits/plant,

yield/plant (g), average fruit weight/plant, chlorophyll and protein content. Dual inoculation proved to be highly influential (Ramakrishna and Selvakumar, 2012). Considerable nitrogen fixation by *Azotobacter* spp. and *Azospirillum* spp in the rice crop rhizosphere was reported repeatedly (Razie and Anas, 2008; Sison, 1999). Phytochrome production in case of rice and a beneficial effect on plant growth were also shown for a range of other microorganisms (Fernando *et al.*, 2010; Difuntorum *et al.*, 2006). *Trichoderma* spp. have been known for decades to increase plant growth and crop yield (Lindsay and Baker, 1967; Chang, *et al.*, 1986; Harman *et al.*, 2000), to improve crop nutrition and fertiliser uptake (Yedia *et al.*, 2001), to speed up plant growth and enhance plant greenness (Harman, 2006), as well as to control numerous plant pathogens (Cuevas *et al.*, 2005).

For various crops different biofertilisers have been recommended as indicated in Table 2.

TABLE 2:- Recommendations of different Bio-fertilizer for various crops

Bio-Fertilizer	Recommended Crops
<i>Rizobium</i>	Pluses, Oilseeds, Fodders
<i>Azospirillum</i>	Rice, Wheat, millets, maize, sorghum, sugarcane
<i>Azotobacter</i>	Rice, Wheat, millets, other cereals, cotton, vegetables, sunflower, mustered, flowers
<i>Blue Green algae</i>	Submerged rice
<i>Azolla</i>	Submerged rice with maximum temperature
<i>PSM</i>	All crops

Source: FAI, 2006-07

Another important characteristic of *Azotobacter* association with crop improvement is secretion of ammonia in the rhizosphere in the presence of root exudates, which help in modification of nutrient uptake by the plants (Narula and Gupta, 1986). Combined inoculation of biofertilisers *Azospirillum*, *Azotobacter* and phosphobacteria also recorded growth increase in black pepper (Bopaiah and K.B.A 1989). While *Rhizobium*, Blue Green Algae (BGA) and *Azolla* are crop specific, bio-inoculants like *Azotobacter*, *Azospirillum*, Phosphorus Solubilizing Bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad spectrum biofertilizers (Gupta, 2004). Examples of some free living nitrogen fixing bacteria are obligate anaerobes (*Clostridium pasteurianum*), obligate aerobes (*Azotobacter*), facultative anaerobes, photosynthetic bacteria (*Rhodobacter*), cyanobacteria and some methanogens. The example of K solubilizer is *Bacillus mucilaginosus* while for P-solubilizer are *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis* and *Pseudomonas straita*.

Sundara *et al.* (2002) observed that the application of phosphate solubilising bacteria (PSB), *Bacillus megatherium* var. *phosphaticum*, increased the PSB population in the rhizosphere and phosphorus availability in the soil. It also enhanced sugarcane growth, its yield and quality. When used in conjunction with P fertilizers, PSB reduced the required P dosage by 25%. In addition, 50% of costly superphosphate could be replaced by a cheap rock phosphate, when applied in combination with PSB. Young *et al.* (2003) studied the effects of a combined treatment of multifunctional biofertilizer plus 50% chemical fertilizer on

lettuce yield. From his results it is observed that there was a 25% increase of lettuce yield for the treatment of ½ chemical fertilizer plus biofertilizer compared to that of the chemical fertilizer treatment, indicating that at least 50% of chemical fertilizer can be saved as multifunctional biofertilizer was used along with chemical fertilizer.

Cyanobacteria are a diverse group of Prokaryotes, widely distributed in fresh water, marine and terrestrial environments. They are free-living photosynthetic bacteria which exist singly or in colonies and some of them are in filament forms (Curtis, 1992, Jacobson *et al.*, 1993). *Cyanobacteria* including *Anabaena* and *Nostoc* species possess normal vegetative cells and specialized heterocyst cells which are involved in photosynthesis and nitrogen fixation respectively (Meckerras *et al.*, 1990; Jacobson *et al.*, 1993; Ehira *et al.*, 2003). *Cyanobacteria* are capable of forming symbiotic association with many plants and fungi (Perter, 1990; Bergman *et al.*, 1992; Meeks and Elahim 2002). In association with plants, cyanobacteria fix atmospheric nitrogen and release organic matter, which is absorbed by plants (Rai *et al.*, 2000). *Cyanobacteria* were also used as biofertilizers in several crop plants (Venkataraman, 1986; Subba Rao, 1998; Gangawar and Thangavelu, 1992; Bose and Majumdar, 1999; Dasappa, 2000). For the last two decades, biofertilizers like *Azotobacter*, *Azospirillum*, *Vesicular Arbuscular Mycorrhiza* (VAM) and *Cyanobacteria* etc.; were used extensively to minimize the frequent use of chemical fertilizers, to improve the soil status and plant growth (Venkataraman, 1986; Subba Rao, 1988; Gangawar & Thangavelu, 1992; Das *et al.*, 1995;

Bose and Majumdar, 1999). El-Komy (2005) demonstrated the beneficial influence of co-inoculation of *Azospirillum lipoferum* and *Bacillus megaterium* for providing balanced nitrogen and phosphorus nutrition of wheat plants. The inoculation with bacterial mixtures provided a more balanced nutrition for the plants and the improvement in root uptake of nitrogen and phosphorus was the major mechanism of interaction between plants and bacteria. Co-inoculation of some *Pseudomonas* and *Bacillus* strains along with effective *Rhizobium* spp. is shown to stimulate chickpea growth, nodulation and nitrogen fixation. Findings of Mohammadi *et al.* (2010) showed that the highest sugar, protein, starch contents, nodule weight and seed nitrogen, potassium, phosphorus of chickpea were obtained from combined application of phosphate solubilizing bacteria, *Rhizobium* and *Trichoderma* fungus.

The fixed phosphorus in the soil can be solubilized by phosphate solubilizing bacteria (PSB), through the process of organic acid production, chelation and ion exchange reactions and make them available to plants. Therefore, the use of PSB in agricultural practice would not only offset the high cost of manufacturing phosphate fertilizers but would also mobilize insoluble in the fertilizers and soils to which they are applied (Banerjee *et al.*, 2010). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002). Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) constitute 1 to 50%, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5% in P solubilization potential (Chen *et al.*, 2006). Crop response to inoculation with symbiotic nitrogen fixer and phosphate solubilizing microorganisms has established their important role in supplementing nitrogen and phosphorus to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers (Tambekar *et al.*, 2009). Habibi *et al.* (2011) strongly suggested that using biofertilizers (combined strains) plus half a dose of organic and chemical fertilizers have resulted in the greatest grain yield and oil yield in medicinal pumpkin. They revealed that 50% of required nitrogen and phosphorus fertilizers could be replaced by bio and organic fertilizers, because bio and organic fertilizers improved the use efficiency of recommended nitrogen and phosphorus fertilizers and reduced the cost of chemical fertilizers, also prevented the environment pollution from extensive application of chemical fertilizers. Radhakrishnan (1996) reported that inoculation of *Azospirillum* and phosphor-bacteria resulted in higher root biomass and more bolls in cotton. Findings of Mohammadi (2010) showed that inoculation of biofertilizers (PSB+ *Trichoderma* fungi) + application of FYM had a great influence on *canola* growth, height and grain yield in compared to control treatment. The application of biofertilizers had significant effects on nutrient uptake of chickpea Mohammadi *et al.* (2011). Prajakta Patil *et al.* (2013) selected some crops such as Ground Nut, Paddy, Maize, Tomato, and Onion for various treatments of biofertilizers, vermicompost. *Rhizobium* etc. Seed treatment has been given to ground nut, *Pseudomonas* to maize while *Azotobacter* and *Azospirillum* to sorghum and paddy @ 250g/10 kg of seeds. It was concluded that Organic formulations and biofertilizers play an important

role in development of soil structure and nutrient availability which supports the entire plant growth system.

Single application of *Azotobacter* and *Mycorrhiza* inoculation and in combination to each other increased significantly spike per square meter in bread wheat cultivar as compared to without inoculation treatment. Maximum kernel weight was found in *Azotobacter* and *Azotobacter+Mycorrhiza* and minimum in control and *Mycorrhiza* treatments. Ammonium nitrate and *Azotobacter* + *Mycorrhiza* treatments gave significantly higher grain yield than the other N sources and biofertilizers. Biologic yield and harvest index were only affected by N sources treatments (Bahrani *et al.*, 2010).

Biofertilisers in mulberry

Mulberry is an important commercial crop grown extensively as a food plant for silkworm (*Bombyx mori* L.) a creature which famously produces very strong strands of protein which can be used to produce fibers and finally the silken fabrics. It continues to grow and produce leaf throughout the year under tropical conditions. The quality of mulberry leaf has profound influence on silkworm rearing. Mulberry fruit is also used commercially in jams, preserves, and fruit wines, among other things. Some cultivars have more interesting fruit than others; the best mulberry fruit is sweet and slightly tangy due to a mild acid content. The quality of leaf has a direct bearing upon the cocoon quality and quantity as well. It is said that 38.2 % of cocoon crop success is directly due to leaf quality fed to silkworms during the course of rearing. Continuous production of leaf for a longer time results in depletion of soil nutrients and unless these nutrients viz., NPK are replenished back into the soil system, the quality of leaf is bound to suffer. Farmers do not generally apply nutrients to soil in their right or recommended dosage viz., 300: 120: 120 kg NPK in the case of mulberry under temperate conditions either due to their non-availability, erratic availability or even because of the fact that they cannot afford to do so.

The co-inoculation of mulberry with phosphate solubilising micro-organisms, nitrogen fixing bacteria and *arbuscular mycorrhiza* has influenced its nutrient uptake through leaf. The data revealed that maximum nitrogen (484.12 kg/ha), phosphorus (59.83 kg/ha) and potassium 244.61 kg/ha uptake through leaf has taken place due to co-inoculation treatments as compared to uninoculated control (Baqual and Das, 2006). Significant beneficial effect was also recorded on growth and yield of V1 mulberry variety due to co-inoculation with microbial consortium containing phosphate solubilising micro-organisms, nitrogen fixing bacteria (*Azotobacter*) and VA-mycorrhiza even after curtailing nitrogen and phosphorus to the extent of 25-50% of the recommended dose (Baqual *et al.*, 2005). The dual inoculation of mulberry with phosphate solubilising microorganisms like *Bacillus megaterium*, *Aspergillus awamori* and nitrogen fixing bacteria *Azotobacter chroococum* under varying levels and sources of phosphorus and nitrogen revealed significant beneficial effect on fresh root biomass/sapling, total above ground biomass/sapling, leaf, stem and root nitrogen content of the saplings of V1 and S36 mulberry varieties (Baqual and Das 2012).The

beneficial effect of inoculation of mulberry plants with *Azotobacter* bio-fertilizer and *Vesicular Arbuscular mycorrhiza* have also been well documented by Das *et al.* (1994 a,b) and Katiyar *et al.* (1995).

Nitrogen is an essential major plant nutrient and is incidentally the most deficient nutrient resulting in lesser yield. In Kanva-2 variety of mulberry the application of *Azotobacter chroococum* resulted in 50% curtailment of nitrogen (Das *et al.*, 1994). Yadav and Kumar (1994) and Balasubramanian (1995) too reported the positive response of mulberry to the application of *Azotobacter* inoculants. Choudhury *et al.* (1995) and Bhogेशha *et al.* (1996) recommended the *Azotobacter* biofertiliser @ 20 kg/ha/yr with a growth promoting vipul @ 250 ml/ha/crop (2 splits) to increase the leaf yield of irrigated mulberry. Das *et al.*, 1996 observed the positive effect of application of *Azotobacter* to different cultivars of mulberry and found the highest response in the variety S⁵⁴ when it was applied with 150 kg N/ha/yr. This clearly demonstrates the differential response of mulberry genotypes to *Azotobacter*. Similarly protein content of mulberry leaf, moisture percentage as well as chlorophyll content also improved significantly due to co-inoculation (Baqual *et al.*, 2005). The uptake of nitrogen, phosphorus and potassium through mulberry leaf was increased significantly due to co-inoculation as compared to the un-inoculated plants (Baqual *et al.*, 2006 a). The cocoon parameters due to co-inoculation with microbial consortium also indicated improved trend (Baqual *et al.*, 2006 b). Incidence of major foliar diseases of mulberry caused by *Cercospora moricola*, *Phyllactinea corylea*, *Cerotelium fici*, *Fusarium plallidroseum* and *Pseudomonas syringae* was found to be effectively reduced when application of *Azotobacter chroococum* was applied (Sharma *et al.*, 1996). Further the inhibitory effect of *Azotobacter* species on phytopathogens is also well established (Sharma and Chahal, 1987, Pandey and Kumar, 1990). This inhibitory effect of *Azotobacter chroococum* on phyto-pathogens is found to be due to production of siderophores which are low molecular weight compounds (Neilands and Leong, 1986). The mulberry plants of both Kanva-2 and Mysore local varieties inoculated with *Azospirillum brasilense* @ 2 kg/ha and supplemented with 150 kg N/ha/yr increased the leaf yield and nitrogen upto 10% (Yadav and Kumar, 1989). Mulberry cuttings treated with *A. chroococum* and *Azospirillum brasilense* prior to planting increased the sprouting and rooting percentage of cuttings thereby ensuring the establishment of more mulberry cuttings (Gangawar and Thangavelu, 1992). Sannappa *et al.* (2005) and Raje Gowda (1996) observed that the application of organic fertilizers to mulberry had a significant influence on cocoon yield, shell ratio, silk productivity and single cocoon filament length.

Phosphorus being a master key element is an essential plant nutrient required for early establishment and better plant growth. Phosphorus exists in soil as inorganic and organic phosphates. The inorganic forms are compounds of Ca, Fe, Al etc. The organic phosphorus containing compounds are derived from plants animals and micro-organisms. Plants are able to absorb phosphorus in available form. The important

P. solubilising microorganisms are *Aspergillus awamori*, *Bacillus megaterium*, *B. polymixa* and *Pseudomonas striata*. The utility of phosphorus solubilising micro-organisms in mulberry is tremendous. *Cyanobacterial* biofertilizer (CBB) along with and without chemical (NPK) fertilizer on soil nutrients and leaf quality traits of mulberry indicated increased soil nutrients with decreased soil PH, CBB treated plots as compared to control plots. Further, the study also revealed that 50% reduction in the dose of chemical fertilizer can be compensated by the addition of higher dose of CBB (Ram Rao, *et al.*, 2009). However, very few reports are available exclusively on the use of *cyanobacteria* as biofertilizers for mulberry (Bose and Majumdar, 1999 and Dasappa 2000).

Mycorrhizal plants of mulberry are found to be superior in survivability, plant growth, biomass production and leaf quality in comparison to non-mycorrhizal plants (Fatima *et al.* 1996). Umakant *et al.* (1998) working on the response of mulberry saplings to inoculation with VAM and *Azotobacter* reported that dual inoculation of nursery bed with *Glomus fasciculatum* and *Azotobacter chroococum* considerably increased the plant growth and mulberry sapling development. Further, inoculation of soil with these organisms enriches rhizosphere microflora which can have a vital influence on plant growth through mineral uptake Sukhada (1988). The available information on the combined use (co-inoculation) of *Vesicular Arbuscular Mycorrhiza*, nitrogen fixing bacteria, phosphate solubilizing bacteria and fungi have indicated their use as enhancement in soil fertility and thereby improved plant growth through their increased biological activity in the rhizosphere Subba Rao (1998). Mulberry plants, whose leaf is exclusively used for rearing silkworms (*Bombyx mori* L.) for subsequent cocoon production, have indicated their positive response towards the application of biofertilisers. The beneficial effect of inoculation of mulberry plants with *Azotobacter* bio-fertilizer and *Vesicular Arbuscular mycorrhiza* have been well documented by Das *et al.*(1994 a,b), Katiyar *et al.* (1995) and Baqual *et al.* (2005). In India, the mulberry is grown in abundance, mostly in the tropical belt where most of the regions are dominated by soils that are low in available nutrients and moisture Osonubi *et al.* (1991). Therefore, it becomes imperative to use low cost biofertilisers with added advantages in terms of crop production and sustainability of sericulture. Integrated organic manures packages of practices which included *Azotobacter*, VAM, seriphos, vermicompost and green manure application to V-1 mulberry and in turn feeding the leaves to CSR2 and CSR4 bivoltine silkworm breeds resulted to improved rearing and grainage parameters of silkworms similar to the standard fertilizers application (Jagadeesh *et al.*, 2005).

Association of fungus with plants roots is termed as mycorrhiza and among the different types of mycorrhiza VAM (*Vesicular arbuscular mycorrhiza*) is the most important as it occurs among the wider range of host. It is observed that about 90% of the vascular plants are mycorrhizal. They form beneficial associations with almost all the crops irrespective of the environment under which

they grow and due to their dependence for carbon, they enter into symbiosis with almost any kind of host and render it resistant to various biotic and abiotic stresses (Dehne, 1987). A plant may have more than one mycorrhiza that can colonize and form symbiosis with it. Among the mycorrhiza, most common association is the vesicular-arbuscular mycorrhiza (VAM) type, which produces fungal structures (vesicles and arbuscules) in the cortex region of the root. The benefits of VAM fungi include:

- Improved uptake of nutrients
- Increased tolerance to abiotic and biotic stress
- Beneficial alterations to plant growth regulators and synergistic interactions.

Muthukrishnan *et al.* (1981) noticed the occurrence of VA mycorrhiza in the feeder roots of mulberry. Ambika *et al.* (1994) observed the occurrence of five genera of VAM (*Glomus*, *Acaulospora*, *Sclerocystis*, *Gigaspora*) in the rhizosphere of different genotypes of mulberry. Baqual *et al.* (2006 c) also observed association of VAM fungi in the rhizosphere of mulberry under temperate climatic conditions. Das *et al.* (1995 a) revealed significantly higher yield of mulberry in Kanva-2 due to *Glomus mosseae* inoculation and application of rock phosphate @ 30 kg/ha/yr. This indicated the possibility of saving 75% of P input through VAM. The nursery inoculated with *G. mosseae* had tremendous improvement of growth development and survivability of mulberry saplings (Das *et al.*, 1995 b). The dual inoculation of mulberry with *Azotobacter* and VAM and application of 50% NPK enhanced the growth and yield under irrigated conditions (Katiyar *et al.*, 1996). Fatima *et al.* (1996) reported significant increase in cocoon yield due to inoculation of mulberry with *Glomus mosseae* and 30 kg P/ha/yr. Mulberry saplings inoculated with VAM (*G. fasciculatum* and *Azotobacter chroococum*) exhibited considerable increase in plant growth and development (Umakanth and Bagyaraj, 1998). The role of VAM as biocontrol agent against plant pathogens in different crops has also been elucidated by many workers (Sampanghi and Bagyaraj, 1989). The reduction of disease incidence in mulberry was encountered in those plants inoculated with VAM (*G. fasciculatum* in combination with 60 or 9 kg P/ha/yr (Sharma, *et al.*, 1995). Mycorrhizal inoculation in case of mulberry also indicated highly significant amounts of chlorophyll a and b (Baqual *et al.*, 2005). These biofertilizers are reported to be ecofriendly economical and beneficial in mulberry (Das *et al.*, 1995; Reddy *et al.*, 2000 and Dasappa, 2000).

How to use biofertilisers in mulberry

a).AZOTOBACTER

The high fertilizer requirement in mulberry cultivation especially nitrogen (300 kg N/ha/year) is leading to increased cost of its cultivation. *Azotobacter* biofertilizer, a cost effective supplement to chemical nitrogenous fertilizers in mulberry cultivation, is recommended to make sericulture more profitable. Besides, it reduces the deleterious effect of chemical fertilizers on soil health and also reduces the water pollution from nitrate contamination through leaching.

Recommendation

- Apply 20 kg *Azotobacter* biofertilizer/ha/year (to compensate 150 kg nitrogen) in 5 split doses @ 4 kg each time after every leaf harvest/pruning and intercultural operations.
- Use phosphorous and potash @ 120 kg/ha/year each in 2 split doses, as per recommendation for irrigated mulberry.
- Farmyard manure (FYM) should be applied @ 20 tonnes/ha/year as recommended for irrigated mulberry. Apply only 150 kg nitrogen/ha/year instead of 300 kg nitrogen in 5 equal split doses @ 30 kg each time after every leaf harvest/pruning and intercultural operations. The mixed culture of VA-mycorrhiza containing spores of *Glomus fasciculatum* and *Glomus mosseae* is applied to mulberry garden by intercropping technique with maize as mycorrhizal host (Katiyar *et al.* 1998).

b).VAM (Vesicular arbuscular mycorrhiza)

Furrows of 10 cm depth are first opened between alternate mulberry rows adjacent to the plant roots and soil based mycorrhizal inoculum is introduced @ 1000 kg /ha having about 10-15 spores /g of inoculum as thin layer by placing in furrows. Maize seeds are sown on the thin layer of inoculum in such a way that a proper contact between maize seeds and the inoculum is established when the seeds are germinated. The furrows are then closed and irrigated. The seeds are allowed to germinate and grown as maize plants between the rows of mulberry. The plants are allowed to grow for a period of one month till the roots of maize develop properly and establish contact with the VAM inoculum placed below. After one month the maize plants are cut at the middle portion with the help of a sickle so as to check the growth and competition of the maize plants with mulberry. In this technique roots of maize plants are colonized quickly by VA-mycorrhiza as soon as the roots come in contact with the VAM inoculum and the population of VA-mycorrhiza increases in the rhizosphere of maize plants.

Points to be considered during biofertiliser application:

- Biofertilizer product must contain good effective strain in appropriate population and should be free from contaminating microorganisms.
 - Select right combination of biofertilizers and use before expiry date.
 - Use suggested method of application and apply at appropriate time as per the information provided on the label.
 - For seed treatment adequate adhesive should be used for better results.
 - For problematic soils use corrective methods like lime or gypsum
 - Ensure the supply of phosphorus and other nutrients.
- #### Precautions for using biofertilizers:
- Right combinations of biofertilizers needs to be used.
 - Biofertilizer packets are stored in cool and dry place away from sun light.
 - Cop specific, biofertilisers be used.

- Other chemicals should not be mixed with the biofertilizers.
- Sufficient gap be given between the application of biofertilisers and chemical fertilizers.
- Use of expired biofertilisers should be avoided.
- Both nitrogenous and phosphatic biofertilizers are to be used to get the best results along with organic manures.

Probable reasons for not getting response from the application of biofertilizers:

On account of quality of product

- Use of ineffective strain.
- Insufficient population of microorganisms.
- High level of contaminants.

On account of inadequate storage facilities

- May have been exposed to high temperature.
- May have been stored in hostile conditions.

On account of usage

- Not used by recommended method in appropriate doses.
- Poor quality adhesive.
- Used with strong doses of plant protection chemicals.

On account of soil and environment

- High soil temperature or low soil moisture.
- Acidity or alkalinity in soil.
- Poor availability of phosphorous.
- Presence of high native population.

In order to maintain soil health and to keep it fertile, it is of greater relevance that balanced approach towards application of fertilisers, bio fertilisers and other organic matter be adopted for the sustainable crop growth. This will not only reduce the burden of stakeholders but will also in long run improve our soil microbial complex for sustainable agriculture.

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