



EFFECT OF CYANOBACTERIAL BIOFERTILIZER ON SOIL NUTRIENTS AND MULBERRY LEAF QUALITY AND ITS IMPACT ON SILKWORM CROPS

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Abstract: *The present field experiment was conducted to study the effect of cyanobacterial biofertilizer (CBB) along with and without chemical (NPK) fertilizer on soil nutrients and leaf quality traits of mulberry. Eleven CBB treated mulberry plots (T1 to T12) and one control plot (T12) were maintained and data on soil pH, soil nutrients and leaf quality traits were recorded in each of these experimental plots for every crop. The soil nutrients were increased whereas; the soil pH was decreased in different CBB treated plots as compared to control plot. Among different CBB treatments, T9 (Av + Nm + Sm (120kg/ha/crop)+ 50% NPK) exhibited superior performance with regard to leaf quality traits and was found on par with control 772 (100% NPK). Bioassay study also revealed no significant difference in silkworm growth and cocoon characters between treatments T9 and T12. Overall results showed that T9 was very effective in terms of improvement in soil nutrients and leaf quality traits in mulberry. Further, it is also clear from the study that 50% reduction in the dose of chemical fertilizer can be compensated by the addition of higher dose of CBB. Therefore, CBB can be recommended to the sericulture farmers with an intension to improve the soil fertility condition and leaf quality traits besides saving 50% cost of chemical fertilizers.*

Keywords: *Cyanobacteria, mulberry leaf quality, leaf yield, soil nutrients and cocoon characters*

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INTRODUCTION

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). The most common filament forms of Cyanobacteria are *Anabaena* and *Nostoc* species as these Cyanobacteria possess normal vegetative cells and specialized heterocyst cells which are involved in photosynthesis and nitrogen fixation respectively (Meckerrase *Jal.*, 1990; Jacobson *et al.*, 1993; *Ehira et al.*, 2003; Huang *et al.*, 2005). Cyanobacteria are capable of forming symbiotic association with many plants and fungi (Perter, 1990; Bergman *et al.*, 1992; Meeks and Elahi, 2002). In association with plants, Cyanobacteria fix atmospheric nitrogen and release organic matter, which is absorbed by plants (Gantar *et al.*, 1995; Rai *et al.*, 2000). Cyanobacteria were also used as biofertilizers in several crop plants (Venkataraman, 1986; Subba Rao, 1988; 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 and Thangavelu, 1992; Das *et al.*, 1995; Bose and Majumdar, 1999). These biofertilizers were reported to be ecofriendly, economical and beneficial in mulberry (Das *et al.*, 1995; Reddy *et al.*, 2000 and Dasappa, 2000). However, very few reports are available exclusively on the use of cyanobacteria as biofertilizers for mulberry (Bose and Majumdar, 1999 and Dasappa 2000). Also the earlier scientists have not emphasized much on the influence of CBB on soil nutrients and leaf quality traits of mulberry. Therefore, the present investigation was carried out to assess the efficiency of cyanobacterial biofertilizers along with or without chemical fertilizer on soil status, leaf yield and leaf quality traits of mulberry and their influence on silkworm rearing parameters under tropical conditions of Mysore.

MATERIALS AND METHODS

Field experiment was conducted in the farmer's field of Budiguppe village, Kanakapura Tq Ramanagara district, Karnataka, India, to analyze the effect of cyanobacterial biofertilizer (CBB) along with and without chemical fertilizer (NPK) on leaf yield and leaf quality traits of



mulberry. Initially, the soil nutrient status viz., soil pH, organic carbon (OC), total nitrogen (N), available phosphorus (P), available potassium (K) and cyanobacteria spp. were recorded before conducting the experiment. For the study, three different doses of CBB (i) 90 kg/ha/crop, (ii) 105 kg/ha/crop and (iii) 120 kg/ha/crop were selected based on the earlier experimental works (Bose and Majumdar, 1999; Dasappa, 2000). Likewise three different doses of chemical fertilizers i.e., (i) 25% NPK: 15:6:6 kg/ha/crop, (ii) 50% NPK: 30:12:12 kg/ha/crop (iii) 100% NPK: 60:24:24 kg/ha/crop (recommended dose of CSR&TI, Mysore) were used. Three species of cyanobacteria viz., *Anabaena variabilis* (Av), *Nostoc muscorum* (Nm) and *Scytonema millei* (Sm) were used as CBB in the study. The pure cultures of these cyanobacteria in slants were obtained from Indian Agricultural Research Institute (IARI), New Delhi; they were sub-cultured and multiplied in the laboratory of the Department of Botany, University of Mysore, India. The large volume of multiplied cyanobacteria were lyophilized and made into powder to avoid secondary contamination. These lyophilized cyanobacteria were mixed individually with farmyard manure (FYM) and made as cyanobacterial biofertilizer (CBB). The field was kept wet for few days immediately after the application of CBB. Further, irrigation schedule was properly maintained in all the experimental plots. The application of CBB to the experimental plots was done by following the standard procedure (Venkataraman, 1972; Anonymous, 1978).

For the study, single and combination treatments of CBB and chemical fertilizers were computed such as:

- T1: Av (90kg/ha/crop) + 50% NPK;
- T2: Nm (90kg/ha/crop) + 50% NPK;
- T3: Sm (90kg/ha/crop) + 50% NPK;
- T4: Av + Nm (90kg/ha/crop) + 50% NPK;
- T5: Av + Sm (90kg/ha/crop) + 50% NPK;
- T6: Nm + Sm (90kg/ha/crop) + 50% NPK;
- T7: Av + Nm + Sm (90kg/ha/crop) + 50% NPK;
- T8: Av + Nm + Sm (105kg/ha/crop) + 50% NPK;
- T9: Av + Nm + Sm (120kg/ha/crop + 50%NPK);
- T10: Av + Nm + Sm (120kg/ha/crop + 25%NPK);
- Til: Av + Nm + Sm (120kg/ha/crop) without NPK and
- T12: 100% NPK (Control).



Two years old (0.20 ha.) M5 mulberry garden was selected and from this garden a total of 2400 plants grown with a spacing of 60cm x 60cm were taken for the study. For each treatment, 4 replications with 50 plants /replication were maintained in randomized block design (RED). Initially the plants were pruned 30 cm above the ground level and CBB was applied 15 days after pruning. Cultural operation and application of FYM was done as per the norms of CSR&TI, Mysore (Krishnaswami, 1978). After 70 days of pruning, 20 plants were randomly selected replication- wise, from each of twelve treatments and leaf yield data were recorded for ten crops. The average of ten crops data on leaf yield was calculated treatment wise and converted to ton/ha/year.

From different CBB treated and control plots mature leaves (12th - 14th leaf from top) were collected replication / treatment wise and different leaf quality traits were estimated. Leaf Moisture Content (LMC%) and Moisture Retention Capacity (MRC%) after 6 hrs from harvest were estimated by following the method of Vijayan *et al.* (1996). Total chlorophyll content (mg/g of fresh weight) was estimated by adopting the procedure of Hiscox and Israelstam (1979). Nitrate reductase (NR) activity was also estimated in mature leaves (Scot and Neyra, 1979). Leaf samples collected were dried and used in triplicate for analysis of total protein content following Lowry *et al.* (1951), total amino acid content estimated by adopting ninhydrin method using leucine as standard (Spies, 1955). Nitrogen content was estimated by micro-kjeldahl method (Jackson, 1973). Sugar and starch contents (soluble carbohydrate) were estimated by the method of Dubios *et al.* (1956) and Me. Cready *et al.* (1960) respectively. Further, to confirm the efficiency of CBB on leaf quality traits, a bioassay study was conducted with silkworm race PM * NB4D2 for three different seasons. Leaves from CBB treated (T9) and control (T12) plots were utilized for feeding the silkworm until spinning. At three different seasons, rearing data *viz.*, larval duration, weight of 10 mature larvae (g), Effective Rate of Rearing (ERR) by number and by weight, Single cocoon weight, Single shell weight and Shell ratio (%) were recorded separately, later average of three seasons data was calculated and analyzed statistically (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The influence of different treatments of CBB with and without chemical fertilizers on soil nutrient parameters and on leaf quality traits was assessed and summarized in Table I a; i II. The soil pH in control (T12) plot was 8.2, but it was decreased to 7.3 to 8.0 in different CBB



(T1 - T11) treated plots. It was reported that soil pH decreased in the cyanobacteria treated plot (Dasappa 2000). Cyanobacteria can change the soil pH towards neutrality (Hashem, 2001). Likewise, in control plot, the soil nutrients i.e., Organic carbon (OC), Nitrogen (N), Phosphorus (P) and Potassium (K) were 0.36%, 0.033%, 7.35kg/ha and 174.20kg/ha respectively. Whereas, in different CBB treated plots, the nutrients were found increased and the range of increase in the nutrients are, OC: 0.38 - 0.52%; N: 0.034 - 0.054%; P: 7.46-11.46kg/ha and K: 151.83 - 194.37kg/ha. The increase in soil nutrients was certainly due to the influence of CBB. Cyanobacteria produce extra cellular polymers of diverse chemical composition, especially exopolysaccharides that enhance microbial growth and as consequence, improve soil structure and exoenzyme activity (Caire *et al.*, 1997). In the present study, soil nutrients were high in T9; this was mainly due to the higher dose of CBB with 50% NPK fertilizers. In contrast, all the nutrients were low in T1 plot, probably due to total avoidance of chemical fertilizer in the treatment. It was observed that application of higher dose of CBB alone (T1) proved less effective in improving the soil nutrients. Cyanobacteria not only maintain the soil pH but also help to enhance soil nutrients, retain soil moisture and liberate different hormones that stimulate plant growth (Hashem, 2001; Meeks and Elahi, 2002).

Leaf quality traits:

The growth of silkworm (*Bombyx mori* L.) and production of quality cocoons depends on timely feeding of good quality mulberry leaves to silkworm. Therefore, it is most imperative to analyze different leaf quality traits such as leaf moisture content (LMC), moisture retention capacity (MRC), total chlorophyll, nitrogen, protein, amino acids and carbohydrate contents. The leaf quality traits estimated in different CBB treated (T1 -T11) and control (T12) plots were represented in Table II. LMC and MRC are two important factors in maintaining the nutrition level in mulberry leaves, which in turn improve its palatability for silkworm. These two traits are influenced by genetic and environmental factors (Vijayan *et al.*, 1997) and are also positively related with an increased growth of silkworm larvae (Paul *et al.*, 1992). In the present study, LMC and MRC were high in T9 (73.5% and 75.0% respectively) and were on par with control T12 (73.6% and 75.2%).



Table I. Effect of different CBB Treatments on soil nutrients

Treatment	Soil Status (pH)	Organic Carbon (%)	Total Nitrogen (%)	Available Phosphorus (Kg/ha)	Available Potassium (Kg/ha)
T1 : Av (90kg/ha/crop) + 50% NPK	7.6	0.42	0.042	8.43	178.41
T2: Nm (90kg/ha/crop) + 50% NPK	7.9	0.44	0.044	8.44	180.56
T3 : Sm (90kg/ha/crop) + 50% NPK	7.8	0.43	0.042	8.32	176.77
T4 : AV + Nm (90kg/ha/crop) + 50% NPK	7.5	0.44	0.044	8.22	175.53
T5 : AV + Sm (90kg/ha/crop) + 50% NPK	8.1	0.43	0.043	8.66	176.33
T6 : Nm + Sm (90kg/ha/crop) + 50% NPK	7.4	0.45	0.056	8.72	177.22
T7 : Av + Nm + Sm (90kg/ha/crop) + 50% NPK	7.3	0.56	0.047	8.60	176.55
T8 : Av + Nm + Sm (105kg/ha/crop) + 50% NPK	7.6	0.66	0.055	9.24	189.66
T9 : Av + Nm + Sm (120kg/ha/crop) + 50% NPK	7.5	0.54	0.045	11.22	195.32
T10 : AV + Nm + Sm (120kg/ha/crop) + 50% NPK	7.8	0.66	0.044	9.45	178.56
T11 : Av + Nm + Sm (120kg/ha/crop)	7.4	0.48	0.043	7.66	152.56
T12 : 100% NPK (Control / Temoin)	8.1	0.35	0.056	7.45	173.23
CD at 5%	NS	0.08	0.047	2.05	9.12



Table II. Effect of different CBB Treatments on leaf quality traits of mulberry

Treatment	LMC (%)	MRC (%) (after 6 hrs of harvest)	Total Chlorophyll (mg/g.f.wt0)	Protein (%)	Nitrogen (%)	Sugar (%)	Starch (%)	Total amino acid (mg/g/dry wt.)	NR activity ($\mu\text{g NO hr/g}$)	Leaf yield (ton/ha/yr)
T1 : Av (90kg/ha/crop) + 50% NPK	70.4	72.66	2.43	18.56	3.12	9.74	10.66	202.5	7.6	28.67
T2: Nm (90kg/ha/crop) + 50% NPK	71.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T3 : Sm (90kg/ha/crop) + 50% NPK	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	30.67
T4 : AV + Nm (90kg/ha/crop) + 50% NPK	70.4	72.66	2.43	18.56	3.12	10.74	10.66	202.5	7.6	28.67
T5 : AV + Sm (90kg/ha/crop) + 50% NPK	72.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T6 : Nm + Sm (90kg/ha/crop) + 50% NPK	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	31.67
T7 : Av + Nm + Sm (90kg/ha/crop) + 50% NPK	72.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T8 : Av + Nm + Sm (105kg/ha/crop) + 50% NPK	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	31.67
T9 : Av + Nm + Sm (120kg/ha/crop) + 50% NPK	73.5	75.2	2.87	20.55	4.12	12.66	11.22	234.6	9.5	35.6
T10 : AV + Nm + Sm (120kg/ha/crop) + 50% NPK	71.4	72.66	2.43	17.56	3.12	8.74	10.66	202.5	7.6	30.67
T11 : Av + Nm + Sm (120kg/ha/crop)	72.4	71.66	2.43	19.56	3.12	10.74	10.66	205.5	7.6	30.67
T12 : 100% NPK (Control / Temoin)	70.4	72.66	2.43	18.56	3.12	10.74	10.66	202.5	7.6	28.67
CD at 5%	NS	0.31	0.12	0.06	0.07	0.05	0.20	2.30	1.21	0.60

Table III. Rearing performance and Cocoon characters of silkworm in T9 and T12.

Treatment	Larval duration (days)	Wt. of 10 mature larvae (g)	ERR		Single Cocoon Wt.	Single Shell Wt.	Shell Ratio (%)
			By No.	By Wt.			
T9 : Av + Nm + Sm (120 kg/ha/crop) + 50% NPK	28.07 \pm 0.98	40.97 \pm 0.62	8197 \pm 32.6	13.95 \pm 1.96	1.687 \pm 0.09	0.298 \pm 0.09	17.66 \pm 1.36
T12 : 100% NPK (control / temoin)	28.12 \pm 1.16	40.55 \pm 1.54	8015 \pm 54.6	13.32 \pm 2.32	1.625 \pm 0.16	0.284 \pm 0.08	17.48 \pm 2.56
t value /	NS	NS	2.14*	2.47	NS	NS	NS



Av : Anabaena variabilis; Nm : Nostoc muscorum, Sm : Scytonema milleio; NPK : Chemical fertilizers / Engrais chimiquet, Kg / ha / crop / kg / ha / , ERR : Effective rate of rearing.

The leaf quality is usually determined based on higher LMC and MRC (Bongale and Chaluvachari, 1995; Sujathamma and Dandin, 2000). Further in T9, the reduced dose (50%) of chemical fertilizer did not affect the LMC and MRC. This may be due to the influence of CBB, which had mediated the moisture availability in the soil rhizosphere, thereby maintaining normal growth, water uptake and other metabolism in plants.

Chlorophyll content is an important quality trait, which determines the photosynthetic efficiency of a variety. Total chlorophyll content was high in T9 (2.87mg/gf.wt) and found on par with T12 (2.87mg/gf.wt.), while it was low in T1 (2.23mg/gf.wt.). The increased chlorophyll content in T9 was mainly due to higher dose of CBB along with 50% NPK fertilizers. It was reported that higher chlorophyll content in leaves indicates the photosynthetic efficiency in mulberry (Sujathamma and Dandin, 2000). Low chlorophyll content in T1 clearly indicates that higher dose of CBB alone may not be able to supply the required nutrients for the normal growth and physiological activities of plants.

Next to leaf moisture content, total nitrogen and amino acid contents are the two important traits, which determine the leaf quality in mulberry (Machii, 1989). These two traits were high in T9 (4.12% and 234.6mg/g.dry wt.) and T12 (2.43% and 202.5mg/g.dry wt.), while these traits were low in T1 (2.84% and 176.9mg/g dry wt.). It was reported that varieties possessing higher nitrogen and amino acid contents in leaves are nutritively superior and positively related to growth and development of silkworm (Machii and Katagiri, 1991; Suryanarayana and Shivashankar Murthy, 2002). In case of T9 though the recommended dose of NPK fertilizers was reduced to half, the total nitrogen and amino acids contents were not affected, this may be due to attributed influence of CBB on the availability of mineral elements in the soil and their translocation in to the plant system, which later in turn enhance the synthesis of amino acids and other hormones etc., in the treatments.

Among the eleven CBB treatments, total protein content was found comparatively high in T9 (20.55%) and was on par with control T12 (20.56%). According to Horie (1980), dietary protein level should be 20-25%, which is required for optimal growth of silkworm larvae. The lower or reduced use of chemical (NPK) fertilizer in T9 did not affect the available quantity of protein content in the leaves; this may be due to influence of CBB which had



compensated the reduced dosage of chemical fertilizers in the treatments. In mulberry leaves, carbohydrates are available in plenty and it was reported to be the main source of energy for silkworm (Hiratsuka, 1917 and Horie, 1978). The quantity of carbohydrate is determined based on the quantity of total sugar and starch available in leaves (Bose and Bindroo, 2001). Sugar and starch content were high in treatment T9 (12.66% and 11.22%) and was almost equal to that of control (12.44% and 11.48%). The results revealed that CBB had pronounced influence on biosynthesis of carbohydrates in the leaves. The low dose of NPK fertilizers in T9 was compensated efficiently by the addition of higher dose of CBB in the treatment.

Nitrate reductase (NR) activity is one of the most important regulatory enzymes in nitrogen metabolism, which catalyses the reduction of nitrate to nitrite, a rate limiting step in the utilization of nitrates in plants (Deckard *et al*, 1973). NR activity was the highest in T9 (9.5ugNo₂/hr/gf.wt.) and lowest in T1 (7.6 ugNo₂/hr/gf.wt). NR activity in T9 was almost equal to that of control T12 (10.4ugNo₂/hr/gf.wt). In the present study, 50% curtailment in the recommended dose of chemical fertilizer did not affect the NR activity in T9 (Table I). This may be probably due to higher dose of CBB in T9, which had compensated the lower (50%) use of chemical fertilizer. Similarly, among the CBB treatments, leaf yield was found high in T9 (35.6 ton/ha/yr), while it was low in T1 (28.67 tons/ha/yr). In the present study, T9 besides showing high NR activity also showed high leaf protein, nitrogen and amino acids. It was reported that NR activity is significantly correlated with leaf protein, leaf yield and other yield attributing characters, thus it can be used as an additional parameter for identifying the superior mulberry genotypes (Rao *et al.*, 2000).

From the study, it is understood that application of reduced (50%) dose of chemical fertilizers can be compensated by applying the required dose of CBB and due to the reason, leaf quality traits in T9 was found on par with T12 (control). In cyanobacteria, nitrogen / carbon fixation mechanism is a unique biochemical process wherein the N fixation is mediated by the enzyme nitrogenase, which is well protected in the heterocyst cell (Hashem, 2001 and Ehira *et al.*, 2003). It is a thick walled, large transparent cell, occurs at regular intervals amidst series of vegetative cells of Cyanobacteria. In symbiosis with plants, heterocyst cell formation in cyanobacteria increases 3 to 10 fold (Meeks and F.lahi, 2002) and this leads to the enhancement of nitrogen fixation process. The fixed nitrogen is first



reduced to ammonium in the heterocyst, later it is converted to glutamate and then passes as amino acids to adjacent vegetative cells (Thomase 1977). In return fixed carbon, probably sucrose flows from vegetative cells to heterocyst cells (Wolk, 1968; Schilling and Ehrensperger, 1985). Cyanobacteria during their intercellular biochemical processes secrete several nutrients, hormones and vitamins etc., which are absorbed by the plants.

Silkworm rearing performance:

The leaves from CBB treated (T9) and control (T12) plots were collected and used for bioassay study to analyze the influence of CBB on silkworm growth and cocoon characters. The average of three seasons rearing data recorded on silkworm growth and cocoon characters are summarized in Table III. In general, significant differences were not observed in rearing parameters between T9 and T12. However, a marginal improvement on silkworm growth and cocoon characters was recorded in T9 as compared to control (Table III). This clearly indicates the efficiency of CBB, which had compensated the lower use of fertilizers by not only fixing the atmospheric N, but also synthesizing required nutrients, vitamins, amino acids, hormones etc., which helps to improve the growth, metabolism and physiological activity of the host plants, with the result leaf quality might have been improved and thus feeding on such quality leaves maintained the normal growth and cocoon characters of silkworm.

Overall study reveals that mixture of three combination species of CBB treatment was found more effective than combination of two or single species CBB treatment. The highest dose of CBB (120kg/ha/crop) with 50% NPK fertilizers in T9 was found effective and showed superior performance with regard to leaf yield, leaf quality and cocoon parameters. In case of T10, the similar highest dose of CBB (120kg/ha/crop) with 25% NPK fertilizers exhibited moderate performance. Finally, the highest dose of CBB (120kg/ha/crop) without NPK fertilizers in T11 showed the least performance with respect to leaf yield and leaf quality traits probably due to total avoidance of chemical fertilizers in the treatment. In case of T8, CBB dose was slightly reduced (105kg/ha/crop) but chemical fertilizer dose was increased when compared to T10 and due to the reason T8 showed better performance than T10. It is clear from the study that higher dose of CBB along with NPK fertilizers to an extent of 50% is essential for maintaining the normal growth, yield and metabolism in plants. The present study confirms the earlier reports that mixture of three species of CBB combination proved



to be the most effective in terms of maintaining the soil pH and improvement in soil environment, leaf yield, leaf quality and also in economizing the chemical fertilizer application by 50 % (Dasappa, 2000). Cyanobacteria cheap source of N do not cause pollution, improve soil fertility condition, water-holding capacity and maintain biodiversity of soil (Hashem, 2001)." Therefore, CBB can be recommended to the sericulture farmers as cost effective and eco-friendly approach to sustain the productivity of both mulberry as well as cocoons. However the major constraint lies in culturing/ production of CBB in large quantities in the laboratory and supply to the farmers.

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