

Efficacy of Cyanobacterial Biofertilizer (CBB) on Leaf Yield and Quality of Mulberry and its Impact on Silkworm Cocoon Characters

Dasappa, D. M. Ram Rao* and S. N. Ramaswamy

Central Sericultural Research and Training Institute, Mysore - 570 008, Karnataka, India.

(Received 24 January 2006; Accepted 1 June 2006)

An experiment was conducted to study the efficiency of cyanobacterial biofertilizer (CBB) with chemical (NPK) fertilizer on quantitative and qualitative characters of mulberry variety Kanva-2. Their influences on silkworm growth and cocoon characters were also studied. Ten different CBB and NPK fertilizer treatments were given to 5000 plants of established mulberry garden. Treatments were of four types viz., (i) T1 to T7: single and combination dose of CBB+50% NPK (ii) T8: combination dose of CBB+25%NPK, (iii) T9: CBB only and (iv) T10: control-100% NPK. Soil pH decreased and nutrients status increased in CBB (T1-T9) treated plots. Average of ten crops data on quantitative traits revealed that T7 (CBB [N. muscorum (1.0 g), A. variabilis (1.0) and S. millei (1.0 g)] + 50% NPK) was very effective in improving growth parameters. Leaf yield was also found high in treatment T7 (32.12 tons/ha/yr.) followed by T10 (31.17 tons/ha/yr.) and T8 (27.67 tons/ha/yr.). Leaf quality characters were found high in T7 and low in T9. Most of the quality traits in T7 are on par with control T10. The results revealed that reduction in the dose of chemical fertilizers in T7 did not affect the leaf yield and leaf quality traits of mulberry. This clearly indicates that the efficiency of CBB (T7) provides nitrogen, increases essential nutrients available in soil, maintain soil pH and supply growth substances required for the improvement of leaf yield and leaf quality of mulberry. Bioassay study also revealed no significant difference in silkworm growth and cocoon characters between treatments T7 and T10. Economics calculated revealed that T7 is highly economical and beneficial over T10

by gaining an amount of Rs. 660/-/acre/crop. Thus, treatment T7 containing N. muscorum (1.0 g), A. variabilis (1.0 g) and S. millei (1.0 g) + 50% NPK fertilizers can be recommended to sericulturists mainly to reduce the use of NPK fertilizers, by saving 50% of its cost and to improve soil fertility conditions, which in turn improves leaf yield and quality of mulberry.

Key words: Cyanobacterial biofertilizers, Chemical fertilizers, Mulberry leaf yield, Leaf quality, Cocoon characters

Introduction

Cyanobacteria (Blue green algae) are free living photosynthetic bacteria contain chlorophyll, carotenoids and some unusual accessory pigments such as phycobilins. They are widely distributed in fresh water, marine and terrestrial environments. Cyanobacteria occur singly or in colonies and often grow under extreme environmental conditions such as high temperature and salinity (Jacobson *et al.*, 1993). Some of them exists in the form of filaments, (*Anabaena* and *Nostoc* species) which possess a specialized cells called heterocyst, arise from normal vegetative cells of cyanobacterium in response to low levels of reduced nitrogen (Rai, 1990; Jacobson *et al.*, 1993). Cyanobacteria have the ability to carry out both photosynthesis in normal vegetative cells and nitrogen fixation in heterocyst cells (Mackerras *et al.*, 1990; Jacobson *et al.*, 1993; Ehiras *et al.*, 2003; Huang *et al.*, 2005). Nitrogen fixing cyanobacteria are capable of forming symbiotic association with various plants and fungi (Stewart *et al.*, 1983; Peters, 1990; Rowell and Kerby, 1991; Bergman *et al.*, 1992). In symbiosis with plant, cyanobacteria fix atmospheric nitrogen and release organic matter, which is taken up by plants (Rai, 1990; Gantar *et al.*, 1995; Rai *et al.*, 2000). The biological activity of cyanobacteria was

*To whom correspondence should be addressed.
Central Sericultural Research and Training Institute, Mysore - 570 008, Karnataka, India. Tel: 0091-(0)821-2362571; Fax: 0091-(0)821-2363757; E-mail: drmr_rao@yahoo.com

not affected even after application of recommended doses of pesticides or herbicides to plants (Venkataraman and Rajyalakshmi, 1972) and cyanobacteria helps to retain soil moisture and improves soil fertility condition (Roy Choudhury *et al.*, 1979; Islam and Hashem, 1995; Hashem, 1997; 2001).

In several crops chemical fertilizers were used frequently and due to that soil fertility condition gradually deteriorated. Therefore, scientists have intensified their efforts to reduce the use of chemical fertilizers by using biofertilizers (Ambica *et al.*, 1994; Reddy *et al.*, 2000; Dubey, 2000). Biofertilizers are cost effective, eco-friendly and a renewable source of plant nutrients to supplement chemical fertilizers (Marwaha, 1995; Dubey, 2000). For the last two decades, biofertilizers such as *Azotobacter*, *Azospirillum*, *Vesicular Arbuscular Mycorrhiza* (VAM) and Cyanobacteria were used extensively in many crops primarily to reduce frequent use of chemical fertilizers, to save the soil fertility condition and to improve the plant growth (Venkataraman, 1986; Subba Rao, 1988; Gangawar and Thangavelu, 1992; Das *et al.*, 1994; Marwaha, 1995; Hashem, 1997; 2001; Reddy *et al.*, 2000). Cyanobacterial biofertilizer (CBB) has been used in different crop plants, however some of the scientist reported that CBB cannot totally replace the addition of chemical fertilizer to improve soil fertility and crops yield (Venkataraman, 1986; Gantar *et al.*, 1991; Jayaram and Shanmugasundaram, 1993; Hashem, 2001).

In mulberry, a very few reports are available on the effect of cyanobacteria on chemical and nutritional properties of salt affected soil of mulberry garden and its productivity (Bose and Majumder, 1999). However, its effect on different yield attributing characters/leaf quality of mulberry and its influence on silkworm cocoon characters are not studied so far. Therefore, the present investigation was undertaken to study in detail on the efficiency of cyanobacterial biofertilizer (CBB) with chemical fertilizer (NPK) on leaf yield and leaf quality characters of mulberry and its impact on silkworm growth and cocoon characters.

Materials and Methods

An experiment was conducted in the farmer's field of Sindualli village, Mysore district to study the efficacy of cyanobacterial biofertilizer (CBB) and chemical fertilizer (NPK) on leaf yield and leaf quality of mulberry. Before conducting the experiment, soil pH, Organic Carbon (OC), total nitrogen (N), available phosphorus (P), potassium (K) and cyanobacteria spp. in the soil were noted. For the study, three species of cyanobacteria *viz.*, *Nostoc*

muscorum (Nm), *Anabaena variabilis* (Av) and *Scytonema millei* (Sm) were used as cyanobacterial biofertilizers. The samples of these nitrogen-fixing cyanobacteria were obtained from Indian Agricultural Research Institute (IARI), New Delhi and subcultured in the laboratory, Department of Botany, University of Mysore. From these stocks, large volumes of cyanobacteria were prepared and lyophilized. Then the powdered cyanobacteria were mixed with Farmyard manure and inoculated to the soil by the standard procedure (Venkataraman, 1972; Anonymous, 1978) was followed. In the present study, two different doses of CBB ((i) 22 kg/acre/crop and (ii) 32.6 kg/acre/crop) and three different doses of NPK *viz.*, (i) N:P:K ratio (25%):6:2.4:2.4 kg/acre/crop (ii) N:P:K (50%):12:4.8:4.8 kg/acre/crop and (iii) NPK (100%) 24:9.6:9.6 kg/acre/crop as per the recommended dose of CSR&TI, Mysore were used (Anonymous, 1999). The doses of CBB and chemical fertilizers were calculated and converted into grams/plant for different treatments. After application of CBB, irrigation was given continuously for three days and there after irrigation was maintained once in four days. For the study, 5000 mulberry plants were utilized for 10 different treatments and for each treatment 5 replications were maintained by following randomized block design and each replication consists of 100 plants. The treatments *viz.*, T1: Nm (2.0gm)+50% NPK; T2: Av (2.0gm)+50% NPK; T3: Sm (2.0gm)+50% NPK; T4: Nm (1.0gm)+Av (1.0gm)+50% NPK; T5: Nm (1.0gm)+Sm (1.0gm)+50% NPK; T6: Av (1.0gm)+Sm (1.0gm)+50% NPK; T7: Nm (1.0gm)+Av (1.0gm)+Sm (1.0gm)+50% NPK; T8: Nm (1.0gm)+Av (1.0gm)+Sm (1.0g)+25% NPK; T9: Nm (1.0gm)+Av (1.0gm)+Sm (1.0g) and T10: 100% NPK as control. These treatments were broadly classified into four types, (i) T1 to T7: single and combinations of CBB+50% NPK; (ii) T8: combination of CBB+25% NPK; (iii) T9: combinations of CBB without NPK and (iv) T10: 100% NPK as control.

Newly established (half an acre) K2 mulberry garden of a progressive sericulture farmer was selected and a total of 5000 plants (grown in 2' x 2' spacing) were taken for the experiment. These plants were pruned 20 cm above ground level and after 15 days CBB was applied and after 15 days NPK was applied as per the recommended dose of Central Sericultural Research and Training Institute, Mysore (Anonymous, 1999). Farmyard manure application and other cultural operation were done according to the norms of CSR&TI, Mysore. After 65 days of pruning, 20 plants were randomly selected replication wise from each treatment and data on different morphological characters *viz.*, Length of the longest shoot as plant height (cm), average shoot length (cm), number of shoots/plant, number of leaves/plant, single leaf area (cm²) using leaf

area meter model CI 203 and weight of 100 leaves (kg) were recorded. Again pruning was done and same procedure was followed for each crop. Ten crops data were recorded as above for two years (2000-2001) and statistically analyzed (Snedecor and Cochran, 1967). Leaf yield data was recorded in each of the ten treatments by harvesting leaves separately in each treated and control plot then weighed and calculated for tons/ha/yr.

From the treatment field, fully expanded mature leaves (14th/15th leaf from top) were collected replication wise and estimated qualitative characters, such as Leaf moisture (%) and moisture retention capacity (%) after 6 hrs of harvest by following the method of Vijayan *et al.* (1996). Total Chlorophyll (mg/gm.f.wt.) was estimated following the procedure of Hiscox and Israelisiam (1979). Leaf samples collected were processed, dried and used in triplicate for analysis of Protein % by Lowry *et al.* (1951), total amino acid following ninhydrin method by using leusine as standard (Spies, 1955). Nitrogen content estimated by micro-kjeldahl method (Jackson, 1973) and Sugar content (soluble carbohydrate) was estimated adopting phenol sulphuric acid method (Dubois *et al.*, 1956).

Further to verify the efficacy of CBB on leaf quality and its influence on silkworm growth and cocoon characters, a bio-assay study was conducted for three seasons with PM × NB₄D₂. The rearings were carried out as per the improved technology of silkworm rearing (Krishnaswami, 1978). Leaves from CBB treated (T7) and control (T10) plots were used for feeding the silkworm till spinning. Average of three seasons data on weight of 10 mature larvae (g), Larval duration, Effective Rate of Rearing (ERR) by number and by weight, Single cocoon weight, Single shell weight and Shell ratio (%) were recorded separately and analyzed statistically (Snedecor and Cochran, 1967). The economics were calculated for treatment T7 and compared with control T10, based on cost of CBB, leaf material, labour charges and income gain over chemical fertilizer.

Results and Discussion

The present experiment was aimed to study the effect of cyanobacterial biofertilizer (CBB) with and without chemical fertilizers (NPK) on leaf yield and leaf quality of mulberry and its influence on silkworm growth and cocoon characters. After crop wise application of CBB for six months, it was observed that soil pH which was initially 8.4 was decreased to 7.0–7.4 in different CBB treated plots. The decrease in soil pH in different treated plots was mainly due to the influence of CBB. The reduction in soil pH by cyanobacterial inoculation has been

reported by few scientists (Hashem, 1997, 2001; Bose and Mujamder, 1999). The nutrients in CBB treated plots were found increased as compared to control plot. Initially the availability of nutrient status in soil was (i) OC: 0.35%, (ii) N: 0.032%, (iii) P: 7.00 kg/ha, (iv) K: 170 kg/ha. After the CBB treatment, the range of different nutrients increased to (i) Organic carbon: 0.42–0.57%, (ii) total nitrogen: 0.048–0.060%, (iii) available phosphorus: 11–16 kg/ha and (iv) available potassium: 190–215 kg/ha. Similar results were reported in mulberry by Bose and Majumdar (1999). Cyanobacteria inoculation helps to retain soil moisture, reduces soil pH, increases organic matter and liberates different hormones that stimulate plant growth (Hashem, 2001; Meeks and Elahi, 2002).

Quantitative characters of Mulberry

The average of 10 crops data on seven quantitative characters *viz.*, Plant height, average of shoot length, no. of shoots/plants, no. of leaves/plant, 100 leaves wt., single leaf area and leaf yield (tons/ha/yr.) were recorded in different CBB treatments and in control (100% NPK) are presented in Table 1. In general all the morphological traits exhibited superior performance in T7 (combinations of CBB + 50% NPK). Whereas, these traits showed low performance in T9 (only CBB). The quantity of CBB used in T7 and T8 are same (3 g/plant) but difference in response of growth parameters were noticed, this may be due to difference in the dose of NPK used in these treatments (Table 1).

Leaf yield recorded was found high in T7 (32.12 tons/ha/yr), while it was low in T9 (18.20 tons/ha/yr). Even after reduction in dose of NPK, higher leaf yield was obtained in T7, this was mainly due to efficiency of CBB and its additional supply of plant nutrients, improvement of soil environment, biological process in soil, liberation of growth promoting substances and vitamins (Hemanth Kumar and Kaushik, 1989; Bose and Majumder, 1999; Hashem, 2001). Lower leaf yield in T9 clearly indicates that CBB alone may not be able to supply the required quantity of essential nutrients and other growth substances for normal plant growth, therefore it was observed that optimum quantity of NPK fertilizers is also needed for effective improvement in growth and leaf yield in mulberry. It was reported in other crops that biofertilizers application couldn't totally replace chemical fertilizers (Venkataraman, 1986; Hashem, 2001).

It was observed in the present study that mixture of three species (*N. muscorum* + *A. variabilis* + *S. millei*) of CBB treatment (T7 and T8) were found more effective in improving the morphological traits than one and two species of CBB treatment (T1 to T6). These results were similar to the findings of Bose and Majumder (1999) in

Table 1. Quantitative characters in different combinations of CBB and chemical fertilizers

Treatments	Plant height (cm)	Average shoot length (cm)	No. of shoots/plant	No. of leaves/plant	100 leaves weight (g)	Single leaf area (cm ²)	Leaf yield Tons/ha/year
T1: Nm (2.0 g) + 50% NPK	107.2	78.4	4.5	64.2	305	187.5	25.63
T2: Av (2.0 g) + 50% NPK	105.4	80.2	4.6	64.5	298	190.2	24.94
T3: Sm (2.0 g) + 50% NPK	102.5	76.4	4.5	63.4	295	185.8	24.56
T4: Nm (1.0 g) + Av (1.0 g) + 50% NPK	110.2	84.3	4.7	66.3	314	192.5	26.48
T5: Nm (1.0 g) + Sm (1.0 g) + 50% NPK	108.7	78.6	5.1	65.6	316	191.8	27.07
T6: Am (1.0 g) + Sm (1.0 g) + 50% NPK	106.5	77.5	4.7	64.8	310	193.4	26.32
T7: Nm (1.0 g) + Av (1.0 g) + Sm (1.0 g) + 50% NPK	122.4	90.6	5.4	71.4	336	207.5	32.12
T8: Nm (1.0 g) + Av (1.0 g) + Sm (1.0 g) + 25% NPK	112.6	82.4	5.0	67.4	318	196.6	27.67
T9: Nm (1.0 g) + Av (1.0 g) + Sm (1.0 g)	80.3	52.7	4.0	50.6	252	172.5	18.20
T10: 100% NPK	120.7	86.4	5.3	69.2	329	205.2	31.17
SE ±	3.655	3.218	0.134	1.754	972	3.127	1.220
CD 5%	4.432	4.025	0.356	3.026	154	4.393	2.806

Nm: *Nostoc muscorum*; Av: *Anabaena variabilis*; Sm: *Scytonema millei*; NPK = Chemical fertilizer**Table 2.** Qualitative characters in different combinations of CBB and chemical fertilizers

Treatments	Leaf moisture content (%)	Moisture retention capacity (%)	Total chlorophyll (mg/g f.wt.)	Protein content (%)	Nitrogen content (%)	Sugar content (%)	Amino acid mg/g dry wt.
T1: Nm (2.0 g) + 50% NPK	72.5	74.4	2.68	20.52	4.10	10.34	3.16
T2: Av (2.0 g) + 50% NPK	71.8	73.3	2.61	20.80	4.22	10.65	3.20
T3: Sm (2.0 g) + 50% NPK	72.7	74.2	2.60	21.18	3.88	10.58	3.17
T4: Nm (1.0 g) + Av (1.0 g) + 50% NPK	72.6	76.5	2.72	21.86	4.31	11.55	3.30
T5: Nm (1.0 g) + Sm (1.0 g) + 50% NPK	71.7	74.6	2.76	22.14	4.20	11.82	3.38
T6: Am (1.0 g) + Sm (1.0 g) + 50% NPK	72.8	73.5	2.73	22.05	4.18	10.96	3.29
T7: Nm (1.0 g) + Av (1.0 g) + Sm (1.0 g) + 50% NPK	73.4	76.3	2.94	23.43	4.52	12.41	3.64
T8: Nm (1.0 g) + Av (1.0 g) + Sm (1.0 g) + 25% NPK	72.8	75.0	2.70	22.64	4.30	11.90	3.40
T9: Nm (1.0 g) + Av (1.0 g) + Sm (1.0 g)	70.6	71.5	2.20	19.18	3.62	9.85	2.62
T10: 100% NPK	73.2	76.2	2.91	23.28	4.43	12.56	3.49
SE ±	0.262	0.499	0.060	0.426	0.800	0.298	0.086
CD 5%	0.529	1.230	0.148	0.914	1.722	0.606	0.204

Nm: *Nostoc muscorum*; Av: *Anabaena variabilis*; Sm: *Scytonema millei*; NPK = Chemical fertilizer

mulberry. Most of the traits in two species of CBB combinations (T4–T6) showed better response than single species of CBB treatments (T1–T3). Higher dose of CBB coupled with 50% NPK fertilizer in T7, proved more effective in enhancing leaf yield and yield attributing characters (Table 1). It was well established that nitrogen fertilization has no negative effect on nitrogen fixation of cyanobacteria (Bose and Majumder, 1999). Nitrogen fixation in cyanobacteria is brought by a high molecular weight metalloprotein enzyme known as nitrogenase and it is well protected in heterocyst cells. Usually in symbiosis with plants, heterocyst cells frequencies are increased 3 to 10 fold (Meeks and Elahi, 2000). The quantity of CBB used in T7 might have increased the number of heterocyst cells in cyanobacteria which in turn convert more quantity of atmospheric N₂ into ammonia with the result more quantity of nitrogen is utilized and ultimately improvement in growth and yield were recorded in the treatment.

Leaf quality characters

The growth of silkworm, *Bombyx mori* L. and production of cocoons mainly depends on proper feeding of good quality mulberry leaves to silkworm. Therefore, it is essential to study leaf quality characters such as leaf moisture %, moisture retention capacity %, total chlorophyll, protein, nitrogen, sugar and amino acid contents. These traits were estimated in leaves of different treatments of CBB with and without chemical (NPK) fertilizer is given in Table 2. The leaf moisture % and moisture retention capacity was found high in T7 followed by T10 (control) and T8 as compared to other treatments. The identification of good quality leaves are primarily based on the higher leaf moisture %, moisture retention capacity % in leaves (Bongale and Chaluvachari, 1995; Sujathamma and Dandin, 2000) and it is positively related with increased growth of silkworm larvae (Paul *et al.*, 1992). These two traits are under the influence of genetic and environmental factor (Vijayan *et al.*, 1997). In the present experiment the T7 was found on par with T10 control. It clearly indicated that the growth and moisture content of leaves are not affected even after reducing the recommended dosage of NPK fertilizers, this may be due to the efficacy of CBB, which had compensated the less use of NPK fertilizer.

Total chlorophyll content in leaves were found high in T7 (2.94 mg/g.f.wt.) and T10 (control 2.91 mg/g.f.wt.) and it was low in other treatments (2.20 to 2.76 mg/g.f.wt.) are presented in Table 2. Higher chlorophyll content in leaves indicates the photosynthetic efficiency, therefore it may be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin,

2000). Among the CBB treatments, total nitrogen and amino acid contents were found high in T7. Whereas, these traits were found low in T9 and in other treatments. Higher contents of both nitrogen and amino acid in leaves proved nutritively superior (Machii, 1989; Machii and Katagiri, 1991). Higher amino acid content increases the growth and development of silkworm, which in turn enhances the synthesis of silk protein (Machii and Katagiri, 1991). Similarly, protein content was found high in T7 (23.43%) and it was low in T9 (19.18%). Horie (1980) reported that optimum dietary protein level should be 20–25%, which is required for better growth of silkworm larvae. In the present study also the level of protein content in T7 and T10 was found high and within the optimum range (Table 2).

Mulberry leaves contain plenty of carbohydrates (Haratsuka, 1971), which are utilized by silkworm as energy source and for synthesis of lipids and amino acids (Horie, 1978). It can be calculated based on total sugar and starch available in leaves (Bose and Bindroo, 2001). In the present experiment, total sugar was found high in T7 (12.41%) and it was on par with control (T10: 12.56%) (Table 2). This clearly indicates that reduction in dose of chemical fertilizer did not affect the quantity of sugar content and may be due to efficiency of combination of CBB. Over all results revealed that combinations of CBB coupled with 50% NPK fertilizers proved effective in enhancing leaf quality characters. Whereas, use of CBB treatment alone proved less effective in improving leaf quality characters. It was observed that use of CBB alone may not fulfill the nutrient/fertilizer requirement of the plants therefore, combined dose of CBB+NPK fertilizer application is most essential for effective improvement on growth and quality of mulberry.

Silkworm rearing performance

The mulberry leaves collected in T7 (CBB + 50% NPK) and control (100% NPK) was utilized to study their impact on silkworm growth and cocoon characters. The average of three seasons data recorded on silkworm growth and cocoon characters are summarized in Table 3. In general, significant difference was not observed in different rearing parameters between two treatments (T7 and T10 control). However, marginal improvement on silkworm growth and cocoon characters was recorded, when silkworm larvae were fed with the leaves of T7 (Table 3). The reduction in the dose of chemical fertilizers (50% NPK) did not affect either leaf quality or cocoon characters and this may be due to the efficiency of CBB which had compensated the less use of chemical fertilizers by supplying required amount of nitrogen and other essential nutrients/growth hormones to maintain normal plant

Table 3. Rearing performance and cocoon characters of silkworm in T7 and T10

Treatments	Larval duration (days)	Wt. of 10 mature larvae (gm)	ERR (No.)	ERR (Wt.)	Single cocoon wt. (g)	Single shell wt. (g)	Shell ratio (%)
T7: CBB + 50% NPK	28.02	41.25	8250	14.00	1.700	0.300	17.64%
T10: 100% NPK (Control)	28.10	40.67	8020	13.20	1.630	0.286	17.54%
SE \pm	0.040	0.290	115.3	0.401	0.035	0.006	0.050

Table 4. Economics calculated for T7 and T10 for one acre mulberry plot

Particulars	T7	T10
1. Fertilizers used for experiment	CBB + 50%NPK	100% NPK
2. No. of plants/acre/crop (2' x 2' spacing)	10,890	10,890
3. CBB used (gm/plant)	3.0gm	-
4. CBB used (kg/acre/crop) (item 2 x 3)	32.60 kg	-
5. Cost of CBB (@Rs.6/Kg) (item 4 x 5)	Rs. 195/-	-
6. Recommended dose of chemical Fertilizer N:P:K (kg/acre/crop)	12: 4.8: 4.8 NPK (50%)	24: 9.6: 9.6 NPK (100%)
7. Types of NPK..fertilizer used: (item 6 x 7 a,b,c)		
A.S. = 20.6% N	58.25 kg	116.50 kg
SSP. = 16% P	30.00 kg	60.00 kg
(c) MOP. = 60% K	8.00 kg	16.00 kg
8. Total NPK. Fertilizer (kg/acre/crop)	96.25 kg	192.50 kg
9. NPK Fertilizer used (gm/plant) (item 8 ÷ 2)	9gm	18gm
10. Cost of NPK Fertilizer: (item 7 x 10 a,b,c)		
(a) A.S. = Rs. 6.43 / kg	Rs. 374.50/-	Rs. 749/-
(b) SSP. = Rs. 3.43 / kg	Rs. 102.90/-	Rs. 205.80/-
(c) MOP. = Rs. 4.45 / kg	Rs. 35.60/-	Rs. 71.20/-
11. Total amount Rs. acre/crop	Rs. 513/-	Rs. 1026/-
12. Labours used for fertilizer application	3	2
13. Labour charges (Rs.50/ manday)	Rs. 150/-	Rs. 100/-
14. Total Expenditure incurred (item 5 + 11 + 13)	Rs. 858/-	Rs. 1126/-
15. Leaf yield (kg/acre/crop)	2570 kg	2494 kg
16. Cost of leaf (@ Rs. 2/kg) (item 15 x 16)	Rs. 5140/-	Rs. 4748/-
17. Income gain per acre/crop (item 16 - 14)	Rs. 4282/-	Rs. 3622/-
18. Gain over chem. fert.	Rs. 660/-	-
19. Net income over chem.fert. (Rs./ha/year)	Rs. 8250/-	-
20. Cost: Benefit ratio	1:1.3	-

AS: Ammonium sulphate (N), SSP: Single super phosphate (P) and MOP: Murate of potash (K)

growth with the result leaf quality was not affected and due to the reason, silkworm growth and cocoon characters were also not affected.

Based on the observations recorded, economics were calculated for T7 (CBB+50% NPK) and T10 (100% NPK) for one acre mulberry garden are presented in Table 4. Expenditure incurred on cost of fertilizers and labour charges for T7 was Rs. 858/-while, for T10 it was Rs. 1126/-. Leaf yield (tons/acre/crop) obtained and cost of leaves (@ Rs.2/kg) in T7 was Rs. 5140/- whereas, in T10 it was Rs. 4748/-. A total gain of Rs. 660/-acre /crop can

be obtained in T7 over control T10. Net income gain after the use of T7 for one hectare per year is Rs. 8250/-. This clearly indicates that T7 is highly economical, ecofriendly and beneficial as compared to T10 control (Table 4).

An overall view of the results indicated that T7 (CBB + 50%NPK) is very effective in enhancing all the quantitative traits. However, with regard to leaf quality, most of the quality traits in T7 are on par with T10 control. Reduction in the dose of NPK fertilizer in T7 did not affect the leaf yield or the leaf quality traits. Further reduction in the dose of NPK fertilizer in T8 (CBB+25%NPK) resulted

with lower leaf yield and leaf quality as compared to T7 and T10. Use of CBB alone (T9) proved less effective in improving the quantity as well as quality traits. Bioassay study between two treatments (T7 and T10) showed no significant difference in silkworm growth and cocoon characters. Economics calculated also revealed T7 (CBB + 50%NPK) is highly economical and beneficial over T10 (100% NPK). Cyanobacteria are cheap source of nitrogen, increases available P and S level in soil, water holding capacity of soil, do not cause pollution and maintain biodiversity of soil (Hashem, 2001). Therefore, it is concluded from the experiment that T7 (3gm of CBB + 50% NPK) can be recommended to sericulture farmers mainly to reduce the use of chemical fertilizer (NPK), by saving 50% of its cost, besides improving the soil fertility condition, which in turn improves the leaf yield, quality of mulberry.

References

- Ambica, P. K. Das, R. S. Katiyar and P. C. Chaudhury (1994) The influence of VAM association on growth, yield and nutrient uptake in some mulberry genotypes (*Morus* spp.) *Indian J. Seric.* **33**, 166-169.
- Anonymous (1978) *Algal Technology for Rice Research Bulletin* N0. 9 Indian Agricultural Research Institute, New Delhi, India.
- Anonymous (1999) Approval norms on farm inputs and disinfectants for Central Silk Board Units, Bangalore. pp.1-39.
- Bergman, B., A. N. Rai, C. Johansson and E. Soderback (1992) Cyanobacterial plant symbiosis. *Symbiosis.* **14**, 61-81.
- Bongale, U. D. and Chaluvachari (1995) Evaluation of 8 mulberry germplasm varieties by leaf biochemical and bioassay moulting studies. *Sericologia.* **35**, 83-94.
- Bose, P. C. and B. B. Bindroo (2001) A comparative biochemical study of seven promising mulberry (*Morus alba* L.) varieties under rainfed condition of subtropical region. *Indian J. Seric.* **40**, 171-173.
- Bose, P. C. and S. K. Majumder (1999) Effect of Blue green algae on chemical and nutritional properties of salt-affected soil of mulberry garden, productivity of mulberry and its economics. *Sericologia.* **39**, 459-465.
- Das, P. K., P. C. Chaudhury, A. Ghosh, R. S. Katiyar, Y. Madhava Rao, V. B., Mathur and Mazumder (1994) Studies on the effect of Bacteria Biofertilizer in irrigated mulberry (*Morus alba* L.) *Indian J. Seric.* **33**, 170-173.
- Dubey, Y. P. (2000) Biofertilizers-A component of plant nutrient supply system. *Farmer and Parliament.* **37**, 13-14.
- Dubios, M. K., K. A. Giller, K. Hamilton, P. A. Reier and F. Smith (1956) Colorimetric methods for the determination of sugar and related substances. *Ann. Chem.* **28**, 350-356.
- Ehiras, S. M., M. Ohmori and N. Sato (2003) Genome wide expression analysis of the responses to nitrogen deprivation in the heterocyst forming cyanobacteria *Anabaena* sp. Strain pcc 7120 DNA. *Res.* **10**, 97-113.
- Gangawar, S. K. and K. Thangavelu (1992) Evaluation of biofertilizers for establishment of mulberry (*Morus alba* L.) *Sericologia.* **32**, 173-181.
- Gantar, M., N. W. Kerby, P. Rowell and Z. Obreh, (1991) Colonization of wheat (*Triticum Vulgare* L.) by N₂ fixing cyanobacteria. I. A survey of soil cyanobacterial isolate forming association with roots. *New Phytol.* **118**, 477-483.
- Gantar, M., Peter Rowell, W. Nigel, Kerby and I. W. Sutherland (1995) Role of Extracellular Polysaccharide in the colonization of wheat (*Triticum Vulgare* L.) roots by N₂-fixing cyanobacteria. *Bio. Fertil Soil.* **19**, 41-48.
- Hashem, M. A. (1997) Role of blue green algae in improving soil fertility and reclaiming salinity of soil *Annual Report of BAURES*, Financial Research Projects BAU: Mymensingh, India.
- Hashem, M. A. (2001) Problems and prospects of cyanobacterial biofertilizers for rice cultivation. *Aust. J. Plant Physiol.* **28**, 881-888.
- Hemanth Kumar and B. D. Kaushik (1989) Bioameliorant blue green algae and salt affected soil. *Proceeding of National Seminar on biofertilizer Technology Transfer* Aurangabad, India. pp. 30.
- Hiratsuka, E. (1971) Researches on the nutritional of the silkworm. Shangi Shikenjo. *Hokoku Tech. Bull.* **2**, 353-412.
- Hiscox, J. D. and H. F. Israelisiam (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**, 1332-1334.
- Horie, Y (1978) Quantitative requirements for growth of the silkworm, *Bombyx mori* L. *J. A. R. Q.* (Japan) **12**, 211-217.
- Horie, Y. (1980) Recent advances in Sericulture. *Annu. Rev. Entomol.* **25**, 49-71.
- Huang, G, Qing Fan, Sigal Lechnoyossef, Elizabeth wojcuich, C. Peter Wolk, Takakazu Kaneko and Satoshi Tabata (2005) Clustered genes required for the synthesis of Heterocyst envelope polysaccharide in *Anabaena* sp. Strain pcc 7120. *Journal of Bacteriology.* **187**, 1114-1123.
- Islam and M. A. Hashem (1995) Role of blue green algae in improving soil environment. *Bangladesh Journal of Training and Development.* **8**, 112-118.
- Jacobson, B. L., Young Kee Chae, L. John, Markley, Ivan Raymut and M. H. Holden (1993) Molecular structure of the oxidized recombinant Heterocyst [2Fe-2S] Ferredoxin from *Anabaena* 7120 determined to 1.7-Å Resolution *Biochemistry* **32**, 6788-6793.
- Jackson, M. L. (1973) Nitrogen determination for soil and plant tissues In: *Soil chemical analysis* Prentice-Hall of India (Ltd.). New Delhi. pp.183-204.
- Jayaram, S. and S. Shanmugasundaran (1993) Algal biofertilizer choice species of *Scytonema*. *Proc. Natl. Sem. On Cyanobacterial Research-Indian Science* (ed.) G. Subramanian NMFC, BARD Tiruchirapalli pp.24-31.
- Krishnaswamy, S. (1978) New technology of Silkworm rearing. *Bulletin, CSRI* **2**, 1-24.

- Lowry, O. H., N. J. Rosenbrough, A. L. Flair and R. C. Randal (1951) Protein measurement with folin-phenol reagent. *J. Bio. Chem.* **93**, 265-275.
- Machii, H. (1989) Varietal differences of nitrogen and amino acid contents in mulberry leaves. *Acta Sericul. Entmol.* **1**, 51-61 I In Japanese.
- Machii, H. and K. Katagiri (1991) Varietal differences in nutritive values of mulberry leaves for rearing silkworm *J. A. R. Q. (Japan)* **25**, 202-208.
- Mackerras, A. H., B. N. Youens, R. C. Weir, G. D. Smith, (1990) In cyanophycin involved in the integration of nitrogen and carbon metabolism in the cyanobacteria *Anabaena* cylindrical and *Gloeothecha* grown on light/dark cycles. *J. Gen. Microbiol.* **136**, 2049-2056.
- Marwaha, B. C. (1995) Biofertilizers-A supplementary source of plant nutrient. *Fert. News.* **40**, 39-50.
- Meeks, J. C. and J. Elahi (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free living and plant associated symbiotic growth status. *Microbiol Mol. Biol. Rev.* **66**, 94-121.
- Paul, D. C., G. Subba Rao and D. C. Deb (1992) Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concomitant larval duration. *J. Insect Physiol.* **38**, 229.
- Peters, G. A. (1990) Azolla and Plant cyanobacteria symbiosis: as pests of form and function In: Polsinelli M. Materassi R. Vincenzini M. (eds.) Nitrogen fixation, Proceedings of the 5th International symbiosis on nitrogen fixation with non-legumes. *Kluwer Academic Dordrecht* pp. 377-388.
- Rai, A. N. (ed.) (1990) *A Hand book of symbiotic cyanobacteria*, CRC Press. Boca Raton-In *Plant biochemistry and Molecular biology*-Peter Lea and Richard C. Leegood. pp 129-153.
- Rai, A. N., C. K. Derba and B. Bergman (2000) Cyanobacterium plant symbiosis REVIEW. *New Phytol.* **147**, 449-481.
- Reddy, M. P., D. M. Ram Rao, R. S. Verma, B. Srinath and R. S. Katiyar (2000) Effect of VAM inoculated and addition of phosphorus on the growth of S-13 mulberry saplings. *Indian J. Seric.* **39**, 12-15.
- Rowell, P. and N. W. Kerby (1991) Cyanobacteria and their symbionts. In: *Biology and Biochemistry of nitrogen fixation* (M.J. Dilworth and A.R. Glenn eds) Elsevier. New York. 373-407.
- Roy Chodhury, P. Kaushik, B. D. Krishnamurthy and G. S. Venkataraman (1979) Effect of Blue green algae and Azolla application on the aggregation status of the soil. *Cur. Sci.* **48**, 454-455.
- Snedecor, G. W. and W. G. Cochran (1967) *Statistical methods*. Oxford and IBH Publishing Co. New Delhi, India.
- Spies, J. R. (1955) Colorimetric procedure for amino acid and phenol. In. *Methods In Enzymology* colonick, S. P. and N. P. O. Kaplan (eds), pp. 461-477.
- Stewart, W. D., P. Rowell and A. N. Rai (1983) Cyanobacteria-Eukaryotic Plant Symbiosis. *Ann. Microbia (Paris)* **134**, 205-228.
- Subba Rao, N. S. (1988) Biofertilizer-Potentialities and problem. In: S. P. Sen and P. Polit (Eds). Biofertilizer potentialities and problems. *Plant Physiology forum* Nayaprakash, Calcutta, 7-16.
- Sujathamma, P. and S. B. Dandin (2000) Leaf quality evaluation of mulberry (*Morus* spp.) through chemical analysis *Indian J. Seric.* **39**, 117-121.
- Venkataraman, G. S. (1972) In: *Algal biofertilizer and rice cultivation* Today and Tomorrow Printers and Publishers, New Delhi 1-75.
- Venkataraman, G. S. and B. Rajyalakshmi (1972) Relative tolerance of blue green algae to pesticides. *Indian J. Agri. Sci.* **42**, 119.
- Venkataraman, L. V. (1986) Blue Green Algae as Biofertilizer In : *CRC. Hand Book of Microalgal Mass Culture* (ed). A. Richmond CRC Press. Boca Roton, Florida, 455-471.
- Vijayan, K., A. Tikader, K. K. Dass, B. N. Roy and T. Pawan Kumar (1996) Genotypic influence on leaf moisture content of moisture retention capacity in mulberry (*Morus* spp.) *Bull. Seric. Res.* **7**, 95-98.
- Vijayan, K., M. K. Raghunath, K. K. Das, A. Tikader, S. P. Chakraborti, B. N. Roy and S. M. H. Qadri (1997) Studies on leaf moisture of mulberry germplasm varieties. *Ind. J. Seric.* **36**, 155-157.