



Effect of Bio-fertilizers on nutrient availability in soil, mulberry leaf quality and silkworm cocoon reeling characters

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ABSTRACT

A field experiment was carried out at Periyar EVR College, Tamil Nadu, India, in basic soil to study the nutrient availability and mulberry leaf quality. Three groups of mulberry plants of MR2 variety were bio-fertilized with *Azospirillum*, *phosphobacteria* and vermicompost respectively. Fourth group of mulberry plants were bio-fertilized with farm yard manure (FYM). The soil and leaves of four groups were analysed for their macronutrient and micronutrient content. The N, P and K content of vermicompost bio-fertilized soil was high 118.5 kg/ac, 4.26 kg/ac, and 178kg/ac respectively. The N, P and K content of vermicompost bio-fertilized mulberry leaves were high 0.89%, 0.54% and 3.48% respectively. The micronutrient like zinc, copper, iron, manganese, calcium, magnesium also were found to be very high in vermicompost bio-fertilized soil and vermicompost bio-fertilized mulberry leaves. The results revealed that the nutrient availability in soil and mulberry leaf quality increased gradually in the order FYM bio-fertilized soil and mulberry leaves, *Azospirillum* bio-fertilized soil and mulberry leaves, *phosphobacteria* biofertilized soil and mulberry leaves and vermicompost bio-fertilized soil and mulberry leaves. It was clear that vermicompost is the best bio-fertilizer for mulberry plants. Among the four bio-fertilizer treatments, soil nutrient content, leaf quality traits of mulberry variety(MR2)& reeling performances of silkworms(LxNB4D2) were found to be improved by vermicompost bio-fertilizer. Thus this technology can be recommended to sericulture farmers.

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Introduction

The term “bio-fertilizers” denote all the nutrient inputs of biological origin for plant growth. They are basically microbial “inoculants and provide nitrogen biologically to the plants which are non hazardous way of fertilization to plants.

The microbes in the bio fertilizer do the following important functions - convert ambient nitrogen into forms that the plants can use (nitrate & ammonia), increase porosity by gluing soil particles together, defend plants against pathogens by out competing pathogens for food and saprophytic fungi in the soil break down leaf litter into usable nutrients.

The production of quality mulberry leaves plays an important role in silk production and its economics. Leaf quality influences not only the growth and development of silk worm, but also the quality and quantity of silk produced (Krishnaswami et al 1970). The main event of sericulture is silk worm rearing and hence the success of silkworm rearing to a great extent depends on the availability of enough quantities of quality mulberry leaves. The quality of leaves is affected by many factors, such as climatic factors, nutrients, water management, pest and pathogens (Singh et al 1992). The earthworms play a multifaceted role in the process of soil improvement by promoting humus formation, improving soil texture and enriching the soil. Das (1997) reported that vermiculture holds a good prospect for transforming the colossal sericultural waste into vermicompost rich in nitrogen, phosphorus, potash and micronutrients. For the last one decade, bio-fertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improves soil fertility status and for enhancement of crop production by their biological activity in

the rhizosphere. Extensive research was carried out on the use of bacteria (*Azotobacter* and *Azospirillum*) and VAM fungi as bio-fertilizers to supplement nitrogen and phosphorus fertilizers and observed considerable improvement in the growth of several crop plants (Marwaha,1995, Selvaraj et al., 1996) including mulberry (Das et al., 1998). In mulberry, few reports are available with regard to use of VAM and bacterial bio-fertilizers wherein they were found to be effective in curtailing the recommended dose of chemical fertilizers (Umakanth and Bagyaraj ,1998; Reddy et al, 2000; and Kashyap et al, 2004). Reddy et al., 2000 have documented considerable improvement in the growth parameters of mulberry. However earlier workers have not emphasized much on the influence of bio-fertilizers on leaf quality characters which plays vital role in governing the feed conversion efficiency in sericulture and thereby quality cocoon production. Therefore, in the present investigation, a comparative study was made to assess the individual effect of FYM, *Azospirillum*, *phosphobacteria* and vermicompost on nutrient availability of soil, mulberry leaf quality parameters and silkworm cocoon reeling performances.

Materials and methods:

A field experiment was conducted at periyar EVR college, Tiruchirappalli, India in basic soil to study the nutrient availability and mulberry leaf quality. Three groups of mulberry plants of MR2 variety were bio--fertilized with *Azospirillum*, *phosphobacteria* and vermicompost respectively. Fourth group of mulberry plant were bio-fertilized with farm yard manure (FYM). The bio-fertilizers were obtained from bio fertilizer unit Mannarpuram, Trichy. After the establishment period of

mulberry plantation, bio-fertilizers were applied @20kg/ha/yr to the soil. A total of 500 plants were imposed with four different treatments. Each treatment consisted of 125 plants. Soil nutrient status of different bio-fertilizer treated mulberry plots were estimated. Leaf quality parameters were determined in fully matured leaves. Leaf moisture content was estimated by following the method of Vijayan et al. (1996). Total chlorophyll content was estimated by the method of Hiscox and Israelstam (1979). Leaf samples collected were dried and used for analyzing total protein content by adopting the method of Lowry et al. (1951), aminoacids following ninhydrin methods (Spies, 1955) using leucine as standard and nitrogen content was estimated following micro-Kjeldhal method (Jackson, 1973). Starch and sugar contents (soluble carbohydrate) were estimated by the method McCready (1960) and Dubios et al. (1956) respectively. Total organic carbon (TOC) was determined by the partial oxidation method (Walkley and Black, 1974). Total extractable phosphorous was determined by using Olsen's sodium bicarbonate extraction method (Olsen et al. 1954). K, Ca and Mg were determined after extraction of the sample using ammonium acetate extraction method (Simard et al. 1993), and the samples were analyzed by Perkin Elmer AA-6300, double beam atomic absorption spectrophotometer (AAS). The experiment was repeated thrice and average data on different leaf quality traits were computed.

Further to confirm the effect of bio-fertilizers on the leaf quality, a bioassay study was conducted with a commercial cross breed silkworm race of LXNB4D2 for 3 different seasons. The rearing was carried out by following the improved silkworm rearing technology (Krishnawami, 1978). Mulberry leaves from four different treatment were used for feeding the silkworms still spinning. Average of three seasons data on reeling performances such as filament length, filament weight, denier, renditta and sericin & fibroin content were recorded separately for different treatments and were analyzed statistically (Gomez and Gomez, 1983).

Results and discussions

The results of the present investigation revealed that the pH of FYM soil was 7.06 and pH of bio-fertilized mulberry plots ranged between 6.89-7.59. This may be due to interaction of bio-fertilizers in the rhizosphere of soil. Likewise electrical conductivity of FYM soil was 0.26 dsm⁻¹ and in the bio-fertilized mulberry plot it ranged from 0.16-0.36 dsm⁻¹. In contrast the mineral nutrients were found to be increased in the bio-fertilized soils than FYM soil. The available nitrogen and potassium were found to be very high in vermicompost bio-fertilized mulberry plots. After bio-fertilizer treatments an improvement in nutrient status ranged for nitrogen 87.6-108.5 kg/ac, available phosphorus 3.56-6.39 kg/ac and K 105-178 kg/ac, available manganese ranged 2.63-3.45 ppm, available iron ranged 4.58-6.25 ppm, available zinc ranged 0.96-1.36 ppm, available copper ranged 0.56-1.09 ppm.

The data revealed that the microbial inoculants influenced the available nutrients status in the rhizosphere of soil in different bio-fertilized mulberry plots. Similar results were reported by Sharma et al., (2002).

Leaf Quality Parameters

In mulberry, leaf moisture content (LMC) is an important factor that maintains the nutritive levels of leaves, which in turn improve its palatability for silkworm. LMC was found high in vermicompost biofertilized mulberry plants (72.89%) followed by *phosphobacteria* biofertilized mulberry plants (72.65), *Azospirillum* bio-fertilized mulberry plants (70.26%) and FYM bio-fertilized plants (69.78%)

Leaves possessing higher LMC are identified as good quality leaves (Bongale and Chaluvachari, 1995; Sujathamma and Dandin, 2000). It was reported that higher leaf moisture content is significantly associated with the growth and nutritional parameters of silkworm (Rahmathulla et al. 2004).

It clearly suggests that influence of microbial inoculants might have mediated the moisture availability in the soil rhizosphere, thereby maintaining normal growth, water uptake and other metabolism in plants.

Chlorophyll content: Significant differences in total chlorophyll content were observed among the four treatments. Increased amount of chlorophyll content in vermicompost bio-fertilized mulberry leaves (316.25 mg%) indicates the photosynthetic efficiency. Chlorophyll content of *phosphobacteria* bio-fertilized mulberry leaves was 286.95 mg% chlorophyll content of *Azospirillum* bio-fertilized mulberry leaves was 279.29% and in FYM bio-fertilized mulberry leaves was 259.63 mg%.

Total nitrogen and amino acid contents are the two important factors, which also determine the leaf quality (Machii and Katagiri, 1989). These two traits were found high (118.5 kg/ac and 189.15 mg%) in vermicompost bio-fertilized mulberry leaves, medium (106.9 kg/ac and 173.20 mg%) in *phosphobacteria* biofertilized mulberry leaves, low (103.2 kg/ac and 172.62 mg%) in *Azospirillum* bio-fertilized mulberry leaves and lowest (87.6 kg/ac and 156.23 mg%) in FYM bio-fertilized mulberry leaves. It was reported that varieties possessing higher nitrogen and amino acid contents in leaves are nutritionally superior and positively related to growth and development of silkworm (Machii and Katagiri, 1991; Suryanarayan and Shivashankar, 2002).

Among the four bio-fertilizer treatments total protein was found comparatively high (5.26 mg %) in vermin compost bio-fertilized mulberry leaves and it was low (4.16 mg %) in FYM bio-fertilized mulberry leaves.

In mulberry leaves, carbohydrates are available in plenty and it was reported to be the chief source of energy for silkworm (Hiratsuka, 1917 and Hori, 1978). Out of the four bio-fertilizer treatments, total starch, total sugar, total carbohydrates, total hormones (6.95 mg%, 3.16 mg%, 0.92 mg% and 115.62 mg%) were high in vermincompost bio-fertilized mulberry leaves. (Table 2).

Leaf nutrient parameters

Total nitrogen, phosphorus and potassium were high (0.89%, 0.54%, 3.49%) in vermincompost bio-fertilized mulberry leaves. All the three parameters were medium (0.56%, 0.36% and 3.26%) in *Azospirillum* bio-fertilized mulberry leaves. All the three parameters were lowest (0.48%, 0.36%, and 3.24%) in FYM bio-fertilized mulberry leaves. The micro nutrients like calcium (19.32%) Magnesium (8.79%), Sulphur (0.94%) Zinc (1.56 ppm) Iron (132.56 ppm), Manganese (19.64 ppm, Boron (0.08 ppm) and Molybdenum (0.03 ppm) which were very high in vermincompost bio-fertilized mulberry leaves. The results revealed that the micro nutrients increased gradually in the order FYM bio-fertilized mulberry leaves, *Azospirillum* bio-fertilized mulberry leaves, *phosphobacteria* bio-fertilized mulberry leaves and vermicompost bio-fertilized mulberry leaves (Table

The result of Das et al (1994) in improving mulberry leaf quality due to *Azotobacter* inoculation also confirms the present finding and the economic potentiality of usage of various microbial inocula in mulberry cultivation.

Table 1. Data on soil nutrient status of different bio-fertilizer treated mulberry plots

S.No	Name of the parameter	Biofertilizers used				F-Test
		FYM	Azospirillum	Phosphobacteria	Vermicompost	
1.	pH	7.06	7.59	7.16	6.89	*
2.	Electrical conductivity(dsm ⁻¹)	0.26	0.36	0.22	0.16	*
3.	Organic Carbon(%)	0.22	0.45	0.45	0.56	*
4.	Organic Matter(%)	0.44	0.90	0.84	1.03	*
5.	Available Nitrogen(kg/ac)	87.6	106.9	103.2	108.5	*
6.	Available Phosphorus(kg/ac)	3.56	4.78	6.39	4.78	*
7.	Available Potassium (kg/ac)	105	125	146	178	*
8.	Available zinc(ppm)	0.96	1.26	1.26	1.36	*
9.	Available Copper(ppm)	0.56	0.85	0.89	1.09	*
10.	Available Iron(ppm)	4.58	5.63	5.63	6.25	*
11.	Available Manganese(ppm)	2.63	3.26	3.26	3.45	*
12.	Cat ion Exchange capacity (c. mole Proton+/kg)	22.6	28.9	28.9	29.6	*
Exchangeable Bases(c. mole proton+/kg)						
13.	Calcium	9.6	13.6	13.0	13.6	*
14.	Magnesium	6.9	8.9	9.2	9.6	*
15.	Sodium	1.26	1.68	1.67	1.68	*
16.	Potassium	0.21	0.28	0.29	0.32	*

*significant

Table 2. Effect of different biofertilizer treatment on the biochemical constituents of mulberry leaves(%)

S.No	Name of the parameter(%)	Biofertilizers used				F-Test
		FYM	Azospirillum	Phophoba bacteria	Vermicompost	
1.	Moisture Content(%)	69.78	70.26]	72.65	72.89	*
2.	Total chlorophyll(mg%)	259.63	279.26	286.5	316.25	*
3.	Total amino acids (mg%)	156.23	172.62	173.20	189.15	*
4.	Total protein(mg %)	4.16	4.89	4.96	5.26	*
5.	Total starch(mg %)	6.28	6.63	6.89	6.95	*
6.	Total sugar(mg %)	2.08	2.89	2.93	3.16	*
	Total Reducing sugar(mg%)	105	125	146	178	*
	Total Non Reducing (mg%)					
7.	Total vitamins (mg %)	249	256	262	275	*
8.	Total Carbohydrates (mg %)	7.65	8.59	8.65	8.96	*
9.	Total Hormones (mg %)	89.63	93.54	102.36	115.62	*

Table 3. Effect of different biofertilizer treatment on the nutrient constituents of mulberry leaves

S.No	Name of the Parameter	Biofertilizers used				F-Test
		FYM	Azospirillum	Phosphobacteria	Vermicompost	
1.	Ash(%)	2.06	2.16	4.15	4.55	*
2.	Organic Carbon(%)	0.52	0.89	1.09	1.26	*
3.	Total Nitrogen(%)	0.48	0.56	0.72	0.89	*
4.	Total Phosphorus(%)	0.36	0.36	0.62	0.54	*
5.	Total Potassium(%)	3.26	3.4	3.48	3.49	*
6.	Total Sodium(%)	0.89	1.06	1.26	1.36	*
7.	Total Calcium(%)	12.36	13.59	15.63	19.32	*
8.	Total Magnesium(%)	8.56	8.69	8.70	8.79	*
9.	Total Sulphur(%)	0.69	0.78	0.89	0.94	*
10.	Total Zinc(ppm)	1.23	1.26	1.48	1.56	*
11.	Total Copper(ppm)	0.52	0.52	0.56	0.60	*
12.	Total Iron(ppm)	102.6	112.65	113.6	132.56	*
13.	Total Manganese(ppm)	17.56	18.96	19.62	19.64	*
14.	Total Boron(ppm)	0.05	0.06	0.07	0.08	*
15.	Total Molybdenum(ppm)	0.01	0.02	0.03	0.03	*

Table 4. Reeling performances of *Bombyx mori* fed with biofertilized mulberry leaves

Biofertilizer used	Filament length(m0)	Filament weight(g)	Renditta (%)	Sericin content (g)	Fibroin content(g)	Denier	F –Test
Control	635±20	1.22±0.12	15.3%	0.21	0.65	3.4±0.04	*
<i>Azospirillum</i>	635±19	1.15±0.14	15.66%	0.24	0.69	3.6±0.03	*
<i>Phosphobacteria</i>	702.5±17	1.65±0.2	15.8%	0.28	0.76	3.8±0.04	*
Vermicompost	725±20	1.74±0.3	16%	0.31	0.91	4.0±0.05	*

Silkworm Cocoon Reeling Performances

The leaves from different bio-fertilizer treated plots were used for bioassay to study the influence of bio-fertilizer on silkworm cocoon reeling performances. The average of three seasons reeling performances are summarized in Table 4. In general significant differences were observed in the reeling performances in all the four treatments. The results revealed that the reeling performances increased gradually in the order worms fed with FYM bio-fertilized mulberry leaves, phosphobacteria biofertilized mulberry leaves, *Azospirillum* bio-fertilized mulberry leaves and vermicompost bio-fertilized mulberry leaves.

In the present investigation the increment in reeling performance in worms fed with vermicompost bio-fertilized mulberry leaves than other treatments was mainly due to the efficiency of microbial inoculants which had fixed atmospheres N, supply of P, synthesis of other nutrients, vitamins, aminoacids, hormones etc which helped to enhance the growth, metabolism and physiological activity of host plants which resulted in leaf quality improvements and due to their reason improvement in silkworm growth and reeling performances were observed. The beneficial effects of *Azotobacter* in combination with VAM have been reported by several workers in different crop plants (Mandhare et al, 1998 and Sreeramalu et al, 2000 and Sumana and Bagyaraj, 2000). Further it is concluded from the experiment that bio-fertilizers, a cheaper supplement to the expensive chemical fertilizers can be used in mulberry cultivation to reduce the use of chemical fertilizers and thus saving 50% cost of chemical fertilizers. Thus this technology can be recommended to Sericultural farmers of semi arid zone as cost effective and eco-friendly approach to sustain the productivity of both mulberry as well as cocoons.

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