

[Research]

## Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semiarid conditions

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### ABSTRACT

The influence of VAM fungi and bacterial biofertilizer (BBF) with 50% reduction in the recommended dose of (N and P) chemical fertilizers on leaf quality traits of mulberry variety (S-13) and its impact on silkworm (PM × NB4D2) growth and cocoon characters were studied under semi-arid conditions. Four different treatments were imposed i.e., T1: Control (only 100% NPK); T2: VAM (50% cut in P); T3: BBF (50% cut in N) and T4: BBF and VAM (50% cut in N and P). The results revealed that reduction (50%) in the dose of chemical fertilizers in T2, T3 and T4 did not affect the leaf quality traits or cocoon parameters, this may be due to the effect of microbial inoculants in these treatments, which had efficiently regulated the normal growth, metabolism and physiological activity in plants. Among the three-biofertilizer treatments, leaf quality, silkworm growth and cocoon parameters were found improved in T4 and was on par with T1 control. The dual inoculation (T4) proved economical and beneficial with regard to saving of 50 % cost of chemical fertilizers and improvement in soil fertility, leaf quality and cocoon parameters, thus this technology can be recommended to sericulture farmers of semi-arid conditions.

**Keywords:** Biofertilizers, S-13 variety, Leaf quality, Silkworm growth, Cocoon characters, Semi-arid condition.

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### INTRODUCTION

Biofertilizers are commonly called as microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes. For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve 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 biofertilizers 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.*, 1993; and Katiyar *et al.*, 1995 and Reddy *et al.*, 1998). Dual inoculation of VAM and Bacterial biofertilizer proved more effective in increasing the growth of different crop plants (Pawar, 1993; Thaker and Panwar, 1995; Sansamma *et al.*, 1998; Sreeramulu *et al.*, 2000 and Sumana and Bagyaraj, 2002).

In mulberry, few reports are available with regard to use of both the biofertilizers 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 both biofertilizers 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 VAM fungi (*Glomus mosseae*) and bacterial (*Azotobacter chroococcum*) biofertilizer (BBF) and also both of them comprehensively on mulberry leaf quality parameters as well their influence on the cocoon characters of silkworm *Bombyx mori* L.

## MATERIALS AND METHODS

A field experiment was conducted at the Regional Sericultural Research Station, Anantapur, a semi arid zone of Andhra Pradesh, India to study the influence of Vesicular arbuscular mycorrhiza (VAM) fungi comprising inoculum of *Glomus mosseae* and bacterial biofertilizer (BBF) prepared with *Azotobacter chroococcum* on the popular improved rainfed mulberry variety S-13. The biofertilizers were obtained from Central Sericultural Research and Training Institute (CSR&TI), Mysore. Before conducting the experiment the native soil pH, Organic carbon (OC), Electric conductivity (EC), available Phosphorus (P) and potassium (K) were recorded as per the standard analytical methods. VAM was applied in the nursery beds and the mulberry cuttings (S-13) were planted as per the method of Katiyar *et al.*, (1995).

A total of 5000 VAM inoculated and uninoculated saplings were raised from the nursery beds and after 6 months of growth, healthy saplings were transplanted to the main field with plant geometry of 60cm × 60cm spacing. After the establishment period of mulberry plantation, bacterial biofertilizer (*Azotobacter chroococcum*) was applied (@ 20kg/ha/yr in five equal splits) to the soil after each crop harvest by following the recommended norms of CSR&TI, Mysore. VAM was applied only once at the nursery level and it was reported to retain at the root zone for many years (Katiyar *et al.*, 1995).

A total of 896 plants were imposed with four different treatments viz., T1: Control (100 % NPK = 300:120:120 kg/ha/yr.), T2:

VAM (50% cut in P fertilizer), T3: BBF (50% cut in N fertilizer) and T4: VAM and BBF (50% cut in both N and P fertilizers). Each treatment consisted of 4 replications and in each replication 56 plants were maintained in a randomized block design (RBD).

A total of five crops per annum @ 70 days crop growth were followed. During 60 - 65 days of crop growth, leaf quality parameters were determined in fully matured (12<sup>th</sup> - 14<sup>th</sup>) index leaves from the shoot apex. Leaf moisture content and moisture retention capacity (after 6 hours of harvest) were estimated by following the method of Vijayan *et al.*, (1996). Total chlorophyll content was estimated by the method of Hiscox and Isreilstam (1979).

Leaf samples collected were dried and used for analyzing total protein content by adopting the method of Lowry *et al.*, (1951), amino acids following ninhydrin methods (Spies, 1955) using leusine 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. Nitrate reductase activity was also estimated adopting the procedure of Scot and Neyra, (1979).

The experiment was repeated thrice and average data on different leaf quality traits were computed. Average of ten crops data for leaf yield was recorded replication wise from each treatment and calculated as tons/acre/crop.

Further, to confirm the effect of biofertilizers (VAM and *Azotobacter*) on the leaf quality, a bioassay study was conducted with a commercial cross breed silkworm race of PM × NB4D2 for 3 different seasons. The rearing was carried out by following the improved silkworm rearing technology (Krishnaswami, 1978). Mulberry leaves from the four different treatments (T1 to T4) were used for feeding the silkworms till spinning. Average of three seasons data on larval growth and cocoon characters such as weight of 10 mature larvae (g), survivability (%), single cocoon weight, single shell weight, and shell ratio (%), filament length (mm) and raw silk recovery (%) were recorded separately for different treatments and analyzed statistically (Gomez and Gomez, 1983).

## RESULTS AND DISCUSSION

The results of the present investigation elucidated that the initial soil pH recorded in control plot was 8.2 and one year after biofertilizer treatments (T2, T3 and T4), the pH was found to be slightly decreased, ranged from 8.0 to 7.6 pH, this may be due to interaction of biofertilizers in the rhizosphere of soil. Likewise, electric conductivity (EC) in the control plot was 0.430 mmhos/cm and one year after VAM and BBF treatments, a marginal decrease in EC was recorded, which ranged from 0.410 to 0.340 mmhos/cm (Table 1.). In contrast, the mineral nutrients in T2, T3 and T4 plots were found slightly increased as compared to control (T1) plot. The nutrients available initially in the soil was OC: 0.26%, available P: 7kg/ha and K: 280kg/ha and after the biofertilizer treatments an improvement in nutrient status ranged for OC: 0.32 - 0.47%, available P: 10 - 14 kg/ha and K: 300 - 350kg/ha was recorded. The data reveals that the microbial inoculants influenced the available nutrients status in the rhizosphere of soil in different treated plots. Similar results were reported by Sharma *et al.*, (2002).

## Leaf quality parameters

In mulberry, Leaf moisture content (LMC) and moisture retention capacity (MRC) are the two important factors that maintain the nutritive levels of leaves, which in turn improve its palatability for silkworm.

These two traits (LMC and MRC) were found high in T4 (74.17% and 80.79%) followed by T1 (73.50% and 80.21%), T3 (73.48 and 80.14%) and T2 (73.16 and 78.84%). Leaves possessing higher LMC and MRC 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, 2004). The reduction in the dose (50 %) of chemical fertilizers in T2, T3 and T4 did not affect the LMC and MRC (Table 2).

It clearly suggests that the 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.

**Table 1 Data on soil nutrient status of different biofertilizer treated mulberry plots.**

Treatment	pH	EC(m mhos/cm)	OC(%)	P(kg/ha)	K(kg/ha)
T1 (C)	8.2	0.430	0.26	7.0	280
T2 (VAM)	8.0	0.410	0.32	10.0	300
T3 (BBF)	7.8	0.365	0.40	11.0	328
T4 (VAM+BBF)	7.6	0.340	0.47	14.0	350
CD 5%	NS	0.09	0.05	1.35	18

C=Control 100% NPK; VAM = Only 50% cut in P fertilizer; BBF = Only 50% cut in N fertilizer; VAM+BBF = 50% cut in both N and P fertilizers.

**Table 2. Effect of different biofertilizers treatments on the biochemical constituents of mulberry leaves.**

Treatment	Leaf moisture Content (%)	Moisture retention capacity (%)	Total Chlorophyll (mg/g) f.wt.	Total Protein (%)	Total Sugar (%)	Total Starch (%)	Total Nitrogen (%)	Total amino acid (mg/g dry wt.)	NR Activity $\mu$ gm No <sub>2</sub> /hr/g.f. wt.	Leaf yield (ton/acre/crop
T1 (C)	73.50	80.21	3.15	20.44	10.54	10.86	3.89	217.5	11.25	3.25
T2 (VAM)	73.16	78.84	2.94	18.84	9.94	9.96	2.98	211.2	10.16	3.07
T3 (BBF)	73.48	80.14	3.07	19.78	10.12	10.38	3.90	215.4	10.77	3.10
T4(VAM+BBF)	74.17	80.79	3.62	21.57	11.46	11.05	4.05	230.7	12.47	3.64
CD 5%	NS	0.11	0.08	NS	0.08	0.05	0.15	0.20	0.50	0.06

C=Control 100% NPK; VAM = Only 50% cut in P fertilizer; BBF = Only 50% cut in N fertilizer; VAM+BBF = 50% cut in both N and P fertilizers.

Chlorophyll content: Significant differences in total chlorophyll content were not observed among the four treatments. However, marginal increase in chlorophyll content was recorded in T4 (3.62 mg/g.f.wt.) when compared to control T1 (3.15 mg/g.f.wt.). The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency, thus it can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin, 2000). The improvement in chlorophyll content might be due to synergistic interaction of both biofertilizers in T4. It was observed that reduced dose of chemical fertilizers can be manipulated by the addition of both nitrogenous and phosphorous biofertilizers.

Total nitrogen and amino acid contents are the two important factors, which also determine the leaf quality (Machii, 1989). These two traits were found high (4.05% and 230.7mg/g.dry wt.) in combined biofertilizer treatment (T4) when compared to single biofertilizer treatments (T2 and T3) and were found on par with control T1 (3.89% and 217.5mg/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, 2002). Further, observations revealed that reduction (50%) in the recommended dose of N and P fertilizers interestingly did not affect nitrogen and amino acids contents in the leaves of T2, T3 and T4, which may be due to attributed influence of microbial inoculants on the availability of mineral elements in the soil and their translocation in to the plant system and thus enhanced the synthesis of vitamins, amino acids and other hormones etc., in the treatments.

Among the three-biofertilizer treatments total protein was found comparatively high in T4 (21.57%) and was on par with control T1 (20.44%). According to Horie (1980), optimum dietary protein level should be 20 – 25%, which is required for better growth of silkworm larvae. The reduction in N and P fertilizer dose in T2, T3 and T4 did not affect the available quantity of protein content in the leaves due to synergistic influence of biofertilizers, which had compensated the reduced dosage of chemical fertilizers in these treatments. In mulberry leaves,

carbohydrates are available in plenty and it was reported to be the chief source of energy for silkworm (Hiratsuka, 1917 and Horie, 1978). Carbohydrate is estimated based on the quantity of total sugar and starch available in leaves. (Bose and Bindroo, 2001). Out of the three-biofertilizer treatments, the sugar and starch content was found slightly higher in treatment T4 (11.46% and 11.05%) and was almost on par with control T1 (10.54% and 10.86%). The results revealed that dual inoculation of biofertilizers (T4), had pronounced influence on biosynthesis of carbohydrates in the leaves. Incidentally, it was also reported that the association of VAM fungi are known to improve the amino acids and carbohydrates in the host plants (Kashyap *et al.*, 2004)

Nitrate reductase (NR) activity is one of the most important regulatory enzyme in nitrogen metabolism, which catalyses the reduction of nitrate to nitrite and thus a rate limiting step in the utilization of nitrates in plants (Deckard *et al.*, 1973). In the present study, 50% curtailment in the recommended dose of chemical fertilizer did not affect the NR activity in all the biofertilizer treatments (Table 2).

NR activity was found comparatively high in T4 (12.47µgNo<sub>2</sub>/hr/g.f.wt.) than T2 and T3 and was on par with T1 (11.25µgNo<sub>2</sub>/hr/g.f.wt.). Similarly, among the biofertilizers treatments leaf yield was found high in T4 (3.64 ton/acre/crop) and was found better over control T1 (3.25 tons/acre/crop). 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).

### Silkworm rearing performance

The leaves from different biofertilizer treated plots were used for bioassay to study the influence of biofertilizer on silkworm growth and cocoon characters. The average of three seasons rearing data recorded on silkworm growth and cocoon characters are summarized in Table 3. In general, significant differences were not observed in rearing parameters in all the four treatments. However, marginal improvements on silkworm growth and cocoon characters were recorded when both (VAM and BBF)

biofertilizers were used in T4. The cocoon yield in T4 (60.72 kg /100dfls) was found slightly higher over control T1 (59.94 kg/100dfls). Fathima *et al.*, 1996 also observed improvement in cocoon yield, when the biofertilizer (VAM - *Glomus mosseae*) treated leaves were fed to the silkworms. The increment in cocoon yield in treatment T4 was mainly due to the efficiency of microbial inoculants (VAM and BBF) which had compensated the less use of chemical fertilizers by fixing atmosphere N, supply of P, synthesis of other nutrients, vitamins, amino acids, hormones etc., which helped to enhance the growth, metabolism and physiological activity of the host plants, with the result leaf quality was improved and due to the reason improvement in silkworm growth and cocoon characters was observed.

In the present investigation in case of T2, the leaf quality traits were unaffected even after reducing 50% of P fertilizers. It clearly indicates that the association of VAM to the plants not only ensure the increased availability of water and nutrients, but also absorbs and enhance the uptake of microelements, alters the physiological activity of host, increases the level of amino acids in the colonized roots of several plant species (Subba Rao, 1982, Katiyar *et al.*, 1995 and Reddy *et al.*, 1998).

Similarly in T3, leaf quality traits were not affected by reducing 50% N fertilizers, which may be due to the influence of BBF (*Azotobacter chroococcum*), which not only fix atmosphere nitrogen, but also synthesize and secrete certain amino acids, vitamins, hormones, anti-fungal antibiotics etc., in turn promote the growth and metabolism in plants (Das *et al.*, 1995). In case of T4, when both the chemical fertilizers (N and P) were

curtailed to 50% and supplemented with both (VAM and BBF) biofertilizers, a marginal improvement was observed in some of the quality traits when compared to the control (T1) and also with other treatments (T2 and T3). The dual inoculation enhanced the plant growth more than the single inoculation and un-inoculated control as reported in sunflower (Sumana and Bagyaraj, 2002). 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, 2002). In mulberry, it was reported earlier that dual inoculation proved effective in terms of economizing N and P fertilizer application by 50 %, without affecting the leaf yield (Reddy *et al.*, 2000).

Overall results confirm that chemical fertilizers can be supplemented with the addition of biofertilizers to some extent, however these biofertilizers cannot totally replace the chemical fertilizers. Further, it is concluded from the experiment that biofertilizers, 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.

Further, use of biofertilizers helps to improve the soil fertility status. The dual inoculation of VAM and BBF (T4) proved beneficial not only in terms of economizing N and P fertilizers by 50% but also improved the leaf quality, silkworm growth and cocoon characters. Thus this technology can be recommended to the sericultural farmers of semi-arid zone of Andhra Pradesh, as cost effective and eco-friendly approach to sustain the productivity of both mulberry as well as cocoons.

**Table 3. Bioassay on the Influence of VAM and Azotobacter on silkworm growth and cocoon characters.**

Treatment	Wt. of 10 mature larvae (g)	Survivability (%)	Cocoon yield/100dfls (kg)	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	Filament length (mm)	Raw silk recovery (%)
T1 (C)	40.16	86.40	59.94	1.52	0.255	16.77	745.42	37.64
T2 (VAM)	40.20	86.05	56.64	1.49	0.251	16.84	741.57	37.54
T3 (BBF)	40.81	86.65	57.78	1.52	0.252	16.57	742.36	37.62
T4 (VAM+BBF)	41.64	87.14	60.72	1.56	0.265	16.98	751.38	38.28
CD at 5%	NS	NS	1.13	NS	NS	NS	0.35	NS

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