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# Influence of Biofertilizers on Macronutrient uptake by the Mulberry Plant and its Impact on Silkworm Bioassay

**M. F. Baqual[1]* and P. K. Das[2]**
1- Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, 674, G.P.O., Srinagar – 190001 (J & K), India.
2- Central Sericultural Research and Trainig Institute, Mysore, Karnataka, India.
*Corresponding Author's E-mail: fbaqual@rediffmail.com*

## ABSTRACT
The co-inoculation of mulberry with phosphate solubilizing micro-organisms, nitrogen fixing bacteria and arbuscular mycorrhiza has influenced its macronutrient uptake through leaf. The data revealed that maximum nitrogen (484.12 kg /ha) , phosphorus (59.83 kg /ha) and potassium (244.61 kg/ha) uptake through leaf has taken place due to co-inoculation treatments as compared to the un inoculated treatments irrespective of different levels and sources of fertilizer. Significantly higher effective rate of rearing (ERR, 95.33%), single cocoon weight (2.06 g) and single shell weight (0.44 g) were recorded by feeding silkworm with the leaves harvested from the treatment T8 receiving 175 kg nitrogen and 70 kg phosphorus/ha/yr with rock phosphate as a source of phosphorus and co-inoculated with beneficial micro-organisms. Maximum filament length of 936.33 meter per cocoon and reel ability of (86%) was also recorded in the treatment T8.

**Keywords**: Biofertilisers, Mulberry, Macronutrients, Bio-assay

## INTRODUCTION

The sustainable production of mulberry leaf and cocoon crop is entirely dependent on the maintenance of the soil fertility of mulberry garden through the periodical application of organic manures and fertilizers in required quantity. Among the fertilizer elements, nitrogen and phosphorus play key role in plant growth and development. Although these fertilizers are applied to the soil, yet their application is not commensurate with their recommended dosage due to their alarming cost and erratic availability. Moreover, the ill effects of inorganic fertilizers on soil health can not also be over looked. The biofertilizers enriched with bacteria and fungi have proven to be of gre importance in improving the yield and quality of various agricultural crops and mulberry which is the sole food for silkworm (*Bombyx mori L.*) rearing is one such crop (Chandrashekar et al, 1996; Baqual et al. 2005). Mulberry has been reported to respond well to the application of nitrogenous and phosphatic fertilisers Pain (1964), Kasivishwanathan et al. (1977). The works of Umakanth and Bagyaraj (1998) on dual inoculation of S34 mulberry variety (suitable for rainfed areas) in the nursery bed with Glomus fasciculatum at (a Vesicular Arbuscular Mycorrhizal fungus) and Azotobacter chroococcum (a nitrogen fixing bacterium) revealed considerable increase in plant growth and development of mulberry saplings. Kundaswamy et al. (1986) reported an increase in nitrogen level of mulberry due to Arbuscular Mycorrhizal fungal inoculation. Studies of Rangarajan and Santhanakrishnan (1995) with Morus alba grown with two different levels of nitrogen and phosphorus showed that combined inoculation of plants with plant growth promoting rhizobacteria (Pseudomonas fluorescens), Glomus fasciculatum and Azospirillum were superior to dual inoculation or single inoculants or uninoculated controls and enhanced the quality of mulberry leaves and consequently resulted in the increase of all economically important characters of silkworm larvae and silk production. The results of co-inoculation studies conducted by Chandrashekar et al. (1996) on mulberry with Bacillus megaterium var phospahticum, Azospi-rillum brasilense and Acaulos-pora laevis with single super phosphate as well as rock phosphate revealed a leaf yield of 31075 Kg/ha/year and 29630 Kg/ha/year respectively. The growth parameters like number of branches and length of branches were also increased when compared to control. With a view to understand the role of combined use of biofertilisers in mulberry leaf nutrient uptake and its subsequent impact on silkworm rearing the present study was taken.

## MATERIALS AND METHODS

The experiment was conducted under field conditions with one year old established irrigated mulberry garden of V1 (Victory 1) variety, situated at CSR and TI farm Mysore, Karnataka India. Randomized block design with twelve treatments and three replications was followed. The treatments comprised of two levels of inoculation (I0: No inoculation & I1: inoculation), two sources of phosphorus (S1: Single super phosphate and S2: Rock phosphate) and three fertilizer doses (F1: full recommended dose, F2: 3/4th of recommended dose and F3; ½ the recommended dose of nitrogen and phosphorus respectively). The recommended doses of nitrogen and phosphorus are 350 kg and 140 kg /ha/yr respectively. The experimental soil type was red loam with pH of 8.29, EC 0.197 m mhos/cm2, OC % 0.517, nitrogen % 0.0208%, available phosphorus 12.90 kg/ha, available potassium 316.66 lbs/acre. The plots were inoculated with a mixed culture of mycorrhiza containing spores of Glomus fasciculatum and Glomus mosseae by intercropping technique with maize as mycorrhizal host (Katiyar et al. 1998). All the plots which were inoculated with arbuscular mycorrhiza were then subsequently inoculated with Azotobacter @ 20 kg/ha/yr and phospha-te soulbilizing bacteria (Bacillus megaterium ) @ 5 kg /ha/yr, phosphate solubilizing fungi (Aspergillus awamori) @ 5kg /ha/yr in five equal splits corresponding to five crop harvests. The bacterial biofertilizer and fungi were used by mixing with powdered FYM and applied near the rhizosphere of mulberry by making 8 to 10 inch deep furrows. The chemical fertilizers of nitrogen and phosphorus were applied in five split doses as per the doses mentioned in the treatments above. However, potassium was applied @ 140 kg /ha/yr in the form of muriate of potash as common dose irrespective of treatments. The fertilizers were applied after a gap of 10-12 days of the application of bio-fertilizers.

### ESTIMATION OF NUTRIENT UPTAKE:

The nutrient uptake in terms of nitrogen, phosphorus and potassium (NPK) contents due to different treatments was assessed in the first year. For this, the initial status of soil nitrogen, phosphorus and potassium were first estimated in kg / ha following standard methods. The amount of nutrients which were supplied through NPK fertilizers and farmyard manure in different cropping periods (5 crops / ha / year) was also added to the calculated available soil nutrients and the total amount of soil NPK contents / ha was calculated. Further, the amount of NPK removed from the soil through five crops in a year via leaf harvests were calculated on dry weight basis. Finally these values (NPK contents of leaf) were subtracted from the values of total NPK contents of soil which indicated the amount of NPK up take by mulberry leaf /ha/yr.

In order to evaluate the nutritional status of mulberry leaf as influenced by the co-inoculation of various beneficial micro-organisms with reduced dose of fertilizers, a rearing of silk worm was also conducted with high yielding CSR hybrids (CSR4 X CSR5 ) following improved silkworm rearing technology Datta (1992 ). Twelve disease free layings (dfls) of CSR hybrids were first brushed in twelve individual trays @ one disease free laying in each tray corresponding to 12 treatments of the experiment. Finally, after fourth in star, the worms in twelve trays were extended to thirty-six trays with 200 larvae in each tray to maintain three replications for each treatment until spinning and the experiment was laid in randomized block design. The worms were fed with the leaf harvested from different treatment combinations from the very first in star onwards to get any variations due to different treatments. The data on effective rate of rearing (ERR) per 10,000 larvae brushed by weight and by number, single cocoon weight (SCW), single shell weight (SSW) and shell percentage were recorded following the methods described below. Finally statistical analysis of data was done.

### EFFECTIVE RATE OF REARING (ERR) BY NUMBER.

The effective rate of rearing is the indication of survival percentage of larvae. This was worked out for total number of cocoons harvested out of 200 larvae maintained in each treatment and replication. The values were then converted to ERR by number based on 10,000 larvae brushed.

### ERR BY WEIGHT:

The ERR by weight indicates the cocoon yield. In this case the ripe 5th instar larvae were collected and mounted on collapsible plastic / rotary mountages which facilitate the worms to start spinning cocoons. Once the spinning was over, the cocoons were harvested after 6-7 days of mounting and weighed. This weight was estimated for 10,000 larvae brushed and expressed as cocoon yield in Kg.

### SINGLE COCOON WEIGHT (SCW).

After harvesting, ten cocoons from each treatment and replication were randomly picked up and weighed individually using an sensitive electronic balance. This weight included the weight of cocoon shell and the pupa present inside the cocoon. The weight of the two indicated the single cocoon weight. The weight was recorded and expressed in g.

### SINGLE SHELL WEIGHT.

The same cocoons which were selected for recording the weight were cut open and pupae were removed.Then each empty shell was weighed *individually using the same balance and the weight was recorded in gram which indicated single shell weight.*

### SHELL PERCENTAGE.

*After recording the shell weight and cocoon weight, the shell percentage was calculated using the formula detailed below.*

$$\text{Shell \%} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

**Table 1. Effect of co-inoculation of biofertilizers on the macro nutrient content in the mulberry leaves.**

| Treatment | † Amount of nutrient present in soil | | | Nutrient up take by leaf | | |
|---|---|---|---|---|---|---|
| | N | P | K | N | P | K |
| Un-inoculated treatments | 788.42 | 177.90 | 525.45 | 457.76 | 54.31 | 214.53 |
| Co-inoculated treatments | 788.42 | 177.90 | 525.45 | 484.12 | 59.83 | 244.61 |

This also includes the nutrients applied through fertilizers and manures.

Treatment details:

$T_0$: $F_1S_1I_0$ $T_1$: $F_1S_2I_0$

$T_2$: $F_2S_1I_0$

$T_3$: $F_2S_2I_0$ $T_4$: $F_1S_1I_1$

$T_5$: $F_2S_1I_1$

$T_6$: $F_3S_1I_1$ $T_7$: $F_3S_2I_1$

$T_8$: $F_2S_2I_1$

$T_9$: $F_1S_2I_1$ $T_{10}$: $F_3S_2I_0$

$T_{11}$: $F_3S_1I_0$

$F_1$: Recommended dose of nitrogen and phosphorus

$F_2$: 3/4$^{th}$ of recommended dose of nitrogen and phosphorus

$F_3$: ½ of recommended dose of nitrogen and phosphorus

$I_0$: No inoculation

$I_1$: Inoculation

$S_1$: Single super phosphate (SSP)

$S_2$: Rock phosphate (RP)

**Table 2. Effect of feeding of V1 variety mulberry co-inoculated with biofertilizers on silkworm (*Bombyx mori L.*) rearing.**

| Treatment | ERR/10,000 By No. | By. Wt. | Single Cocoon Wt.(g) | Shell Wt. (g) | SR % | Av. Filament length (m) | Reel ability % |
|---|---|---|---|---|---|---|---|
| T0 | 9416 | 17.01 | 1.94 | 0.43 | 22.25 | 908.33 | 85.00 |
| T1 | 7750 | 14.62 | 1.86 | 0.39 | 21.05 | 873.33 | 80.00 |
| T2 | 7766 | 15.64 | 1.88 | 0.39 | 22.21 | 880.00 | 79.66 |
| T3 | 7950 | 14.40 | 1.75 | 0.40 | 21.03 | 773.00 | 77.00 |
| T4 | 8216 | 16.66 | 1.94 | 0.42 | 22.32 | 890.00 | 82.00 |
| T5 | 9150 | 16.01 | 1.94 | 0.41 | 22.27 | 913.33 | 82.66 |
| T6 | 8633 | 15.41 | 1.90 | 0.44 | 21.39 | 901.66 | 85.00 |
| T7 | 9333 | 16.71 | 1.88 | 0.42 | 21.25 | 870.66 | 85.00 |
| T8 | 9533 | 18.86 | 2.06 | 0.44 | 22.62 | 936.33 | 86.00 |
| T9 | 8850 | 16.53 | 1.93 | 0.41 | 22.08 | 863.33 | 80.33 |
| T10 | 7666 | 14.42 | 1.87 | 0.40 | 21.71 | 889.00 | 82.66 |
| T11 | 8533 | 16.05 | 1.88 | 0.40 | 22.14 | 791.66 | 81.00 |

**Table 3. Economics of using phosphate solubilizing microorganisms (PMS) azotobacter, va-mycorrhiza and rock phosphate mulberry cultivation using 1/2 dose of nitrogen and phosphorus.**

| TREATMENTS | | | CONTROL | | |
|---|---|---|---|---|---|
| Inputs | Quantity. | Cost/ ha/yr ( Rs)‡ | Inputs | Quantity. | Cost/ ha / yr (Rs)‡ |
| Farm Yard Manure | 20 MT§ | 9600.00 | Farm yard manure | 20 MT | 9600.00 |
| Ammonium Sulphate | 875 kg | 4358.00 | Ammonium sulphate | 1750 kg | 8715.00 |
| Rock phosphate | 350 kg | 910.00 | Single super phosphate | 875 | 2800.00 |
| Muriate of potash | 233 kg | 962.00 | Muriate of potash | 233 kg | 962.00 |
| Phosphate solubilising bacteria | 5 kg | 275.00 | ------ | ----- | ---- |
| Vesicular arbuscular mycorrhiza | 1000 kg | 200.00¶ | ------ | ----- | ---- |
| Azotobacter | 23 kg | 1265.00 | ----- | ----- | ---- |
| TOTAL | | 17,570.00 | | | 22,077.00 |

The economics is worked out for V1 mulberry variety under 350: 140: 140 NPK fertilizer dosage difference (Control - Treatment) = 22,077 – 17570 = 4500.00 net saving over control.
‡ The currency is in Indian rupees, to convert it in US $ divide the figures by 42.
§ Metric tones
¶ The depreciation cost of VAM for 15 years.
Treatment details same as in Table 1.

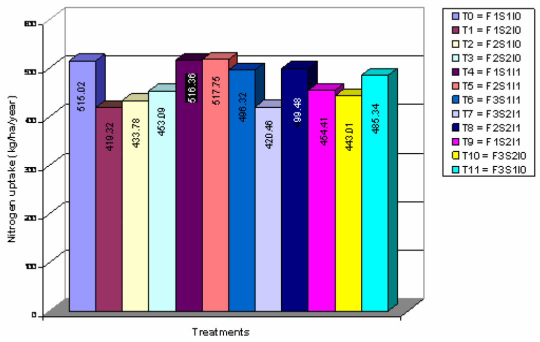


**Fig. 1. Effect of co inoculation on nitrogen uptake through mulberry leaf (kg/ha/year) Treatment details same as in Table 1.**

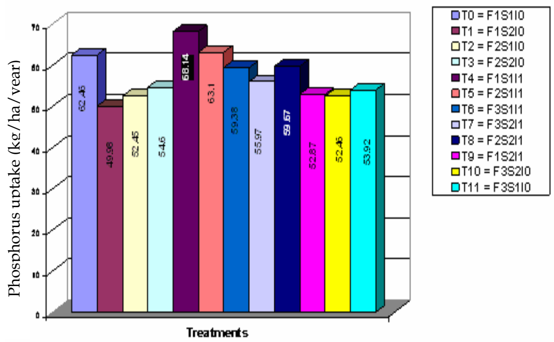


**Fig. 2. Effect of co inoculation on phosphorus uptake through mulberry leaf (kg/ha/year).Tre-atment details same as in Table 1.**

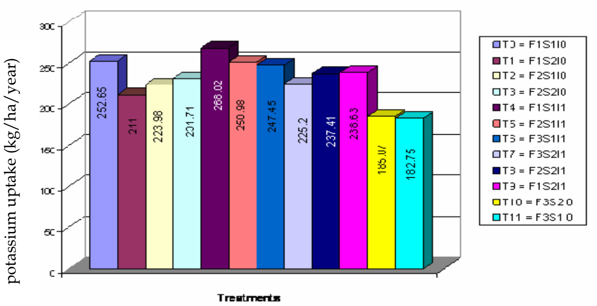


**Fig. 3. Effect of co inoculation on potassium uptake through mulberry leaf (kg/ha/year).**

## RESULTS AND DISCUSSION

The inoculation of biofertilisers with reduced dose of inorganic fertilizers has resulted in significant improvement in uptake of nitrogen, phosphorus and potassium through mulberry leaf as compared to the un-inoculated plants. The data on macronutrient uptake through the leaf has revealed that maximum nitrogen (484.12 kg /ha) , phosphorus (59.83 kg /ha) and potassium (244.61 kg/ha) uptake through leaf has taken place due to co-inoculation treatments as compared to the un inoculated treatments irrespective of different levels and sources of fertilizer (Table – I). The maximum nitrogen uptake (517.75 kg/ha) through leaf has taken place due to the treatment T5 with co-inoculation. Maximum phosphorus uptake (68.14 kg/ha) and potassium uptake (268.02 kg /ha) through leaf was recorded with the treatment T4. (Figs:1-3). Thus it has been clearly realized that co-inoculation of mulberry has also influenced the status of macronutrient uptake of mulberry through leaf. The result of Das et.al (1994) in improving mulberry leaf quality due to Azotobacter inoculation also confirms the present findings and the economic potentiality of using various microbial inocula in mulberry cultivation. There was a clear indication of improvement in various cocoon characters of mulberry silkworms because of feeding leaf from the co-inoculated plots. This is attributed to the increase in the nitrogen, phosphorus and potassium contents in mulberry leaf due to inoculation. The studies of Baqual et al (2005) have revealed increased NPK and chlorophyll content of mulberry leaf due to co-inoculation with microbial consortium. The increased leaf nutrient content in turn might have contributed to better economic characters of silkworm (Bombyx mori L.) as has been observed in the present study. Significantly higher (ERR) effective rate of rearing by number (9533 the number of cocoons harvested out of 10,000 larvae brushed), single cocoon weight (2.06 g ) and single shell weight (0.44 g) were recorded by feeding silkworm with the leaves harvested from the treatment T8 co-inoculated with beneficial micro-organisms (Table 2).

The role of quality mulberry leaf in production of increased quantity of quality cocoons have been well established by many workers Krishnaswami 1978; Datta 1992; Ravikumar, 1988). Similarly, the importance of nutritive care for young age silkworm rearing and its influence on cocoon crop production is also widely accepted (Chaluvachari, 1995).

Though the treatment T8 was significant over many other treatments, it was also at par with the treatments T0, T5, T6 and T9 in respect of ERR by number, T0, T4 and T5 in respect of single cocoon weight and T6 in respect of single shell weight. However, a non-significant variation was also observed with ERR by weight and shell ratio due to different treatments. Significant variation was also observed in the silk filament length and cocoon reel ability due to different treatments. Maximum filament length of 936.33 meter per cocoon was recorded in the treatment T8 as compared to other treatments except T0, T5 and T6. Similarly, the reel ability of cocoon was also significantly increased in the treatment T8 (86%). Since, the young age silkworm needs more amount of sugar and protein in mulberry leaf for their better survivability in adult age, the increased protein and other essential nutrients in mulberry leaf due to co-inoculation further justifies the importance of the use of eco-friendly and beneficial micro-organisms as bio-inoculants in mulberry cultivation.

The studies on economics of using bio-inoculants in mulberry also indicated that approximately Rs 2000 – 4500 /ha/yr could be saved only on the input cost of nitrogen and phosphorus in mulberry cultivation.

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