Mysore J. Agric. Sci., 51 (3) : 660-665, 2017

Bioresponses of Mulberry to Foliar Spray of Silkworm (*Bombyx mori* L.) Pupal Protein

D. S. Mahesh and T. K. Narayanaswamy
Department of Sericulture, College of Agriculture, UAS, GKVK, Bengaluru-560 065
E-mail : tkns56@rediffmail.com

Abstract

A field experiment was conducted to study the effect of foliar spray of silkworm pupal protein on yield and quality parameters of V-1 Mulberry at Department of Sericulture during 2016-17. The results of the study indicated that, application of recommended dose of fertilizers with foliar spray of silkworm pupal protein @ 2 per cent (T_6) significantly increased the mulberry yield attributing parameters *viz*., number of leaves per plant (226), leaf area (196.55dm^2), leaf yield per plant (1047.92g) and leaf yield per hectare per year (64.68 MT) compared to control. Significant improvement in leaf moisture (75.69 %), total chlorophyll (2.48mg / g), total sugars (13.22 %), total soluble protein (12.18 %) and crude protein (21.14 %) was observed in T_6 treatment compared to all the other treatments. Application of silkworm pupal protein at 2 per cent concentration along with recommended dose of fertilizers significantly increased both biochemical constituents and yield parameters of mulberry leaves.

Keywords: Mulberry, pupal extract, pupal protein, biochemical constituents, yield parameters

Mulberry is a perennial, deep rooted, fast growing and high biomass producing foliage plant. Mulberry leaf is a sole source of food for silkworm (*Bombyx mori* L.). The quality of mulberry leaf is being considered as the prime factor governing the production of good cocoon. Improved nutrient management practices coupled with improved agronomic management technologies substantially increased the harvest index of mulberry. However, owing to the increasing demands for silk, stress has to be laid on the further improvement of leaf productivity both in terms of quality and quantity (Geetha *et al.,* 2017). Due to excessive use of synthetic chemical fertilizers a serious threat is caused not only to mulberry crop but also to the environment (Shashikanta, 2016). So, an alternative measure is necessary for enhancing mulberry production without causing any serious damage to the ecosystem. A foliar spray is generally used for increasing crop production, yield and leaf quality effectively. Therefore, it is necessary that the nutrients applied to the mulberry through foliar nutrition are made easily available even at later stages of leaf development and it should be cost effective. Venkataramana *et al.* (2009) opined that, the leaf quality alone contributes to about 38.2 per cent among the factors influencing the success of silkworm cocoon crop.

Thus, the mulberry leaf quality plays a predominant role in healthy growth of silkworm and the economic traits such as larval, cocoon and grainage parameters which are influenced largely by the nutritional status of the leaves fed to silkworm (Krishnaswami *et al*., 2011). Hence, nutrition of silkworm, *Bombyx mori* L. is of primary importance as the cocoon production is directly influenced by the nutritive status of mulberry leaves.

The productivity of mulberry not only varies according to the different geographical area but also according to mineral potential of soil. Among the factors responsible for low yield, inadequate nutrient supply plays an important role. The quality and quantity of mulberry can be increased by adopting the physiological manipulations through the foliar sprays of nitrogen sources *i.e.,* silkworm pupal extract, silkworm pupal protein and urea. Hence, the present study was undertaken to find out the effect of different concentrations of (0.5, 1 and 2 per cent) silkworm pupal extract, silkworm pupal protein and urea to improve the biochemical constituents and yield attributing parameters of mulberry.

Material and Methods

A field experiment was conducted in an established irrigated V-1 mulberry garden planted at a spacing of 90 x 90 cm in order to study the effect of foliar application of silkworm pupal extract, silkworm pupal protein and urea on yield and quality of mulberry in randomized block design with fourteen treatments and three replications during 2016-17 at Department of Sericulture, UAS, GKVK, Bengaluru (Plate 1). All the cultural practices were followed as per the package of practices for irrigated mulberry garden (Dandin *et al.,* 2010). Three foliar sprays of silkworm pupal extract, silkworm pupal protein and urea were given as per treatments at 30, 40 and 50 days after pruning (DAP) the mulberry garden. The recommended doses of farm yard manure (20 MT / ha / yr) and chemical fertilizers (350 kg N: 140 kg P: 140 kg K / ha / yr were applied to all treatments. The treatments were T_1= Silkworm pupal extract (SPE) @ 0.5 per cent , T_2= Silkworm pupal extract (SPE) @ 1 per cent , T_3= Silkworm pupal extract (SPE) @ 2 per cent , T_4= Silkworm pupal protein (SPP) @ 0.5 per cent , T_5= Silkworm pupal protein (SPP) @ 1 per cent , T_6= Silkworm pupal protein (SPP) @ 2 per cent, T_7= SPE @ 0.5 per cent + SPP @ 0.5 per cent, T_8 = SPE @ 1 per cent + SPP @ 1 per cent, T_9= SPE @ 2 per cent + SPP @ 2 per cent , T_{10}= Urea spray @ 0.5 per cent, T_{11}= Urea spray @ 1 per cent, T_{12}= Urea spray @ 2 pe rcent, T_{13}= Aqueous extract and T_{14}= Control (RDF + 20 MT FYM / ha / year). At the time of harvest, yield attributing parameters *viz*., number of leaves per plant, leaf area (dm^2), leaf yield per plant (g) and leaf yield per hectare (MT) was recorded. After harvest of the crop, the quality parameters like leaf moisture, total chlorophyll, total sugars, total soluble protein and crude protein was estimated by using standard procedures.

Plate 1: General view of foliar sprayed mulberry plot

Estimation of biochemical constituents of V-1 mulberry

Leaf moisture

Moisture percentage in leaf was estimated through gravimetric method by taking the difference between fresh and dry weights and expressed in percentage on fresh weight basis (A.O.A.C., 1980).

$$\text{Moisture percentage} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}}$$

Estimation of total Chlorophyll

Chlorophyll content in mulberry leaves was determined by the following procedure described by Hiscox and Israelstam (1979). The total chlorophyll content of leaf was computed using the formula suggested by Arnon (1949).

Fresh leaves in each treatment were plucked at 60th day for estimating chlorophyll content. 100 mg of leaf tissue was placed in a vial containing 7 ml of di-methyl sulphoxide (DMSO) and chlorophyll was extracted in to the fluid by incubating at 65^0C overnight. The extract was then transferred to a graduated tube and made up to a total volume of 10 ml with DMSO, assayed immediately or transferred to vials and stored between 0-4^0C until required for analysis. Assay was done by transferring 3 ml of chlorophyll extract to a cuvette and the OD values at 645nm and 663nm were read in an ELICO UV spectrophotometer against a DMSO blank. The total chlorophyll content in different treatments was calculated by using the following formula,

$$\text{Total Chlorophyll (mg/g fresh weight)} = \frac{20.2\ (\text{O.D. } 645) - 8.02\ (\text{O. D. } 663) \times \text{Volume}}{1000 \text{ g weight of leaves (g)}}$$

Estimation of crude protein

The crude protein estimation was carried out by micro kjeldahl method (A.O.A.C., 1980). A known weight of the sample was digested with concentrated sulphuric acid using potassium sulphate and mercuric sulphate as catalyst, until a clear solution was formed. The digested samples were distilled in microkjeldahl distillation apparatus after addition of 10ml of 40 per cent sodium hydroxide. The ammonia liberated was collected in 2 per cent boric acid containing 2 to 3 drops of mixed indicator of methyl red and methylene blue. This ammonium borate was titrated against 0.02 N sulphuric acid and the total nitrogen was calculated. The nitrogen content was multiplied with 6.25 to obtain crude estimate of protein.

Estimation of total soluble proteins

Total soluble proteins were estimated by using the procedure of Lowry *et al.* (1951). The fresh plant sample was transferred to a blender; pre chilled acetone (kept at -20^{0}C) was added sufficiently to cover the sample and then blended for 2 to 3 minutes at low speed followed by high speed for 3 to 5 minutes with an intermittent break. Then the mixture was filtered through a Buchner funnel with Whatman No. 1 filter paper under suction. Finally, the powder was spread on Whatman No. 1 filter paper and air dried for one hour and kept at -20^{0}C till further use. 0.5 gm of acetone powder was extracted with 5 ml of 0.2 M Tris-HCl buffer (pH 8.4) containing 0.056 M β-mercapto-ethanol. The resultant slurry was centrifuged at 12000 rpm for 10 minutes at 4^{0}C. The supernatant was used for the estimation of total soluble proteins. Known aliquots of the samples were made up to 1 ml using distilled water. 5 ml of solution C was added and mixed and allowed to stand for 10 minutes for colour development. The colour developed was read at 660 nm after 30 minutes, against reagent blank. Known aliquots of extracts were made up to 1 ml with distilled water and to this 0.5 ml of phenol reagent was added and mixed well. Then 5 ml of 96 per cent sulphuric acid was added and placed in a water bath at 30^{0}C for 20 minutes and the absorbance was read at 490 nm. The amount of total sugars in the sample was estimated by comparing the results with a standard glucose curve (0-100 μg / ml) prepared in similar way.

Estimation of total sugars

The total sugars in all the samples were estimated by the phenol sulphuric acid method (Dubios *et al.*, 1956). 0.25 gm of dry sample was extracted with 10 ml of 80 per cent warm ethanol in a pestle and mortar and the extract was centrifuged and the supernatant was retained. The procedure was repeated for two more times and the extracts were pooled and evaporated to dryness in a hot water bath and the residue was dissolved in 5 ml of distilled water. Interfering coloured pigments were removed by using activated charcoal. 5 ml of extract was mixed with activated charcoal and kept overnight after which the extract was centrifuged and the clear supernatant was used for estimation of total sugars.

Yield parameters of mulberry (V-1) at 60 days after pruning

Number of leaves per plant

The leaves were counted from five labeled plants at 60th days after pruning and mean number of leaves per plant was calculated by using the formula.

$$\text{Number of leaves per plant} = \frac{\text{Total No. of leaves}}{\text{No. of plants}}$$

Leaf area (dm^2)

Calculate the leaf area by measuring the length and breadth of leaf and multiply with 0.69 (correction factor) on the 60th day after pruning from five labeled plants.

Fresh leaf yield (g / plant and MT / hectare)

Five labeled plants were maintained exclusively for estimation of leaf yield were harvested individually plant wise and then leaf yield was recorded.

The total leaf yield obtained from net plot was recorded as the leaf yield and expressed in g / plot and converted to the leaf yield in metric tonnes ha^{-1}.

Results and Discussion

There were significant changes in the yield and biochemical levels of mulberry due to foliar application of different concentrations of silkworm pupal protein was observed on mulberry. The values for leaf moisture content was higher in the treatment T_6 (75.69 percent) of 2 per cent SPP foliar application (Table I), this may be attributed to the stimulatory effect in leaf anatomical parameters such as stomatal size, frequency, mesophyll tissue, cuticle thickness and leaf thickness could have resulted in higher moisture content. Dhiraj and Venkatesh Kumar (2012) found that, the formation of wax, which has been considered to play an important role in water conservation of plants, was proportional to the thickness of cuticle. The thickness of the leaf is also physiologically important, since thicker leaves tend to have greater

TABLE I

Biochemical constituents of Mulberry (V_1) leaf as influenced by foliar spray of silkworm pupal protein at 60 DAP.

	Treatments	Leaf moisture (%)	Total chlorophyll (mg / g)	Total Sugar (%)	Total Soluble protein (%)	Crude protein (%)
T_1	Silkworm pupal extract (SPE) @ 0.5%	71.68	2.20	12.28	10.89	19.44
T_2	Silkworm pupal extract (SPE) @ 1%	72.50	2.26	12.42	11.04	19.71
T_3	Silkworm pupal extract (SPE) @ 2%	74.01	2.46	12.80	12.55	19.82
T_4	Silkworm pupal protein (SPP) @ 0.5%	72.00	2.30	12.60	11.74	19.96
T_5	Silkworm pupal protein (SPP) @ 1%	73.15	2.23	12.94	11.39	20.68
T_6	Silkworm pupal protein (SPP) @ 2%	75.69	2.58	13.22	12.18	21.14
T_7	SPE @ 0.5% + SPP @ 0.5%	72.90	2.29	13.06	11.90	21.50
T_8	SPE @ 1% + SPP @ 1%	74.33	2.50	13.13	12.06	20.59
T_9	SPE @ 2% + SPP @ 2%	73.04	2.41	12.29	11.98	20.80
T_{10}	Urea spray @ 0.5 %	71.93	2.21	12.11	11.06	20.06
T_{11}	Urea spray @ 1 %	73.11	2.30	12.08	11.24	20.22
T_{12}	Urea spray @ 2 %	72.18	2.26	12.36	11.57	20.48
T_{13}	Aqueous extract	71.22	2.32	12.21	10.84	19.72
T_{14}	Control (Recommended dose of fertilizers)	70.18	2.13	11.84	9.96	18.06
	F-Test	*	*	*	*	*
	SEM±	0.48	0.012	0.10	0.19	0.56
	CD @ 5 %	1.46	0.037	0.30	0.57	1.64

Note : %- Percent

mesophyll surface unit area and hence facilitate to high photosynthetic rate.

The data revealed that the maximum total chlorophyll (2.58 mg / g), total sugars (13.22 per cent), total soluble protein (12.18 per cent) and crude protein (21.14 per cent) were recorded in T_6 which recieved 2 per cent silkworm pupal protein, followed by the treatment (T_8) of SPE @1 per cent + SPP @1 per cent over all other treatments and control (Table I). The foliar application of silkworm pupal protein and silkworm pupal extract supplemented the additional essential nutrients and are the direct source of nitrogen. Similarly, the foliar application enhances the absorption of nutrients by the leaf at the site of application. This might be the reason for increasing chlorophyll and crude protein contents in the leaf. These findings are supported by the observations of Nandakumar *et al.* (2008), who have reported that, protein rich sources of foliar sprays contains multifunctional organic acids (amino acids, citric acid and humic acid) with the necessary major nutrients, micro elements and

TABLE II.

Effect of foliar application of silkworm pupal protein on yield parameters of Mulberry (V_1) at 60DAP.

	Treatments	Number of leaves / plant	Leaf Area (dm2)	Leaf yield / Plant (g)	Leaf yield / ha (MT)
T_1	Silkworm pupal extract (SPE) @ 0.5%	206	158.55	931.14	57.47
T_2	Silkworm pupal extract (SPE) @ 1%	210	157.77	921.52	56.88
T_3	Silkworm pupal extract (SPE) @ 2%	216	159.55	899.78	55.53
T_4	Silkworm pupal protein (SPP) @ 0.5%	202	162	920.07	56.79
T_5	Silkworm pupal protein (SPP) @ 1%	215	176.88	973.99	60.11
T_6	Silkworm pupal protein (SPP) @ 2%	226	196.55	1047.92	64.68
T_7	SPE @ 0.5% + SPP @ 0.5%	208	149.88	887.56	54.04
T_8	SPE @ 1% + SPP @ 1%	219	181.7	995.76	61.46
T_9	SPE @ 2% + SPP @ 2%	213	151.66	888.13	54.81
T_{10}	Urea spray @ 0.5 %	204	164.88	986.1	60.86
T_{11}	Urea spray @ 1 %	211	161.44	899.09	55.49
T_{12}	Urea spray @ 2 %	207	160.44	858.93	53.01
T_{13}	Aqueous extract	206	176	897.21	55.38
T_{14}	Control (20 MT FYM + Recommended NPK)	202	144.88	839.94	51.84
	F-Test	*	*	*	*
	SEM±	3.90	1.40	6.02	0.40
	CD @ 5 %	11.68	4.21	18.10	1.22

vitamins. It is known to promote the uptake of nutrients, stimulate photosynthesis, protein synthesis and activate enzyme action. Foliar application of protein rich sources has significantly increased the chlorophyll-a, chlorophyll-b, total chlorophyll and soluble protein while crude protein was higher.

Foliar application with silkworm pupal protein @ 2 per cent (T_6) significantly increased the leaf area (196.55 dm^2) followed by foliar application with silkworm pupal extract @ 1 per cent + silkworm pupal protein @ 1 per cent (T_8) (181.7 dm^2) treatments compared to other treatments (Table II). Higher leaf area was recorded in T_6, which may be due to complete solubility of protein in water, better penetration and availability of nutrient for plant absorption which makes increase in the dimension of leaf (Dhiraj and Venkatesh Kumar, 2012).

Higher leaf yield per plant was recorded in T_6, which recieved silkworm pupal protein (SPP) @ 2 per cent (1047.92 g) followed by T_8, which received SPE @ 1 per cent + SPP @ 1 per cent (995.76 g) and T_5 (973.99 kg) treatments compared to other treatments (Table II). Foliar spray induced promontory effect due presence of growth inducing substances, essential amino acids, vitamins and plant nutrients. The results are in accordance with Singhvi *et al.* (2010) who have reported that leaf yield was increased by the foliar application of protein sources in mulberry. Increased leaf area and also other bioconstituents of leaves contributed for increased leaf yield. The yield increase in T_6 may be the fact that the amino acid based foliar spray has got higher solubility, penetration and hence better absorption by the crop. Silkworm pupal protein also more soluble in water and provides the good amount of nutrient for the plant absorption.

Basal dose of Recommended NPK+FYM along with each time foliar addition of silkworm pupal prtein @ 2 per cent / plant through three times at ten days intervals starting from 30 DAP significantly improved the leaf yield of established V-1 mulberry in irrigated condition.

Foliar feeding is a technique of feeding plants by applying nutrients directly to the leaves. It has been known for many years that, plants are able to absorb essential nutrients through their leaves. Present study concluded that, foliar nutrients improved crop quality and yield of mulberry. The evaluation of silkworm pupal protein as a plant growth stimulator for mulberry under field conditions showed that the application of silkworm pupal protein @ 2 per cent along with recommended dose of NPK + FYM has significantly increased the yield attributing and quality parameters of mulberry (V-1).

REFERENCES

A.O.A.C., 1980, *Official Methods of Analysis.* (Ed. Daniel banes), published by A.O.A.C., Washington, D.C., Bangalore, p. 105.

Arnon, O. I., 1949, Copper enzymes in isolated chloroplasts polyphenoxidase in *Beta vulgaris. Pl. Physiol.,* **24**: 1-15.

DANDIN, S. B., JAYASWAL, J. AND GIRIDHAR, K., 2010, *Handbook of Sericulture Technologies,* CSB, Bengaluru, p. 25.

DHIRAJ, K. AND VENKATESH KUMAR, 2012, Application of foliar nutrients to increase productivity in sericulture. *J. Entomology,* **9** : 1-12.

DUBIOS, M., GILES, K. A., HAMILTON, T. K., ROBEOS, R. A. AND SMITH, R., 1956, Calorimetric determination of sugars and related substances. *Anal. Chem.*, **28**: 350-356.

GEETHA, T., RAMAMOORTHY, R. AND MURUGAN, N., 2017, Effect of foliar spray of micronutrients applied individually and in combination on mulberry leaf production, cocoon productivity and profitability. *Statistical Approaches on Multidisciplinary Research,* **1** : 12-17.

HISCOX, J. D. AND ISRAELSTAM, G. F., 1979, A method for the estimation of chlorophyll from leaf tissue without maceration. *Can. J. Bot.*, **57**: 1332-1334.

KRISHNASWAMI, S., KUMARARAJ, S., VIJAYARAGHAVAN, K. AND KASIVISWANATHAN, K., 2011, Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing and nitrogen fertilizers. *Indian Silk*, **10** : 79-89.

LOWRY, O. H., ROSEBROUGH, N., FARR, A. AND RANDALL, R., 1951, Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193** : 265-275.

NANDAKUMAR, R., SARVANAN, A., SINGARAM, P. AND CHANDRASEKARAM, 2008, Effect of lignite humic acid on soil nutrient availability of different growth stages of rice grown on vertisol and alfisols. *Acta Agronomica Hungarica*., **52** : 227-236.

SHASHI KANTA, 2016, Effect of foliar spray B-995 on mulberry growth and yield parameters. *Inter. J. Innovative Res. and Rev.,* **4**(2) : 26-28.

SINGHVI, N. R., KODANDARAMAIAH, J., REKHA, M., SARKAR, A. AND DATTA, R. K., 2010, Effect of salicylic acid on leaf yield and pigment content in mulberry *Morus* spp. *Indian Silk*, **2** (1) : 100-102.

VENKATARAMANA, P., KRISHNA RAO, J. V. AND KAMBLE, C. K., 2009, Efficacy of foliar sprays of vermin wash and cow dung wash on biochemical and yield attributes of mulberry (*Morus alba* L.). *Karnataka J. Agric. Sci.,* **22**(4) : 921-923.

(*Received :* May, 2017 *Accepted :* August, 2017)