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Potala Harshita Mala
Department of Sericulture, College of Agriculture University of Agricultural Sciences GKVK, Bengaluru, Karnataka, India

Chandrashekhar S
Department of Sericulture, College of Agriculture University of Agricultural Sciences GKVK, Bengaluru, Karnataka, India

Corresponding Author:
Potala Harshita Mala
Department of Sericulture, College of Agriculture University of Agricultural Sciences GKVK, Bengaluru, Karnataka, India

Influence of Seri-waste bio-digester on biochemical constituents, growth and yield of mulberry

Potala Harshita Mala and Chandrashekhar S

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Abstract
A field experiment was conducted to study the influence of seri waste biodigester on growth and yield parameters of V_1 mulberry at department of sericulture during 2017-18. The results indicated that, application of 50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF (T_4) increased the mulberry yield attributing parameters *viz.*, plant height (163.19 cm), number of branches per plant (13.32), number of leaves per branch (33.00), leaf area (208.33 dM^2) and leaf yield (1012.33 g/plant) per plant compared to control. Significant increase in leaf moisture, total chlorophyll, crude protein and crude fibre was observed in T4 treatment compared to all other treatments. Application of 50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF significantly increased both biochemical constituents and yield parameters of mulberry leaves.

Keywords: Mulberry, Seri waste bio digester, biochemical constituents, yield parameters

Introduction
Sericulture is an agro-based industry which is mainly practiced by small and marginal farmers in India for regular source of income and it generates enormous quantity of bio-waste. In this regard the use of Seri-waste in the biogas production has come up recently, the use of Bio-digester effluent on the growth of mulberry may add the nutrition to the soil and improves the fertility levels may in turn contribute to the higher growth and yield of mulberry.
Karnataka as a sericulturally leading state producing enormous quantity of biomass which provides an alternative source of energy as the biogas generated by bio-digester and also it is good organic sources of nutrient to the soil. Realization of the fertility level of the soil nutrients is the most important factor in sericulture to obtain good cocoon harvest and inturn resulting in good quality silk. However, microbial conversion of organic matter to methane has become attractive as a method of waste treatment and resource recovery. Bio-digesters are however, good solutions for rural agrarian communities, intensive livestock farms, towns and cities where large volumes of domestic and animal wastes can produce significant quantities of methane. Indiscriminate use of chemical fertilizers and pesticides leads to the increased soil fertility depletion. One of the ways to solve the problem is the promotion of integrated farming systems, with minimal external inputs and recycling of all wastes. (Preston and Leng, 1989) [8]. The most important feature of this approach is the recycling of animal and plant wastes in order to prevent deterioration of soil fertility through loss of nutrients and organic matter, erosion and salinity. (Rodriguez and Preston 1996) [11]. Hence, application of manures to the soil can reduce environmental pollution and also improve the soil fertility through recycling of plant nutrients.

Materials and Methods
A field Experiment was conducted during Kharif season from August – February, 2017-18 at the Department of Sericulture, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bengaluru. The type of soil is clay loam.

Treatment Details
T_1: 100% Recommended Dose of Fertilizers

T_2: 75% Seri Bio-digester effluent+ 25% RDF
T_3: 50%Seri Bio-digester effluent + 25% Compost + 25% RDF
T_4: 50%Seri Bio-digester effluent+25% Bio-digester effluent + 25% RDF
T_5: 25%Seri Bio-digestereffluent + 25% Bio-digestereffluent + 25% Vermicompost +
25% Compost
T_6: 75% Seri Bio-digester effluent + 25%Vermicompost
T_7- 50%Compost+50%Seri bio-digester effluent
T_8- 100% Vermicompost
T_9- 100% Bio-digester effluent
T_{10}-100%Compost
T_{11}-100% Seri Bio-digester effluent

Leaf analysis
Mulberry leaf samples were collected from every treatment at 60 days after pruning for analysis.

Total nitrogen
Nitrogen content of leaves was estimated by adopting Micro Kjeldhal digestion distillation method.

Digestion of plant samples with di-acid mixture
A powdered sample of 0.5g was pre-digested with 5ml of concentrated HNO3 and again digested with a di-acid mixture (HNO3: HClO4 in the proportion of 10:4 ratio). Volume of the digest was made up to 100ml with distilled water and preserved for total elemental analysis (Jackson, 1967) [4].

Total phosphorus
The total phosphorus content was determined by taking a known volume of the digested materials by adopting the Vanadomolybdophosphoric yellow colour method as described by Jackson (1967) [4].

Total potassium
Using the respective di-acid, the total potassium content of the above samples was estimated by atomizing the diluted digest to a calibrated flame photometer under suitable measuring conditions as described by Jackson (1967) [4].

Secondary nutrients
Calcium and magnesium were determined by the EDTA titration or Versonate-titraton method. Sulphur content in the di acid digested sample was estimated by turbidometric method as outlined by Jackson (1967) [4].

Micronutrients
The content of Zn, Mn, Cu and Fe in the residues was determined by using atomic absorption spectrophotometer with appropriate hallow cathode lamps (Lindsey and Norwell, 1978) [5].

Estimation of biochemical constituents of V1 mulberry
The collected mulberry leaf samples at the time of harvest were dried at 60° C in hot air oven, powdered using a grinder fitted with stainless steel blades and preserved in polythene bags for further analysis (Jackson, 1967) [4]. The leaf moisture content, protein and fibre contents were carried out using standard reference of AOAC (1990) protocol.

Leaf moisture
Moisture content of mulberry leaf expressed in% wet basis, was carried out by hot air oven method at105±1°c for 24 hours and continue the same procedure till constant results was obtained and then percent moisture content was determined by following formula

$$\text{Moisture content(\% w. b)} = \frac{\left(\begin{matrix}\text{Initial weight}\\ \text{of leaf}\end{matrix}\right) - \left(\begin{matrix}\text{Final weight}\\ \text{of leaf}\end{matrix}\right)}{\text{Initial weight leaf}} \times 100$$

Chlorophyll estimation
Chlorophyll content in mulberry leaf was determined by the following procedure described by Hiscox and Isrealstam (1979) [3]. The total chlorophyll content of leaf was computed using the formula suggested by Arnon (1949) [2].

Total Chlorophyll

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

Protein
The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method involving digestions, distillation and titration of the sample. The nitrogen in protein or any other organic material is converted to ammonium sulphate by H_2SO_4 during digestion.

Reagents required
- Mixed indicator: prepared by using 0.1% bromocresol green and 0.1% methyl red indicator in 95% alcohol.
- 4% boric acid
- 40% sodium hydroxide
- 1N HCl
- Catalyst for digestion: digestion mixture (copper sulphate and potassium sulphate)

Digestion: weigh 250 mg of sample and placed in 250ml Kjeldahl flask add 1 to 2 g of catalyst mixture and 7ml of conc.H_2SO_4 and placed in digestion chamber for an hour at 300°C and 2 hours at 400°C or until the colour of the digest was clear white.

Distillation: distillation of sample was carried out by automatic distillation unit, place the Kjeldahl flask in one chamber and conical flask in another chamber and press the button run. The distillation unit was first furnished with boric acid (4%) and it was done for about 13sec. and after that furnished with NaOH (40%) and it was done for about 9min, during this process the liberated ammonia gets trapped. During the distillation process the colour of the digests in the Kjeldahl flask was turned to pale blue.

Titration: the solution in the conical flask was titrated against the 1N Hcl by adding 1or 2 drops of mixed indicator till the brick red colour will come. The protein content of the sample was calculated by multiplying the nitrogen value by a factor 6.25.

The result was calculated using the formula:

$$\text{Protein (\%)} = \frac{14 * \text{Titre value} * \text{Normality of Hcl}}{\text{Sample weight(g)}} * 6.25$$

Fibre: The crude fibre of the sample was estimated by using moisture and fat free sample by Fibra plus apparatus. Transfer the 2-3g of moisture and fat free sample to fibre estimating thimbers and placed in the digestion chamber. Add 150ml of boiling sulphuric acid solution to the chamber and set the temperature to 500°C for 10min after that reduce the temperature to 400°C and leave it for 30min. after that drain out the acid by using suction pump and washed with boiled distilled water after acid wash the digestion chamber was filled with 150ml of boiling sodium hydroxide solution for about 30min at 400°C. Then drain out the alkali solution by using suction pump again the tubes were washed with boiled distilled water. After that place the crucibles in oven at 100°C for about one hour and take the weight of crucible after that place the crucible in muffle furnace at 550°C for 3 hours or until the white ash was formed and take the final weight.

The fibre content of sample was calculated by:

$$\text{Crude fibre } \left(\frac{g}{100g}\right) = \frac{[100 - (\text{moisture} + \text{fat content of sample})] * We - Wa}{\text{weight of sample taken}}$$

W_e = Pre-weighed ash (g)
W_a = Weight of dish after washing (g)

Growth and Yield parameters of mulberry

Plant height (cm): Plant height was recorded from the base of the main shoot to the top most fully opened leaf in five randomly selected plants under each treatment, in three replications. The mean of five plants was worked out to obtain the plant height.

Number of branches per plant

The number of branches in five randomly selected plants was counted in each treatment under each replication and the mean was worked out.

Number of leaves per shoot

The total number of leaves in each shoot of the plant was counted from five randomly selected mulberry plants.

Leaf area (dm^2 plant^{-1}):

The area of third fully opened leaf from top was determined by multiplying length× breadth with a constant factor 0.6898. The product was then multiplied with number of green leaves per plant to get leaf area per plant.

Leaf yield (g plant^{-1})

Leaf yield per plant was recorded replication wise by harvesting fresh leaves from five randomly selected plants under each treatment and mean yield was calculated.

Results and Discussion

Table 1: Influence of Seri waste bio digester on biochemical constituents of V1 mulberry on 60th day after pruning

Treatments	Moisture content (%)	Total chlorophyll (mg/g)	Crude protein (%)	Crude fibre (%)
T_1	70.32	2.21	18.36	1.22
T_2	72.59	2.39	19.67	3.40
T_3	73.10	2.40	20.09	4.65
T_4	74.46	2.41	20.19	4.78
T_5	73.82	2.44	19.69	3.78
T_6	72.56	2.27	19.56	2.73
T_7	72.19	2.25	19.52	2.26
T_8	70.53	2.23	18.80	1.48
T_9	71.49	2.22	19.30	1.76
T_{10}	70.65	2.24	19.10	1.67
T_{11}	71.55	2.25	19.51	2.13
F- test	*	*	*	*
S.Em±	0.113	0.005	0.116	0.022
C.D@ (5%)	0.332	0.014	0.342	0.064

Moisture percentage of V1 leaves varied among different treatments. Mulberry raised with T_4 (50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF) recorded highest leaf moisture (74.46%) over other treatments followed by T_5 (25% Seri Biodigester effluent + 25% Bio-digester effluent + 25% Vermicompost + 25% Compost) recorded as 73.83% of leaf moisture. Similarly, the lowest percentage of moisture (70.32%) was recorded in T_1(100% Recommended dose of fertilizers). Increase in moisture content of leaves might be due to water retention capacity and slow and steady supply of moisture from seri biodigester liquid. Yokoyama (1974) [14] reported that, usually moisture content of mulberry leaves varied from 64 to 83%. Application of organic manures supplied all the nutrients to mulberry which inturn increased the moisture content (Ravikumar 2003) [9].

Significant improvement was recorded in chlorophyll content of V1 leaves (Table 1). Maximum amount of total chlorophyll (2.44 mg/g) was encountered in T_5-(25% Seri Bio-digester effluent + 25% Bio-digester effluent + 25% Vermicompost + 25% Compost) followed by T_4 (50% Seri Bio-digester effluent +25% Bio-digester effluent + 25% RDF) which recorded, 2.41 mg/g of total chlorophyll. Similarly, the minimum chlorophyll was recorded in T_1 (100% Recommended dose of fertilizers) with 2.21 mg/g of total chlorophyll. Adequate supply of nutrients through seriwaste biodigester liquid may be attributable for increase in chlorophyll content in leaves. These observations are in agreement with findings of Shivakumar *et al.* (2000) [13] where, organic manures supplemented in different forms caused an increase of chlorophyll content of mulberry.

Varied amounts of crude protein and crude fibre contents were noticed in V1 mulberry among different treatments (Table 1). Maximum crude protein (20.19) and crude fibre (4.78) were recorded in T_4 (50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF). The next best treatment was T_3 (50% Seri Bio-digester effluent + 25% Compost + 25% RDF) which showed 20.09% of crude protein and 4.65 of crude fibre% followed by T_5 (25% Seri

Bio- digester effluent + 25% Bio-digester effluent + 25% Vermicompost + 25% Compost) that recorded 19.69% of crude protein and 3.78 of crude fibre percentage. Increase in crude protein and crude fibre might be due to the availability of nitrogen in the plants. Similar kinds of results were observed in studies of Ray *et al.* (1973) [10] who reported that, application of organic and inorganic nutrients resulted in increase in crude protein and crude fibre contents of mulberry. Shankar *et al.* (2002) [12] revealed that, slow release of nutrients from organic matter mulberry supplied garden might result in higher crude protein and crude fibre in leaf.

Table 2: Influence of Seri waste bio digester on growth and yield parameters in V1 mulberry at 60th days after pruning.

Treatments	Plant height (cm)	Number of Branches/plant	Number of Leaves/Branch	Leaf area(dm^2)	Leaf yield (g/plant)
T_1	147.05	9.92	23.67	184.50	897.33
T_2	158.45	12.50	26.81	200.00	998.33
T_3	161.37	12.65	29.15	208.00	1010.33
T_4	163.19	13.32	33.00	208.33	1012.33
T_5	160.05	12.52	28.17	204.00	999.00
T_6	154.31	12.5	26.37	191.28	949.00
T_7	153.99	10.88	26.18	190.18	940.00
T_8	149.09	10.03	24.74	184.67	909.00
T_9	152.41	10.24	25.59	185.67	916.00
T_{10}	151.19	10.24	25.08	184.83	916.00
T_{11}	153.83	10.69	26.09	185.00	926.00
F- test	*	*	*	*	*
S.Em±	0.487	0.054	1.663	0.982	4.762
C.D@ (5%)	1.436	0.159	4.907	2.897	14.048

The maximum plant height (163.19 cm), number of branches per plant (13.32) and more number of leaves per plant (33.00) at 60th day after pruning recorded significantly higher values in mulberry raised with (50% Seri Bio-digester effluent+25% Biodigester effluent + 25% RDF. The next best treatment was application of T_3 (50%Seri Bio-digester effluent + 25% Compost + 25% RDF) where in plant height recorded was 161.37 cm, number of branches per plant recorded was 12.65 and number of leaves per plant was 29.15. However, lowest traits were recorded when mulberry was raised with T_1 (100%Recommended dose of fertilizers) with 147.05 cm of plant height, 9.92 of number of branches per plant and 23.67 leaves per plant. Plant height profoundly increased due to nitrogen addition to the soil through seri waste biodigester liquid along with recommended dose of fertilizers and biodigester liquid. More the plant height, more was the number of branches and leaves. Similar results were observed by Shivakumar *et al.* (2000) [13] as per whom the combination of organic manures and inorganic fertilizers helped to increase the plant height and number of branches and leaves.

Significant variation was noticed with regard to leaf area and leaf yield per plant of V1 mulberry among the different treatments (Table 2). Among the different treatments, leaf area (208.33 dM^2)and leaf yield (1012.33 g/plant)were significantly higher in T_4(50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF) followed by T_3 (50% Seri Bio-digester effluent + 25% Compost + 25% RDF) recorded leaf area of 208.00 dM^2 and leaf yield of 1010.33 g/plant and T_5 (25% Seri Bio-digester effluent+25%Bio-digester effluent + 25% Vermicompost + 25% Compost) recorded leaf area of (204 dM^2) and leaf yield of plant(909 g/plant). The lowest values of leaf area (184.50) and leaf yield (897.33) were recorded in T_1(100% Recommended dose of fertilizers). The leaf area and leaf yield per plant increased due to positive influence of seriwaste bio digester liquid. The combination of Seri waste bio digester liquid along with biodigester liquid and recommended dose of fertilizers might have helped in slow release of macro and micro-nutrients. The lowest leaf area and leaf yield in T_1 (100% Recommended dose of fertilizers) may be due to shorter plant height and lowest number of leaves and may be due to insufficiency of nutrients to the root zone. The present findings are comparable to the results of Narayanaswamy *et al.* (2006) [7] who reported that, application of organic manures with combination of organic manures and inorganic fertilizers recorded higher yield compared to NPK alone in S_{36} mulberry.

Macro nutrients have significantly increased with the application of seriwaste biodigester liquid in V1 leaves (Table 3). The significantly higher leaf nitrogen, phosphorus and potassium contents of 3.33%, 1.80% and 1.63% was noticed in T4 (50% Seri Bio-digester effluent +25% Biodigester effluent + 25% RDF) followed by T_5 (25% Seri Bio-digester effluent + 25% Bio-digester effluent + 25% Vermicompost + 25% Compost) which recorded 3.29%N, 1.74%P and 1.61%K and T3 (50% Seri Bio-digester effluent + 25% Compost + 25% RDF) that resulted in 3.26, 1.70 and 1.61% of NPK, respectively. Increase of macronutrients of leaf may be mainly due to the application of seriwaste biodigester liquid which is the rich source of NPK. Murali *et al.* (2006) [6] reported that uptake of NPK was increased by integration of organic manures and inorganic fertilizers in S36 and M5 mulberry.

Table 3: Influence of Seri waste bio digester on macronutrient and secondary nutrient contents of V1 mulberry leaves on 60th day after pruning

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulphur (%)
T_1	3.14	0.90	1.11	0.78	0.55	0.26
T_2	3.26	1.68	1.57	1.12	0.69	0.31
T_3	3.26	1.70	1.61	1.13	0.71	0.35
T_4	3.33	1.80	1.63	1.19	0.73	0.73
T_5	3.29	1.74	1.61	1.14	0.72	0.69
T_6	3.23	1.63	1.52	1.08	0.69	0.30
T_7	3.23	1.57	1.51	0.95	0.64	0.30
T_8	3.19	1.20	1.15	0.78	0.57	0.28
T_9	3.21	1.53	1.41	0.91	0.60	0.28
T_{10}	3.21	1.51	1.39	0.84	0.59	0.28

T_{11}	3.22	1.53	1.47	0.93	0.62	0.29
F- test	*	*	*	*	*	*
S.Em±	0.010	0.038	0.011	0.011	0.007	0.006
C.D@ (5%)	0.030	0.112	0.031	0.034	0.021	0.017

Table 4: Influence of Seri waste bio digester on micronutrient contents of V1 mulberry leaves on 60th day after pruning

Treatments	Iron (ppm)	Manganese (ppm)	Copper (ppm)	Zinc (ppm)
T_1	245.54	50.50	29.69	37.64
T_2	286.53	52.24	31.93	40.94
T_3	289.47	54.18	32.74	42.81
T_4	288.52	53.33	32.39	41.41
T_5	301.79	54.28	33.10	43.02
T_6	278.35	51.82	31.79	40.58
T_7	245.98	51.36	31.74	40.52
T_8	194.53	49.50	28.65	37.57
T_9	233.50	50.64	30.63	38.87
T_{10}	176.32	41.43	28.60	36.84
T_{11}	245.54	51.34	31.70	39.67
F- test	*	*	*	*
S.Em±	0.986	0.342	0.573	0.574
C.D@ (5%)	2.909	1.010	1.692	1.695

Secondary nutrients *viz*., calcium, magnesium and sulphur and micronutrients such as iron, manganese, copper and zinc of V1 mulberry leaves were highly influenced by application seri waste biodigester liquid (Table 3, 4). Among all the treatments, mulberry raised with T4 (50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF) recorded higher calcium content (1.19%), magnesium content (0.73%) and sulphur content (0.73%) followed by T_5 (25% Seri Bio-digester effluent + 25% Bio-digester effluent + 25% Vermicompost + 25% Compost) which registered 1.14%Ca, 0.72%Mg and 0.69%S. Among all the treatments, the micronutrients were significantly higher in T_5 (25% Seri Bio-digester effluent + 25% Bio-digester effluent + 25% Vermicompost + 25% Compost) with 301.79ppm iron, 54.28ppm manganese, 33.10ppm copper and 43.02ppm zinc followed by T4 (50% Seri Bio-digester effluent + 25% Biodigester effluent + 25% RDF) that recorded 288.52, 53.33, 32.39 and 41.41ppm of iron, manganese, copper and zinc, respectively. The increase in secondary and micro nutrients might be due to application of seriwaste biodigester liquid at different levels to supply the recommended dose of nutrients for quality leaves and productivity. Murali *et at.* (2006) [6] too observed that the combination of organic manures with inorganic fertilizers have yielded significantly higher calcium, magnesium and sulphur contents in S36 and M5 leaves of mulberry.

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