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Effect of *Kappaphycus alvaerezii* SLF on Growth and Biochemicals in *Morus Alba* L. and *Bombyx Mori* L

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Abstract: *This study was undertaken to determine the effect of Kappaphycus alvaerezii SLF on growth, biomass and biochemicals in mulberry leaves, Morus alba L. and its impact on Bombyx mori L. It was found that soil application of SLF of Kappaphycus alvaerezii has enhanced the shoot length, number of leaves, leaf area, circumference of stem, root growth and total biomass by 107%, 100%, 135% , 91%, 140% and 140% respectively at third month after the application of SLF of Kappaphycus alvaerezii in comparison with control. Application of SLFof Kappaphycus alvaerezii also increased the level of total soluble protein by 190%, carbohydrate by 108% and total free amino acid by 131% with respect to control. The impact of SLF grown leaves on overall growth of Bombyx mori was analysed. First instar silkworm larvae were reared on SLF grown leaves as test and leaves normally grown as control. Length and weight of larvae increased at second instar stage by 21% and 18% and fourth instar stage by 11% and 24% over the control . At fifth instar stage, the weight was increased by 37% on compared to control. At first instar stage, larvae fed with test leaves showed an increased total soluble protein level by 49%, carbohydrate by 10% and total free amino acid by 32% more in relation to the control. Moreover at fifth instar stage protein content was more by 41% with respect to the control. From the study it is evidenced that application of SLF of Kappaphycus alvaerezii improved the quality and quantity of mulberry leaves, the only food for Bombyx mori. This impact inturn had a direct bearing on the growth, biomass and biochemical status of Bombyx mori and the quality of cocoon.*

Keywords: *Morus alba L., Bombyx mori L., Kappaphycus alvaerezii (Doty) Doty ex P.C. Silva), growth, biochemicals, instar stages*

Abbreviation:

SLF- Seaweed Liquid Fertilizer

1. INTRODUCTION

Mulberry foliage is the only food for the silkworm (*Bombyx mori)* and is grown under varied climatic conditions ranging from temperate to tropics. Mulberry leaf is a major economic component in Sericulture since the quality and quantity of leaf produced per unit area has a direct bearing on cocoon harvest. Cocoon characters both quantitative as well as qualitative, depend largely on the quality and quantity of leaves used as feed [1], [2], [3]. Hence application of manures and fertilizers is one of the important inputs for increasing mulberry leaf yield. Synthetic chemicals, fertilizers, pesticides, herbicides, growth promoters and other outputs though enhanced productivity of mulberry they adversely affect the ecosystem [4]. In recent years, Seaweed liquid fertilizers derived from marine seaweeds were found superior than chemicals because of the presence of high level of organic matter, micro nutrients, vitamins and fatty acids and also growth regulators such as auxins, cytokinin and gibberellins [5]. Their beneficial effects include enhanced seed germination, plant yield, root growth, tolerance to different plant stresses and increase in plant resistant to infections or insect attack [6], [7], [8]. Moreover application of seaweed extract increased chlorophyll content [9]. Unlike, chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds [10]. Therefore it is suggested that rearing silkworm with SLF grown mulberry leaves provide sufficient quantity of essential component to the larva for more growth and cocoon production. In this study an attempt has been made to analyse the effect of *Kappaphycus alvaerezii* SLF on mulberry and *Bombyx mori* reared on SLF grown leaves.

2. MATERIALS AND METHODS

2.1. Collection of Seaweeds

Seaweed belonging to Red algae (*Kappaphycus alvaerezii* (Doty) Doty ex P.C. Silva) was collected from Hare Island, Thoothukudi coast, Tamil Nadu during low tide in the early morning hours in November, 2014 – January, 2015. The material was washed thoroughly with running tap water to remove all the unwanted impurities and adhering sand particles. It was shade-dried for seven days. The dried material was finely powdered and stored.

2.2. Preparation of Seaweed Liquid Extract

Powdered seaweed and distilled water were taken in a conical flask in the ratio of 1:10 (w/v).The mixture was cooked in a pressure cooker and filtered through cheese cloth. The filtrate was centrifuged at 10,000 rpm to remove the suspended impurities. The filtrate thus obtained was considered as 100% SLF. 1% SLF was prepared by adding distilled water and used for soil application.

2.3. Cultivation of Plant

Morus alba L. cuttings (5 cm) were collected from Demonstration cum training center-V.M.Chathram, Tirunelveli, Tamil Nadu, India. The cuttings were potted during November to February (2014-2015) at Plant Research Centre, St. Mary's College campus, Thoothukudi, Tamil Nadu, India. Each pot was filled with 3 kg of garden loamy soil and a single cutting of *Morus alba* was planted in each pot. They were kept under diffuse light till the buds sprouted, there after they were kept under natural light conditions. Seaweed liquid fertilizer (1%) was applied through soil along with irrigation after expansion of third leaf and was continued for three months. Control plants were irrigated with water.

2.4. Silkworm Rearing

Silkworm eggs were collected from Demonstration cum training centre, V.M. Chathram, Tirunelveli district, Tamil Nadu, India. Rearing was carried out under laboratory condition. Removal of fecal matter and diseased larvae was done at regular interval. Larvae were divided into two groups. One group is reared on leaves obtained from *Kappaphycus alvaerezii* SLF grown plant (test plant) and another group is reared on leaves from control plant irrigated with water. Growth parameters like length, weight and biochemical parameters such as total soluble protein, carbohydrate and total free amino acid were analysed at different instar stages.

2.5. Analysis

Chemicals of analytical grade were used for all the analyses. total soluble protein [11], carbohydrate [12] and total free amino acid [13] were analysed. Leaves harvested from plants after 3 months of SLF treatment were used for all analyses.

3. RESULTS AND DISCUSSION

3.1. Growth

Data presented in (Table 1) shows the effect of SLF on growth of *Morus alba*. It was found that soil application of SLF of *Kappaphycus alvaerezii* enhanced the shoot length, number of leaves, Leaf area, circumference of stem, root growth and total biomass by 107%, 100%, 135%, 91%, 140% and 140% respectively at third month after the application of SLF *Kappaphycus alvaerezii* in comparison with control . SLF contained macronutrients, trace elements, organic substances like amino acids and plant growth regulators such as auxin, cytokinn, gibberellins [5] which might be responsible for the observed biostimulative role of seaweeds *Kappaphycus alvaerezii.* It could also be accounted to the alginate and phycocolloids present in it. The phycocolloids retain soil moisture, porosity, favour rhizosphere micro organisms, there by caused more nutrients and nitrate absorption resulted into higher overall growth performance as established in the present investigation. Liquid extract obtained from seaweeds has recently gained much interest as soil and foliar spray for inducing shoot growth and yield in orchards and horticultural plants [14-19]. Seaweed or its products are commonly used in agriculture to stimulate plant growth and enhance productivity. Their beneficial effects include improved seed germination [20], plant yield, root growth, shoot growth, [21], [9] biomass and crop yield [22] and increase in plant resistance to infections and stress [6], [7], [8]. The result obtained in the present investigation is in confirmity with earlier reports.

Table1. *Effect of SLF Kappaphycus alvaerezii on growth parameters in Morus alba L.*

Growth Parameters	Control	Treated
Shoot growth (cm)	14.8 ± 0.4	30.7 ± 0.6 (107)
Stem circumference (cm)	1.1 ± 0.8	2.1 ± 0.5 (91)
No. of leaves	20 ± 3	40 ± 6 (100)
Leaf area (cm^2)	140.5 ± 1.5	330.25 ± 6.5 (135)
Root growth (cm)	13.6 ± 0.2	32.6 ± 0.3 (140)
Fresh Biomass (mg)	43.51 ± 0.13	104.27 ±0.19 (140)

Values represent the means of five replicates ± standard deviation

Values in parentheses indicate % increase over control

Control = control plants were irrigated with water

Treated= plants were irrigated with 1% SLF

3.2. Biochemicals

Carbohydrate, total soluble protein, and total free amino acid play a vital role as dietary constituents of *Bombyx mori*. Soil application of SLF of *Kappaphycus alvaerezii* increased the level of total soluble protein by 190%, carbohydrate by 108% and total free amino acid by 131% with respect to control (Table 2). The observed results of the present study coincided with earlier reports [23-26]. The increase in total soluble protein in the test plant is due to the availability of most of the necessary elements present in SLF for protein synthesis [27]. This result indicated that biochemicals like protein (r=0.9935, P>0.01%), carbohydrate (r=0.7221, P>0.01%) and amino acid (r=0.6754, P>0.01%) contributed to increasing biomass in *Morus alba*. Silkworms should be fed with good quality mulberry leaves in abundant quantity for the successful cocoon production [28]. Increase of leaf biomass rich in total soluble protein, carbohydrate and total free aminoacid by SLF application is a desirable feature in Sericulture industry which is obtained by *Kappaphycus alvaerezii* SLF application.

Table2. *Effect of SLF Kappaphycus alvaerezii on biochemicals in Morus alba L.*

Biochemicals (mg/g) FW)	Control	Treated
Total soluble protein	15.5901 ± 0.0327	45.6269 ± 0.0569 (96)
Carbohydrate	24.7306 ± 0.0327	51.5407 ± 0.1829 (108)
Total free amino acid	19.6871 ± 0.4398	45.5824 ± 0.0932 (131)

Values represent the means of five replicates ± standard deviation

Values in parentheses indicate % increase over control

Control = control plants were irrigated with water

Treated= plants were irrigated with 1% SLF

3.3. Larval Growth

Enrichment of the mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and the quality of the larvae can be enhanced and maintained. Mulberry leaves grown with applicarion of SLF (*Kappaphycus alvaerezii*) was tested for its impact on larval growth parameters. First instar larvae were reared on SLF grown leaves as test and leaves normally grown as control. The length and weight of larvae were increased in test when compared to control. Length of larvae increased at second instar stage and was 21% more than the control. Weight of larvae of first instar stage increased by 7% and second instar stage by 18% in comparison with control (Figure 1 and 2). At third instar stage, the length of (test) larvae was increased by 17% with respect to control and insignificant positive correlation existed between *Morus alba* leaf biochemicals and *Bombyx mori* weight at third instar stage (r=0.39190, P>0.01%). Length and weight of larvae was increased at fourth instar by 11% and 24% respectively in relation to control. At fifth instar stage,the larval length was not much differed between test and control however, the weight was increased by 37% on compared to control. Highly positive correlation existed between *Morus alba* leaf biochemicals and *Bombyx mori* weight at fifth instar stage (r=0.86679, P>0.01%).

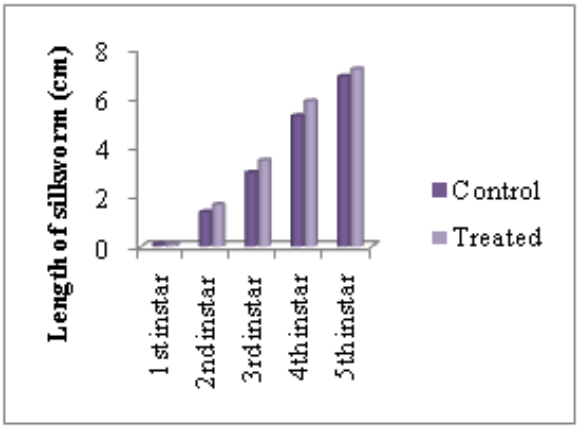

Fig1. *Effect of SLF treated Morus alba leaves on length of Bombyx mori*

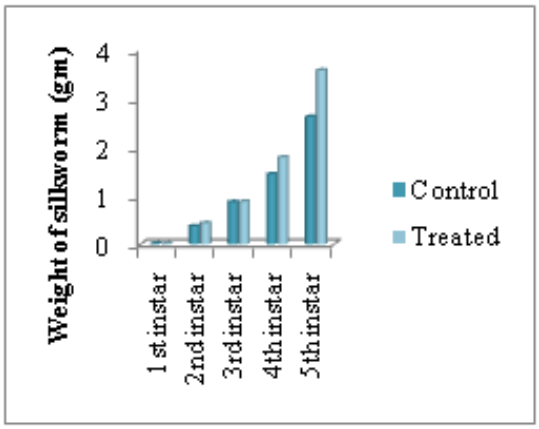

Fig 2. *Effect of SLF treated Morus alba leaves on weight of Bombyx mori*

3.4. Biochemicals

The larvae fed with test leaves increased their total soluble protein content at 5 different instar stages. At first instar stage, larvae fed with test leaves showed an increased total soluble protein (49%), carbohydrate (10%) and total free amino acid (32%) in relation to the control (Table 4). At second and third instar stage, protein level was 24%, 28% greater respectively, where as carbohydrate and amino acid level were increased only by 10% and 7% respectively over the control. The larvae fed with test leaves showed considerable increase in protein and was 41% more than the control at fifth instar stage. The data obtained during the study clearly expressed that test leaves augmented silk worm protein more rather than amino acids and carbohydrate. Nyiira [29] found that about 70% of the silk protein produced by the silkworm is directly derived from the protein of the mulberry leaves and it is directly correlated with production efficiency of cocoon shell in silkworms [28], [30]. Enrichment of the mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and the quality of the larvae can be enhanced and maintained. The result of this study clearly indicated the positive effect of *Kappaphycus alvaerezii* SLF treated plant leaves on growth and nutritional parameters of *Bombyx mori*. Higher leaf protein, carbohydrate and amino acid contents are desirable for the healthy growth of silkworm larvae and better cocoon production.

Table3. *Effect of Kappaphycus alvaerezii SLF grown Morus alba leaves on protein, carbohydrate and amino acid in Bombyx mori*

Instar stages	**Total soluble Protein (mg/g FW)**		**Carbohydrate (mg/g FW)**		**Total free Amino acid (mg/g FW)**	
	Control	Treated	Control	Treated	Control	Treated
1st instar	12.6782 ±0.1123	18.9782 ± 0.1738 (49)	29.8920 ±0.0370	32.8920± 0.0139 (10)	21.0019 ±0.0967	27.6490± 0.5631 (32)
2nd instar	18.5023 ±0.0178	22.9974 ± 0.0098 (24)	35.5518 ±0.0511	40.7183 ±0.0621 (15)	30.6722 ±0.6740	31.6920±0.03487(3)
3rd instar	31.7816 ±0.0226	40.7729 ± 0.0073 (28)	35.0632 ±0.1331	38.6638± 0.0211 (10)	27.3781 ±0.3542	29.3693±0.08121(7)
4th instar	17.5802 ±0.1833	31.9021 ± 0.0367 (81)	41.4233 ±0.0482	47.6925±0.0672 (15)	31.3376 ±0.6537	32.5674± 0.0664(3)
5th instar	27.9918 ±0.1474	39.6701 ± 0.0210 (41)	36.6451 ±0.0980	40.5930± 0.0415 (11)	30.5699 ±0.0863	32.4553± 0.4471(2)

Values are the means of ten replicates ± standared deviation

Values in parentheses indicate % increase over control

Control = larvae fed with control plant leaves

Treated = larvae feed with SLF treated leaves

4. CONCLUSION

SLF is cost effective and environmentally healthy option to improve the growth, antioxidants, biochemical components which will meet the requirement of organic produce in the present scenario. Rearing silkworm with SLF grown mulberry leaves provide sufficient quantity of essential components to the larvae for more growth and cocoon production which inturn will become a boon to the silk industry.

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