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# Efficacy of sea plant extract (*Kappaphycus* sp) in growth of mulberry and subsequently boosting the immunity against *Bm*NPV in silkworm, *Bombyx mori* L

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## Abstract
Field experiment was conducted to evaluate the performance of sea plant extract sprayed at different interval of time on mulberry and the treated leaves were fed to the silkworm *Bombyx mori* which were infected with *Bm*NPV. It was found that foliar application of sea plant extract from LBS 13 at 1.5 ml/l at 45$^{th}$ day after pruning enhanced the leaf yield per plant by 308.81 g/pl, average plant height by 150.42 cm, number of shoots present (14.32) and leaves per plant (400 leaves per plant) when compared with control. When the treated leaves were fed to the *Bm*NPV infected silkworms, LBS13 at 1.5 ml/l recorded highest larval weight of 3.33 g/larvae with larval duration of 7.67 days followed by least larval mortality (3.33%) and percent disease incidence (11.33%) when compared to control. The study evidenced that application of sea plant extract of *Kappaphycus* sp. has improved the growth attributes of mulberry, the only food for *B mori* and inturn had a direct positive impact on development of *B. mori*.

**Keywords:** Sea plant extract, *Kappaphycus* sp., mulberry, *Bm*NPV

## Introduction
Mulberry (*Morus alba* L.) foliage is the only food for the silkworm, *Bombyx mori* L. It has been analysed that, nearly 70 percent of the silk proteins present in the cocoon are derived from mulberry leaves. Hence, it's necessary for the silkworms to be fed with good quality mulberry leaves which in turn will be responsible for successful cocoon production [17]. Though synthetic chemicals, fertilizers, pesticides, herbicides, growth promoters and other inputs escalate the productivity of mulberry, they do adversely affect the ecosystem. Continuous production of mulberry crop for a long time will result in gradual reduction of leaf yield and also its quality [12]. In the current global scenario it is very much needed to adopt eco-friendly agricultural practices for a better and sustainable Sericulture.

Organic farming can be stated as the remedy to the ills of modern chemical agriculture. Many viable options have to be explored to meet the increasing demand of organic inputs day by day and one of the option among those is the use of sea plant extract serving as source of nutrient for the plants [19]. Extracts procured from marine sea plants could be used to replace the synthetic chemicals as they contain high levels of organic matter, micro nutrients, vitamins, fatty acids in them along with growth regulators such as Auxins, Cytokinin and Gibberellic acid. Unlike, the extensively used synthetic chemicals, extracts which are derived from the sea plants are known to be biodegradable, nontoxic to ecosystem, non-polluting and non-hazardous to the environment [7]. Apart from being eco friendly their beneficial effects to the growing plants include enhanced root growth, leaf growth of the plant, increased yield, tolerance to different plant stress conditions and increased resistance to pathogens and pests. The application of sea plant extract from *K. alvaerezii* boosted the shoot length, number of leaves per plant, leaf area, circumference of growing stem, root growth of the plant and total biomass in mulberry [10]. Application of different sea plant extracts were also found beneficial in other crops like black gram [4] and chilly [1, 6]. Therefore, in the present research it is proposed to study the effect of cultivated sea plant (*Kappaphycus* sp.) extract on growth and development of mulberry. *B mori*, which is being reared domestically from the past 5000 years has a significant economic status and is considered as a model organism in scientific research, molecular biology and genetics. Silkworm is attacked by many of the pathogenic fungi, bacteria and viruses which cause loss of almost 20–30 percent of the potential cocoon production every year [8]. Among the diseases caused to silkworms, *Bm*NPV (*B. mori* Nuclear Polyhedrovirus) accounts for almost 80 percent of the total cocoon loss [9]. Heavy use of chemicals to prevent the disease may result in causing toxicity to the silkworms. Therefore, the present study was conducted to search for a natural, cost effective and eco friendly alternative which would boost the mulberry leaf quality as well as aid in managing *Bm*NPV.

## Materials and Methods

### a) Experimental layout

The experimental design was set up at the Department of Sericulture, University of Agriculture Sciences, Bangalore with well established mulberry garden of $V_1$ variety. Package of practices suggested for irrigated condition of mulberry was followed. Recommended levels of fertilizer application (300:140:140 NPK kg/ha/year) was applied along with 20 tonnes of FYM/ha [2]. The field experiment done was laid according to the Randomized Block Design with 14 treatments and 3 replication each.

### b) Treatment details

Cultivated tropical sea plant extracts of *Kappaphycus* sp. (*a.i.* sulfated galactans) with four different formulations were procured from Sea6 Energy Pvt. Ltd. (Centre for Cellular and Molecular Platforms, Bangalore) *viz*., LBD3, LBD12, LBS6 and LBS13. The sea plant extracts selected were used as foliar sprays in freshly pruned mulberry garden at three different concentrations on 21 and 30 Days After Pruning (DAP) and care was taken to ensure that there was no drifting of chemicals due to wind by holding a polythene cover along the treated plants while spraying.

| Treatments | Description |
|---|---|
| $T_1$ | Foliar application of LBD3 at 2 ml/l |
| $T_2$ | Foliar application of LBD3 at 4 ml/l |
| $T_3$ | Foliar application of LBD3 at 6 ml/l |
| $T_4$ | Foliar application of LBD12 at 0.5 ml/l |
| $T_5$ | Foliar application of LBD12 at 1 ml/l |
| $T_6$ | Foliar application of LBD12 at 1.5 ml/l |
| $T_7$ | Foliar application of LBS6 at 0.5 ml/l |
| $T_8$ | Foliar application of LBS6 at 1 ml/l |
| $T_9$ | Foliar application of LBS6 at 1.5 ml/l |
| $T_{10}$ | Foliar application of LBS13 at 0.5 ml/l |
| $T_{11}$ | Foliar application of LBS13 at 1 ml/l |
| $T_{12}$ | Foliar application of LBS13 at 1.5 ml/l |
| $T_{13}$ | Distilled water control |
| $T_{14}$ | Control |

LBD=Liquid Bio Defence: LBS= Liquid Bio Stimulant

### c) Observations taken

Plant height (cm), number of shoots per plant and number of leaves per plant were calculated on 45th Day After Pruning (DAP). The shoot height was measured from the base of the plant to the tip of the fully opened leaf of all the shoots of labelled plants and mean shoot height was calculated.

$$\text{Mean shoot height} = \frac{\text{Total shoot height}}{\text{Number of shoots}}$$

Leaf area was calculated at 45 DAP from the plants using a leaf area meter. Five labelled plants were maintained exclusively for estimation of leaf yield. Leaves harvested individually plant wise and their yield was recorded. The total leaf yield obtained from net plot was recorded as the leaf yield and expressed in g/plant which was recorded on 45th DAP.

### d) Silkworm rearing

The popular commercial cross breed PM X $CSR_2$ was used for the study. The larvae were provided with chopped mulberry leaves of required quantity and quality. The experimental design was CRD with 14 treatments and 3 replications each. Purified Polyhedral Occlusion Bodies (POBs) of *Bm*NPV were received from Karnataka State Sericulture Research and Development Institute, Thalaghattapura. The concentration of occlusion bodies were determined by using Neubaure's haemocytometer. Infection of the worms was carried out orally by feeding them with viral suspension of $10^{-8}$ ($7.3 \times 10^{-3}$ POBs/ml) smeared on mulberry leaves of standard size ($10 \times 12$ $cm^2$) soon after the third moult at 0.5 ml/leaf/replication. The sea plant extracted mulberry leaves were fed to the worms three times per day. Different rearing parameters along with mortality rate, percent disease incidence and Effective rate of rearing (ERR) were recorded by using the formula given.,

$$\text{Larval mortality (\%)} = \frac{\text{No.of dead larvae}}{\text{Total no.of worms per treatment}} \times 100$$

$$\text{Effective Rate of Rearing (\%)} = \frac{\text{Number of cocoons harvested}}{\text{Number of worms brushed}} \times 100$$

The data obtained were statistically analysed by using Web Based Agricultural Statistics Software Package WASP 2.0 (Web Agri Stat Package) and also using one way ANOVA (Randomized Block Design and Completely random design) and CRD (Completre Random Design) with DMRT (Duncan's Multiple Range Test) to determine significant differences among different treatments.

## Results and Discussion

**Table 1:** Growth attributes of sea plant extract treated mulberry at 45 Days after Pruning

| Treatments | | Plant height (cm) | No. of shoots / plant | No. of leaves / plant | Leaf area ($cm^2$) | Leaf yield / plant (g) |
|---|---|---|---|---|---|---|
| $T_1$ | Foliar application of LBD3 at 2 ml/l | 128.37$^{def}$ | 14.05$^{b}$ | 374.00$^{d}$ | 106.47$^{d}$ | 284.53$^{bc}$ |
| $T_2$ | Foliar application of LBD3 at 4 ml/l | 134.94$^{cde}$ | 13.26$^{cd}$ | 232.33$^{g}$ | 84.07$^{f}$ | 258.18$^{d}$ |
| $T_3$ | Foliar application of LBD3 at 6 ml/l | 138.31$^{bc}$ | 13.5$^{d}$ | 325.33$^{f}$ | 103.69$^{d}$ | 236.87$^{g}$ |
| $T_4$ | Foliar application of LBD12 at 0.5 ml/l | 128.41d$^{ef}$ | 13.04$^{de}$ | 203.67$^{j}$ | 84.50$^{f}$ | 238.67$^{g}$ |
| $T_5$ | Foliar application of LBD12 at 1 ml/l | 137.73b$^{cd}$ | 13.50$^{c}$ | 225.00$^{h}$ | 97.53$^{e}$ | 245.74$^{e}$ |
| $T_6$ | Foliar application of LBD12 at 1.5 ml/l | 138.53$^{bc}$ | 14.05$^{b}$ | 356.33$^{e}$ | 107.60$^{d}$ | 253.59$^{d}$ |
| $T_7$ | Foliar application of LBS6 at 0.5 ml/l | 140.86$^{abc}$ | 14.06$^{b}$ | 385.0$^{bc}$ | 121.72$^{b}$ | 284.78$^{bc}$ |
| $T_8$ | Foliar application of LBS6 at 1 ml/l | 113.11$^{g}$ | 12.83$^{ef}$ | 199.67$^{j}$ | 65.90$^{h}$ | 220.59$^{i}$ |
| $T_9$ | Foliar application of LBS6 at 1.5 ml/l | 121.53f$^{g}$ | 13.25$^{d}$ | 381.00$^{c}$ | 74.27$^{g}$ | 245.12$^{ef}$ |
| $T_{10}$ | Foliar application of LBS13 at 0.5 ml/l | 140.39$^{bc}$ | 14.05$^{b}$ | 389.33$^{b}$ | 102.85$^{d}$ | 282.72$^{c}$ |
| $T_{11}$ | Foliar application of LBS13 at 1 ml/l | 145.98$^{ab}$ | 14.12$^{ab}$ | 398.67$^{a}$ | 116.47$^{c}$ | 289.53$^{b}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| $T_{12}$ | Foliar application of LBS13 at 1.5 ml/l | $150.42^{a}$ | $14.32^{a}$ | $400.00^{a}$ | $135.68^{a}$ | $308.81^{a}$ |
| $T_{13}$ | Distilled water control | $127.27^{ef}$ | $13.13^{d}$ | $222.00^{h}$ | $77.29^{g}$ | $239.74^{fg}$ |
| $T_{14}$ | Control | $128.25^{def}$ | $13.05^{de}$ | $216.00^{i}$ | $74.72^{g}$ | $228.61^{h}$ |
| | F-test | * | * | * | * | * |
| | SE.m ± | 3.39 | 0.084 | 1.654 | 1.794 | 1.856 |
| | CD at 5% | 9.851 | 0.244 | 4.808 | 5.215 | 5.395 |

*Significant at 5%, DAP- Days after Pruning.

Plant height of mulberry exhibited significant difference among the treatments wherein the highest mulberry plant growth was noticed in the treatment LBS13 at 1.5 ml/l (150.42 cm) and the shortest mulberry plant was observed in the treatment LBS6 at 1 ml/l (113.11 cm). LBS13 at 1.5 ml/l was on par with LBS13 at 1 ml/l (145.98 cm) and LBS6 at 0.5 ml/l (140.86 cm). The treatments LBS13 at 0.5 ml/l (140.39 cm), LBD12 at 1.5 ml/l (138.53 cm) and LBD3 at 6 ml/l (138.31 cm) were on par with each other as well as exhibited higher growth of mulberry when compared to that of the absolute control (128.25 cm) and distilled water control (127.27 cm) (Table. 1). Sea plant products provide growth-stimulating activities, and the use of them as bio stimulants in crop production is well established. Biostimulants are defined as "materials, other than fertilizers, which promote plant growth when applied in small quantities" and they are also referred to as "metabolic enhancers" [18].

The treatment LBS13 at 1.5 ml/l was found to enhance the number of shoot per plant by (14.32) as well as number of leaves per plant was found to be more in it (400 leaves / pl). The least number of shoot per plant and number of leaves per plant were found in the mulberry plants treated with LBS6 at 1 ml/l 12.83 and 199.67, respectively (Table. 1). The treatments LBS13 at 1 ml/l (14.12), LBS13 at 0.5 ml/l (14.05), LBS6 at 0.5 ml/l (14.06) and LBD12 at 1.5 ml/l (14.05) also exhibited better and on par results regarding number of shoots per plant in mulberry ecosystem and proved to be better than the control (13.05). Higher number of leaves per plant were also found in the treatments LBS13 at 1 ml/l (398.67), LBS13 at 0.5 ml/l (389.33) and LBS6 at 0.5 ml/l (385) which were on par with each other as well as better than absolute control (216).

The treatment LBS13 at 1.5 ml/l recorded larger leaf area of about (135.68cm2) on 45th DAP, followed by the treatment LBS6 at 0.5 ml/l (121.72 cm2) and LBS13 at 1 ml/l (116.47 cm2). Lesser leaf area was recorded in the treatment LBS6 at 1 ml/l (65.90). Since the growth of mulberry treated with LBS6 at 1 ml/l was less, lesser leaf area can be observed in the plants (Table. 1).

Greater yield potential of (308.81 g/pl) was recorded in LBS13 at 1.5 ml/l treated mulberry plants which was followed by LBS13 at 1 ml/l (289.53 g/pl), LBS13 at 0.5 ml/l (282.72 g/pl), LBS6 at 0.5 ml/l (284.78 g/pl) and LBD3 at 2 ml/l (284.53 g/pl). The mulberry plants treated with LBS6 at 1 ml/l exhibited lesser yield of 220.59 g/pl when compared to that of the control (228.61 g/pl). Present findings strengthens the observations reporting that leaf yield was found to increase when lesser concentration of sea plant extract (0.5ml/l) from *Dictyota dichotoma* and *Kappaphycus alvaerezii* were applied as foliar sprays [16]. The application of the marine sea plant extract from *K. alvaerezii* boosted the shoot length of plant, number of leaves per plant, leaf area, circumference of stem, root growth and total biomass by 107%, 100%, 135%, 91%, 140% and 140% respectively in mulberry at third month after the application in comparison with control[10].

Crop yield in plants are enhanced due to - and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances affect cellular metabolism in sea plant extract treated plants [3] [15] [11]. Yield increases in seaweed-treated plants are thought to be associated with the hormonal substances present in the extracts, especially cytokinins. Cytokinins in vegetative plant organs are associated with nutrient partitioning, whereas in reproductive organs, high levels of cytokinins may be linked with nutrient mobilization.

**Table 2:** Larval parameters of *Bm*NPV worms treated with sea plant extract

| Treatment | Larval duration of V instar silkworms (days) | Larval weight (g/larvae) | Larval mortality (%) | Percent disease incidence | Effective rate of raring (%) |
|---|---|---|---|---|---|
| $T_1$ | $10.30^{bc}$ | $2.03^{g}$ | $9.67^{abc}$ | $33.33^{a}$ | $46.00^{f}$ |
| $T_2$ | $7.00^{g}$ | $3.10^{b}$ | $4.33^{cd}$ | $12.67^{d}$ | $81.33^{a}$ |
| $T_3$ | $8.67^{e}$ | $2.51^{de}$ | $7.67^{bcd}$ | $25.33^{abc}$ | $62.00^{bcd}$ |
| $T_4$ | $8.00^{ef}$ | $2.68^{c}$ | $9.00^{abcd}$ | $20.67^{bcd}$ | $72.67^{abc}$ |
| $T_5$ | $10.67^{ab}$ | $1.88^{gh}$ | $11.33^{ab}$ | $30.00^{ab}$ | $60.00^{cde}$ |
| $T_6$ | $11.00^{a}$ | $1.78^{h}$ | $14.33^{a}$ | $35.33^{a}$ | $56.00^{def}$ |
| $T_7$ | $10.67^{ab}$ | $2.22^{f}$ | $10.33^{abc}$ | $31.33^{a}$ | $54.67^{def}$ |
| $T_8$ | $10.33^{abc}$ | $2.20^{f}$ | $10.00^{abc}$ | $30.00^{ab}$ | $57.33^{def}$ |
| $T_9$ | $8.00^{ef}$ | $3.00^{b}$ | $4.67^{cd}$ | $12.67^{d}$ | $82.67^{a}$ |
| $T_{10}$ | $9.50^{d}$ | $2.66^{cd}$ | $7.67^{bcd}$ | $19.33^{cd}$ | $74.67^{ab}$ |
| $T_{11}$ | $9.67^{cd}$ | $2.60^{cde}$ | $4.67^{cd}$ | $14.67^{d}$ | $78.00^{a}$ |
| $T_{12}$ | $7.67^{fg}$ | $3.33^{a}$ | $3.33^{d}$ | $11.33^{d}$ | $80.67^{a}$ |
| $T_{13}$ | $10.48^{ab}$ | $2.50^{de}$ | $11.67^{ab}$ | $35.33^{a}$ | $48.67^{def}$ |
| $T_{14}$ | $10.83^{ab}$ | $2.48^{e}$ | $13.33^{ab}$ | $32.00^{ab}$ | $46.67^{ef}$ |
| F-test | * | * | * | * | * |
| SE.m ± | 0.238 | 0.057 | 2.144 | 3.528 | 4.680 |
| CD at 5% | 0.688 | 0.164 | 6.210 | 10.219 | 13.558 |

*Significant at 5%

The treatment LBS13 at 1.5 ml/l recorded shorter larval duration of 7.67 days when compared to that of distil water control (10.48 days) and absolute control (10.83 days) among the silkworms infected with *Bm*NPV were fed with sea plant extract treated mulberry leaves (Table 2). Longest duration was noticed in the treatment LBD12 at 1.5 ml/l (11.00 days). The infected silkworms does exhibit extended larval duration as seen in control batch, whereas the treatment LBS13 at 1.5 ml/l has considerably prevented the extension of duration and the worms have spun the cocoon within the expected stipulated time.

All the treatments exhibited significant difference in the larval weight (Table 2). The treatment LBS13 at 1.5 ml/l recorded maximum of 3.33 g/larvae followed by LBD3 at 4 ml/l recording 3.1 g/larvae, which were comparatively higher than that of the distilled water control (2.50 g/larvae) and absolute control (2.48 g/larvae). Minimum larval weight was recorded from treatment LBD12 at 1.5 ml/l (1.78 g/larvae). As the nutrient quality of the mulberry leaves is enhanced, the silkworms feeding on them will inturn exhibit an increased body weight, but the control batch exhibit worms which do not feed and eventually starve to death. Effect of *Kappaphycus alvaerezii* SLF on silkworm recorded increased Length and weight of larvae at second instar stage by 21% and 18% and fourth instar by 11% and 24% over the control. They also recoded that fifth instar weight was increased by 37 percent when compared to control [15].

The treatment LBS13 at 1.5 ml/l exhibited least larval mortality (3.33%) and percent disease incidence (11.33%) when compared to other treatments (Table 2). The treatment LBD12 at 1.5 ml/l recorded highest larval mortality of 14.33 percent with 35.33 percent disease incidence. It is found that the maximum silk protein which is produced by the silkworm is directly derived from the mulberry leaf protein and it is directly correlated with production efficiency of silkworms [5].

Maximum Effective Rate of Rearing was recorded in LBD3 at 4 ml/l (81.33%) and LBS13 at 1.5 ml/l (80.67%) which were on par with each other and were way higher than that of distilled water control (48.67%) and absolute control (46.67%). Extracts from many marine algae expressed antibacterial, antifungal, antiviral, antioxidant and antibiotic activity [13]. Antiviral activity of selected medicinal plants and marine seaweeds on the grasserie infected larvae of silkworm, *B.mori* revealed decrease in the polyhedrons due to several phytochemical constituents with significantly higher peak areas for some compounds and these compounds were reported to be antimicrobial in nature [14].

## Conclusion

The mulberry growth parameters such as plant height, number of shoots per plant, number of leaves per plant and leaf area were found higher than the control in Foliar application of LBS13 (0.5, 1 and 1.5 ml/l). Application of seaweed extracts can be beneficial for the growth and yield, the resistance to pests and diseases and the quality of crops. However, the bio stimulatory potential of many sea plants have not been fully exploited due to the lack of scientific data on growth factors present in seaweeds and also their mode of action in affecting growth of the plant. LBS13 at 1.5 ml/l along with LBS13 at 1 ml/l and LBS6 at 1.5 ml/l have exhibited increase in the larval weight, decreased larval mortality and increased ERR percentage in the *Bm*NPV infected silkworms. The results of present study clearly indicates that the positive effect of *Kappaphycus* sp. treated mulberry leaves on growth and nutritional parameters of *B mori*. Since sulphated galactan are known to be antiviral in nature, being the main active ingrediant of the sea plant extract could be aiding in decreasing the *Bm*NPV infection to the silkworms and will be a advantageous to sericulture farmers.

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