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RESEARCH PAPER

Sea plant extract (*Kappaphycus* sp) - A boon to sericulture industry

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Abstract : A field experiment was conducted to evaluate the performance of sea plant extract sprayed on mulberry plant at different interval of time 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, LBS 13 @ 1.5 ml/l on 45th day after pruning enhanced the leaf yield per plant by 308.81 g, average plant height by 150.42 cm, number of shoots (14.32) and leaves per plant (400 leaves) compared with control. When the treated leaves were fed to the *Bm*NPV infected silkworms, LBS13 @ 1.5 ml/lrecorded highest larval weight (3.33 g/ larvae) with larval duration (7.67 days), least larval mortality (3.33 %) and 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, which inturn had a direct positive impact on development of*B. mori*.

Key Words : Sea plant extract, *Kappaphycus* sp., Mulberry, *Bm*NPV

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INTRODUCTION

Mulberry (*Morus alba* L.) foliage is the only food for the silkworm, *Bombyx mori* L. Approximately 70 % of the silk proteins are reported to have beenderived from mulberry leaves. Therefore, feeding silkworms with quality mulberry leaves is imperative for successful cocoon production (Vijaya *et al*., 2009). Being perennial, continuous harvest of foliage will result in gradual reduction of yield and quality in mulberry (Rashmi *et al*., 2009).Though synthetic chemicals *viz.,* fertilizers, pesticides, herbicides, growth promoters and other inputs escalate plant productivity, they do adversely affect the mulberry ecosystem. Hence adopting eco-friendly agricultural practices is imminent for a sustainable sericulture inthe current global scenario.

Organic farming can be a remedy to the ills of modern, chemical based agriculture. In this direction, more viable options have to be explored to meet the increasing demand for organic inputs and the use of sea plant extract is found as one of the options as a source of nutrients for plant growth and development (Zodape, 2001). Extracts of marine sea plants contain high levels of organic matter, micro nutrients, vitamins, fatty acids

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along with growth regulators such as Auxins, Cytokinin and Gibberellic acid. Unlike, the extensively used synthetic chemicals, these extracts are biodegradable, nontoxic, non-polluting and non-hazardous to the environment (Khan *et al*., 2009). Apart from being eco friendly, their impact is reflected in terms of enhanced root growth, leaf expansion, increased yield and tolerance to biotic and abiotic stress in the plants. The shoot length, number of leaves per plant, leaf area, and circumference of growing stem, root growth and total biomass accumulation was enhanced in mulberryupon using sea plant extract from *K. alvaerezii*(Maria and Evanjaline, 2015). The application of sea plant extracts were also found beneficial in other crops like black gram (Ganesh *et al*., 2015) and chilly (Arunkumar *et al*., 2015; Jayasinghe *et al*., 2016).Therefore, in the present research it is proposed to study the effect of cultivated sea plant (*Kappaphycus* sp.) extracton growth and development of mulberry.

The silkworm *B.mori*, feeding on mulberry leaves has been completely domesticated since 5000 years and is considered as a model organism in scientific research, molecular biology and genetics. During different life stages, the silkworm is attacked by many pathogenic microbes causing a loss of almost 20–30 percent cocoon production every year (Lekha *et al*., 2015). Amongstdifferent diseases, the virus,*Bm*NPV accounts foralmost 80 percent of the total crop lossin sericulture (Liang and Qing, 2014). It is said that prevention is better than cure and the same holds good even in disease management in silkworm rearing. Further, use of chemicals to prevent the disease incidence adds to environmental pollution and may also lead to toxicity to the silkworms. With this in view, 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.

MATERIAL AND METHODS

The experiment was conducted at the Department of Sericulture, University of Agriculture Sciences, Bangalore during 2016-19. The mulberry (Var. V_1) garden was maintained following the package of practices suggested for irrigated condition (Dandin *et al*., 2003). The field experiment was laid according to Randomized Block Design with 14 treatments each in 3 replications.

Treatment details:

Cultivated tropical sea plant extracts of *Kappaphycus* sp. (*a.i.* sulfated galactans) with four different formulations *viz*., LBD3, LBD12, LBS6 and LBS13 were procured from Sea6 Energy Pvt. Ltd. (Centre for Cellular and Molecular Platforms, Bangalore). The selected sea plant extracts were used at three different concentrations as foliar spray in freshly pruned mulberry garden. The spraying was carried out on 21 and 30 Days After Pruning (DAP) with due care to avoid drifting of spray solution on adjacent plants.

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

LBD=Liquid Bio Defence: LBS= Liquid Bio Stimulant

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

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

Total leaf area was measured using a leaf area meter (LI-3100C). The total leaf yield was recorded on 45th DAP by harvesting the leaves separately from each of the five plants maintained exclusively for yield estimation and expressed as g/plant.

Silkworm rearing:

The popular commercial hybrid PM X CSR_2 (cross breed-CB) was used for rearing experiment. The larvae were fed with mulberry leaves harvested from treated

plants as per recommendation (Dandin *et al.,* 2003) following CRD with 14 treatments in 3 replications each. Purified *Bm*NPV Polyhedral Occlusion Bodies (POBs) were collected from Karnataka State Sericulture Research and Development Institute, Thalaghattapura. Neubaure's haemocytometer was used to determine the concentration of POBs. The silkworms were inoculated *per oral* by feeding them with viral suspension of 10^{-8} (7.3×10^{-3} POBs/ml) smeared on mulberry leaves of standard size (10×12 cm^2) soon after the third moult. The treated mulberry leaves were fed to the worms three times per day. Different rearing parameters along with mortality, percent disease incidence and Effective Rate of Rearing (ERR) were recorded as follows:

$$\text{Mortality (\%)} = \frac{\text{No. of dead larvae}}{\text{Total no. of worms per treatment}} \text{x100}$$

$$\text{Effective rate of rearing (\%)} = \frac{\text{Number of cocoons harvested}}{\text{Number of worms brushed}}$$

The data obtained were statistically analysed by using Web Based Agricultural Statistics Software Package WASP 2.0 (Web Agri Stat Package).The one way ANOVA with DMRT (Duncan's Multiple Range Test) was carried out to determine significant differences among different treatments.

RESULTS AND DISCUSSION

The plant height measured maximumin the treatment LBS13 @ 1.5 ml/l (150.42 cm) and the shortest mulberry plant was observed in the treatment LBS6 @ 1 ml/l (113.11 cm). LBS13 @ 1.5 ml/l was on par with LBS13 @ 1 ml/l (145.98 cm) and LBS6 @ 0.5 ml/l (140.86 cm). The treatments LBS13 @ 0.5 ml/l (140.39 cm), LBD12 @ 1.5 ml/l (138.53 cm) and LBD3 @ 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. Bio-stimulants 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"(Zhang and Schmidt, 1997).

The treatment LBS13 @ 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 @ 1 ml/l 12.83 and 199.67, respectively (Table 1). The treatments LBS13 @ 1 ml/l

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 @ 2 ml/l	128.37^{def}	14.05^{b}	374.00^{d}	106.47^{d}	284.53^{bc}
T_2	Foliar application of LBD3 @ 4 ml/l	134.94^{cde}	13.26^{cd}	232.33^{g}	84.07^{f}	258.18^{d}
T_3	Foliar application of LBD3 @ 6 ml/l	138.31^{bc}	13.5^{d}	325.33^{f}	103.69^{d}	236.87^{g}
T_4	Foliar application of LBD12 @ 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 @ 1 ml/l	137.73b^{cd}	13.50^{c}	225.00^{h}	97.53^{e}	245.74^{e}
T_6	Foliar application of LBD12 @ 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 @ 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 @ 1 ml/l	121.53f^{g}	13.25^{d}	381.00^{c}	74.27^{g}	245.12^{ef}
T_9	Foliar application of LBS6 @ 1.5 ml/l	113.11^{g}	12.83^{ef}	199.67^{j}	65.90^{h}	220.59^{i}
T_{10}	Foliar application of LBS13 @ 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 @ 1 ml/l	145.98^{ab}	14.12^{ab}	398.67^{a}	116.47^{c}	289.53^{b}
T_{12}	Foliar application of LBS13 @ 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 @ 5 %		9.851	0.244	4.808	5.215	5.395

*Significant @ 5 %; DAP- Days after Pruning

(14.12), LBS13 @ 0.5 ml/l (14.05), LBS6 @ 0.5 ml/l (14.06) and LBD12 @ 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 @ 1 ml/l (398.67), LBS13 @ 0.5 ml/l (389.33) and LBS6 @ 0.5 ml/l (385) which were on par with each other as well as better than absolute control (216).

The treatment LBS13 @ 1.5 ml/l recorded larger leaf area of about (135.68cm2) on 45th DAP, followed by the treatment LBS6 @ 0.5 ml/l (121.72 cm2) and LBS13 @ 1 ml/l (116.47 cm2). Lesser leaf area was recorded in the treatment LBS6 @ 1 ml/l (65.90). Since the growth of mulberry treated with LBS6 @ 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 @ 1.5 ml/l treated mulberry plants which was followed by LBS13 @ 1 ml/l (289.53 g/pl), LBS13 @ 0.5 ml/l (282.72 g/pl), LBS6 @ 0.5 ml/l (284.78 g/pl) and LBD3 @ 2 ml/l (284.53 g/pl). The mulberry plants treated with LBS6 @ 1 ml/l exhibited lesser yield of 220.59 g/pl when compared to that of the control (228.61 g/pl).

The growth stimulating activity of sea plant products and their utilization in crop production is well established in several crops. Biostimulants are the "materials, other than fertilizers, which promote plant growth when applied in small quantities" and they are also referred to as "metabolic enhancers"(Zhang and Schmidt, 1997), which is well established by Tiwari *et al.* (2014) who observed an increased leaf yield in mulberry when low concentration of sea plant extract (0.5 ml/l) frim *Dictyotadichotoma* and *K. alvarezii* were used as foliar spray in mulberry. Elongated shoot length, increased number of leaves per plant, leaf area, circumference of stem, root growth and total biomass by 107%, 100%, 135%, 91%, 140% and 140%, respectively were reported in mulberry at third month after the application of extracts from *K. alvarezii* (Maria and Evanjaline, 2015). The increased biomass accumulation in plants is thought to be associated with the hormonal substances *viz.,* cytokinins, auxins, and abscisic acid (ABA)-present in the extracts apart from several micro-nutrients, amino acids and vitamins that affect cellular metabolism in treated plants (Durand *et al*., 2003; Stirk *et al*., 2003; Ordog *et al*., 2004). The cytokinins in vegetative organs are associated with nutrient partitioning while those in reproductive organs may be linked with nutrient

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

Treatments	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^{e}	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 %

mobilization thereby enhancing the growth, development and productivity. Further accumulation of micro nutrients, amino acids and vitamins improve the leaf quality in mulberry thus opening a new dimention for use of sea plant extracts in sericulture.

The treatment LBS13 @ 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 @ 1.5 ml/l (11.00 days). The infected silkworms does exhibit extended larval duration as seen in control batch, whereas the treatment LBS13 @ 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 @ 1.5 ml/l recorded maximum of 3.33 g/larvae followed by LBD3 @ 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 @ 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.

The treatment LBS13 @ 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 @ 1.5 ml/l recorded highest larval mortality of 14.33 per cent with 35.33 per cent disease incidence. The studies conducted by Stirk *et al*. (2003) using *K. alvaerezii* extracts on silkworm recorded increased larval body length and weight. The fifth instar larval weight recorded an increase of 37 %. It is observed that the silkworms derive maximum silk proteins directly from the mulberry leaf and hence the leaf protein content may be directly correlated with silk production efficiency of silkworms (Jadav *et al*., 2000). The stimulant molecules in the sea plant extracts might enhance the protein metabolism in silkworms thus supporting more body mass accumulation. Further, the antimicrobial, antioxidant and antibiotic activity expressed by the extracts of many marine algae (Ravikumar *et al*., 2009) enhance the defence mechanism in the silkworms to fight against the biotic and abiotic stresses. The phytochemical constituents showing higher peaks in the grasserie infected larvae of *B. mori* reported to be antimicrobial in nature (Somu *et al*., 2017) thus resulting in reduced larval mortality and hence increased ERR. The infected larvae fed with sea plant extracts survived even though the pathogencity existed mainly due to the defence responses stimulated by the antimicrobial compounds.

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 @ 1.5 ml/l along with LBS13 @ 1 ml/l and LBS6 @ 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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