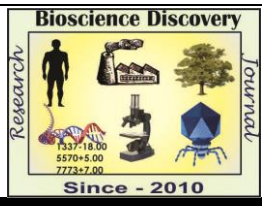
Bioscience Discovery, 8(2): 235-240, April - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)

Research Article



Effect of application of *Ascophyllum nodosum* extract on the yield and quality of Mulberry leaves

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Article Info

Received: 05-01-2017,
Revised: 25-02-2017,
Accepted: 27-02-2017

Keywords:
Ascophyllum nodosum, Mulberry, Seaweed extract

Abstract

An experiment was conducted to evaluate the potential of seaweed extract from *Ascophyllum nodosum* in enhancing the growth, yield and quality of mulberry leaves under field condition. Foliar sprays of different concentrations (0.5-1.0 ml/L) of seaweed extract (*A. nodosum*) were sprayed on the foliage of mulberry plants at different time intervals. It was found that foliar application of seaweed extract from *A. nodosum* 0.5 ml/L at 21, 28 and 35 days after pruning enhanced leaf yield per plant by 72.47 %, average plant height by 92.36 % and protein content (mg g^{-1} of fresh weight) by 67.30 % when compared to control. Results of the present investigation indicate that application of seaweed extract from *A. nodosum* at lower concentration significantly increases both quality and quantity of mulberry leaves.

INTRODUCTION

Mulberry (*Morus* spp.) is one of the most important commercial crops grown extensively as food plant for silkworm (*Bombyx mori* L.) and it is grown under varied climatic conditions ranging from temperate to tropics. In India, mulberry covers three lakh hectares in different agro climatic conditions varying from temperate to tropical. Silkworm being monophagous insect derives almost all the nutrients for growth and development from the mulberry leaf. It has been estimated that, nearly 70% of the silk proteins are derived from mulberry leaves. Hence, silkworms should be fed with good quality mulberry leaves for the successful cocoon production (Vijaya *et al*., 2009). The leaf yield and quality of mulberry depends on the soil type, plant variety, and availability of plant nutrients and agro-ecological conditions, which reflects on the quality of silk production. In India, mulberry contributes to an extent of 38.20 per cent for successful cocoon production (Miyashita, 1986). Though synthetic chemicals, fertilizers, pesticides, herbicides, growth promoters and other inputs enhance productivity of mulberry, they adversely affect the ecosystem. The continuous production of mulberry for a long time results in gradual reduction in leaf yield and quality (Rashmi *et al*., 2009).

The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable Sericulture. Chemicals have made an adverse impact on not only soil but also on the beneficial soil microbial communities and the plants cultivated in those soils. Organic farming is proving as a remedy to the ills of modern chemical agriculture. To meet the increasing demand of organic inputs many viable options have to be explored (Chhaya, 1997) and one such option is the use of seaweed extracts as source of nutrient (Zodape, 2001). Extracts from marine seaweeds could replace synthetic chemicals because they contain high levels of organic matter, micro nutrients, vitamins and fatty acids and also growth regulators such as Auxins, Cytokinin and Gibberellins.

Unlike, synthetic chemicals, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous (Khan *et al*., 2009). Their beneficial effects include enhanced root growth, leaf growth, yield, tolerance to different plant stresses and increase in plant resistant to infections or insect attack. Maria Victorial Rani and Evanjaline, 2015 found that application of seaweed extract from *K. alvaerezii* enhanced the shoot length, number of leaves, leaf area, circumference of stem, root growth and total biomass in mulberry. Application of seaweed extracts were also found beneficial in other crops like black gram (Ganesh *et al.,* 2015) and chilly (Arunkumar *et al.,* 2015 and Jayasinghe *et al*., 2016).

Seaweed extracts are considered as an organic agriculture input as they are environment friendly and safe for the health of animals and humans (Khan *et al*., 2009). Application of seaweed extracts as organic biostimulant is fast becoming accepted practice in farming due to their beneficial effect (Russo and Beryln, 1990 and Verkleij, 1992). There are several examples of seaweed application as organic biostimulant in several horticultural plants, food grain crops, trees etc. However, no much effort has been made in the field of mulberry research for the exploitation of seaweed as biostimulant although seaweed extracts are one of cheapest effective organic plant nourishing agents. Since studies on utilization of various seaweed extracts in Sericulture is scarce, it is necessary to study the effect of seaweed extracts in enhancing the quality and productivity of mulberry leaves for the optimal utilization of seaweeds in Sericulture. Therefore present study attempts to evaluate the potential of *A. nodosum* in enhancing the growth, yield and quality of mulberry under field condition.

MATERIALS AND METHODS

A field experiment was conducted at Central Sericultural Research & Training Institute, Berhampore, (Latitude - 24° 5'28.01"N and Longitude 88°15'56.37"E) West Bengal with foliar application of different concentrations of seaweed extract on mulberry. Experiment was conducted during July-August, 2016 at the institute. The plantation of S1635 with the spacing of (150+90 cm) × 60 cm (Paired row) was used for the study and each subplot consisted of twenty four mulberry plants. Package of practice recommended for irrigated condition was followed. Recommended dose of fertilizer 336:180:112 NPK kg ha^{-1} $year^{-1}$ was applied along with 20t FYM/ha. *A. nodosum* extract (Swarna - manufactured by Rahul Bio Agro, Ranaghat, Nadia) was used in the present investigation. Treatments were 0.5 ml/ L (T_1) and 1.0 ml/ L (T_2) *A. nodosum* extract at 21, 28 and 35 DAP (Days After Pruning); 0.5 ml/ L (T_3) and 1.0 ml/ L (T_4) *A. nodosum* extract at 21 and 28 DAP ; 0.5 ml/ L (T_5) and 1 ml/ L (T_6) *A. nodosum* extract at 21 DAP along with T_7 as control (water spray).

Randomized Block Design (RBD) was used and each treatment was replicated three times. *A. nodosum* extract was mixed with water as per the treatment specification and uniformly applied to the mulberry foliage with Knapsack sprayer. 60 days after pruning five plants were collected from each subplot and data on different growth attributes *viz.*, leaf yield per plant, Average plant height, internodal length, number of branches per plant and harvest index was recorded. Leaf quality parameters Moisture content, Chlorophyll content and protein content were estimated.

Calculation growth parameters

$$\text{Internodal length (cm)} = \frac{\text{Total shoot length (cm)}}{\text{Total number of leaves}}$$

$$\text{Harvest Index} = \frac{\text{Leaf yield}}{\text{Total biological yiled (Leaf + shoot)}} \text{ X } 100$$

Estimation of Moisture Content (%): Calculated by oven dry process. Fresh and dry weight of twenty leaves of each treatment was recorded.

$$\text{Moisture content (\%)} = \frac{\text{(Fresh weight - Dry weight)}}{\text{Fresh weight}} \text{ X } 100$$

Estimation of Chlorophyll content: Biochemical parameters Chlorophyll A, Chlorophyll B, total Chlorophyll and Carotenoid were estimated following the method of Arnon (1949) using 3 replications of all the treatments and the control. Chlorophyll was extracted in 80% Ethyl alcohol and the absorption at 663 nm and 645 nm and 470 were read in a spectrophotometer. Using the absorption coefficients the amount of chlorophyll was calculated.

Chlorophyll A = [(12.7×OD663)-(2.69×OD645)] 0.1
Chlorophyll B = [(22.9×OD645)-(4.68×OD663)] 0.1
Total Chlorophyll = Chlorophyll A + Chlorophyll B
Carotenoids = (OD470×100)/2500

Estimation of Protein content: Protein content of leaves was estimated by Lowry's method (Lowry *et al.*, 1951). The amount of protein (mg) per gram of the fresh weight is expressed.

Analysis of data: Data was subjected to statistical analysis using Web Based Agricultural Statistics Software Package WASP 2.0 (Web Agri Stat Package). The data was analyzed statistically using one way ANOVA (Randomized Block Design) with DMRT (Duncan's Multiple Range Test) to determine significant differences among different treatments.

RESULTS AND DISCUSSION

Data pertaining to growth parameters were statistically analyzed and presented in the table 1. It was found that foliar application of *A. nodosum* extract enhanced the leaf yield per plant (g) and plant height (cm) in comparison with control.

Highest leaf yield per plant (376.60 g) was recorded when 0.5 ml/ L of *A. nodosum* extract was applied three times at weekly interval (T_1) followed by two times foliar application of 1.0 ml/ L of *A. nodosum* extract (T_4) while control recorded lowest leaf yield per plant (218.00 g). T_1 enhanced per plant leaf yield by 72.47 % compared to control. No significant differences were observed when number of shoots per plant, internodal length and harvest index were compared and all the treatments were on par with each other. Application of *A. nodosum* extract significantly increased plant height. Plant height was longest (76.04 cm) when 0.5 ml/ L of *A. nodosum* extract was applied three times at weekly interval (T_1) followed by three times foliar application of 1.0 ml/ L of *A. nodosum* extract (T_2) while shortest plants were recorded in control (39.53 cm). T_1 enhanced average plant height by 92.36 per cent compared to control.

Table 1: Effect of *Ascophyllum nodosum* extract on mulberry yield parametrs

Treatment	Leaf Yield per plant (g)*	No. of shoots per plants	Internodal length (cm)	Plant height (cm)	Harvest index
T1	376.60[a**]	15.93	5.40	76.04[a]	0.50
T2	319.00[a]	17.73	6.25	67.52[ab]	0.47
T3	323.32[a]	18.67	5.59	62.73[bc]	0.59
T4	357.66[a]	17.33	6.04	60.01[bc]	0.47
T5	353.26[a]	16.93	6.63	60.77[bc]	0.48
T6	340.66[a]	16.53	5.96	54.15[c]	0.48
T7	218.00[b]	16.80	6.11	39.53[d]	0.47
SEm ±	52.019	1.422	0.386	4.006	0.037
CD at 5% level of significance	91.32	N.S	N.S	12.34	N.S

*Mean of three replications

** Treatments with same letters are on par with each other

Data presented in (Table 2) shows the effect of *Ascophyllum nodosum* extract on mulberry leaf quality. Foliar application of *A. nodosum* extract significantly enhanced protein content. Control recorded lowest protein content (45.45 mg g^{-1} of fw) while highest protein content of 76.04 mg g^{-1} of fw was recorded when 0.5 ml/ L of *A. nodosum* extract was applied three times at weekly interval (T_1) followed by three times foliar application of 1.0 ml/ L of *A. nodosum* extract (67.52 mg g^{-1} of fw). Protein content was enhanced by 67.30 % in T_1 when compared with control. No significant difference was observed when Chlorophyll A (mg g^{-1} of fw), Chlorophyll B (mg g^{-1} of fw), total Chlorophyll (mg g^{-1} of fw), Carotenoid (mg g^{-1} of fw) and moisture content (%) of different treatments were compared and all the treatments were on par with each other.

Table 2: Effect of *Ascophyllum nodosum* extract on mulberry leaf quality

Treatment	Protein Content (mg g^{-1} of fw)	Chlorophyll A (mg g^{-1} of fw)	Chlorophyll B (mg g^{-1} of fw)	Total Chlorophyll (mg g^{-1} of fw)	SPAD unit	Carotenoid (mg g^{-1} of fw)	Moisture Content (%)
T1	76.04[a]	2.41	0.85	3.26	16.91	0.080	76.00
T2	67.52[ab]	2.49	0.78	3.26	18.08	0.078	76.99
T3	62.73[ab]	2.33	0.77	3.10	17.86	0.078	76.05
T4	60.01[abc]	2.24	0.77	3.00	17.64	0.078	75.63
T5	60.77[abc]	2.36	0.85	3.21	17.14	0.084	76.64
T6	54.15[bc]	2.39	0.84	3.22	17.67	0.084	75.93
T7	45.45[c]	2.20	0.60	2.80	17.19	0.070	75.23
SEm±	5.368	0.178	0.066	0.156	0.795	0.002	0.706
CD at 5% level of significance	16.54	N.S	N.S	N.S	N.S	N.S	N.S

*Mean of three replications

** Treatments with same letters are on par with each other

Results of the present investigation indicate that application of seaweed extract from *A. nodosum* at lower concentration significantly increases both quality and quantity of mulberry leaves. Increase of leaf biomass rich in total soluble protein content is desirable which could be obtained with the application of *A. nodosum* extract. Seaweed extract contains macronutrients, trace elements, organic substances like amino acids and plant growth regulators such as Auxin, Cytokinin, Gibberellins (Crouch and Van Staden., 1994) which may be responsible for the observed biostimulative role of seaweed extract. Present findings strengthens the observations of Tiwary *et al.*, 2014 who reported that lower concentration of seaweed extract (0.5ml/L) from *Dictyota dichotoma* and *Kappaphycus alvaerezii* as foliar spray was most suitable for increasing leaf yield. Maria Victorial Rani and Evanjaline, 2015 also observed that application of seaweed extract from *K. 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 in comparison with control.

Plant hormones are active at extremely low concentration and seaweed extracts contain plant growth regulators (PGR) like Auxins, Cytokinins, and Gibberlins which control the growth and structural development of plant. Seaweeds enhance photosynthesis *via* increasing a plants chlorophyll levels. By increasing the level of chlorophyll, plant would be able to efficiently harness the sunlight increasing yield. Whapham *et al*., 1993 reported that application of a low concentration of *A. nodosum* extract to soil or on foliage of tomatoes produced leaves with higher chlorophyll content than those of untreated controls. Increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract. Rayorath *et al.*, 2008 also observed that Extracts of seaweed *A. nodosum* have been shown to affect the root growth of *Arabidopsis* at very low concentrations (0.1 g / L). Zewail, 2014 reported that foliar application of commercially available seaweed and mixture of

free amino acids significantly increased Photosynthetic pigments, total chlorophyll and crude protein in leaves of common bean. It was also found that aqueous concentrates of *Padina pavonia, Acanthophora spicifera* and *Ulva lactuca* as foliar spray significantly reduced the severity of fungal blast of rice caused by *Pyricularia oryzae* (Flora and Maria Victorial Rani, 2012). *P. pavonia* was reported to be abundant in all the seasons (pre-monsoon, monsoon and post-monsoon) in Hare Island, Gulf of Mannar (Mary Josephine *et al.,* 2013).

Liquid extract obtained from seaweeds has recently gained much interest as soil and foliar spray for enhancing shoot growth and yield in orchards and horticultural plants like black gram (Ramamoorthy and Sujatha, 2007; Sethi and Adhikary, 2008), green gram (Zodape *et al.,* 2010) cluster bean (Xavier *et al.,* 2007), okra (Jothinayagi, 2009), grape (Norrie and Keathley, 2006). Seaweed or its products are commonly used in agriculture to stimulate plant growth and enhance productivity. Their beneficial effects include enhanced yield, root growth, shoot growth, (Thirumalthangam *et al.,* 2003 and Thirumaran, 2009), biomass and crop yield (Eyras *et al.,* 1998). *K. alvarezii* and *Gracilaria edulis* extracts were very effective in enhancing the yield, growth and improved the quality of the produce in black gram. Number of pods/plant, pod weight, seed weight/plant and test weight of seed and quality of seeds were augmented due to the application of seaweed extracts (Ganesh *et al.,* 2015). Application of seaweed extracts prepared from *Gracilaria corticata* var. *corticata* and *Sargassum wightii* significantly promoted the growth of chilly plants (Arunkumar *et al.,* 2015). Seaweed liquid fertilizer from *Ulva lactuca, Sargusum wightti, K. alvarezii* and *Gracilaria verrucosa* in combination with recommended rate of chemical fertilizer also enhanced the growth, yield and quality of *Capsicum annum* (Jayasinghe *et al*., 2016). The results of the present investigation are in agreement with the earlier reports.

Present study was an attempt to evaluate the potential of *Ascophyllum nodosum* in enhancing the growth, yield and quality of mulberry leaves under field condition. It was found that foliar application of seaweed extract from *A. nodosum* 0.5 ml/L at 21, 28 and 35 days after pruning (Three times application at weekly interval) enhanced leaf yield per plant by 72.47 %, average plant height by 92.36 % and protein content (mg g^{-1} of fw) by 67.30 % compared to control. Results of the present investigation indicate that application of seaweed extract from *A. nodosum* at lower concentration significantly increases both quality and quantity of mulberry leaves.Seaweed extract is an environmentally healthy option to improve the growth, biochemical components which will meet the requirement of organic produce in the present scenario.

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How to Cite this Article:

Anil Pappachan, Lungjo Bariampan and Kanika Trivedy, 2017. Effect of application of *Ascophyllum nodosum* extract on the yield and quality of Mulberry leaves. *Bioscience Discovery,* **8**(2):235-240.