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Influence of *Halimeda macroloba* Decsne SLF on seed germination, growth and protein profiles of *Vigna radiata* (L.) Wilczek. var. K851

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Abstract

The present study was aimed to evaluate the effect of seaweed liquid fertilizers (SLF) obtained from *Halimeda macroloba* Decsne on germination, shoot and root growth and protein profile of *Vigna radiata* (L.) Wilczek. var. K851. For the protein profile studies, the young leaves of SLF treated seedlings of *Vigna radiata* were washed once in deionised water and mashed in a pre-chilled mortar in 500 µl of phosphate buffer (pH 7.0). The protein samples were separated in SDS-PAGE and stained using coomassie brilliant blue.

Abbreviations: **SLF** - Seaweed Liquid Fertilizers; SDS-PAGE – Sodium Deodyl Sulphate Poly Acrylamide Gel Electrophoresis;

Keywords: Seaweed Liquid Fertilizers Bio-fertilizer; SDS-PAGE

Introduction

Seaweeds are utilized in the agricultural practices due to the rich content of macro and micronutrients (Anantharaj and Venkatesalu, 2001) and plant growth promoting hormones like auxins, gibberellins and cytokinins (Stirk *et al.,* 2004). In India the use marine macroalgae in modern agriculture has been investigated by many workers (Johnson *et al.,* 2014; Narasimha Rao and Reshmi, 2014; Maria Victorial Rani and Usha, 2013; Kalaivanan *et al.,* 2012; Sridhar and Rengasamy, 2011; Bai *et al.,* 2011; Zodape *et al.,* 2011; Sridhar and Rengasamy, 2010; Thirumaran *et al*., 2009; Khan *et al*., 2009; Sethi and Adhikary, 2008; Xavier and Jesudass, 2007). Globally, the coastal peoples use the seaweeds as manure or in the form of compost (Strik *et al*., 2004). The present study was aimed to evaluate the effect of *Halimeda macroloba* Decsne SLF on germination, shoot and root growth and protein profile of *Vigna radiata* (L.) Wilczek. var. K851.

Materials and Methods

Halimeda macroloba Decsne was harvested from the coastal area of Idinthakarai, India (8° 10'37"N and 77^0 44' 48" E). The collected thallus of *H. macroloba* was washed thoroughly using tap water and spread on blotting paper to remove excess water. SLF was prepared by following the method of Bhosle *et al.,* (1975). Viable seeds of *Vigna radiata* (L.) Wilczek. var. K851 (green gram) were obtained from the Agricultural College and Research Institute, Killikulam, Thoothukudi district, Tamil Nadu. The Seaweed liquid fertilizer of *H. macroloba* at various percentages viz., 0 (T_0), 10 (T_1), 25 (T_2), 50 (T_3), 75 (T_4) and 100 (T_5) were prepared. The seeds were soaked in various concentrations of *H. macroloba* SLF for
12 h separately. The mean shoot and root length of SLF treated seedlings of *Vigna radiata* were observed at the end of 7^{th} day after seed sowing and the results were tabulated. Triplicate samples were used for all the parameters. For the protein studies, the young leaves of SLF treated seedlings of *Vigna radiata* were washed once in deionised water and mashed in a pre-chilled mortar in 500 µl of phosphate buffer (pH 7.0). The resultant slurry was centrifuged at 10000 rpm for 10 min at 4°C in a Micro 22 R centrifuge and the supernatant was stored at -70°C before use. SDS-PAGE was carried out at 25°C.

Results

Maximum percentage of seed germination (90%) was in 25% treatment of *H. macroloba* SLF and minimum (56%) was exhibited in 100% of *H. macroloba* SLF (Fig. 1). Seed treated with high concentration of *H. macroloba* SLF seeds were unsuccessful to enhance germination rate. The effect of various concentrations of *H. macroloba* SLF on growth parameters such as shoot length and root length are demonstrated in Fig. 2. The highest shoot length (11.85 cm) was observed in 25% SLF of *H. macroloba* and lowest shoot length (3.05 cm) was displayed in 100% SLF. The maximum root length (3.87 cm) was observed in 25% SLF of *H. macroloba* and lowest level of growth (1.31 cm) was recorded in 100% SLF (Fig. 2).

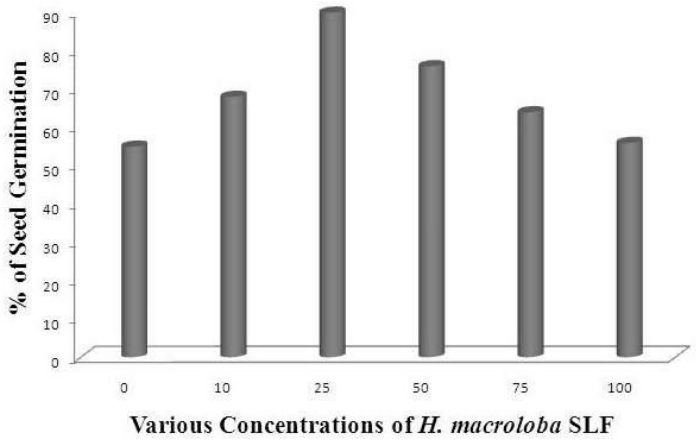

Fig. 1: Effect of *H. macroloba* SLF on Seed Germination of *Vigna radiata* var. K851

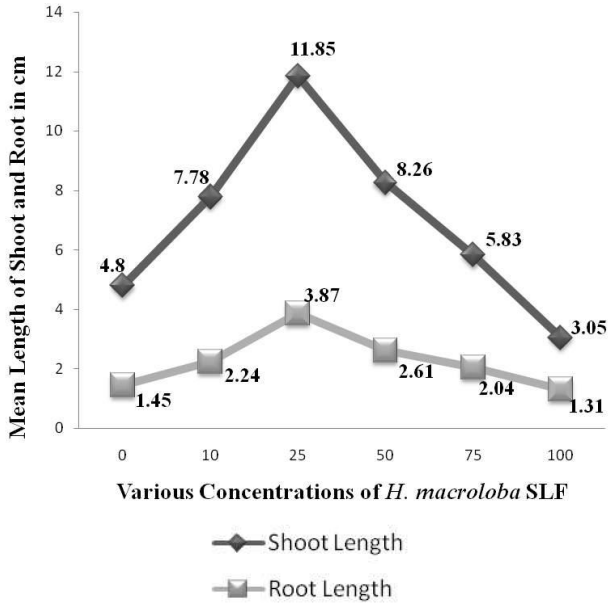

Fig. 2: Effect of *H. macroloba* SLF on vegetative growth of *Vigna radiata* var. K851

In case of *V. radiata*, the number of observable polypeptides bands increased gradually from 0 - 25% concentration of SLF treated seedlings and further increase in SLF concentration reduced the number of observable bands (Table 1) Multiple regions of actively stained gel systems were obtained for SDS-PAGE. On the 7th day, a total number of 52 bands were observed with 21 MW-Rf values ranging from 0.125 to 0.821. Due to the influence of seaweed liquid fertilizer of *H. macroloba* the protein profile of *V. radiata* elicited the following *H. macroloba* SLFs induced proteins (P_1) viz., MW-Rf values 0.125, 0.263, 0.312, 0.400, 0.421, 0.473, 0.505, 0.526, 0.547, 0.564, 0.652, 0.684 and 0.821. Among these protein with MW-Rf value 0.125 showed its presence in all SLF treated seedlings. The SLF treated seedlings of *V. radiata* showed total number of 20 proteins (P_2) with the MW-Rf value 0.180, 0.289, 0.554 and 0.807. Among these MW-Rf 0.180 and 0.289 were observed in T_0, T_1, T_2, T_3, T_4.and T_5 seedlings. The MW-Rf value 0.554 and 0.821 showed their presence in T_0, T_1, T_2 and T_3 seedlings. The following protein bands with MW-Rf 0.216, 0.457, 0.650 and 0.759 were present only in the control; hence they may be SLF sensitive bands (P_3). T_1 seedlings showed ten bands, out of which six bands with MW-Rf values 0.125, 0.421, 0.473, 0.564, 0.684 and 0.821 were induced protein (P_1 protein). Out of which the protein band with MW-Rf 0.821 expressed its restricted presence in T_1 seedlings. The following proteins with the Rf 0.180, 0.289, 0.554 and 0.807 were P_2 proteins. T_2 seedlings showed thirteen protein bands, among these nine bands with MW-Rf value 0. 125, 0.263, 0.312, 0.421, 0.473, 0.505, 0.526, 0.564 and 0.684 were P_1 proteins. The proteins with MW-Rf 0.263 and 0.312 showed their presence in T_2 seedlings. The following four protein bands with MW-Rf 0.180, 0.289, 0.554 and 0.807 were P_2 proteins**.** T_3 seedlings showed total number of eight proteins. Among these four proteins were P_1 proteins. The P_1 protein with MW-Rf 0.547 expressed their occurrence only in T_3 seedlings. The following four protein bands with MW-Rf 0.180, 0.289, 0.554 and 0.807 were P_2 proteins**.** T_4 seedlings showed seven bands. Among these five protein bands were P_1 proteins. Only two proteins with the MW-Rf 0.180 and 0.289 were the P_2 protein. T_5 seedlings showed six protein bands in the gel system.

Among these four proteins with the MW-Rf 0.125, 0.400, 0.526 and 0.652 were P_1 proteins. T_5 seedlings showed two proteins with MW-Rf 0.180 and 0.289 were P_2 proteins.

Table 1: SDS – PAGE banding pattern of different concentration of *H. macroloba* treated *V. radiata* seedlings on 7th day

MW-Rf	Positions	Regions	SLF concentration of *H. macroloba* (%)					
			0	10	25	50	75	100
0.125	PP 1^1	**1**	-	+	+	+	+	+
0.180	PP 1^2		+	+	+	+	+	+
0.210	PP 2^1	**2**	-	+	-	-	-	-
0.216	PP 2^2		+	-	-	-	-	-
0.263	PP 2^3		-	-	+	-	-	-
0.289	PP 2^4		+	+	+	+	+	+
0.312	PP 3^1	**3**	-	-	+	-	-	-
0.400	PP 4^1	**4**	-	-	-	+	+	+
0.421	PP 4^2		-	+	+	-	-	-
0.457	PP 4^3		+	-	-	-	-	-
0.473	PP 4^4		-	+	+	-	-	-
0.505	PP 5^1	**5**	-	-	+	-	+	-
0.526	PP 5^2		-	-	+	+	-	+
0.547	PP 5^3		-	-	-	-	+	-
0.554	PP 5^4		+	+	+	+	-	-
0.564	PP 5^5		-	+	+	-	-	-
0.650	PP 6^1	**6**	+	-	-	-	-	-
0.652	PP 6^2		-	-	-	-	+	+
0.684	PP 6^3		-	+	+	+	-	-
0.759	PP 7^1	**7**	+	-	-	-	-	-
0.807	PP 8^1	**8**	+	+	+	+	-	-
0.821	PP 8^2		-	+	-	-	-	-

Discussion

Anantharaj and Venkatesalu (2001 and 2002) reported the positive effect of *Caulerpa racemosa* and *Gracilaria edulis* SLF on *Vigna catajung* and *Dolichos biflorus* in seed germination, seedling growth, fresh and dry weight. Similarly in the present study also the seed germination percentage was increased in the SLF soaked seeds of *Vigna radiata*. Murugalakshmi Kumari *et al.,* (2002) reported that the treatment of *Gracilaria corticata* fertilizer on black gram and kambu had increased the growth parameters such as shoot length and root length with optimum concentration of seaweed extract. The results of the present study also directly coincided with Murugalakshmi Kumari et al. observations.

Thirumaran *et al.,* (2009) observed that lower concentrations of SLF increase the germination percentage and vegetative growth than the higher concentration in green chilies and turnip. Sunarpi *et al.,* (2010) also observed similar surveillance in *Oryza sativa*; Thirumaran *et al.,* (2007) in *Raphanus sativus*, Thirumaran *et al.,* (2009) in *Cyamopsis tetrogonolaba*. Similarly in the present study also we observed the enhancement of seed gerimination and seedling growth of *Vigna radiata* in lower concentrations of *H. macroloba* SLF than the higher concentration. Sridhar and Rengasamy (2002) prepared the seaweed liquid fertilizer from *Ulva lactuca* and studied their effect on *Spirulina platensis*. They observed the rate of enhancement in the total protein content at lower concentration. Venkataraman and Mohan (2003) also reported that the SLF treated plants showed a marked increase in soluble protein and soluble sugar contents. Similar to the previous observation, in the present study also the protein profiles are varied with refernce to the concenttrations of SLF treatment. Thambiraj *et al.,* (2012) studied the influence of *Sargassum wightii* and *Hypnea musciformis* seaweed liquid fertilizer on the growth and biochemical constituents of *Cyamopsis tetragonoloba* (L). The seeds of *C. tetragonoloba* treated in SLF performed better growth and certain biochemical attributes than water soaked.

The effect of crude seaweed extracts from *Ulva lactuca* and *Sargassum wightii* was studied on germination and protein profile of five different crops viz., *Amaranthus roxburghinus*, *Amaranthus tricolor*, *Arachis hypogea*, *Capsicum annum* and *Tagetes erecta*. Among the five different crops treated with 1.0% SLF of both seaweeds, except *A. roxburghinus*, the other four studied speceis showed additional bands. A maximum of five additional bands appeared in *C. annum* under *U. lactuca* SLF. Further, one or two bands appeared in *A. tricolor*, *A. hypogea* and *T. erecta* (Sridhar and Rengasamy, 2011). In the present study also we observed the following bands 0.125, 0.263, 0.312, 0.400, 0.421, 0.473, 0.505, 0.526, 0.547, 0.564, 0.652, 0.684 and 0.821 in the protein profile of *V. radiata* by the treatment of *H. macroloba* SLFs. Sridhar and Rangasamy

(2011) suggested that the induced appearance of the protein profile may be due to regulation of plant growth regulators like auxin and cytokinin as well as macro and micro elements present in the SLFs. The results of the present study supplemented the previous observations and *H. macroloba* is eco-friendly easily manageable input to farming.

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