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Effect of *Ulva fasciata* Deile seaweed liquid fertilizer on growth and protein profile of *Vigna radiata* (L.) Wilczek. var. K851.

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Abstract

In the present investigation, an attempt has been made to study the effect of SLF from *Ulva fasciata* Deile on seed germination, growth and protein profile of *Vigna radiata* (L.) Wilczek. var.K851. Seed germination, shoot length, root length and protein profile was analyzed with different concentrations (0%, 10%, 25%, 50%, 75% and 100%) of SLF. The results showed that 25% SLF has higher level of seed germination, growth and protein profile compared to other concentrations.

Key words:

Ulva lactuca, Seaweed Liquid Fertilizer, *Vigna radiata*, SDS – PAGE, Protein profile.

Introduction

Seaweeds form an integral part of marine coastal ecosystems. They include the macroscopic, multicellular marine algae that commonly inhabit the coastal regions of the world's oceans where suitable substrata exist. It has been estimated that there are about above 9,000 species of macroalgae broadly classified into three main groups based on their pigmentation viz., Phaeophyta, Rhodophyta and Chlorophyta respectively. Most of them are the red (6000 species), brown (2000 species) or green (1200 species). A wide range of beneficial effects have been reported from the use of liquid seaweed extracts (Blunden, 1991). Seaweed liquid fertilizers were found superior than chemical ones because of the presence of high levels of organic matter, thus accounting a reduction of 50% cost for chemical fertilizer (Aitken and Senn, 1965). Seaweeds are one of the most important marine resources of the world and being used as human food, animal feed and raw material for many industries, they are also used as manure for agricultural and horticultural crops (Chapman and Chapman, 1980). The importance due to the presence of minerals, trace elements and plant growth regulators which occur in water soluble form (Moller and Smith, 1998 & 1999) and enhances the disease resistance in field crops (Verkleij, 1992). Seaweeds contain a diverse range of organic compounds. Atleast seventeen of the common aminoacids occur in the macroalgae (Munda and Gubensek, 1975). Seaweeds also contain a wide range of vitamins which might be utilized by crops (Giiven *et al*., 1976; Kanazawa, 1963). Vitamins C, B, (thiamine), B2 (riboflavin), B12, D3, E, K, niacin, pantothenic, folic and folinic acids occur in algae. Although vitamin A is not present, the presence of its precursor ß-carotene and fucoxanthin has been demonstrated (Stephenson, 1968). Liquid extracts derived from marine algae have been used over the past forty years on a variety of crops to promote plant growth and development (Crouch and Van Staden, 1994). Recent reports showed that seaweed concentrates increase plant vigour and yield have resulted in a renewed interest in modern day application of commercial preparations (Sivasankari *et al*., 2006, Thirumaran *et al.,* 2009, Sridhar *et al*., 2010). With this knowledge the present study was aimed to study the effect of SLF from *Ulva fasciata* Deile on seed germination, growth and protein profile of *Vigna radiata* (L.) Wilczek. var.K851.

Materials and Methods

Collection of Seaweeds

Ulva fasciata Deile was collected from the coastal area of Idinthakarai, India (8º 10' 37"N and 77º 44' 48" E). The thallus was colected by hand picking method and washed thoroughly with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes. The thallus of *U. fasciata* was placed in new polythene bags and kept in an ice box containing slush ice and transported to the laboratory. It was then washed thoroughly using tap water and maximum care was taken to remove the salt and epiphytes on the surface of the sample. The water was drained off and the thallus was spread on blotting paper to remove excess water.

Preparation of seaweed liquid fertilizers

One kg of seaweed was cut into small pieces and boiled separately with 1: l of distilled water for an hour and weltered. The weltered was taken as 100% concentration of the seaweed extract and from this divergent concentrations (10%, 25%, 50%, 75% and 100%) were prepared using distilled water (Bhosle *et al.*, 1975). As the seaweed liquid fertilizer contains organic matter, it was refrigerated at 4°C.

The crop plant selected for the present study was *Vigna radiata* (L.) Wilczek. belonging to the family Fabaceae. The seeds of *V. radiata* were collected from Agricultural College, Tuticorin, Govt. of Tamil Nadu, India. The seeds with uniform size, colour and weight were chosen for the experimental purpose. The selected seeds were stored in a metal tin (Rao, 1976).

SDS-PAGE analysis

The seedlings were grown in the culture room for a period of 7 days. On 7th day, the randomly collected whole plants were used as a source for protein isolation. 500 mg of freshly harvested tissues were taken and homogenized with 3.5 ml of ice-cold 0.1M phosphate buffer (pH 7.0) in a pre-chilled mortar and pestle. It was centrifuged at 10,000 rpm for 10 min and the supernatant was collected and used for protein separation. SDS-PAGE was performed by the method described by Anbalagan (1999). Electrophoresis was carried out at 25°C in the air conditioned room. Separation of protein was carried out at 50v till the tracking dye reaches the separating gel and at 100v thereafter for 3-5 hours or until the tracking dye had migrated to the bottom of the gel. After running the electrophoresis, the gels were carefully removed from the mold and subjected to activity staining.

Results

Ulva fasciata SLF treated seeds of *V. radiata* showed different percentage of seed germination ranged from 40 to 90. Highest percentage of germination (90%) was observed in 25% of *U. fasciata* SLF and lowest percentage of germination (40%) was recorded in 100% of *U. fasciata* SLF (Fig- 1). Seeds treated with high concentration of *U. fasciata* SLF failed to enhance the germination rate. The effect of various concentrations of *U. fasciata* SLF on growth parameters such as shoot length and root length are demonstrated in Fig-1. Highest shoot length (11.72 cm) was observed in 25% SLF of *U. fasciata* and lowest shoot length (2.22 cm) was observed in 100% SLF. Maximum length of root (3.93 cm) was demonstrated in 25% SLF of *U. fasciata* and lowest level of growth (1.06 cm) was recorded in 100% SLF.

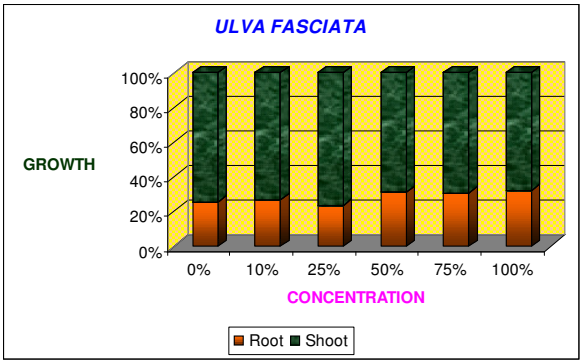

Fig.1 Effect of different consentrations of *U. fasciata* SLF on 7 days seedlings of *Vigna radiata*

The relative position of various concentrations of *U. fasciata* SLFs treated seedlings of *V. radiata* protein profiles revealed by SDS-PAGE. The results of *U. fasciata* SLFs treated seedlings of *V. radiata* protein profiles were showed in Fig-1 (Table 1). In case of *V. radiata*, the number of observable polypeptides increase gradually from 0-25% concentration of SLF treated seedlings and further increase in SLF concentration reduced the number of observable bands. Multiple regions of actively stained gel systems were obtained for SDS- PAGE. Based on the expression of protein in the SDS-PAGE gel system the proteins were classified as follows induced proteins (P_1), tolerant proteins (P_2) and sensitive proteins (P_3).

On 7th day, a total of 56 bands were observed with various Rf values ranging from 0.428 to 0.857. Due to the influence of seaweed liquid fertilizer of *U. fasciata* the protein profile of *V. radiata* elicited the following Rf values viz., 0.373, 0.429, 0.548, 0.571, 0.583, 0.595, 0.607, 0.631, 0.643, 0.667, 0.679, 0.690, 0.714, 0.726, 0.738, 0.774, 0.833, 0.845 and 0.857. Of which Rf value 0.607 and 0.726 showed their unique presence in T_1, T_2, T_3 and T_4 concentration. The Rf value 0.690 showed its restricted presence in T1, T3 and T_4 plants. Rf 0.429 and 0.833 showed their presence in T_1 and T_2 plants. Rf 0.857 showed its presence in T_2 and T_3 plant.

The SLF treated seedlings of *Vigna radiata* showed a total number of 20 tolerant proteins (P_2) with the Rf value 0.457, 0.554, 0.650, 0.759 and 0.807. Among these Rf 0.457 was observed in T_0, T_1, T_2, T_3 and T_4. The Rf 0.554, 0.650 and 0.807 were observed in T_0, T_1, T_2 and T_3 plants in the protein gel system of *V. radiata*. Protein bands with Rf 0.759 were found in T_0, T_1 and T_2 plants. The following protein bands with Rf-0.180, 0.216 and 0.289 were present only in the control; hence they may be considered as SLF sensitive bands (P_3).

T_1 plants showed twelve bands, of which seven bands with Rf values 0.429, 0.607, 0.619, 0.667, 0.690, 0.726 and 0.833 were observed to be in the protein (P_1). Of which Rf value 0.619 and 0.667 showed their restricted presence only in the protein gel system of *V. radiata* of T1 plant. The following proteins with Rf 0.457, 0.554, 0.650, 0.759 and 0.807 are P_2 proteins of T_1 plant. T_2 plants showed thirteen protein bands, Among these, eight bands with Rf value 0.429, 0.595, 0.607, 0.643, 0.714, 0.726, 0.833 and 0.857 were P_2 proteins. Of which only three proteins with Rf 0.595, 0.643 and 0.714 showed their unique presence in T_2 plants. T_3 plants showed total number of nine proteins. Among these five proteins were P_1. Of which the following P1 protein with Rf 0.571 express only in T_3 plants. T_4 plants showed seven bands. Among these, six protein bands were P_1 proteins. Of which Rf 0.738 and 0.845 were showed their unique presence in the gel system. In T_4 Plants gel system only one P_2 protein was observed with the Rf 0.457. T_5 plants showed seven protein bands in the gel system. All the protein bands were P_1 proteins. The following proteins with Rf 0.373, 0.548, 0.583, 0.679 and 0.774 were expressed their restricted presence in T_5 plant.

Figure 2. Zymogram of *U. fasciata* SLF Treated and Control Seedlings of *Vigna radiata var.*K851.

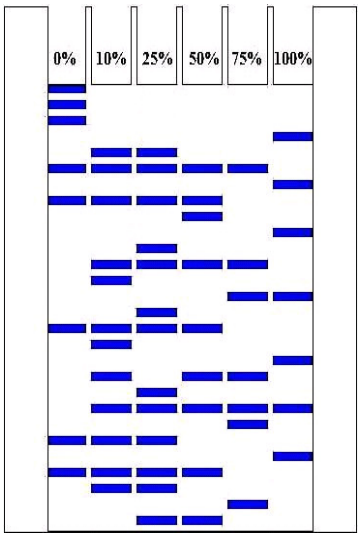

Table 1: SDS – PAGE protein profile of 7 days old seedlings of *V. radiata*

			Concentration of					
MW-Rf	**Positions**	**Regions**	(***U. fasciata***) **in %**					
			0	10	25	50	75	100
0.18	PP 1^1	1	+	-	-	-	-	-
0.216	PP 2^1	2	+	-	-	-	-	-
0.289	PP 2^2		+	-	-	-	-	-
0.373	PP 3^1	3	-	-	-	-	-	+
0.429	PP 4^1	4	-	+	+	-	-	-
0.457	PP 4^2		+	+	+	+	+	-
0.548	PP 5^1		-	-	-	-	-	+
0.554	PP 5^2		+	+	+	+	-	-
0.571	PP 5^3	5	-	-	-	+	-	-
0.583	PP 5^4		-	-	-	-	-	+
0.595	PP 5^5		-	-	+	-	-	-
0.607	PP 6^1		-	+	+	+	+	-
0.619	PP 6^2		-	+	-	-	-	-
0.631	PP 6^3		-	-	-	-	+	+
0.643	PP 6^4		-	-	+	-	-	-
0.65	PP 6^5	6	+	+	+	+	-	-
0.667	PP 6^6		-	+	-	-	-	-
0.679	PP 6^7		-	-	-	-	-	+
0.69	PP 6^8		-	+	-	+	+	-
0.714	PP 7^1		-	-	+	-	-	-
0.726	PP 7^2		-	+	+	+	+	+
0.738	PP 7^3	7	-	-	-	-	+	-
0.759	PP 7^4		+	+	+	-	-	-
0.774	PP 7^5		-	-	-	-	-	+
0.807	PP 8^1		+	+	+	+	-	-
0.833	PP 8^2		-	+	+	-	-	-
0.845	PP 8^3	8	-	-	-	-	+	-
0.857	PP 8^4		-	-	+	+	-	-

DISCUSSION

The results of vegetative growth parameters as influenced by different concentrations of seaweed liquid fertilizers of *Ulva fasciata* in *Vigna radiata* are presented in **Fig. 1**. In the present study, lower concentration of *U. fasciata* SLF treated plants showed maximum growth and higher concentrations showed a decreasing trend in *Vigna radiata*. Similar results were recorded in *Padina* which induced maximum seedling growth at lower concentrations in *Cajanas cajan* (Mohan *et al*., 1994) *Vigna radiata*, *Zea mays* and *Phaseolus mungo* (Lingakumar *et al*., 2004). Dhargalkar and Untawale (1983) also reported similar findings with *Hypnea musciformis, Spatoglossum asperum, Stoechospermum marginatum* and *Sargassum* sp. on

the vegetative growth of crop plants like green chillies, turnips and pineapple. The results of the present study revealed that low concentration of both seaweed extracts (SLF) significantly enhanced the vegetative growth parameters such as shoot length, root length and dry weight of the seedlings (El-Sheekh and El-Saied, 1999).

Growth of any organ is associated with an additional synthesis of proteins which are building blocks of protoplasm and are again the resultant on inter-mediatory metabolism. Proteome analyses have resulted in the identification of many proteins that are inducible by seaweed liquid fertilizer including the metabolism related genes. The results of the present study also revealed the expression of number of proteins which are induced by seaweed liquid fertilizer. The present observation directly coincides and supplements with the previous observations. When the seedlings were treated with above 25% of *U. fasciata* SLF, the number of protein bands and strength of bands were decreased. The banding positions and regions of activity are variable based on the developments of plants and concentrations of seaweed liquid fertilizer supplementations. Similar to the previous observations, the banding positions of *U. fasciata* liquid fertilizer treated seedlings of *V. radiata* showed the variation in the banding profile and expression in the protein gel system. Some proteins and isoenzymes show their marked presence by the supplementation (SLF Induced Proteins), some are failed to express due to SLF supplementations (SLF sensitive proteins) and some showed their presence in the normal plants and SLF treated supplemented seedlings also (SLF tolerant proteins). The results of the present study also revealed the expression of SLF sensitive, induced and tolerant proteins in the *V. radiata* SLF treated seedlings. The analysis of protein content of root and shoot system of *Vicia faba* showed that *Cladophora dalmatica, Enteromorpha intestinalis, Ulva lactuca, Corollina mediterranea, Jania rubens* and *Pterocladia pinnata* extracts increased protein content in both root and shoot system.

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