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EFFECT OF *CAULERPA CORYNEPHORA* MONTAGNE SEAWEED LIQUID FERTILIZER ON GROWTH AND PROTEIN PROFILE OF *VIGNA RADIATA* (L.) WILCZEK. VAR. K851

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ABSTRACT

The present study was aimed to evaluate the impact of *Caulerpa corynephora* Montagne SLF on *Vigna radiata* seed germination, growth and protein expression. *Caulerpa corynephora* SLF was prepared by following the method of Bhosle. The seaweed liquid fertilizer was prepared with different doses viz., 0 (T_0), 10 (T_1), 25 (T_2), 50 (T_3), 75 (T_4), 100% (T_5). Then the seeds were soaked in particular doses of SLF for 12 hr. The crop plants *Vigna radiata* var. K851 (green gram) was treated with the seaweed liquid fertilizers at different concentration. T_0, T_1, T_2, T_3, T_4 and T_5 SLF were analyzed at the end of 7th day after seed sowing. Triplicate samples were used for all the parameters. 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. Maximum percentage of germination (80) was displayed in 25% treatment of *C. corynephora* SLF and minimum percentage of germination (35) was observed in 100% of *C. corynephora* SLF. Highest length of shoot (11.05 cm) was expressed in 25% SLF of *C. corynephora* and lowest shoot length (2.88 cm) was observed in 100% SLF. Highest root length (3.93 cm) was observed in 25% SLF of *C. corynephora* and lowest root length (1.35 cm) was observed in 100% SLF. On the 7th day, a total number of 51 bands were observed with 21 MW-Rf values ranging from

0.80 to 0.891. Due to the influence of seaweed liquid fertilizer of *C. corynephora,* the protein profile of *V. radiata* elicited the following *C. corynephora* SLFs induced proteins (P_1) viz., MW-Rf values 0.237, 0.293, 0.302, 0.358, 0.375, 0.500, 0.576, 0.651, 0.673, 0.750, 0.771, 0.782 and 0.891. The SLF treated seedlings of *V. radiata* showed total number of 24 tolerant proteins (P_2) with the MW-Rf value 0.216, 0.289, 0.457, 0.554, 0.650, 0.759 and 0.807. The SLF senstive proteins (P_3) with MW-Rf 0.180, 0.759 and 0.807 were present only in the control seedlings. In general, in the present study, the seaweed liquid fertilizer prepared from the *C. corynephora* applied to *V. radiata* plants showed better results in all aspects of growth at lower concentration.

KEYWORDS: Protein; *Vigna radiata*; Seaweed Liquid Fertilizer; SDS-PAGE; *Caulerpa corynephora***.**

INTRODUCTION

The fast growing population is mounting tremendous pressure in food production in the world. To meet out this increasing demand, farmers use chemical fertilizers to enhance the crop production. The toxic chemicals (arsenic and cadmium) from the chemical fertilizers accumulate in plant products causing health problems in human by biomagnifications (Hansra *et al*., 1993). In recent years the use of natural seaweed products as substitutes to the conventional synthetic fertilizers has assumed importance. In agriculture, the application of seaweeds are so many as soil conditioners, fertilizers and green manure due to the presence of high amount of potassium salts, micronutrients and growth substances. The growing agricultural practices need more fertilizers for higher yield to satisfy food for human beings. There are many growth hormones, regulators and promoters available to enhance yield attributes. The developed countries utilized such growth hormones in cultivation of crops. In India utilization of seaweeds and their extracts, seaweed liquid fertilizers will be useful for achieving higher agricultural production. The use of marine macro algae as fertilizer in crop production has a long tradition in coastal areas all over the world. The use of these marine macroalgae in modern agriculture has been investigated by many workers (Rama Rao *et al*., 1990 and 1991; Manimala and Rengasamy, 1993; Whapham *et al*., 1993; Lopez-Musquera *et al*., 1997).

In the present day world, the seaweed fertilizers are often found to be more successful than the chemical fertilizer. Marine algae have been utilized directly as manure or

in the form of compost by coastal peoples (Rajkumar and Subramanian, 1999; Strik *et al*., 2004). Besides their application as Farm Yard Manure (FYM), liquid extract obtained from seaweeds popularly known as SLF/LSF has recently gained much interest as foliar spray for inducing faster growth and yield in cereal crops, vegetables, fruits, orchards and horticultural plants (FAO, 2006; Khan *et al*., 2009). Seaweed extracts are now available commercially under the names, such as Maxicrop (Sea born), Algifert (marinure), Goemar GA14, Kelpak 66, Seaspray, Seasol, SM-3, Cytex and Seacrop 16. Recently researchers proved that seaweed fertilizers are better than other fertilizers and are very economical (Gandhiyappan and Perumal, 2001). Any improvement in agricultural system that results in higher production should reduce the negative environmental impact of agriculture and enhance the sustainability of the system. One such approach is the use of biostimulants, which can enhance the effectiveness of conventional mineral fertilizers. Marine bioactive substances extracted from marine algae are used in agricultural and horticultural crops, and many beneficial effects, in the terms of enhancement of yield and quality have been reported. Liquid extracts obtained from seaweeds have recently gained importance as foliar sprays for many crops including various grasses, cereals, flowers and vegetable species (Crouch and Van Staden, 1993). With this knowledge the present study was aimed to evaluate the impact of *Caulerpa corynephora* Montagne SLF on *Vigna radiata* seed germination, growth and protein expression.

MATERIALS AND METHODS

Collection of Seaweeds

Caulerpa corynephora Montagne was collected from the coastal area of Punchal, India (8° 8’44 N and 77^0 36 11” 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 *C. corynephora* 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 *C. corynephora* 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).

The seaweed liquid fertilizer was prepared with different doses viz., 0 (T_0), 10 (T_1), 25 (T_2), 50 (T_3), 75 (T_4), 100% (T_5). Then the seeds were soaked in particular doses of SLF for 12 hr. Then the seeds were sowed and observed for germination and early growth.

Seed treatments: The crop plants *Vigna radiata* var. K851 (green gram) was treated with the seaweed liquid fertilizers at different concentration. T_0, T_1, T_2, T_3, T_4 and T_5 SLF were analyzed at the end of 7^{th} day after seed sowing. Triplicate samples were used for all the parameters.

SDS-PAGE analysis

The seedlings were grown in the culture room for a period of 7 days. On 7^{th} 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

C. corynephora SLF treated seeds of *V. radiata* showed different percentage of seed germination ranging from 35 to 80. Maximum percentage of germination (80) was displayed in 25% treatment of *C. corynephora* SLF and minimum percentage of germination (35) was observed in 100% of *C. corynephora* SLF (Fig. 1). Seeds treated with high concentration of *C. corynephora* SLF failed to enhance

germination percentage. The effect of various concentrations of *C. corynephora* SLF on growth parameters such as shoot length and root length are depicted in Fig. 2. Highest length of shoot (11.05 cm) was expressed in 25% SLF of *C. corynephora* and lowest shoot length (2.88 cm) was observed in 100% SLF. Highest root length (3.93 cm) was observed in 25% SLF of *C. corynephora* and lowest root length (1.35 cm) was observed in 100% SLF.

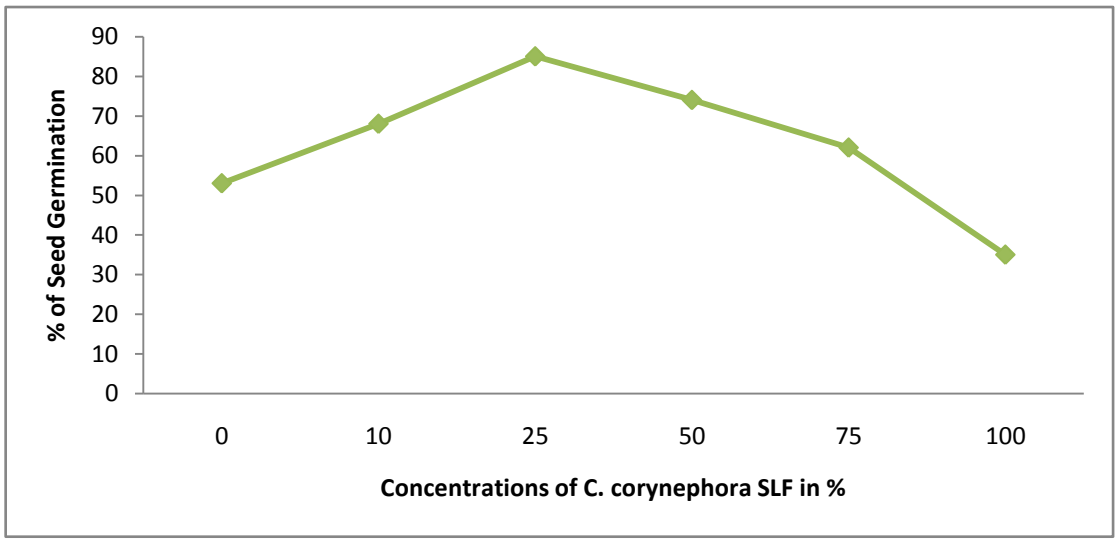

Fig. 1: Effect of *C. corynephora* SLF on *Vigna radiata* Seed germination

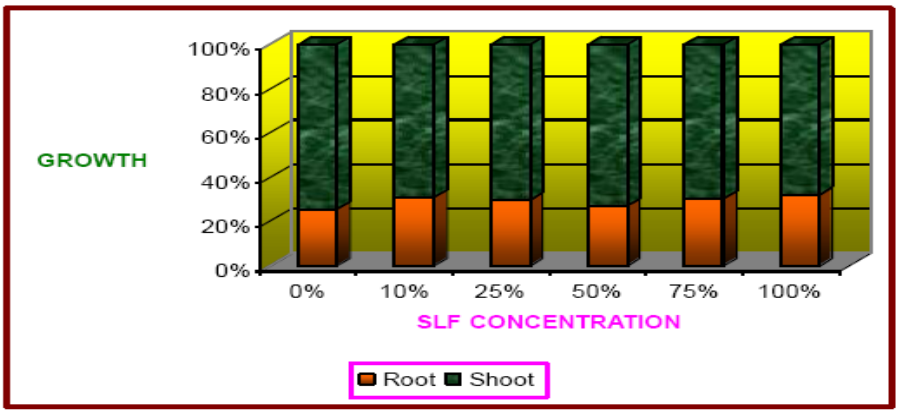

Fig. 1: Effect of different concentrations of *C. corynephora* SLF on 7 Days old

Seedlings of *Vigna radiata*

The effect of *C. corynephora* SLF on the protein expression of *V. radiata* was revealed by SDS-PAGE and the results were illustrated in Fig. 3; Table 1. In case of *V. radiata,* the number of observable polypeptide bands increased 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.

On the 7^{th} day, a total number of 51 bands were observed with 21 MW-Rf values ranging from 0.80 to 0.891. Due to the influence of seaweed liquid fertilizer of *C. corynephora,* the protein profile of *V. radiata* elicited the following *C. corynephora* SLFs induced proteins (P_1) viz., MW-Rf values 0.237, 0.293, 0.302, 0.358, 0.375, 0.500, 0.576, 0.651, 0.673, 0.750, 0.771, 0.782 and 0.891.

Out of which MW-Rf value 0.375 showed its presence in all treated seedlings. MW-Rf 0.673 showed its presence in all *C. corynephora* SLF treated seedlings except T_5 seedlings. The SLF treated seedlings of *V. radiata* showed total number of 24 tolerant proteins (P_2) with the MW-Rf value 0.216, 0.289, 0.457, 0.554, 0.650, 0.759 and 0.807. Among this MW-Rf 0.289 was observed in all *C. corynephora* SLF treated seedlings. The following MW-Rf values 0.457 was illustrated in T_0, T_1, T_2, T_3 and T_4 seedlings. The MW-Rf 0.216, 0.554 and 0.807 showed their presence in T_0, T_1 and T_2 seedlings. The following protein bands with MW-Rf 0.180, 0.759 and 0.807 were present only in the control seedlings; hence they may be SLF sensitive bands (P_3). T_1 seedlings showed ten bands, of which six bands with MW-Rf values 0.293, 0.358, 0.375, 0.576, 0.673 and 0.771 were induced protein (P_1 protein). The following proteins with MW-Rf value 0.216, 0.289, 0.457 and 0.554 were P_2 proteins. T_2 seedlings showed thirteen protein bands. Among these nine bands with MW-Rf value 0.293, 0.358, 0.375, 0.576, 0.651, 0.673, 0.750, 0.771 and 0.891 were P_1 proteins.

Out of which two proteins with MW-Rf 0.651, 0.750 and 0.891 showed their unique presence in T_2 seedlings. T_3 seedlings showed total number of eight proteins. Among these three proteins were P_1 proteins, of which the following P_1 protein with MW-Rf 0.302 expressed in T_3 seedlings. T_4 seedlings showed six bands. Among these three protein bands were P_1 proteins. MW52 Rf 0.237 was showed its unique presence in T_4 seedlings. T_5 seedlings showed six protein bands in the gel system. Among these three proteins with the

MW-Rf 0.375, 0.500 and 0.782 were P_1 proteins, among this MW-Rf 0.500 and 0.782 expressed their unique presence in T_5 seedlings. MW-Rf 0.216, 0.289 and 0.650 were P_2 proteins in T_5 seedlings.

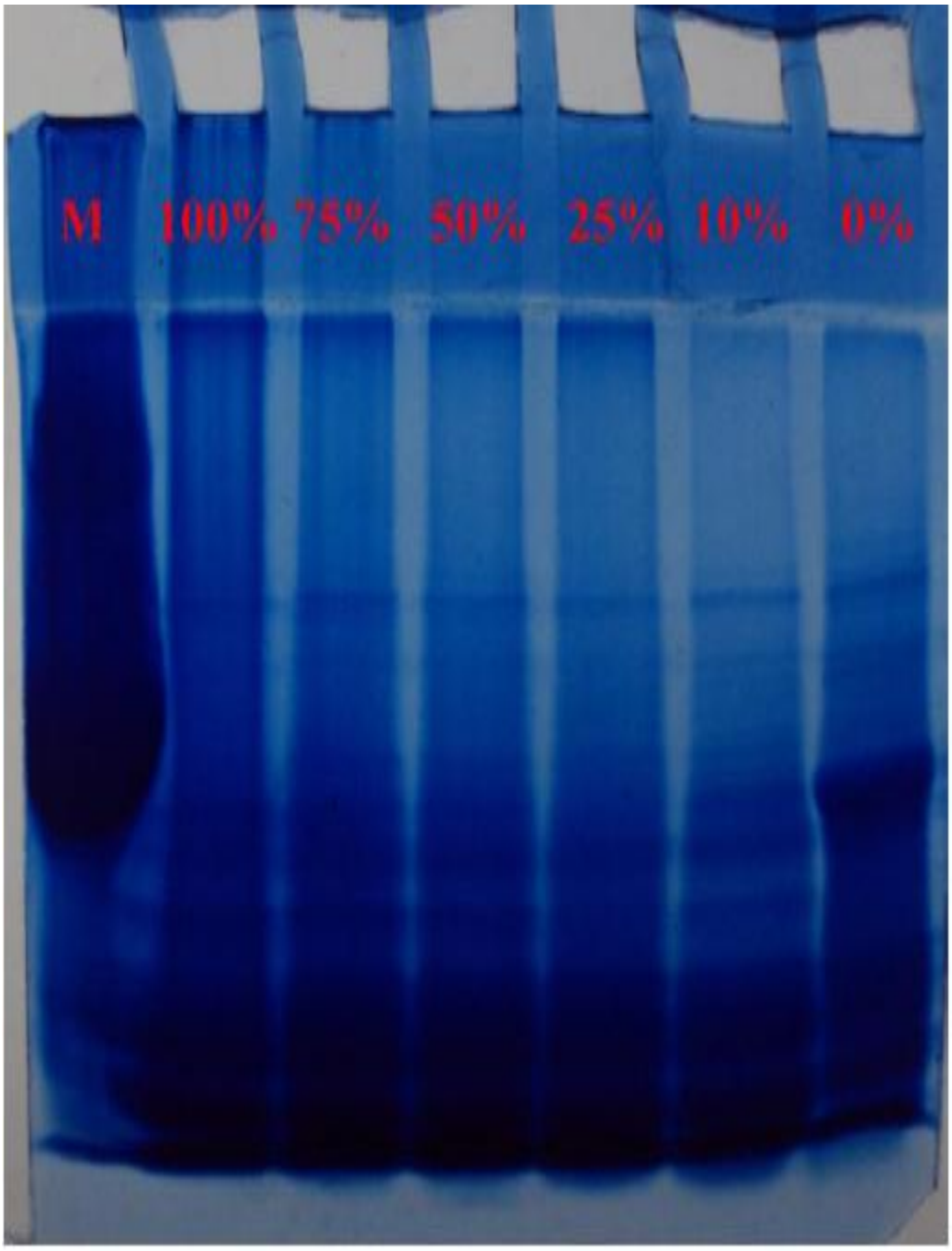

Fig. 3: SDS – PAGE protein profile of 7 days old seedlings of *V. radiata* treated with different concentration of *C. corynephora* SLF

DISCUSSION

The effect of seaweed extract on germination creeping red fescue has been reported by Button and Noyes (1964), the dilute solution increases the rate of germination but stronger solutions are detrimental. The effect of seaweed extracts on green chillies and turnip shows that the low concentrations of an SLF enhance the rate of seed germination (Dhargalkar and Untawale, 1980). In the present study also the maximum percentage of germination (80) was displayed in 25% treatment of *C. corynephora* SLF and minimum percentage of germination (35) was observed in 100% of *C. corynephora* SLF. 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. Similar observation was made in *Cajanus cajan* (Mohan *et al*., 1994), maize, ragi and Kambu (Rajkumar Immanuel and Subramanian, 1999), *Sesmum* (Gandhiyappan and Perumal, 2001), *Oryza sativa* (Asirselvin *et al*., 2004; Sunarpi *et al*.,2010) Cowpea (Sivasankari *et al*., 2006a), *Raphanus sativus* (Thirumaran *et al*., 2007) and *Cyamopsis tetrogonolaba* (Thirumaran *et al*., 2009). In the present study also highest length of shoot (11.05 cm) and root length (3.93 cm) was observed in 25% SLF of *C. corynephora*.

Featonby-Smith and Van Staden (1983) reported that the application of the SLF *Ecklonia maxima* on *Arachis hypogea* increases the protein content. Anandharaj and Venkatesalu (2001) reported the low concentrations of *Caulerpa recemosa* and *Gracilaria edulis* aqueous extract promoted the seedling growth and biochemical constituents of *V. catajung.* Different concentrations (15, 20, 25 and 30%) of SLF were used in seed treatment and better results were obtained in lower doses (15, 20 and 25%) reported by Selvaraj *et al.,* 2004. The results of present study also directly coincided with Selvaraj et al.observations. In case of *V. radiata,* the number of observable polypeptide bands increased gradually from 0 - 25% concentration of SLF treated seedlings and further increase in SLF concentration reduced the number of observable bands. The lowest concentrations of SLF from *Ulva lactuca* to be responsible for enhancing the growth characteristics of *Pirulina flatensis* was reported by Sridhar and Rengasamy (2002). It increased the pigment content, protein, amino acid, total sugar content, catalase, peroxidase and polyphenol oxidase activities also. Similar to the earlier observations in the present study also seeds treated with low concentration (25%) of SLF showed better response in terms of shoot and root length than the higher concentrations of SLF. The results of the present study directly coincided and supplemented the previous

observations. The seaweed liquid fertilizer from *Ulva lactuca* on *Spirulina platensis* enhances the total protein content at lower concentration (Sridhar and Rengasamy, 2002). Venkataraman Kumar and Mohan (2003) reported that the SLF treated plants showed a marked increase in soluble protein and soluble sugar contents. 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, *A.roxburghinus* did not show any additional bands in contrast to the rest of crops. 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 investigation in the effect of different concentrations (0-100%) of SLF of *C. corynephora* on the banding profile was studied. Among these 25% showed more bands than the other concentration. *C. corynephora* SLF treated *Vigna radiata* seedlings showed additional bands. Similarly a maximum of five additional bands appeared in *Capsicum annum* under *U. lactuca* SLF. Further, one or two bands appeared in *Amaranthus tricolor, Araches hypogea* and *Tagetes erecta,* it was suggested that the possible role of plant growth regulators like auxin and cytokinin as well as macro and micro elements present in the SLFs for the appearance of those induced proteins (Sridhar and Rengasamy, 2011**)**. The plant growth regulators play a significant role in cell division, cell elongation and cell differentiation factors and cell type specific gene (Johri and Mitra, 2001). In general, in the present study, the seaweed liquid fertilizer prepared from the *C. corynephora* applied to *V. radiata* plants showed better results in all aspects of growth at lower concentration.

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