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The potential of seaweed liquid fertilizer on the growth and antioxidant enhancement of *Helianthus annuus* L.

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ABSTRACT

A field experiment was carried out during the chithiraipattam (April-May) in India in 2010 to study the effects of foliar applications of different concentrations of seaweed liquid fertilizer (SLF) of *Sargassum wightii* on growth, biochemical constituents and antioxidant enzymes of *Helianthus annuus* L**.** to explore the possibilities of reducing or avoiding chemical fertilizers and to obtain the highest growth and productive parameters. The foliar spray was given twice in 15days interval at two concentrations (2.5%, 5.0%) of SLF. The aqueous extract of *Sargassum wightii* was found to promote almost all the parameters such as shoot length, root length, leaf length, leaf breadth, fresh weight, dry weight and number of leaves. The biochemical contents such as chlorophyll a, b, total chlorophyll, carotenoids, carbohydrate, total phenols and antioxidant enzymes such as catalase, peroxidase and glutathione peroxidase (GPX) were also found to be higher in the leaves. Among the two concentrations 2.5% was found to produce better results in almost all the parameters. The results revealed that foliar application of seaweed extract could be a promising option for pollution free non toxic growth enhancement of *Helianthus annuus* L**.**

Key words: *Sargassum wightii,* growth, biochemical constituents, antioxidant enzymes, Seaweed Liquid Fertilizer.

INTRODUCTION

Any improvement in agricultural system resulting in higher production should reduce the negative environmental impact on agriculture and enhance the sustainability of the system. One such approach is the use of biostimulants, which can successfully replace harmful and costly inorganic fertilizers. Marine bioactive substances extracted from marine algae are used in agricultural and horticultural crops (Blunden, 1991).

Seaweeds are the macroscopic marine algae found at the bottom in relatively shallow coastal waters. They grow in shallow and deep sea areas up to 180 meter depth and in backwaters on the solid substrate such as rocks, dead corals and pebbles. The seaweeds are totally different from higher plants as they neither have true leaves, stems and roots or vascular systems nor specialized sex organs.

Seaweeds have been used as manure, cattle feed, food for human consumption and as a source of phycocolloids such as agar, alginic acid and carrageenan (Chapman, 1970). Besides their application as farmyard manure (FYM), liquid extracts obtained from seaweeds (LSF/SLF) have recently gained importance as foliar sprays for several crops (Metha *et al.*, 1967; Bokil *et al.*, 1974).

The application of seaweed fertilizer for different crops was of great importance to substitute the commercial chemical fertilizers and to reduce the cost of production. Liquid fertilizers derived from seaweeds are found to be superior to chemical fertilizers due to high level of organic matter, macro and micro nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances (Mooney and Van Staden, 1986). It was also used to develop tolerance to environment stress (Zhang *et al*., 2003) and increase nutrient uptake from soil (Verkleij, 1992; Turan and Kose, 2004). The present study was undertaken to investigate the effect of seaweed liquid fertilizers on the growth, biochemical constituents and antioxidant enzymes of *Helianthus annuus* L**.**

MATERIAL AND METHODS

Collection of Seaweeds

The seaweed used in the present study was *Sargassum wightii* belonging to the class Phaeophyceae. They were collected from the coastal area of Rameswaram, India during March 2010. The algal species were hand picked and washed thoroughly with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes. It was kept in an ice box containing slush ice and transported to the laboratory. Samples were washed thoroughly using tap water to remove the salt on the surface of the sample. The water was drained off and the algae were spread on blotting paper to remove excess water.

Preparation of seaweed liquid fertilizer

One kg of seaweed was cut into small pieces and boiled separately with distilled water for an hour and filtered. The filtrate was taken as 100% concentration of the seaweed extract and from this different concentrations (2.5%, 5.0%) were prepared using distilled water (Bhosle *et al*., 1975). As the SLF contain organic matter, the seaweed liquid fertilizer were refrigerated between 0 and 4^0C.

Test crop plant

The crop plant, selected for the present study was *Helianthus annuus* L. belonging to the family Asteraceae.

Seeds

Viable seeds were obtained from the Tamil Nadu Agricultural University, Coimbatore. Care was taken in selecting the seeds of uniform size and they were stored in a metal tin as suggested by Rao (1976).

Experimental design and treatment

The experiment comprised of four treatments, viz, 0 (control, water spray), 2.5%, 5% (volume/volume) of seaweed extract in water and 0.1% NPK spray. Two sprays of *Sargassum wightii* derived extract and NPK were applied, one at the seedling stage (15^{th} days after sowing) and the second at 15days after first spray (30^{th} days after sowing). The treatments were distributed in a randomized block design with three replications.

Soil analysis

The soil sample used for field experiment was subjected to the analysis of physico-chemical properties. The parameters like coarse sand, texture class, nitrogen, phosphorus, potassium, EC, Ph, organic carbon, organic matter were found out.

Morphological parameters

The morphological characters such as shoot length, root length, leaf length, leaf breadth, fresh weight, dry weight, number of leaves were measured on 30^{th} and 45^{th} day after sowing.

Biochemical estimation

The biochemical constituents such as chlorophyll a, b, total chlorophyll (Arnon, 1949), carotenoid (Kirk and Allen, 1965), carbohydrate (Hedge and Hofreiter, 1962) and total phenols (Malic and Singh, 1980) were also determined.

Antioxidant enzyme assay

Catalase activity was estimated by the method of Barber (1980), peroxidase was determined by Rodney and Boyer (1996) and glutathione peroxidase were analyzed by Overbaugh and Fall (1982).

Plants from each treatment were randomly drawn for various analysis. Plant from the field were uprooted carefully and washed in tap water. They were then processed for different analysis. All the growth parameters, biochemical constituent and antioxidant enzymes were analysed on 30th and 45th day after sowing.

RESULTS

A physico-chemical analysis of the experimental soil is presented in Table -1. The soil of the site was clay loam with pH 8.0. The experimental soil was found to contain 0.19% organic carbon, Nitrogen (60ppm), Phosphorous (11.1ppm), Potassium (90ppm) and organic matter 2.8%.

Table 1: Physico - chemical analysis of the experimental soil

Properties	Values
Physical analysis	
Coarse sand (%)	8.4
Texture class	clay loam
Chemical analysis	
N (ppm)	60
P (ppm)	11.1
K (ppm)	90
EC (dsm^{-1})	0.14
pH	8.0
Organic carbon %	0.19
Organic matter %	2.8

The effect of SLF of *Sargassum wightii* on growth parameters such as shoot length, root length, leaf length, leaf breadth, fresh weight, dry weight and number of leaves recorded at 30th and 45th days after sowing (DAS) is presented in Table-2.

The data of various biochemical constituents such as chlorophyll a, b, total chlorophyll, carotenoid, carbohydrate and total phenols observed on 30th and 45th days after sowing (DAS) are presented in Table-3. Even though both the concentrations enhanced the formation of all the biochemical constituents, comparatively a marked increase in all biochemical content have been observed in 2.5% concentration of SLF.

The effect of SLF of *Sargassum wightii* on antioxidant enzymes of the leaves of *Helianthus annuus*.L measured on 30th and 45th days after sowing (DAS) is presented in Table-4. The activities of antioxidant enzymes such as catalase, peroxidase and glutathione peroxidase were found to be much higher in plant treated with 2.5% concentration when compared to control, 5% concentration of SLF and chemical fertilizer.

DISCUSSION

The present investigation has been undertaken to achieve higher production of *Helianthus annuus*.L with eco-friendly and completely safe SLF in the place of costly, harmful and toxic chemical fertilizers. Bhosle *et al*. (1975) prepared a seaweed liquid fertilizer and studied its effect on *Phaeseolus vulgaris*. Rama Rao (1991) reported good yields of *Zizyphus rugosa* fruits, where leaf spray of SLF obtained from *Sargassum wightii* was used.

In the present investigation it is quite interesting to mention here that 2.5% concentration of SLF has remarkable influence on all the growth parameters such as shoot length, root length, leaf length, leaf breadth, fresh weight, dry weight and number of leaves which is in conformity with a earlier report of Metha *et al*. (1967). It is also worth mentioning here that 5% SLF has also been found to have more or less equal growth promoting influence.

Stephenson (1974) recorded that lower concentration of SLF prepared from *Ascopyllum* and *Laminaria* accelerated the growth in maize. These results observed by us are in agreement with those of Chandrasekar *et al*. (2005) who reported that the maximal plant height, leaf area and leaf length and number of leaves were observed in the plot treated with the biofertilizer.

Rama Rao (1991) reported that the dry powder and liquid formulators of the seaweed *sargassum wightii* increase the fresh weight of tomato plants. The number of leaves and leaf area are important factors, because the leaves are the structures bearing photosynthetic machinery and an increased in leaf number and area, may promote

Table 2: Effect of SLF of Sargassum wightii on growth parameters of *Helianthus annuus*.L

Parameters	30DAS				45DAS			
	Control	SLF (2.5%)	SLF (5.0%)	Chemical fertilizer	Control	SLF (2.5%)	SLF (5.0%)	Chemical fertilizer
Shoot length(cm)	45.3±0.2	56.2±0.3 (24.06)	55.3±0.3 (22.07)	52±0.1 (14.79)	89.3±0.2	106±0.4 (18.70)	101.3±0.03 (13.43)	99.1±0.1 (10.99)
Root length (cm)	8.8±0.1	9.8±0.1 (11.36)	8.5±0.1 (-3.40)	8.9±0.1 (1.13)	12.5±0.1	18±0.1 (44.0)	17.1±0.1 (36.8)	17.8±0.1 (42.4)
Leaf length (cm)	9±1.0	15±1.0 (66.66)	14.5±1.5 (61.11)	13.5±1.3 (50.0)	11.5±1.2	16.2±1.0 (40.86)	14.5±1.0 (26.08)	13.4±1.3 (16.52)
Leaf breadth (cm)	4.7±0.2	9.1±0.2 (93.61)	7.3±0.05 (24.06)	7.5±0.1 (24.06)	6.7±0.4	12.1±0.2 (80.59)	11.8±0.0 (76.11)	12.0±0.3 (79.10)
Fresh weight (g)	30±1.0	58.5±1.5 (95.0)	56.3±1.0 (86.66)	48±1.6 (60.0)	60.2±1.0	224±1.2 (272.09)	219±1.0 (263.78)	208±1.6 (245.51)
Dry weight (hg	3.93±0.01	6.82±0.01 (75.53)	5.76±0.01 (46.56)	5.79±0.02 (47.32)	6.03±0.04	30±0.01 (397.51)	27±0.01 (347.76)	24±0.02 (298.0)
No of leaves	10±2.0	17±1.0 (70.0)	15±1.0 (50.0)	14±1.0 (40.0)	18±1.0	24±2.0 (33.33)	21±1. 0 (16.66)	22±0.03 (22.22)

- Values are expressed as mean ± SD
DAS - days after sowing
SLF - Seaweed Liquid Fertilizer

Table 3: Effect of SLF of *Sargassum wightii* on biochemical composition of the leaves of *Helianthus annuus*.L

Parameters	30DAS				45DAS			
	Control	SLF (2.5%)	SLF (5.0%)	Chemical fertilizer	Control	SLF (2.5%)	SLF (5.0%)	Chemical fertilizer
Chlorophyll "a" ($mg\ g^{-1}{}_{FW}$)	1.65±0.005	2.07±0.005 (25.45)	1.90±0.07 (15.15)	1.92±0.01 (16.36)	2.56±0.05	2.95±0.03 (15.23)	2.90±0.06 (13.28)	2.40±0.01 (6.25)
Chlorophyll "b" ($mg\ g^{-1}{}_{FW}$)	0.65±0.02	0.74±0.01 (13.84)	0.66±0.01 (1.53)	0.64±0.05 (1.53)	1.01±0.02	1.21±0.04 (19.80)	1.19±0.01 (17.82)	1.08±0.07 (6.93)
Total Chlorophyll ($mg\ g^{-1}{}_{FW}$)	2.33±0.01	2.73±0.03 (17.16)	2.53±0.03 (8.58)	2.48±0.01 (6.43)	3.50±0.005	4.06±0.01 (16.0)	3.99±0.02 (14.0)	2.68±0.05 (23.42)
Carotenoid ($mg\ g^{-1}{}_{FW}$)	2.72±0.05	3.12±0.05 (14.70)	3.01±0.05 (10.66)	3.04 ±0.01 (11.76)	2.70±0.04	2.74±0.003 (1.48)	2.72±0.005 (0.74)	2.68±0.02 (0.74)
Carbohydrate ($mg\ g^{-1}{}_{FW}$)	0.45±0.03	0.61±0.01 (35.55)	0.58±0.06 (28.88)	0.56±0.03 (24.44)	0.14±0.03	0.43±0.02 (207.14)	0.41±0.03 (192.85)	0.43±0.06 (207.14)
Total Phenols ($mg\ g^{-1}{}_{FW}$)	1.06±0.10	1.94±0.03 (83.01)	1.69±0.04 (59.43)	1.72±0.06 (62.26)	2.57±0.02	3.54±0.05 (37.74)	3.44±0.03 (33.85)	3.47±0.01 (35.01)

- Values are expressed as mean ± SD

DAS - days after sowing

SLF - Seaweed Liquid Fertilizer

Table 4: Effect of SLF of *Sargassum wightii* on antioxidant enzymes of the leaves of *Helianthus annuus*.L

Parameters	30DAS				45DAS			
	Control	SLF (2.5%)	SLF (5.0%)	Chemical fertilizer	Control	SLF (2.5%)	SLF (5.0%)	Chemical fertilizer
Atalase	1.90±0.07	2.88±0.06	2.49±0.02	2.52±0.02	0.47±0.02	0.78±0.03	0.75±0.05	0.72±0.02
(Units/g protein)		(51.57)	(31.05)	(32.63)		(65.95)	(59.57)	(53.19)
Peroxidase	1 .52±0.005	2.90±0.05	2.61±0.05	2.72±0.05	1.63±0.05	2.81±0.06	1.97±0.01	1.99±0.01
(Units/g protein)		(90.78)	(70.71)	(78.94)		(72.39)	(20.85)	(22.08)
Glutathione	1.02±0.01	1.71±0.06	1.53±0.01	1.59±0.01	0.97±0.03	1.66±0.04	1.63±0.01	1.65±0.01
Peroxidase		(67.64)	(50.0)	(55.88)		(71.13)	(68.04)	(70.10)

- Values are expressed as mean ± SD
DAS - days after sowing
SLF - Seaweed Liquid Fertilizer

the better translocation of water uptake and deposition of nutrients and yield (Jeevajyothi *et al*., 1993). It is indeed worth mentioning that there is a marked improvement in all the above parameters with SLF foliar spray administration.

The SLF had also influence in improving biochemical constituents such as chlorophyll a, b, total chlorophyll, carotenoids and total phenols content of experimental plants at lower concentration of 2.5%. The marked increase in the chlorophyll content observed in the present study has also been reported in the observations made by Whapman *et al*. (1993) and Blunden *et al*. (1997). Mostafa and Zheekh (1999) reported that the plant growth substance present in seaweed liquid fertilizer might have enhanced the chlorophyll content in the leaves. The seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in plants (Blunden *et al*., 1996).

In the present investigation, plants treated with lower concentration (2.5%) of seaweed liquid fertilizers were found to show better response in terms of enzyme activities such as catalase, peroxidase and glutathione peroxidase. These results coincide with the studies on *Vigna Zeamays* (Linga Kumar *et al*., 2002 and Asir Selin Kumar *et al*., 2007). Normally the higher activity of the enzyme appears to be the result of greater mobilization of storage compound and energy sources (Bardzik *et al*., 1971).

It is concluded from the above discussion that in *Helianthus annuus*.L seaweed liquid fertilizer stimulates significantly almost all parameters of growth, biochemical constituents and antioxidant activities in field conditions, when compared to control and chemical fertilizer. This simple practice of application of eco-friendly seaweed liquid fertilizers to *Helianthus annuus*.L is recommended to the farmers for attaining better growth, biochemical constituents and antioxidant enhancement.

REFERENCES

1. Arnon, D.I, Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physoil.*, **2**: 1-15 (1949).
2. Asir Selin Kumar, R., Effie, A. and Saravanababu, S., Studies on the effect of seaweed and seagrass liquid fertilizers on the fruit length and weight of *Abelmoschus esculentus* L. var. Hybrid-10. *Seaweed Research and Utilization*, **29**(1&2): 171-175 (2007).
3. Barber, J.M., Estimation of catalase. *Z. Pflazen Phsiol.*, **97**: 135 (1980).
4. Bardzik,J.N., Marsh, H.V. and Haris, J.R., Effect of water stress on the activities of three enzymes in maize seedlings. *Plant Physiology*, **47**: 828-831 (1971).
5. Bhosle, N.B., Untawale, A.G., Dhargalker,V.K, Effect of seaweed extract on growth of *Phaseolus vulgaris*. *Indian J. Mar. Sci.*, **4**: 208-210 (1975).
6. Blunden, G., Agricultural uses of seaweeds and seaweed products. *Seaweed Resources in Europe uses and potential.* 65-81 (1991).
7. Blunden, G., Jenkins, T., and Liu, Y,W., Enhanced chlorophyll levels in plants treated with seaweed extract. *J. Appl. Phycol.*, **8**: 535-543 (1996).
8. Blunden, G.T., Jenkins and Liu-yan-Wen, Enhanced leaf chlorophyll levels in plants treated with seaweed extract. *Jornal of Applied Phycology*, **8**(b): 535-543 (1997).
9. Bokil, K.K., Mehta, V.C. and Datar, D.S., Seaweeds as manure: II pot culture manorial experiments on wheat. *Phykos.*, **13**(1): 1-5 (1974).
10. Chandrasekar, B.R., Ambrose, G. and Jeyabalan, N., Influence of biofertilizers and nitrogen source level on the growth and yeld of Echinochloa frumentacea (Roxb.) Link. *J. Agri. Technol.*, **1**: 223-234 (2005).
11. Chapmam, G.J., Seaweeds and their uses. Methuen and Co.ltd. London, 66 (1970).
12. Hegdge, J.C and Horfreiter, B.T., In:carbohydrate chemistry (Eds Whistler, R.L and Be Miller, J.N.). Academic Press, New York, 120 (1962).
13. Jeevajyothi, L., Mani, A.K., Pappiah, C.M. and Rajagopalan, R, Influence of NPK and *Azospirillum* on the yield of cabbage. *South Indian Horticulture,* **41**: 270-272 (1993).
14. Kirk, J.T.O. and Allen, R.L., Dependence of chloroplast pigments synthesis on protein synthetic effects of actilione. *Biochem. Biophysics Res.J. Canada*, **27**: 523-530 (1965).
15. Linga Kumar, K., Jeyaprakash., Manimuthu, R.C. and Haribaskar, A., Gracillaria edulis an effective alter native source as a growth regulator for legume crops. *Seaweed Reasearch and Utilization*, **24**(1): 117-123 (2002).
16. Malic, C.P and Singh, M.B., Plant enzymology and Histo enzymology. Kalyani publishers, New Delhi, 286 (1980).
17. Metha , V.C., Trivedi, B.S., Bokil, K.K. and Narayana, M.R., Seaweed as manure, studies on nitrification. *Proc. Semi. Sea. Salt and plants* (CSMCR), 357-365 (1967).
18. Mohan ,V.R., Venkataraman Kumar , R., Murugeswari and muthuswami, S., Effect of crude and commercial seaweed extract on seed germination and seedling growth in green gram and black gram. *Seaweed Research and Utilization*, **16**: 23-28 (1994).
19. Mostafa, M.E., and Zheekh, L., Effect of seaweed extracts on seed germination, seedling growth and some metabolic process of Vicia faba L. *Cytobios*, **100**: 23-25 (1999).
20. Mooney, P.A. and Van Staden, J., Algae and cytokinins. *Journal of plant physiology*, **12**: 1-2 (1986).
21. Overbaugh J.M. and Fall, R., FEMS Microbiology letters, **13**: 271-272 (1982).
22. Radney and Boyer, R., Activity and thermal stability of acrylamide Gel-Immobolized peroxidase. An experiment in biotechnology. *Modern experimental biotechnology,* **2**: 441-450 (1992).
23. Rama Rao, K., Effect of aqueous seaweed extract on *Zizlpus mauritioana* Lam. *Botanical society*, **71**: 19-21 (1991).
24. Rao, R.S.N., Seed viability studies under different storage condition. *J. Res.,* **2**: 253 (1976).
25. Sekar ,R.N. Thangaraju and Rengasamy, R., Effect of seaweed liquid fertilizer from *Ulva*

lactuca on *Vigna unguiculata* L.(Walp). *Phykos*., **34**: 49-53 (1995).

26. Sivasankari, S., Venkatesalu, V., Aanantharaj, M. and Chandrasekaran, M., Effect of seaweed extracts on the growth and biochemical constituents of *Vigna Sinensis. Bioresource technology*, **97**: 1745-1751 (2006).
27. Stephenson, W.A., Seaweeds in agriculturae and horticulture Rateaver, peruma valley 3rd edition. *Cal. California*, 241 (1974).
28. Thirumalthangam, R.S. Maria Victorial Rani and Peter marian, M., Effect of seaweed liquid fertilizer on the growth and biochemical constituents of *Cyamposis tetragonoloba* (h.). Taub. *Seaweed Reasearch and Utilisation*, **25**: 99-104 (2003).
29. Turan, M. and Kose, C., Seaweed extracts improve copper uptake of grapevine. *Soil and plant science,* **54**: 213-220 (2004).
30. Verkleij, F.N., Seaweed extracts in agriculture and horticulture: a review. *Biological Agriculture and Horticuture*, **8**: 309-324 (1992).
31. Whapham, C.A., Blunden, G., Jenkins, T. and Wankins, S.D., Significance of betanines in the increased chlorophyll content of plants treated with seaweed extract. *Applied Physiology*, **5**: 231-234 (1993).
32. Zhang, X., Ervin, E.H. and Schmidt, E.R., Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. *Crop science*, **43**: 952-956 (2003).