



Document heading doi: 10.1016/S2305-0500(14)60019-1

Potential of liquid extracts of *Sargassum wightii* on growth, biochemical and yield parameters of cluster bean plant

N. Vijayanand¹, S. Sivasangari Ramya², S. Rathinavel^{3*}¹Department of Botany, Arumugam Pillai Seethai Ammal College, Tiruppattur, Affiliated to Alagappa University, Karaikudi, Tamilnadu, India²Department of Biochemistry, Sri Sarada Niketan College for Women, Amaravathipuram, Karaikudi, Affiliated to Alagappa University, Tamilnadu, India³Centre for Research in Botany, Saraswathi Narayanan College, Madurai, Affiliated to Madurai Kamaraj University, Tamilnadu, India

ARTICLE INFO

Article history:

Received 23 November 2013

Received in revised form 15 December 2013

Accepted 20 December 2013

Available online 20 June 2014

Keywords:

Seaweed extract

Sargassum wightii

Brown alga

Growth, Biochemical

Yield

Cluster bean

ABSTRACT

Objective: To explore biofertilizing efficiency of seaweed liquid extracts of brown alga *Sargassum wightii* (*S. wightii*) on growth, biochemical and yield parameters of *Cyamopsis tetragonoloba* (*C. tetragonoloba*). **Methods:** Seaweeds were made to coarse powder and stock solution was prepared. Different concentrations such as 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 5.0% were prepared and given as foliar spray. **Results:** Seaweed Liquid Extract (SLE) at low concentration (1.5%) exhibited promoting effect on growth and yield parameters. Differential responses in the content of photosynthetic pigments, protein, reducing sugar, ascorbic acid and in the activity of nitrate reductase were also observed in the leaves of SLE treated seedlings when compared to untreated seedlings. Higher concentrations (above 1.5%) of SLE were found to show inhibitory effect. **Conclusion:** The presence of micro and macro nutrients, vitamins, growth hormones and other constituents in the seaweed extract might be very much useful to the crops but their level should be appropriate to enhance growth and productivity. It may be concluded that liquid seaweed extracts could serve as cost effective eco-friendly product for sustainable agriculture.

1. Introduction

Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health including biodiversity, biological cycles, and soil biological activity. Synthetic fertilizers were used widely in those days for agricultural purposes which had led to cause severe health and environmental hazards such as soil erosion, water contamination, pesticide poisoning, water logging, salinisation, depletion of biodiversity, etc. Thus, farmers are switching over to organic fertilizers for

sustainable agriculture. Unlike chemical fertilizer, manure derived from living resources is biodegradable, non-toxic, non-polluting and non-hazardous to soil ecosystem [1].

Seaweeds are the macroscopic marine algae found attached to the bottom in relatively shallow coastal water. Liquid fertilizers derived from natural sources like seaweeds are found to be viable alternatives to fertilizing input for agricultural crops due to its high level of organic matter, micro and macro elements, vitamins, fatty acids and also rich in growth regulators [2]. Seaweed extract is a new generation of natural organic fertilizers containing highly effective nutritious source and promotes faster germination of seeds, increase in yield and resistant ability of many crops [3]. The organic matter of seaweeds increases humus content of the soil, thereby ameliorating the soil texture and preservation of its moisture.

*Corresponding author: S. Rathinavel, Centre for Research in Botany, Saraswathi Narayanan College, Madurai, Affiliated to Madurai Kamaraj University, Tamilnadu, India.

Tel: +91- 9843172712

E-mail: rathinavel_sn@yahoo.com

Seaweed liquid fertilizer is a blend of both plant growth regulators and organic nutrient input is eco-friendly promoting sustainable productivity and maintaining soil health. In recent years, the use of natural seaweed products as substitutes to the conventional synthetic fertilizers has assumed importance [4–6]. Thus, the extracts are applied to improve nutritional status, vegetative growth, yield and fruit quality in crop plants [7–9]. Liquid crude extracts derived from marine algae such as *Cladophora* [10], *Gracilaria* [11] were reported to have manurial efficiency. The present study was undertaken to evaluate the fertilizing efficiency of seaweed liquid extracts of brown marine alga *Sargassum wightii* on cluster bean (*S. wightii*).

2. Materials and methods

2.1. Preparation of seaweed liquid extracts (SLE)

The marine alga *S. wightii* belongs to Phaeophyceae was collected from Mandapam (Lat 9 ° 45'N; Long 79 ° 15'E) located in South East Coast of Tamilnadu. The alga was brought to the laboratory and washed thoroughly in tap water for 3 times to remove all epiphytes, sand particles and associated fauna. The wet weight of sample of collected brown alga was taken, shade dried and then the sample dry weight was determined. Different concentrations of boiled extracts were prepared by mixing appropriate level of liquid extracts with distilled water [12]. The SLE concentrations used in this experiment were ranged from 0.5% to 5.0%.

2.2. Physico-chemical and hormone analyses of SLE

The physical characteristics such as colour and pH were observed using standard method. The composition of elements such as copper, manganese, iron, zinc, cobalt, potassium, magnesium and sodium were estimated using Atomic Absorption Spectrophotometer [13]. Further, the liquid extract of marine alga was also subjected for estimation of auxin [14], gibberellin [15] and cytokinin [16].

2.3. Growth promoting efficiency of SLE on cluster bean seedlings

Seeds of cluster bean were purchased from Agriculture College and Research Institute, Madurai. Healthy seeds free from visible infection, with uniform size were segregated. They were surface sterilized with 0.1% mercuric chloride and then sown in earthen ware pots (9 cm dia) filled with sterilized standard soil mix supplemented with sufficient quantity of NPK. The seed to seed distance in pot was

maintained as 3–5 cm and the pots were irrigated regularly. Foliar application of different concentrations (0.5% to 5.0%) of liquid extracts was given to potted plants after 20 days. Routinely, separate set of potted plants were used for each concentrations. About 50 mL of different concentrations of extracts was given at interval of 3 days for a period of 15 days. Growth parameters viz., shoot length, root length, total height, total fresh and dry weight, leaf area and moisture content were determined. Biochemical profiles such as photosynthetic pigments [17], protein [18], reducing sugar [19], ascorbic acid [20] and nitrate reductase activity were assessed in the leaves of treated plants. Growth and biochemical parameters were observed in 5 weeks old treated and control plants. Yield characteristics were measured in 90 days old plants. All pot experiments were done in four replicates each under natural uniform conditions.

2.4. Statistical analysis

Data were subjected to one-way ANOVA and means were separated by Duncan's test ($P < 0.05$, $n = 5$). Statistical analysis was carried out using IRRISTAT ver. 4.0 (IRRI, Manila, Phillipines) [21].

3. Results

The manurial analyses of liquid extract of our experimental brown marine alga revealed the presence of potassium (1.37 mg/L), copper (2.2 mg/L), manganese (1.53 mg/L), zinc (1.8 mg/L), iron (0.88 mg/L), cobalt (1.103 mg/L), sodium (5.3 mg/L) and magnesium (16.31 mg/L) in appreciable level. Among the elements estimated, magnesium (16.31 mg/L) was found to be abundant in the extract. Similarly, in case of phytohormones analysis, cytokinin (5.5 mg/L) was found to be more when compared to auxin (2.5 mg/L) and gibberellins (2.8 mg/L).

In our experiments, use of seaweed liquid extracts of *S. wightii* significantly promoted the rate of growth and physiology of cluster bean. There was a noticeable increase in growth and biochemical parameters when 1.5% of seaweed liquid extracts of *S. wightii* applied to cluster bean plant. Higher concentrations (2.0% and above) were found to show inhibiting effect on all the above parameters studied. Total plant height (33%), total fresh weight (155%), dry weight (140%), leaf area (61%) and moisture content (55%) were enhanced when 1.5% concentrations of liquid extracts was applied. Further, the retarding effect (reduction by 2% to 27%) in growth parameters was corresponding to increase in the concentrations (2.0%, 2.5% and 5.0%) (Table 1). Statistically significant differences were noticed in total

plant height fresh and dry weight, leaf area and moisture content. Similarly, cluster bean plants when treated with different concentrations of *S. wightii* extracts showed differential responses in biochemical parameters also. (Table 2). The amount of photosynthetic pigments (by 78%), protein (by 73%), sugar (by 101%) and the activity of nitrate reductase (by 159%) were found to be enhanced in cluster bean plants when 1.5% concentrations of extract were given. Other treatments such as 2.0%, 2.5% and 5.0% showed reduced levels of these parameters in the treated plants.

The observations on yield attributes revealed that *S. wightii* at 1.5% concentration enhanced the formation of number of clusters/plant (56%), number of flowers/plant (93%) and number of pods/plant (55%). Moreover, pod weight (by

53%), pod length (40%) number of pods/plant (by 55%) were also increased when compared to untreated plants (Table 3). Inhibitive effect (reduction by 20%–48%) was observed when the plants were treated with higher concentrations (2.0%, 2.5% and 5.0%).

In general, low concentrations of liquid extracts of seaweed extracts had maximum positive influence on growth, biochemical and yield characteristics of cluster bean as reported in previous studies due to the presence of micro and macro elements, growth hormones and vitamins [58–60]. Further, presence of phycocolloids and other ingredients in brown algae may also be responsible for better enhancement in growth and yield production.

Table 1

Influence of liquid extracts of *S. wightii* on growth characteristics of cluster bean.

Treatments	Shoot length (cm)	Root length (cm)	Total height (cm)	Total fresh wt (mg)	Total dry wt (mg)	Leaf Area (mm ²)	Moisture content (%)
Control	8.725 ^b	12.250 ^a	20.000 ^{ab}	1.015 ^a	0.400 ^a	42.115 ^b	41.533 ^a
0.5%	9.325 ^b (106)	13.050 ^{ab} (106)	21.400 ^{bc} (107)	1.130 ^{bc} (111)	0.492 ^b (123)	44.183 ^b (104)	54.685 ^{bc} (131)
1.0%	10.000 ^c (114)	13.400 ^{bc} (109)	23.325 ^{bc} (116)	1.620 ^{de} (159)	0.620 ^c (155)	50.790 ^c (120)	63.645 ^{bc} (152)
1.5%	11.400 ^{cd} (130)	15.250 ^d (124)	26.650 ^{bc} (133)	2.595 ^f (255)	0.962 ^d (240)	68.165 ^d (161)	64.825 ^c (155)
2.0%	10.300 ^c (118)	14.450 ^{cd} (117)	24.750 ^c (123)	1.800 ^e (177)	0.605 ^c (151)	58.400 ^d (138)	64.808 ^c (155)
2.5%	8.950 ^b (102)	14.200 ^{cd} (115)	23.150 ^{bc} (115)	1.365 ^{cd} (134)	0.515 ^b (128)	43.425 ^b (102)	53.720 ^b (128)
5.0%	6.425 ^a (73)	12.300 ^a (100)	18.725 ^a (93)	0.915 ^{ab} (90)	0.400 ^a (100)	35.450 ^a (84)	55.785 ^{bc} (133)

Means sharing different letters within the columns are significantly different ($P < 0.05$ level), different letters followed in each column statistically significant based on DMRT, values in parenthesis are per cent over control.

Table 2

Influence of liquid extract of *S. wightii* on biochemical characteristics of cluster bean.

Seaweed extract treatments	Chlorophyll-a (mg/g fr.wt)	Chlorophyll-b (mg/g fr.wt)	Total chlorophyll (mg/g fr.wt)	Protein (mg/g fr.wt)	Reducing sugar (mg/g fr.wt)	Ascorbic acid (mg/g fr.wt)	NRA (μ moles No-2/g fr.wt/hr)
Con	0.5225 ^a	0.4200 ^a	0.9425 ^a	19.200 ^b	41.650 ^a	0.568 ^a	0.820 ^a
0.5%	0.6500 ^a (124)	0.4450 ^b (105)	1.095 ^a (116)	24.550 ^b (127)	55.850 ^b (134)	0.656 ^{ab} (115)	1.090 ^b (131)
1.0%	0.6650 ^{ab} (127)	0.515 ^c (122)	1.180 ^c (125)	28.250 ^c (146)	62.650 ^c (150)	0.700 ^c (123)	1.635 ^d (190)
1.5%	0.975 ^d (186)	0.710 ^d (168)	1.685 ^d (178)	33.30 ^d (173)	84.10 ^d (201)	0.775 ^{bc} (136)	2.125 ^e (259)
2.0%	0.805 ^c (154)	0.530 ^b (126)	1.335 ^b (141)	26.625 ^{bc} (138)	66.80 ^c (160)	0.637 ^{ab} (112)	1.725 ^d (210)
2.5%	0.650 ^c (124)	0.442 ^b (105)	1.092 ^a (115)	21.350 ^a (111)	51.50 ^b (123)	0.593 ^a (104)	1.345 ^c (164)
5.0%	0.590 ^b (112)	0.315 ^a (74)	0.905 ^a (96)	19.90 ^a (103)	39.10 ^a (93)	0.562 ^a (98)	0.970 ^{ab} (118)

Means sharing different letters within the columns are significantly different ($P < 0.05$ level), different letters followed in each column statistically significant based on DMRT, values in parenthesis are per cent over control.

Table 3Influence of liquid extracts of *S. wightii* on yield parameters of cluster bean.

Seaweed treatments	Number of clusters/ plant	Number of flowers/ plant	Number of Pods/ plant	Pod length (cm)	Pod weight (g)	Number of seeds/ pod
Control	4.000 ^{bc}	4.000 ^{bc}	2.250 ^a	6.250 ^{bc}	3.075 ^{ab}	6.250 ^c
0.5%	4.750 ^{cd} (118)	5.000 ^{cd} (125)	2.500 ^{ab} (111)	6.750 ^{cd} (108)	3.350 ^{ab} (108)	6.750 ^{cd} (108)
1.0%	5.500 ^{de} (137)	6.500 ^{de} (162)	3.250 ^{bc} (144)	8.250 ^e (132)	3.750 ^{bc} (121)	8.250 ^e (132)
1.5%	6.250 ^e (156)	7.750 ^e (193)	3.500 ^c (155)	8.750 ^{de} (140)	4.725 ^c (153)	8.750 ^e (140)
2.0%	5.000 ^{cd} (125)	4.750 ^{cd} (118)	2.500 ^{ab} (111)	6.750 ^{cd} (108)	4.125 ^{bc} (134)	7.750 ^{de} (124)
2.5%	3.500 ^b (88)	3.500 ^{ab} (87)	2.500 ^{ab} (111)	5.500 ^b (88)	3.500 ^{ab} (113)	5.000 ^b (80)
5.0%	2.500 ^a (62)	3.000 ^a (75)	1.750 ^a (77)	3.750 ^a (60)	2.600 ^a (84)	3.250 ^a (52)

Means sharing different letters within the columns are significantly different ($P < 0.05$ level), different letters followed in each column statistically significant based on DMRT, values in parenthesis are per cent over control.

4. Discussion

The presence of phytohormones is in accordance with the earlier findings that reported auxins in the extracts of *Ascophyllum nodosum* (*A. nodosum*) [22] and cytokinins in the extracts of *Ulva* sp. [23], *Durvillaria potatorum* (*D. potatorum*) and *A. nodosum* [24]. Our findings also corroborated with the previous reports made on *Dolichos biflorus* (*D. biflorus*) [11], *Solanum melongena* (*S. melongena*) [25], *Triticum aestivum* (*T. aestivum*) [26], *Abelmoschus esculentus* (*A. esculentus*) [27; 28], *Cajanus cajan* (*C. cajan*) [5], *Brassica nigra* (*B. nigra*) [29], *Lycopersicon esculentum* (*L. esculentum*) [30], *Cyamopsis tetragonoloba* (*C. tetragonoloba*) [31] and *Vigna mungo* (*V. mungo*) [3]. Moreover, lower concentration of 1% *Padina boergesenii* (*P. boergesenii*) and *Ulva lactuca* (*U. lactuca*) extract significantly increased the shoot length, leaf breadth, leaf length, root length and number of roots in *Rhizophora mucronata* (*R. mucronata*) [32] and *Arachis hypogea* (*A. hypogea*) plants [33] respectively. On the contrary, it has been reported that concentration at 20% of *S. wightii* [34] and *Rosenvingea intricate* (*R. intricate*) [35] promoted shoot length, root length, fresh and dry weight of *Abelmoschus esculentus* (*A. esculentus*) and *Cyamopsis tetragonoloba* (*C. tetragonoloba*) respectively. Growth enhancement by seaweed extracts may be due to components such as macro- and microelement, amino acids, vitamins, cytokinins, auxins and abscisic acid (ABA)-like growth substances which affects cellular metabolism in treated plants leading to enhanced growth and crop yield [36–38]. The increased growth of these crops may be due to the occurrence of some growth promoting substances present in the seaweed extract [39, 40]. These hormones play an important role in enhancement of cell size and cell division and together they complement each other as cytokinins are effective in shoot formation and auxin in root development, while micronutrient improve soil health [41]. Several studies have shown that kelp extracts increased nutrient uptake to plants by chelating nutrients due to presence of some organic acids [9, 42]. Therefore, the

higher growth rate in lower concentration of extracts may be directly attributed by the presence of optimum level of essential nutrients and phytohormones as observed during chemical analysis of SLE of *S. wightii*.

Lower concentrations of *S. wightii* (1.5%) enhanced the biochemical components also. This is in accordance with the earlier reports that lower concentrations of seaweed extracts enhanced the biochemical constituents in *C. cajan* [5], *B. nigra* [29], *Citrullus lanatus* (*C. lanatus*) [43], *Trigonella foenum-graecum* (*T. foenum-graecum*) [32], *Solanum melongena* (*S. melongena*) [44], *Abelmoschus esculentus* (*A. esculentus*) [28]. The increase in photosynthetic pigments may be due to the presence of betaines [40], increase in number and size of the chloroplast and better grana development [45]. The increase in the protein content at lower concentrations of SLE confirmed the efficiency of foliar spray as it enhanced the absorption of most of the necessary elements by the seedlings [11]. It would also be associated with increased nitrate reductase activity in the treated plants. The increase in chlorophyll content could also be a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed liquid extract [46]. It may also be attributed with abundance of elements like magnesium which plays vital role in organization of chlorophyll pigments. However, it has been reported that seaweed liquid fertilizer at 10% extracted from brown alga *S. wightii* increased the content of photosynthetic pigments, protein and total sugars in *Vigna radiata* (*V. radiata*) [47] and SLE of *Rosenvingea intricate* (*R. intricate*) at 20% enhanced the photosynthetic pigments and carotenoids in *C. tetragonoloba* [35]. Moreover, in a study, 1% *Ulva lactuca* (*U. lactuca*) extract along with 50% recommended rate of chemical fertilizers enhanced the content of protein, carbohydrate and lipid in *Tagetes erecta* (*T. erecta*) [48].

In many crops, yield is associated with the number of flowers at maturity. As the onset and development of flowering and the number of flowers produced are linked to the developmental stage of plants, seaweed extracts probably encourage flowering by initiating robust plant

growth. Yield increases in seaweed extract treated plants are thought to be associated with hormonal substances present in the extracts, especially cytokinins [53]. Cytokinins have been implicated in nutrient mobilization in vegetative plant organs [54] as well as reproductive organs [55]. Such a response indicates the seaweed extracts are involved in inducing the mobilization of cytokinins from the roots to the reproductive organ or more likely by inducing the amount or synthesis of endogenous fruit cytokinin [56]. This increase in cytokinin availability will eventually result in a greater supply of cytokinins to the later stages (reproductive) of plants and thus initiated flowering and triggered yield.

It may be concluded that the growth and biochemical characteristics of vegetable crop *C. tetragonoloba* could be promoted by the presence of micro and macro elements, growth hormones, vitamins etc. in the SLE of *S. wightii*. Cytokinin and magnesium, which are considered as essential growth promoting constituents in chlorophyll biosynthesis might have played a key role in enhancement of growth and physiology of cluster bean. However, optimum concentration of seaweed liquid extracts is necessary as in this study 1.5% SLE had better influence on growth and productivity of cluster bean plants. The study also emphasizes that seaweed extracts can be effectively used as organic biostimulants to the agricultural crops and also much useful in the practices of organic farming.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors express their heartfelt thanks to the Dr. R. Rengasamy, Director, Centre for Advanced Studies in Botany, Madras University for his valuable help and critical suggestions during the period of the study. Further, thanks are also extended to Central Electro Chemical Research Institute, Karaikudi for chemical analysis of our experimental samples.

References

- [1] Dhargalkar VK, Pereira N. Seaweed : Promising plant of the millennium. *Sci Cul* 2005; **71**: (3–4): 60–66.
- [2] Crouch IJ, VanStaden J. Evidence for the presence of plant growth regulators in commercial seaweed products. *Plant Growth Reg* 1993; **13**: 21–29.
- [3] Ganapathy Selvam G, Sivakumar K. Effect of foliar spray form seaweed liquid fertilizer of *Ulva reticulata* (Forsk.) on *Vigna mungo* L. and their elemental composition using SEM– energy dispersive spectroscopic analysis. *Asian Pac J Rep* 2013; **2**(2): 119–125.
- [4] Eman A, El–Moniem A, Abd–Allah ASE. Effect of green algae cells extract as foliar spray on vegetative growth, yield and berries quality of superior grapevines. *Am. Euras. J Agric Environ Sci* 2008; **4**(4): 427–433.
- [5] Erulan V, Soundrapandian P, Thirumaran G, Ananthan G. Studies on the effect of *Sargassum polycystum* (C. Agardh) extract on the growth and biochemical composition of *Cajanus cajan* (L.) Mill sp. *Amer Eur J Agri & Environ Sci* 2009; **6**: 392–399.
- [6] Sangeetha V, Thevanathan R. Biofertilizer potential of traditional and panchagavya amended with seaweed extract. *J Amer Sci* 2010; **6**: 61–67
- [7] Abd El–Migeed AA, EL–Sayed AB, Hassan HAS. Growth enhancement of olive transplants by broken cells of fresh green algae as soil application. *J Agric Res* 2004; **29** (3): 723– 737.
- [8] Spinelli F, Giovanni F, Massimo M, Mattia S, Guglielmo C. Perspectives on the use of a seaweed extract to moderate the negative effects of alternate bearing in apple trees. *J Hort Sci Biotechn* 2009; **17**(1): 131–137.
- [9] Jannin L, Arkoun M, Etienne P, La1ne P, Goux D, Garnica M, et al. Brassica napus growth is promoted by *Ascophyllum nodosum* (L.) Le Jol. seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. *J Plant Growth Regul* 2013; **32**: 31–52.
- [10]El–Sheekh MM, El–Saied AEF. Effect of seaweed extracts on seed germination, seedling growth and some metabolic process of Fabe beans (*Vicia faba* L.). *Phykos* 1999; **38**: 55– 64.
- [11]Anantharaj M, Venkatesalu V. Studies on the effect of seaweed extracts of *Dolichos biflorus*. *Seaweed Res Utiln* 2002; **24**(1): 129– 137.
- [12]Rama Rao K, Effect of aqueous seaweed extract on *Ziziphus mauritioana* Lam. *J Indian Bot Soc* 1992; **71**: 19–21.
- [13]Humphries EC. Mineral components and ash analysis. In: Rach K, Tracer MV (eds.), *Modern methods of plant analysis*. Berlin:Springer Valeg; 1956, p. 468–502.
- [14]Gordon SA, Paleg LG. Quantitative measurement of indole acetic acid. *Physiol Plant* 1957; **10**: 37–48.
- [15]Graham HD, Henderson JHM. Reaction of gibberellic acid and gibberellins with Folin–Wu Phosphomolybdic acid reagent and its use for quantitative assay. *Plant Physiol* 1961; **36**: 405– 408.
- [16]Syono K, Torrey JG. Identification of cytokinins of root nodules of the Garden Pea, *Pisum sativum* L. *Plant Physiol* 1976; **57**: 602– 606.
- [17]Arnon DI. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 1949 ; **24**: 1–15.
- [18]Lowry OH, Rosebrough H, Farr AL, Randall RJ. Protein measurement by folin–phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
- [19]Nelson N. A photometric adaptation of Somogyis method for the determination of reducing sugar. *Anal Chem* 1944; **31**: 426–428.
- [20]Roe JH. Chemical determination of ascorbic, dehydroascorbic and diketogluconic acids. *Meth Biochem Anal* 1954 ; **1**: 115–139.
- [21]Duncan BD. Multiple range and multiple test. *Biometric* 1965;**11**: 1–42.
- [22]Sanderson KJ, Jameson PE. The cytokinins in a liquid seaweed extract: Could they be the active ingredients? *Acta Hort* (ISHS) 1986; **179**: 113–116.
- [23]Sekar R, Thangaraju N, Rengasamy R. Effect of seaweed liquid fertilizer from *Ulva lactuca* on *Vigna unguiculata* L. (Walp). *Phykos* 1995; **34**: 49–53.
- [24]Craft CA, Hiltz D, Hankins S, MacKinnon SL. Detection of plant growth hormones in *Ascophyllum nodosum* and seaweed products.

- Int Symp Mar Nat Products* 2007; **12**:P074.
- [25]Thirumalthangam R, Maria Victorialrani S, Petermarian M. Effect of seaweed liquid fertilizers on the growth and biochemical constituents of *Cyamopsis tetragonolaba* (L.) Taub. *Seaweed Res Utiln* 2003; **25**: 99–103.
- [26]Zodape ST, Mukherjee S, Reddy MP, Chaudharya DR. Effect of *Kappaphycus alvarezii* (Doty) ex silva. extract on grain quality, yield and some yield components of wheat (*Triticum aestivum* L.) *Int J Plant Prod* 2009; **3**: 97–101.
- [27]Zodape ST, Kawarkhe VJ, Patolia JS, Warade AD. Effect of liquid seaweed fertilizer on yield and quality of okra (*Abelmoschus esculentus* L.) *J Sci Ind Res* 2008; **67**: 1115–1117.
- [28]Sasikumar K, Govindan T, Anuradha C. Effect of seaweed liquid fertilizer of *Dictyota dichotoma* on growth and yield of *Abelmoschus esculentus* (L). *Eur J Exp Biol* 2011; **1**: 223–227.
- [29]Kalidass C, Jayarani S, Glory M. Effect of seaweed liquid fertilizers on growth and biochemical constituents of *Brassica nigra* (L.) *Intl J Agri Environ Biol* 2010; **3**:307–311.
- [30]Zodape ST, Gupta A, Bhandari SC. Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato. *J Sci Ind Res* 2011; **67**: 215–219.
- [31]Thambiraj J, Lingakumar K, Paulsamy S. Effect of seaweed liquid fertilizer (SLF) prepared from *Sargassum wightii* and *Hypnea musciformis* on the growth and biochemical constituents of the pulse, *Cyamopsis tetragonoloba* (L.). *J Res Agri* 2012; **1**: 065–070.
- [32]Pise NM, Sabale AB. Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella foenum-graecum*. *J Phytol* 2010; **2**(4): 50–56.
- [33]Sridhar S, Rengasamy R. Significance of seaweed liquid fertilizers for minimizing chemical fertilizers and improving yield of *Arachis hypogaea* under field trial. *Recent Res Sci Tech* 2010b; **2**(5): 73–80.
- [34]Jothinayagi N, Anbazhagan C. Effect of seaweed liquid fertilizer of *Sargassum wightii* on the growth and biochemical characteristics of *Abelmoschus esculentus* (L.) Medikus. *Recent Res Sci Tech* 2009; **1**: 155–158.
- [35]Thirumaran G, Arumugam M, Arumugam R, Anantharaman P. Effect of seaweed liquid fertilizer on growth and pigment concentration of *Cyamopsis tetragonoloba* (L.) Taub. *Am Euras J Agron* 2009; **2**: 50–56
- [36]Ordog V, Stirk WA, van Staden J, Novak O, Strnad M. Endogenous cytokinins in the three genera of macroalgae from the chlorophyta. *J Phycol* 2004; **40**: 88–95.
- [37]Stirk WA, van Staden J. Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus*. *J Appl Phycol* 1997; **9**: 327–330.
- [38]Durand N, Briand X, Meyer C. The effect of marine bioactive substances(NPRO) and exogenous cytokinins on nitrate reductase activity in *Arabidopsis thaliana*. *Physiol Plant* 2003; **119**: 489–493.
- [39]Mooney PA, Van Staden J. Algae and cytokinins. *J Plant Physiol* 1986; **123**: 1–21.
- [40]Blunden G, Jenkins T, Wan LY. Enhanced leaf chlorophyll levels in plants treated with seaweed extracts. *J Appl Phycol* 1997; **8**(6): 535–543.
- [41]Liu Z, Lijun L. Effects of Plant growth regulators and saccharide on invitro plant and tuberous root regeneration of Cassava. *J Plant Growth Reg* 2011; (30)**1**: 11–19
- [42]Crouch IJ, Beckett RP, VanStaden J. Effect of seaweed concentrate on the growth and mineral nutrition of nutrient stressed lettuce. *J Appl Phycol* 1990; **2**: 269–272.
- [43]Abdel–Mawgoud AMR, Tantaway AS, Hafez MM, Habib HAM. Seaweed extract improves growth, yield and quality of different watermelon hybrids. *Res J Agri Biol Sci* 2010; **6**(2): 161–168.
- [44]Bozorgi HR. Effects of foliar spraying with marine plant *Ascophyllum nodosum* extract and nano iron chelate fertilizer on fruit yield and several attributes of *Solanum melongena* (L.) *ARPJ Agri & Bio Sci* 2012; **7**(5): 200–206.
- [45]Atzmon N, Van Staden J. The effect of seaweed concentrate on the growth of *Pinus pinea* seedlings. *New Forests* 1994; **8**(3): 279–288.
- [46]Whapman CA, Blunden G, Jenkins T, Hankins SD. Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *J Appl Phycol* 1993; **5**: 231–234.
- [47]Sivasankari S, Chandrasekaran M, Kannathasan K, Venkatesalu V. Studies on the biochemical constituents of *Vigna radiata* Linn. treated with seaweed liquid fertilizer. *Seaweed Res Utiln* 2006; **28** (1): 151–158.
- [48]Sridhar S, Rengasamy R. Effect of seaweed liquid fertilizer on the growth, biochemical constituents and yield of *Tagetes erecta*, under field trial. *J Phytol* 2010a; **2**(6) :61–68.
- [49]Aldworth SJ, Van Staden J. The effect of seaweed concentrate on seedling transplants. *S Afr J Bot* 1987; **53**: 187–189.
- [50]Ramamoorthy K, Sujatha K, Sivasubramaniam K. Utilization of seaweed extracts for enhancing yield in black gram (*Vigna mungo*) (L.) hepper. *Seaweed Res and Utiln* 2007; **29**: 97–100.
- [51]Ahmed MY, Sehrawy EL. Effect of seaweed extract on fruiting of Hindy Bisinnara mango trees. *J Amer Sci* 2013; **9**(6): 539–544.
- [52]Parthiban C, Saranya C, Hemalatha A, Kavitha B, Anantharaman P. Effect of seaweed liquid fertilizer of *Spatoglossum asperum* on the growth and pigment content of *Vigna radiata*. *Int J Recent Sci. Res* 2013; **4**(9): 1418–1421.
- [53]Featonby–Smith BC, Van Staden J. Identification and seasonal variation of endogenous cytokinin in *Ecklonia maxima* (Osbeck) Papen F. *Bot Mar* 1984; **17**: 527–531.
- [54]Gersani M, Kende H. Studies on cytokinin–stimulated translocation in isolated bean leaves. *J Plant Growth Regul* 1982; **1**:161–171.
- [55]Letham DS. Cytokinins as phytohormones: sites of biosynthesis, translocation, and function of translocated cytokinins. In: Mok DWS, Mok MC. (eds.) *Cytokinins: chemistry, activity and functions*. Boca Raton: CRC Press;1994, p. 57–80.
- [56]Arthur GD, Stirk WA, van Staden J. Effect of a seaweed concentrate on the growth and yield of three varieties of *Capsicum annum*. *S Afr J Bot* 2003; **69**:207–211.
- [57]Jaworski EG. Nitrate reductase in intact plant tissue. *Biochem Biophys Res Commun* 1971; **43**: 1274–1279.
- [58]Ahmed MY, Sehrawy EL. Effect of seaweed extract on fruiting of Hindy Bisinnara mango trees. *J Amer Sci* 2013. **9**(6): 539–544.
- [59]Parthiban C, Saranya C, Hemalatha A, Kavitha B, Anantharaman P. Effect of seaweed liquid fertilizer of *Spatoglossum asperum* on the growth and pigment content of *Vigna radiata*. *Int J Recent Sci Res* 2013. **4**(9): 1418–1421.
- [60]Pramanick B, Brahmachari K, Ghosh A. Effect of seaweed saps on growth and yield improvement of green gram. *Afr J Agric Res* 2013; **8**(13): 1180–1186