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Synergistic Effect of Seaweed Manure and *Bacillus* sp. on Growth and Biochemical Constituents of *Vigna radiata* L.

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Abstract

Seaweeds are one of the major marine renewable resources of the world. Seaweed fertilizers have often been more beneficial to the crop plants than the conventional chemical fertilizers. Seaweed meals provide nitrogen, phosphorus, potassium and salts besides some readily available micro elements to the plants. The seaweeds are also known to contain plant growth regulators such as cytokinin and auxin in appreciable quantities. In the present study, different physico-chemical characteristics of *Sargassum* manure obtained from the Seaweed Natural and Alginate Products Ltd, Ranipet, India have been assessed. Studies on the effect of different concentrations of *Sargassum* manure on the photosynthetic pigment composition, biochemical make-up and plant growth regulators of *Vigna radiata* L. were made for the *Sargassum* manure itself besides the same in combination with a Bacillus sp. added to the soil as five different combinations (0.1 %). Those plants applied with the *Sargassum* manure and the bacterium demonstrated superior growth in relation to the plants supplied with the *Sargassum* manure alone. Therefore, the use of the bacterium along with the *Sargassum* manure appeared to make this combination an efficient eco- friendly alternative to the conventional chemical fertilizers.

Keywords: *Sargassum manure*; *Bacillus*; Growth; Biochemical constituents; *Vigna radiata* L.; Plant Growth Regulators

Introduction

Two thirds of today's world population depends upon agriculture for livelihood. Sixty five percent of Indian population mostly depends on farming for livelihood. The abuse of pesticides or fungicides has been causing soil pollution besides exerting harmful effects in humans. Chemical fertilizers generally deteriorate soil quality there being disturb the homeostasis of the ecosystems, eventually leading to habitat loss.

Seaweeds constitute one of the important biotic components of the ocean and might serve as an alternative to inorganic fertilizers [1]. Seaweed fertilizers are often found to be more effective in promoting productivity rather than the chemical fertilizers [2]. Seaweed extract contains macro nutrients, trace elements, organic substances such as carbohydrate, amino acids and Plant Growth Regulators [3]. Use of organic manure in agriculture particularly, for seed treatment could be eco-friendly and cost effective. Seaweed extracts are marketed as fertilizer additives [4]. It has been reported that, seaweeds offer several benefits such as increased crop yield, improved growth, induced resistance to frost, fungal and insect resistance, reduced spider, aphid, nematode infestation and increased nutrient uptake from the soil [5]. Seaweed meals provide an approximately equivalent amount of N, less P, but more K and total salts and readily available micro elements compared to most animal manures [6]. Other than the macro and micro nutrients, seaweeds contain many plant growth regulators such as cytokinin, gibberellins and auxin [7-12]. The seaweed sludge does not degrade rapidly owing to the presence of certain complex substances like phenolic derivatives. The plant growth regulating and productive roles of a bacterium isolated from the organic manure was confirmed by culturing the same in a variety of media and their supernants were checked for the presence of bacterial secondary metabolites [13].

The aerobic spore forming *Bacillus* that constitutes the genus, *Bacillus*, majority of which are harmless saprophytes plays a significant role in the breakdown of complex polysaccharides and helps in the cycling of nutrients in nature. *Bacillus* that plays a major role in spore resistance, germination of seed apparently possesses enzymatic machinery that probably leads to entering into association with other organisms in the environment [14]. Owing to their genetic make-up, *Bacillus* spp. resist adverse conditions and can remain viable in soil for long periods and offer sustainable crop protection against pathogens.

More recently, it has been discovered that several microbes in the rhizhosphere produce Indole Acetic Acid (IAA) [15]. *Bacillus* populations also promote growth and health of crops by the action of plant hormones [16]. The strain, *Bacillus subtilis* AFI increased shoot length ranging between 4% and 15% and also raised the plant biomass by up to 15% [17]. *Bacillus cereus* QQ 308 produced growth enhancing substances in Chinese cabbage [18]. *Bacillus* sp. MRF produced IAA at a concentration of 3.71 µg / mL [19]. The rhizobacterial strains of *Bacillus pumilus* INR7 and *Bacillus subtilis* GBO3 when given as seed treatment and soil drenchment for cucumber were found to enhance plant growth gradually under green house conditions [20].

The main objective of this study is to evaluate the efficacy of *Sargassum manure* in combination with a *Bacillus* sp. isolated from the manure on plant growth and its influence on the biochemical composition of *Vigna radiata* L. under green house conditions.

Material and Methods

Collection of *Sargassum manure*.

The manure was collected after extraction of the alginate from *Sargassum* spp. from the Seaweed Natural and Alginate Products Ltd., Ranipet, India. The manure samples were stored in airtight containers and were later analyzed for different parameters.

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The physico - chemical characteristics of *Sargassum manure*

The physico-chemical characters such as colour, odour, pH, macronutrients and micro elements were estimated by the methods as described by the American Public Health Association [21].

In the biochemical analysis total carbohydrate was estimated by the method as described by [22] total protein [23] total lipids [24] total phenol [25] and organic carbon by [26]. Humic acid was estimated by the method given by [27] Plant Growth Regulators [PGRs] such as, auxin, gibberellins and cytokinin were also measured [28].

Isolation of plant growth promoting bacteria

One gram of *Sargassum manure* was mixed with 10 mL sterile distilled nutrient agar plates. The plates were then incubated at 37°C for 24 h. The dominant bacterium from the culture plate was isolated and identified as a *Bacillus* sp.

Growth Study

The bacterial isolate was inoculated in nutrient broth and was incubated at 37°C for 24 h and growth was assessed by measuring the OD at 3h intervals at 600 nm.

Antagonistic activity of *Bacillus* sp. against phytopathogens

The test bacterium, *Bacillus* sp. was screened for the antagonistic activity against the phytopathogen, *Rhizoctonia solani*, on Potato Dextrose Agar (PDA) by dual culture technique.

Screening of the bacterial isolate for auxin production

The bacterial isolate was screened for IAA production [29] by inoculating it in the NB with and without tryptophan (1mg / mL) and incubated at 37°C for 7 days and analysed for auxin by following the method [30].

Sargassum manure and *Bacillus* sp.

Samples were prepared as follows: i] control, ii] unsterilized *Sargassum manure*, iii] sterilized *Sargassum manure*, iv] *Sargassum manure* inoculated with 25mL of 18 hr old *Bacillus* sp. v] Sterilized *Sargassum manure* inoculated with 25 mL of 18 h old *Bacillus* sp. and vi] 50 ml of 18 h old were chosen for the following study.

Test plants

The crop plant, *Vigna radiata* (Fabaceae) was chosen as the test plant for the present study. The seeds of the plant were procured from the local market. Seeds of uniform size, colour and weight were chosen for use in the experiments.

The experimental plants were raised in pots in a glass house. Six different experiments were conducted with four replicates. Seeds soaked in tap water overnight were used in further study. Experiments were conducted in earthen pots (15 cm dia., 13 cm depth and 14 cm height) filled with 700 g of the sterilized red soil.

Experimental

T1 – Control Plants without any set of treatment or application were irrigated with fifty mL of sterile water at 50 mL per day.

T2 *Sargassum manure* Fifty mL of 2% *Sargassum manure* (w/v) on 3rd, 6th, 9th day was applied.

T3 Autoclaved *Sargassum manure* - 50 mL of autoclaved 2% *Sargassum manure* (w/v) on 3rd, 6th, 9th day was applied.

T4 *Sargassum manure* with bacteria 50 mL of 2% non-autoclaved *Sargassum manure* (w/v) with *Bacillus* sp. twenty 5 mL of 18h old on 3rd, 6th, 9th day was applied.

T5 *Sargassum manure* autoclaved with bacterial culture Applied twenty five mL of 2 % autoclaved *Sargassum manure* (w/v) and *Bacillus* sp. twenty five mL of 18h old on 3rd, 6th, 9th day.

T6 - *Bacterial culture* of only fifty mL of 18 h old culture on *Bacillus* sp. on 3rd, 6th, 9th day was applied.

Observations were made on the test plants over a period of 10 days.

After the experiments, the plants were uprooted and analysed against the following morphometric parameters: shoot length (cm), root length (cm), fresh weight (g), dry weight (g), number of lateral roots (Nos.) and number of leaves (Nos). The plants were analyzed for the photosynthetic pigments Chl a, Chl b, total chlorophyll [31] and carotenoids [32]. The amounts of total carbohydrate, total protein, and total lipids were also estimated. The levels of Plant Growth Regulators (PGRs) such as, auxin, gibberellins and cytokinin were also measured [28].

Results

The manure looks brown in color with pH 9.0. Among the various nutrients, it has been found that the level of calcium (50 mg/g) was found to be maximum followed by nitrate (14 mg/g), magnesium (18.0 mg/g), sulphate (31.0 mg/g), potassium (8.0 mg/g) and total Kjeldhal nitrogen (54.64 mg/g) in the manure (Table1). Similarly, Organic substances like total carbohydrate (7.0 µg/mg) total protein (8.2 µg/mg), total lipids (1.5 µg/mg), and total phenol (42 µg/mg) were also quantified. Besides humic acid (35.4 %), total organic carbon (13.3 µg/g) occurring at a C: N ratios (1:5) were also detected in the manure (Table 2).

Growth promoters, auxin (135 µg/g), gibberellin (50 µg/g) and cytokinin (115 µg/g) were also recovered from the manure (Table 3).

The predominant bacterium that occurred in association with the

S.No.	Characters	Quality/Quantity
1	Appearance	Brown
2	Odour	Fermented Odour
3	pH 1g / 10mL 1g / 1000 mL	 9.80 **9.05**
4	Alkalinity Total (as $CaCO_3$) mg/g	96
5	Calcium (as Ca) mg /g	50
6	Magnesium (as mg) mg/g	18
7	Sodium (as Na) mg/g	105
8	Potassium (as K) mg/g	8
9	Iron (as Fe) mg/g	2.12
10	Manganese (as Mn) mg/g	0
11	Free Ammonia (as NH_3) mg/g	1.12
12	Nitrite (as NO_2) mg/g	0.06
13	Nitrate (as NO_3) mg/g	14
14	Chloride (as Cl) mg/g	90
15	Fluoride (as F) mg/g	0.36
16	Sulphate (as SO_4) mg/g	31
17	Phosphate (as PO_4)mg/g	1.71
18	Silica (as Si) mg/g	34.40
19	Total Kjeldhal Nitrogen mg (TKN) mg/g	52.64

Table 1: Physico-chemical characters of *Sargassum* manure.

S. No	Substances	µg / mg
1	Total carbohydrate	7.0
2	Total Protein	8.2
3	Total lipids	1.5
4	Humic acid (%)	35.4
5	Total carbon (%) dry weight*	13.3
6	C:N ratio	1:5
7	Total Phenol (µg/mg)	42.0

(* Total carbon on dry weight)

Table 2: Biochemical composition of *Sargassum* manure.

S.No	Plant Growth Regulators	µg / mg of. F. wt
1	Auxin	135
2	Gibberellin	50
3	Cytokinin	115

Table 3: Plant Growth Regulators of *Sargassum* manure.

Test	Results
Colony morphology	Pale yellow, irregular, smooth surface
Gram's staining	Positive
Cell Shape	Rod
Motility	Motile
Catalase	Postive
Oxidase	Postive
Indole Production	Postive
Methyl red	Postive
Voges Proskauer	Negative
Citrate Utilization	Postive
Urease	Postive
Triple Sugar Iron	Glucose fermented
Nitrate Reduction	Negative
Starch Hydrolysis	Postive
Protease	Postive

Table 4: Physicochemical characters of the Bacterial isolate.

manure was identified as *Bacillus* sp. based on its morphological and biochemical characteristics (Table 4).

The selected test plant, *Vigna radiata* L. was exposed to different combinations of manure and *Bacillus* sp. It was noted when treated with at 25 mL of 2% manure and 25 mL of 18h *Bacillus* sp. culture, the test plants have shown enhanced growth (Figure 1b) compared to control (Figure1a) in terms of the shoot length (20.3 cm) (Figure 2), root length (5.4 cm) (Figure 3), fresh weight (0.87 g) (Figure 4), dry weight (0.12 g) (Figure 5), number of roots (27) (Figure 6) and number of leaves (31) (Figure 7 and Table 5).

On fresh weight basis, the addition of manure and *Bacillus* sp. culture had significantly enhanced the content of chl a, chl b, total chl. and total carotenoids in the test plants. Maximum concentration of Chl a (7.099 mg/g), Chl b (2.860 mg/g), total Chl (9.95 mg/g) (Figure 8) and total carotenoids (0.309 mg/g) (Figure 9), total carbohydrate (2.45 mg/g) (Figure 10), total protein (3.16 mg/g) (Figure 11) and total lipids (23.47 mg/g) (Figure 12) were realized from *Vigna radiata* L. treated with 25 mL of 0.1% manure and 25 mL of 18h *Bacillus* sp. culture (Table 6). It was noticed than all this parameter were higher than7%, 47.64%, 18.28%, 59.19%, 68.36%, 44.19% and 77.42% respectively in relation to the control. Plant growth regulators such as auxin, gibberellin and cytokinin were assessed in *Vigna radiata* L. Maximun auxin (94.33 µg/g), gibberellin (257.71 µg/g) and cytokinin (97.0 µg/g)

Figure 1a: Control - Treated with water.

Figure 1b: Treated - Treated *Sargassum* Manure With *Bacillus* Sp Culture.

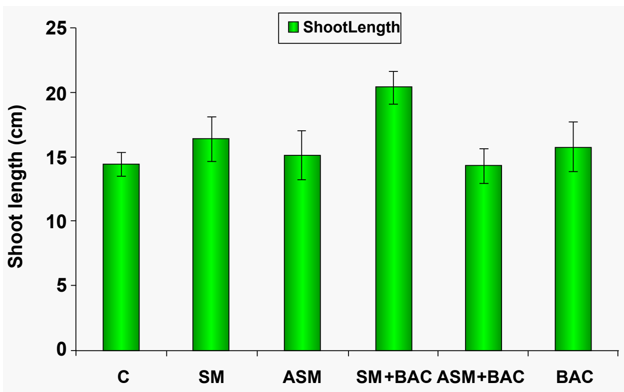

Figure 2: Effect of different proportions of *Sargassum* manure on shoot length of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure + Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

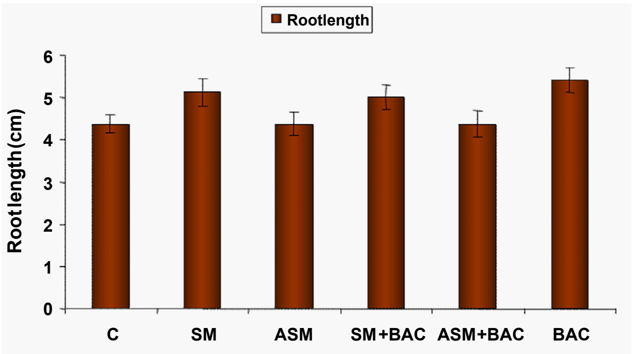

Figure 3: Effect of different proportions of *Sargassum* manure on root length of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure + Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

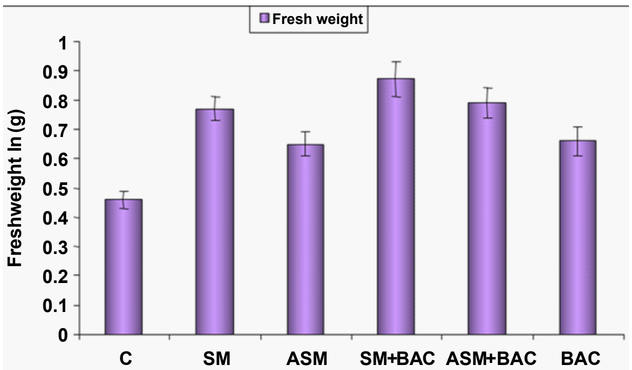

Figure 4: Effect of different proportions of *Sargassum* manure on fresh weight of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

were observed in the plants treated with 25 mL of 2% manure and the 18h *Bacillus* sp culture and the increase over the control was found to be to the tune of 78.73%, 57.0% and 53.0% respectively (Figure 13 and Table 7).

Discussion

The brown seaweed, *Sargassum* is commercially exploited for its principal polysaccharide, alginate. It is extensively used as emulsifier, gelling agent in food, cosmetics and pharmaceutical industries [33]. After extraction of alginate from seaweed, the waste is converted into *Sargassum manure*.

Seaweed manure application increases the availability of trace elements to the crop plants [4]. The beneficial effect of seaweeds on seed germination and plant growth were reported by [34,35]. Seaweed manure contains Fe, Cu, Zn, Co, Mo, Mn, Ni, vitamins and amino acids.

Application of the seaweed manure has raised the seed germination percentage even at lower concentration perhaps suggesting the presence of some plant growth promoting substances such as IAA and IBA, gibberellins (A and B) and cytokinin [36].

The growth enhancement potential of seaweed has been attributed to phenyl acetic acid [PAA] and micro and macro elements [37],

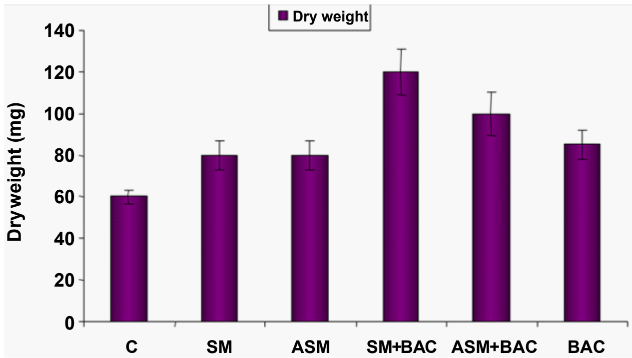

Figure 5: Effect of different proportions of *Sargassum* manure on dry weight of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

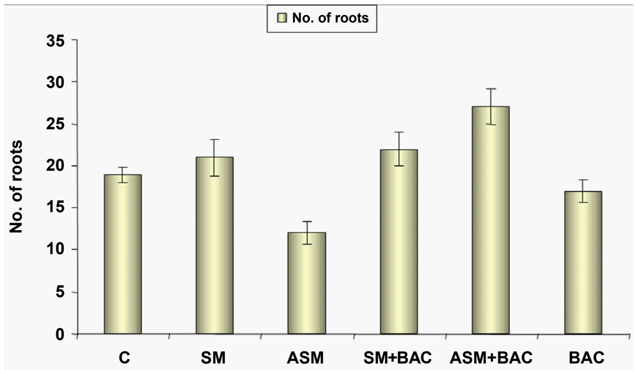

Figure 6: Effect of different proportions of *Sargassum* manure on number of lateral roots of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure + Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

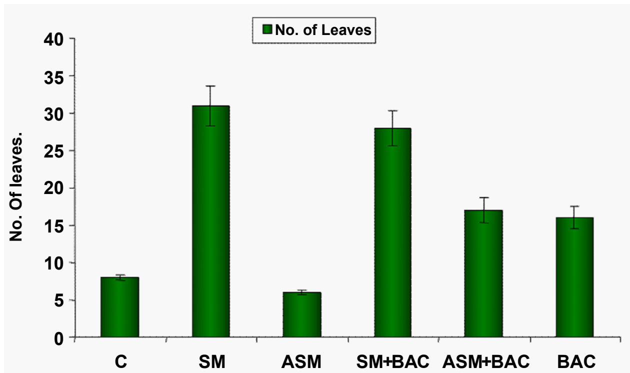

Figure 7: Effect of different proportions of *Sargassum* Manure on number of leaves of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

vitamins and plant growth regulators such as gibberellins and cytokinin [39]. Maximum shoot length of 23.0 cm was observed in 10% Seaweed Liquid Fertilizer treated *Solanum* sp. seedlings [40]. The potential of *Bacillus* sp. to synthesis a wide range of metabolites with antibacterial

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S. No	Parameter	Control	*Sargassum* manure	*Sargassum* manure + *Bacillus* sp.	Autoclaved *Sargassum* manure	Autoclaved *Sargassum* manure + *Bacillus* sp.	*Bacillus* sp.
1	Shoot length (cm)	14.37 ± 0.9	16.37 ± 1.7	20.37 ± 1.23	15.12 ± 1.9	16.25 ± 1.34	15.75 ± 1.96
2	Root length (cm)	4.37 ± 0.22	5.12 ± 0.32	5.42 ± 0.29	4.37 ± 0.29	4.37 ± 0.27	5.00 ± 0.31
3	Number of roots	19 ± 0.9	21 ± 2.2	19 ± 1.99	12 ± 1.32	27 ± 2.11	17 ± 1.38
4	Number of leaves	8.0 ± 0.34	31 ± 2.63	28 ± 2.36	6.0 ± 0.31	17 ± 2.63	16 ± 2.49
5	Fresh weight(g)	0.57 ± 0.03	0.77 ± 0.04	0.87 ± 0.06	0.65 ± 0.04	0.79 ± 0.05	0.66 ± 0.05
6	Dry weight (g)	0.06 ± 3.4	0.08 ± 7.2	0.12 ± 11.0	0.08 ± 6.9	0.10 ± 10.4	0.08 ± 6.8

Table 5: Effect of *Sargassum* manure and *Bacillus* sp. on the growth of *Vigna radiata* L.

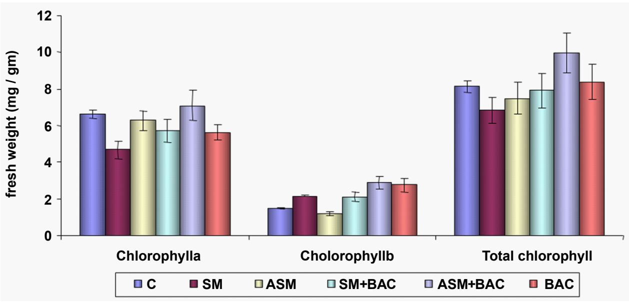

Figure 8: Effect of different proportions of *Sargassum* manure on photosynthetic pigments of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

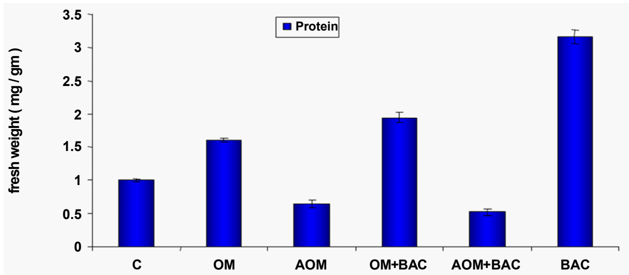

Figure 11: Effect of different proportions of *Sargassum* manure on total protein of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

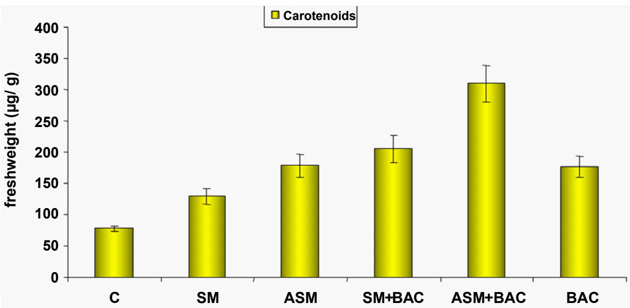

Figure 9: Effect of different proportions of *Sargassum* manure on carotenoids of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

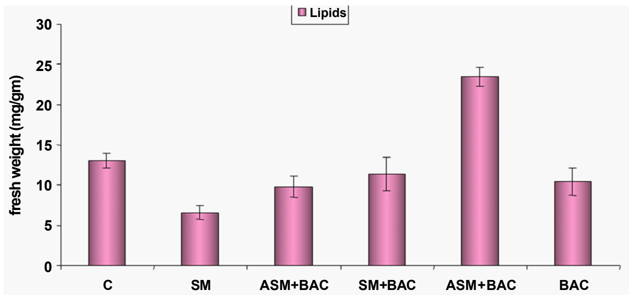

Figure 12: Effect of different proportions of *Sargassum* manure on total lipids of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

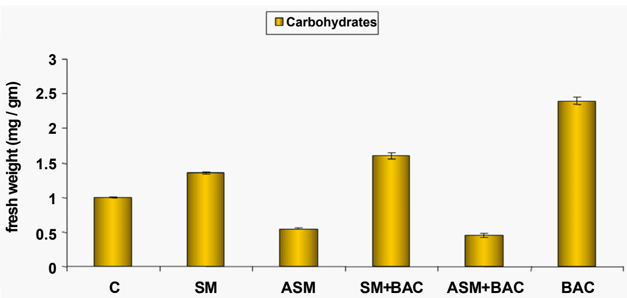

Figure 10: Effect of different proportions of *Sargassum* manure on total carbohydrate of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

and antifungal activity has been extensively studied in medicine and industry and are well known for their ability to control plant disease when applied as biological control agents [41-43]. Numerous *Bacillus* strains have been implicated in activities that suppress pest and pathogen or otherwise promote plant growth. A number of these strains have already been developed commercially as biological fungicides, insecticides, and nematicides or plant growth promoters and their use in agriculture has been thoroughly reviewed [44-47]. Improvement in plant health and productivity are mediated by three different biological mechanisms namely, antagonism of resistant pathogens, promotion of host nutrition and growth and stimulation of plant host defenses.

In the present study, *Vigna radiata* L., showed maximum shoot length of (20.37 cm) and root length [5.42 cm] when it was treated with 2% *Sargassum manure* and *Bacillus* sp. culture. The maximum shoot length (33.10 cm/plant)root length (2.10 cm/plant) fresh weight (6.38 g/plant) dry weight (2.75 g/plant), number of lateral roots (39 numbers/

S. No	parameters	Control	*Sargassum* manure	*Sargassum* **manure +** *Bacillus* **sp.**	**Autoclaved** *Sargassum* **manure**	**Autoclaved** *Sargassum* **manure +** *Bacillus* **sp.**	*Bacillus* **sp.**
1	Chlorophyll a (mg/g)	6.640 ± 0.22	4.667 ± 0.5	7.099 ± 0.83	6.285 ± 0.54	5.703 ± 0.83	5.618 ± 0.43
2	Chlorophyll b (mg/g)	1.496 ± 0.01	2.157 ± 0.03	2.860 ± 0.34	1.211 ± 0.4	2.126 ± 0.34	2.749 ± 0.38
3	Total Chlorophyll (mg/ g)	8.138 ± 0.32	6.823 ± 0.71	9.958 ± 1.1	7.494 ± 0.89	7.828 ± 0.92	8.365 ± 0.95
4	Total Carbohydrate (mg/g)	1.0 ± 0.01	1.350 ± 0.02	2.450 ± 0.05	0.550 ± 0.01	0.450 ± 0.03	1.600 ± 0.04
5	Total Protein (mg/g)	1.0 ± 0.02	1.604 ± 0.03	3.160 ± 0.19	0.644 ± 0.06	0.520 ± 0.05	1.940 ± 0.08
6	Total Lipids (mg/g)	13.1 ± 0.9	6.570 ± 0.89	23.47 ± 2.17	9.820 ± 1.33	11.32 ± 0.09	10.44 ± 1.65
7	Carotenoids (mg / g. f wt)	0.0781 ± 4.2	0.129 ± 12.9	0.310 ± 29.03	0.1784 ±18.27	0.205 ± 21.99	0.176 ±16.42

Table 6: Effect of *Sargassum* manure and *Bacillus* sp. on biochemical constituents of *Vigna radiata* L.

S. No	parameters	Control	*Sargassum* **manure**	*Sargassum* **manure +** *Bacillus* **sp.**	**Autoclaved** *Sargassum* **manure**	**Autoclaved** *Sargassum* **manure +** *Bacillus* **sp.**	*Bacillus* **sp.**
1	Auxin	20 ± 0.8	14.65 ± 1.5	94.33 ± 9.44	14.54 ± 1.7	19.03 ± 1.9	24.91 ± 2.6
2	Gibberellins	110.28 ± 2.17	63.42 ± 1.9	257.71 ± 9.34	94.85 ± 4.28	114.28 ± 4.8	86.57 ± 3.1
3	Cytokinin	46.0 ± 7.22	21.80 ± 6.9	97 ± 23.84	40.2 ± 10.11	46.4 ± 11.8	31.80 ± 8.9

Table 7: Effect of *Sargassum* manure and *Bacillus* sp. on Plant Growth Regulators of *Vigna radiata* L.

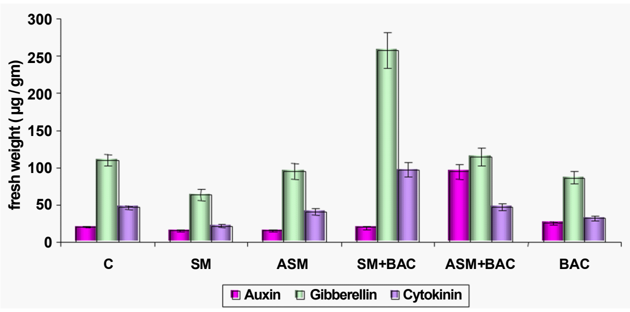

Figure 13: Effect of different proportions of *Sargassum* manure on Plant growth regulators of *Vigna radiata* L.
C- Control; SM – *Sargassum* Manure; ASM- Autoclaved *Sargassum* Manure; SM+BAC- *Sargassum* Manure with Bacterial culture; ASM+BAC- Autoclaved *Sargassum* Manure with Bacterial culture; BAC- Bacterial culture only.

plant) were observed at 20% in the case of *Turbinaria decurrens* plants. The lowest values were observed at 100% seaweed concentration [48].

In the present study, maximum fresh weight (0.87 g) and dry wt. (0.12 g) occurred with the plants treated with 2% *Sargassum manure* and *Bacillus* sp culture. Application of manure raised the contents of the photosynthetic pigments in *Vigna radiata*. Similar trends were observed in SLF treated crop plant, *Vigna unigiculata, Phaseolus radiata* [49], *Cymposis tetragonaloba* [50] and Zea *mays* [51]. The increase in the photosynthetic pigment contents were probably due to the presence of minerals such as Fe, Ni, Cu and Mg in the seaweeds [52] Iron, copper, and magnesium are essential elements which act as catalysts for the synthesis and efficient function of the chlorophyll [53]. The plant growth regulators from the seaweed may also have been responsible for the elevated synthesis of plant pigments [54]. Cytokinin inhibits degradation of chlorophyll, breakdown of protein molecules and aids in the increase of chl a, chl b in *Sorghum bicolor*. Seaweed extracts are known to promote seed germination and plant growth [55-57]. In the present study, elevated chl *a* and chl *b* contents were observed in the case of plants treated with manure. Maximum concentrations of chl *a*, chl *b* and total chlorophyll were observed in *Vigna radiata* L. treated with 25mL 0.1% of manure and 25 mL of *Bacillus* sp. culture .

The application of Seaweed Liquid Fertilizer (SLF) even at low concentration has been reported to increase the pigment content in Cucumber [58]. The enhanced biochemical constituents of the plant could be due to the increased availability of the essential elements available in the SLF [59].

Thus, the manure in combination with the culture of *Bacillus* sp. has been found to be promising for its growth promoting ability. Hence, this simple application of eco-friendly seaweed fertilizer to crop plants is recommended to the growers for better harvest.

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References

1. Metting B, Zimmerman WJ, Crouch I, Van Staden J (1990) Agronomic uses of seaweed and microalgae. In: Introduction to Applied Phycology Ed. Akatsuka, I.SPB, Academic Publishing, The Hague 590-627.
2. Bokil KK, Metha VC, Datar DS (1972) Seaweeds as Manure III. Field Manurial Trials on Pennisetum typhoids S. H. (Pearl millet) and Arachis hypogea (Groundnut). Botanica Marina 15: 148-150.
3. Willams DC, Brain KR, Blunden G, Wildgoes PB (1981) Commercial seaweed extracts. Proc. 8th International Seaweeds Symposium, Bango, Marine science Laboratories, Menai Bridge 761-763
4. Booth E 1965 The Manurial value of Seaweed. Botanica Marina 8: 138-143.
5. Mooney PA, Van Staden J (1985) Effect of seaweed concentrate on the growth of wheat under conditions of water stress. S Afr J Sci 81: 632-633.
6. Simpson K, Hayes S F (1958) The effect of soil conditioners on plant growth and soil structure. J Sci Food Agric 9: 163-170.
7. Tay AAB, Palni LMS, Mac Leod JK (1987) Identification of cytokinin glucosides in a seaweed extract. J Plant Growth Regul 5: 133-138
8. Patier P, Yvin JC, Kloareg Lienart B, Rochas C (1993) Seaweed liquid fertilizer from Ascophyllum nodosum contains Elicitors of plants D-gluconases. Journal Applied Phycology 5: 343-350.
9. Duan D, Liu X, Pan P, Liu H, Chen N, et al. (1995) Extraction and identification of cytokinin from Laminaria japonica Aresch. Botanica Marina 38: 409-412.
10. Përez – Sanz A, Eymar E, Lucena JJ (1996) Effect of foliar sprays on Turf grass of an extract of peat and kelf amended with iron. Journal of Plant and Nutrition 19: 1179-1188.
11. Stirk WA, Van Staden J (1997) Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus* L. Journal of Applied Phycology 9: 327-330.
12. Moller M, Smith ML (1998) The significance of the mineral component of

seaweed suspension on lettuce (*Lactuca sativa* L.) Seedling growth. Journal of Plant Physiology 153: 658-663.

13. Boyd KG, Mearns-Spragg A, Burgess JG (1999) Screening of marine bacteria for the production of microbial repellents using a spectrophotometric chemotaxis assay. Marine Biotechnology 1: 359-363.
14. Driks A (2002) Protein of the spore coat and coat. In *Bacillus subtilis* and its closest relatives, American Society for microbiology (Washington) 527-536.
15. Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42: 207-220
16. Priest F (1993) Systematics and ecology of *Bacillus*. In: *Bacillus subtilis* and Other Gram- Positive Bacteria (Sonenshein, AL, Hoch, JA and Losick R Eds.) American Society for Microbiology, Washington, DC. 3-16
17. Manjula K, Podile AR (2001) Chitin supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF1. Can J Microbiol 47: 618- 625.
18. Wen-Teish Chang, Yu-Chung Chen, Chia-Ling Jao (2007) Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes Bioresour Technol 98: 1224-1230.
19. Paul SD, Nongkynrih (1996) Enzyme activity - a diagnostic tool for toxicity/ deficiency of trace elements in rice plant. Indain Journal of Plant Physiology 1: 225- 257.
20. Raupach GS, Kloepper JW (1998) Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88: 1158-1164.
21. APHA, AWWA and WPCF 1995 Standards Methods for Examination of water and waste water analysis. 19th ed., Washington.
22. Dubios M, Gilles KA, Hamilton TK, Rebers PA, Smith F (1956) Calorimetric method for determination of sugars and related substance. Annuals of Chemistry 28: 350-356.
23. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram qualities of protein utilizing the principle of protein dye binding. Anal Biochem 72: 248-254.
24. Folch J, Less M, Stoare Stanley GH (1957) A simple methods for the isolation and purification of total lipids from animal tissues. J Bio Chem 226: 497-508.
25. Waterman PG, Mole S (1994) Analysis of phenolic plant metabolites. Blackwell Scientific, London, UK.
26. SK el Wakeel, Riley JP (1957) The determination of organic carbon in marinemuds. J Cons Int Explor Mer 22: 180-183
27. Welte (1952) Determination of humic acid. Anjew chemistry 67: 153.
28. Unyayar S, Faith Topcuoglu, Ali Unyaya (1996) A modified method for extraction & identification of IAA, GA3, ABA & Zeatin produced by *P.chrysoporium* ME446 Bulg. Journal of Plant Physiology 22: 105-110.
29. Loper JE, and Schroth MN (1986) Influence of bacterial sources of indole -3-acetiec acid on root elongation of sugar beet. phytopathology and Biochemistry 76: 386-389.
30. Hirte W (1961) Glyzerin-Pepton-Agar, ein vorteilhafter Nährboden für bodenbakteriologische Arbeiten. Zbl Bakteriology 114: 141-146.
31. Mackinney G (1941) Absorption of light by chlorophyll solutions. J Biol Chem 140: 315-322.
32. Wellburn AR, Lichtenthaler H (1984) Formulae and program to determine total carotenoids and Chlorophylls a and b of leaf extracts in different solvents. In Advances in Photosynthesis Research, Vol 2. C. Sybesma, editor. Martinus Nijhoff, The Hague, The Netherlands. 9-12.
33. Joseph T, Baker OBE (1984) Seaweeds in pharmaceutical studies and applications. Hydrobiologia 116/117: 29- 40.
34. Rajeshwari M, Lakshmanan KK, Chitra AS (1983) Effect of seaweed on tomato In : Proc. of National Seminar on the Production Technology of Tomato and Chillies, TNAU, Coimbatore 87-89.
35. Kannan L, Tamil Selvan C (1990) Effect of seaweed manures on *Vigna radiatus* L.(Fabaceae) In: *Perspective in phycology* (Prof. MOP Iyenger Centenary Celebration Volume) VN Today and Tomorrow's Printers and Publishers, New Delhi, India. 427- 430.
36. Challen SB, Hemingway JC (1965) Growth of higher plants in response to feeding with Seaweed extracts. Proc. 5th Indian Seaweed Symposium.
37. Taylor IEP, Wilkinson AJ (1977) The Occurrence of gibberellins - like substance in algae. Phycologia 16: 37- 42.
38. el-Sheekh MM, el-Saied A el-D (1999) Effect of seaweed extracts on seed germination, seedling growth and some metabolic processes of Fabae beans (*Vicia faba* L) Cytobios 101: 23-35.
39. Rajalakshmi MC, Sudha SPS, Sebasthiar S, Sudha N, Raju S (2008) Growth promoting effect of commercial Seaweed Liquid Fertilizer on *Solanum melongena* and *Mucuna pruriens*. Seaweed Research and Utilization 30: 183-187.
40. McKeen CD, Reilly CC, Pusey PL (1986) Production and Partial Characterization of Antifungal Substances Antagonistic to *Monilinia fructicola* from *Bacillus subtilis*. Phytopathology 76: 136-139.
41. Silo-suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, et al. (1994) Biological activities of two fungistatic anti-biotics produced by *Bacillus cereus* UW85. Appl Environ Microbiol 60: 2023-2030.
42. Leifort C, Sigee DC, Epton HAS, Stanley R, Knight C (1992) Isolation of bacterial antagonistic to post harvest fungal disease of cold stored *Brassica* spp. Phytoparas 20:158-163.
43. Siddiqui ZA, Mahmood I (1999) Role of bacteria in the management of plant parasitic Nematodes: a review Bioresource Technology 69: 167-179.
44. Paulitz TC, Blanger RR (2001) Biological control in greenhouse systems. Annual Review of Phytopathology 39: 103-133.
45. McSpadden Gardener B, Weller DM (2001) Changes in populations of rhizosphere bacteria associated with take-all disease of wheat. Appl Environ Microbiol 67: 4414-4425.
46. Jacobson CB, Pasternak JJ, Glick BR (1994) Partial purification and characterization of 1-amino- cyclopropane-1-carboxylate deaminase from the plant growth promoting rhizobacterium Pseudomonas putida GR 12–2. Can J Microbiol 40: 1019–1025.
47. Sivasankari S, Chandrasekaran M, Kannathasan K, Venkatesalu V (2006) Studies in the biochemical constitiuents of *Vigna radiate* L. Treated with seaweed liquid fertilizer. Seaweed Resesrch and Utilization 28: 151-158.
48. Lingakumar K, Jayaprakash R, Manimuthu C, Haribaskar A (2002) *Gracillaria edulis* an effective alternative source as growth regulators for legumm crops. Seaweeds Research Utilization 24: 117-123.
49. Thirumal Thangam R, Maria Victorial Rani S, Peter marian M (2003) Effect of seaweed liquid fertilizers on the growth and biochemical constituents of *Cyamopsis tetragonaloba* (L) Taub. Seaweed Research Utilization 25: 99 -103.
50. Asir selin kumar R, Edwin James J, Saravana Babu S (2004) Comparative studies on the impact of seaweeds and seagrass liquid fertilizer on the chlorophyll content of *Zea mays*. Seaweeds Research Utilization 26: 167 - 170.
51. Ramavat B K, Dosh YA, Parekh RG, Chauhan VD (1986) Concentration of poly valent metals by seaweeds from Okha Coast. Phykos 25: 44-50.
52. Paul SD, Nongkynrih (1996) Enzyme activity - a diagnostic tool for toxicity/ deficiency of trace elements in rice plant. Indian Journal of Plant Physiology 1: 225- 257.
53. Blunden G, Wildgoose PB (1977) The effect of aqueous seaweed extract and kinetin on potato yields. Journal of Science Food Agriculture 28: 121- 125.
54. Bhosle NB, Dhargalkar VK, Untawale AG (1975) Effect of seaweed extracts on the growth of *Phaseolus vulgaris* L (*Fabaceae*) Indian Journal of Marine Science 4: 208-210.
55. Venkataraman Kumar V, Mohan VR, Murugeswari R, Muthuswamy M (1993) Seaweed Research Utilization 16: 23-27.
56. Whaphan CA, Blunden G, Jenkins T, Hankins SD (1993) Significant of betaines in the increased Chlorophyll content of plant treated with seaweed extract. Journal of Applied Phycology 5: 231-234.
57. Kannan L, Tamil Selvan C (1990) Effect of seaweed manures on *Vigna radiates* L. (Fabaceae) In: *Perspective in phycology* (Prof. M.O.P. Iyenger Centenary Celebration Volume) (ed.) Raja Rao, VN Today and Tomorrow's Printers and Publishers, New Delhi, India. 427-430.
58. Raj Kumar Immanuel S Subramanian SK (1999) Effect of fresh extracts and seaweed liquid fertilizers on some cereals and millets 21: 91-94.
59. Anantharaj M, V Venkatesalu (2001) Effect of seaweed liquid fertilizer on *Vigna catajung* seaweed Research and Utilization 23: 33-39.