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Effects of Basal Application of Mulch and Foliar Spray of *Sargassum wightii* Extract on Certain Vegetable Crops

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Abstract

A liquid fertilizer for foliar spray as well as a dry seaweed mulch for soil application have been prepared from *Sargassum wightii* and was evaluated on-farm for okra and cowpea in three farmers' fields. Among the two crops tried, Cowpea responded better than Okra on basal application of Sargasssum mulch as well as foliar spray of its extract especially on the number of fruits per plant and wet weight of fruits. Mulching Sargassum powder to the base of okra and cowpea (T1) increased the root biomass significantly ($p<0.05$), 255% increase in organic carbon content in soil and 895% increase in soil microflora over the control. Foliar spray of Sargassum extract (T2) increased the number of leaves in both the crops and remarkably increased the number of pods, wet weight of pods and finally the yield in Cowpea than mulching (T1). In Okra foliar spray resulted a marginal increase only in wet weight of fruits. When the Sargassum mulch was mixed with two strains of bacteria (CMSS-1 and CMSS-4) associated with the decaying Sargassum thallus and later identified through 16s rRNA sequence analysis as *Bacillus endophyticus* with a similarity score of 98% and *Bacillus infantis* with a similarity score of 99%, the mulch became more effective and could assimilate in soil faster. Considerable increase in number and the fresh weight of fruits in Brinjal were also observed due to the value addition in the mulch.

Keywords: Seaweed manure, foliar spray, value added mulch, *Bacillus infantis*

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Introduction

Seaweeds are one of the renewable marine resources cultivated and exploited worldwide as raw material mainly for phycocolloid industries as well as for food, pharmaceuticals and chemical industries (Mathew et al., 2008; Naganathan et al., 2008). These are known to contain minerals, essential nutrients and growth promoters at levels fairly higher than its terrestrial and freshwater counterparts (Blunden et al.1996; Kaladharan & Sridhar, 1999; Gandhiappan & Perumal, 2007; Ashok Kumar et al., 2012); Jesmi et al. (2016). Also, seaweed extracts exhibit antibacterial and antimicrobial properties (Kotnala et al., 2009; Senthil et al., 2008). Due to strong wave action and rough weather it is common to find huge quantities of seaweeds being drifted and washed ashore. Estimates available from Gujarat coast indicate average fresh biomass of washed ashore seaweeds ranging from 3.1 to 6.60 kg/m^2 per day (Thakur et al., 2008). It is known that huge quantities of spent biomass of seaweeds are being discarded from industries after extracting only 10 to 15% polysaccharides (Kaladharan et al., 1998; Reddy et al., 2006). Here we attempt to bring out the beneficial effects of application of mulch and extract of seaweed, *Sargassum wightii* on vegetable crops and to find some means to utilize the washed ashore resources and the spent biomass from seaweed industries.

Materials and Methods

Samples of seaweed *Sargassum wightii* collected from Pampan-Rameswaram coast of Tamil Nadu were sundried after cleaning and thorough washing with fresh water. Semi dried Sargassum (75% moisture) were transported to the laboratory in ICAR-CMFRI, Kochi. Sargassum mulch was prepared by drying the semi dried Sargassum in a hot air oven at 60°C for 24 h, crushed and pulverized in a cross beating mill (Pulverisette 16, FRITSCH, Denmark) and stored airtight. Seaweed extract for foliar spray was prepared by boiling the finely chopped, semi dried Sargassum (10% wt/v) with distilled water in an autoclave (15 lbs for 1 h). At room temperature, the seaweed extract was decanted, filtered and preserved with 2% (wt/v) sodium benzoate in refrigerator until use. For foliar spray the extract was diluted to 5% (v/v) with distilled water just before application.

Vegetable crops; Okra (*Abelmoshus esculentus*, cv. Arka Anamika) and Cow pea (*Vigna unguiculata*, cv. Kashi Kanchan) were selected for the performance evaluation of mulch and foliar spray and carried out for 75 days in the field of three progressive farmers within the Ernakulam district who were identified with the help of ICAR- Krishi Vigyan Kendra (Ernakulam). Plants of cow pea and okra were raised from seeds in grow-bags filled with potting mixture consisting of composted coir pith and mineral sand. Each crop had 40 grow bags (10- spray, 10- basal, 10- combination of these two and 10- control) and each of the three farmers were provided with 80 grow bags and for three farmers, a total of 240 grow-bags were used. The following are the details of experimental treatments conducted:

T0/Control- Growing plants using potting mixture and watering + spraying distilled water at 5 days interval and forking the base thrice during 60 days.

T1- Growing plants using potting mixture and watering + Sargassum mulch (basal application) @ 60 g/ plant in three regular intervals during 60 days

T2- Growing plants using potting mixture and watering + Foliar spraying of Sargassum extract at 5 days interval @ 5% (50 ml/l of water)

T3- Combination of mulching and foliar spray (T1 + T2)

Parameters such as number of leaves, number of branches, flower induction, fruit setting, number of fruits, number of picking, flower and fruit drop were observed weekly. Length of shoot and root and their wet biomass were determined after 75 days. Soil samples from the root zone were sampled using a corer before and after soil amendments for determining any change in soil microflora (Adam & Duncan, 2001) and soil organic carbon (Walkley & Black, 1934) due to application of seaweed mulch. The data obtained were statistically analysed using SPSS software (version 13.0). To add value to the mulch, cultures of two gram negative bacterial strains (CMSS-1 and CMSS-4) isolated from decaying thallus bits of *Sargassum wightii* were mixed with the Sargassum mulch (5 ml broth/100 g) and incubated for 6 days at 25°C as value addition before application. Brinjal (*Solanum melongena*, L.) plants grown in 10 pots filled with similar potting mixture and 20 g value added seaweed mulch per plant were maintained in the laboratory for evaluating the performance of value added mulch.

Results and Discussion

Among the two crops tried, Cowpea responded better than Okra on basal application of Sargasssum mulch as well as foliar spray of its extract (Table 1). Significant increase in number of fruits per plant and wet weight of fruits were observed in cowpea. Mulching Sargassum powder to the base of okra (T1) and cowpea (T3) increased the root biomass significantly ($p<0.05$). Foliar spray of Sargassum extract resulted in significant increase in the number of leaves in both the crops. Number of branches and height of the plants also showed significant increase due to the basal and foliar application of seaweed manure. Mulching Sargassum at the root zone has helped increase root biomass in both the crops significantly. Foliar spray of Sargassum extract (T2) effectively increased the number of pods, wet weight of pods and the yield in cowpea than mulching (T1). The statistical analysis carried out with data on number of fruits, fruit weigth, pod yield and root biomass of cowpea through oneway analysis of variance revealed that there is no significant difference between the effects of treatments on these characters of cowpea ($p>0.05$). Two way analysis of variance carried out for examining the effects of treatments on number of branches in cowpea with duration as the second source of variation revealed that both treatments as well as duration have significant effects on number of branches. Further analysis using SNK tests identified two homogenous subgroups of treatments (To, T1, T2) and T2, T3). Also each growth periods are one another significantly different with regards to number of branches. Treatment T4 had maximum average number of branches of 2.16. The two-way analysis with plant height as the measured variable showed that all the treatments have homogenous effects on plant height but duration of application have significantly different effects on plant height.

In okra plants foliar spray treatment showed a marginal increase only for wet weight of pods (Table 1). The one-way analysis of variance carried out individually for examining the effects of treatments on number of fruits, fruit weight, pod yield and total yield of okra revealed that there is no significant difference between the effects of all the four treatments on these variables. When the root biomass of okra was measured and used to examine the effects of four treatments, it was found significant ($p<0.05$) and treatments, T1, T2 and T3 formed a homogenous subgroup which is significantly different from the second homogenous sub groups T0 and T2. A two-way ANOVA with plant height as the measured variable with treatments and durations as the two factors, the results obtained revealed that both the treatments as well as durations have significant effects on height of plants. Treatments T0, T1 and T2 formed a subgroup with uniform effects on plant height, whereas the treatments T1 and T3 formed another separate homogenous group. Similar analysis with number of leaves showed that the four treatments have uniform effects. Duration also have significantly different effects on number of leaves. The seaweed *Sargassum wightii* is known to contain high levels of NPK and essential minerals (Rama Rao, 1991; Naganathan et al., 2008).

The quantitative increase obtained in the growth and yield parameters of cowpea as a result of application of Sargassum mulch and foliar spray of its extract can be due to the presence of NPK and the mineral nutrients and minerals and the results we report here agree well with the findings of Arumugam et al. (2008) who reported that similar results in green gram with 1% extract of *Sargassum wightii.* Earlier study involving the agar factory discharge on cowpea plants reported the length of seedlings to 85-87%, wet weight of plants to 194-329%, number of leaves per plant to 31- 38% and the number of root nodules to 42-92% (Kunda & Kaladharan, 2003). Seaweed liquid fertilizer prepared from different seaweeds do exhibit different growth enhancing activities. Sethi & Adhikary (2008) have found that 1% extract of *Chaetomorpha linum* (green seaweed) was better than that of a red seaweed *Gracilaria verrucosa*. Application of seaweed

Table 1. Effect of Sargassum mulch and foliar spray of its extract on growth and yield of cowpea and okra

Characters/ Treatments	Cowpea T0	Cowpea T1	Cowpea T2	Cowpea T3	Okra T0	Okra T1	Okra T2	Okra T3
No.of leaves on 45th day	10.1±0.86	9.9±1.07	10.9±0.83	11.3±0.29	6.97±0.47	6.8±0.69	7.13±0.42	7.77±0.75
No. of branches on 45th day	1.63±0.17	1.7±0.04	1.8±0.41	2.16±0.19	1.66±0.17	1.73±0.09	1.76±0.13	1.73±0.12
Plant height (cm) on 75th day	41.53±1.08	44.2±2.55	45.0±2.45	46.13±3.72	47.63±3.6	51.9±1.66	51.6±2.0	52.67±2.3
Root weight (g) on 75th day	40.17±4.68	57.43±10.9	52.96±10.5	59.5±13.7	29.63±3.95	41.83±3.44	35.3±2.58	41.4±1.35
No of fruits	217±25.5	230±17.5	235±65	225±44	89±7.13	87±11.43	77.66±1.88	76.3±4.92
Fruit weight (g)	12.23±0.25	12.33±1.05	13.7±0.73	13.33±0.49	19.23±2.48	18.97±1.88	19.57±1.88	18.3±0.88
Yield (kg/ plant)	2.63±0.28	2.81±0.7	3.27±1.07	3.02±0.08	1.71±0.19	1.65±0.16	1.52±0.09	1.4±0.08

Mean ± SD, n = 30, T0 - Control, T1 - Mulch (basal), T2 - Foliar spray, T3 - Spray + Mulch

Table 2. Effect of *Sargassum* mulch on the levels of organic carbon and soil bacteria

Treatments	Organic carbon (%)	% increase over Control	Microflora (CFU/g)	% increase over Control
T0	1.28 ± 0.26		2.02±0.12 $\times 10^6$	
T1	4.55 ±0.14	255	2.01±0.14 $\times 10^7$	895
T2	2.12 ±0.12	65.6	2.01±0.15 $\times 10^6$	-
T3	5.28 ±0.28	312	2.10±0.11 $\times 10^7$	895

mulch to the base of Okra and Cowpea increased the levels of organic carbon and the soil microflora in the root zone of T1 and T3 treatments (Table 2). Possinger (2013) obtained significant increase in active Carbon content in corn field soil due to the seaweed amendment. Wang et al., 2017 reported that seaweed amendment in apple fields could increase the soil microflora and soil fertility besides improving the overall plant growth, rate of photosynthesis and activating antioxidant enzymes.

The two strains of bacteria (CMSS-1 and CMSS-4) which were believed to have aided in faster assimilation and better utilization of seaweed mulch were identified later through 16s rRNA sequence analysis. These bacterial strains capable of adding value to the seaweed mulch were identified as *Bacillus endophyticus* with a similarity score of 98% and *Bacillus infantis* with a similarity score of 99%. Considerable increase in number and the fresh weight of fruits were also could be observed due to the value addition in the mulch (Table 3). Brinjal plants which received the value added seaweed mulch had early onset of flowering and fruit setting than in the control (mulch without value addition, $p<0.01$).

Table 3. Effect of value added Sargassum mulch on yield of Brinjal plant

Treatment	No of fruits	Fruit weight (g/no)
Value added mulch	18±3	51.54±5.02
Control mulch (mulch without bacterial incubation)	16±2	40.60±4.81

Mean±SD, n= 10

Soil amendment with seaweeds or their extracts is one of the safe and cost effective process of increasing soil fertility and thus adverse effects of adding chemical fertilizers to soil can be reduced. For the large scale production of mulch and other forms of seaweed manure, raw material available along our coastline becomes too inadequate as they are exploited for phycocolliod production and for production of various nutraceuticals. Alternatively the huge quantities of cast ashore seaweeds can be collected, processed and utilized.

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