Pak. J. Bot., 50(6): 2409-2412, 2018.

SEAWEED BIO-FERTILIZER FOR THE MANAGEMENT OF ROOT ROTTING FUNGI AND ROOT KNOT NEMATODES AFFECTING COTTON CROP

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Abstract

Seaweed can promote plant growth and also enable them to tolerate drought, disease or frost. In this study, eight seaweeds *Stokeyiaindica*, *Stoechospermum marginatum*, *Sargassum binderi, Spatoglossum variabile* (brown), *Melanothamnus afaqhusainii, Solieriarobusta* (red), *Rhizocloni umimplexum* and *Halimeda tuna* (green) collected from Buleji beach, Karachi were evaluated for their role in protecting the cotton from soilborne pathogenic fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *Meloidogyne javanica,* a root knot nematode. Both in screen house and in field experiments application of seaweed as soil amendment caused marked reduction of pathogenic fungi and root knot nematode on cotton roots. Suppressive effect of seaweed on root pathogens is comparable with commercial fungicide and nematicide in reducing the fungal root infection, nematode's galls on roots and nematode's population in soil around roots. For plant growth and suppression of root pathogens of cotton seaweeds offer a non-chemical means in agriculture in the replacement of chemical fungicides and nematicides.

Key words: Seaweed, Cotton, Root rotting fungi, Root knot nematode.

Introduction

Organic amendments has now became an effective method for controlling soilborne pathogens on various crops (Badar *et al.,* 2015; Shafique *et al.,* 2015ab; Zhou & Everts, 2004). Among the various organic matters used for soil amendment, seaweeds are gaining popularity due to their positive effect on plant growth and yield (Sultana *et al.,* 2011). Substances extracted from seaweeds have been reported to enhance plant growth and productivity (Stirk& van Staden, 1997ab, Rathore *et al.,* 2009; Shafique *et al.,* 2016). Treatment with aqueous extract of *Ascophyllum nodosum* enhanced fruit earliness as measured by maturity index (Fornes *et al.,* 1995). An increase in citrus yield was also reported, when treated with seaweed extract of *Ascophyllum nodosum* (Fornes *et al.,* 2002). Soil amendment with seaweed also increased tomato yield under filed condition (Sultana *et al.,* 2011). Improved growth of *Eucalyptus* species has been reported by the foliar spray of seaweed (Kelpak) concentrate (van Staden *et al.,* 1995) and increased grain yield in wheat (Beckett *et al.,* 1991). These extracts could affect cell metabolism through the induction of the synthesis of antioxidant molecules which could favour plant growth and plant resistance to stress (Rahman *et al.,* 2017; Zhang & Schmidt, 2000).

Beneficial effect of algal extract as foliar spray is well documented, however, application of seaweed as soil amendment is now gaining popularity due to raising awareness about the hazardous effect of pesticides (Sultana *et al.,* 2007, 2008, 2009; 2011; Shafique *et al.,* 2016). In our previous studies we have reported protection of roots of chili, sunflower, tomato, soybean (Sultana *et al.,* 2007; 2008; 2009; 2011) eggplant and watermelon (Baloch *et al.,* 2013) from root diseases by the application of seaweed. In this study efficacy of eight seaweeds was evaluated in screen house and also under field condition against root diseases of cotton. The efficacy of seaweeds was also compared with chemical pesticides, carbofuran and topsin-M (thiophanate-methyl).

Materials and Methods

Screen house experiment: Sandy loam soil was mixed with dry powder of seaweed *Solieria robusta, Stokeyia indica, Stoechospermum marginatum, Sargassum binderi, Rhizoclonium implexum, Halimeda tuna, Melanothamnus afaqhusainii* and *Spatoglossum variabile* at 1% w/w. The soil had natural infestation of *Macrophomina phaseolina* (4-13 sclerotia g^{-1} of soil), 5-12% colonization of *Rhizoctonia solani* on sorghum seeds and a mixed population of *Fusarium solani* and *Fusarium oxysporum* (3500 cfu g^{-1} of soil) as determined byusing methods of Sheikh & Ghaffar (1975), Wilhelm, (1955) and Nash & Snyder, (1962) respectively.Then the amended soil was transferred to clay pots at 1 kg per pot and water holding capacity was kept at 50%.After two weeks, six seeds of cotton (*Gossypium hirsutum* L.) were sown in each pot. Where untreated plants served as control, while topsin-M (200 ppm at 50 ml/pot) and carbofuran (0.5 gm/pot) served as positive control for fungi and nematode respectively. Each treatment was replicated four times and randomized in block design. Four plants were kept in each pot and excess were removed after germination. Each pot were inoculated with *Meloidogyne javanica* eggs/juveniles at 2000/pot. Effect of seaweed on soilborne pathogens and plant growth was evaluated after six weeks of nematode inoculation. Date on plant growth such as, shoot length, fresh weight of shoots, root length and root weight were recorded. Whereas, incidence of root infecting fungi was determined as described by Habiba *et al.,* (2016). Number of knots per root system was counted and nematode's population in soil around the roots were estimated to determine the nematode's infestation (Ayoub, 1980).

Field plot experiment: Efficacy of seaweeds was also examined in 2 x 2 meters field plots at Crop Diseases Research Institute, Pakistan Agricultural Research Council, Karachi University Campus. Dry powder of seaweeds: *Solieria robusta*, *Stokeyia indica*, *Stoechospermum marginatum*, *Sargassum binderi*, *Rhizoclonium implexum*, *Melanothamnus afaqhusainii* and *Spatoglossum variabile* were mixed in planting rows at 35 g per meter and watered at 2-3 days interval to allow the decomposition of seaweeds. The soil had infestation of *M. phaseolina* (6-18 sclerotia g^{-1} of soil), *R. solani* (5-12 % colonization on sorghum seeds) and mixed population (2800 $cfug^{-1}$ of soil) of *Fusarium oxysporum* and *F. solani*.Seeds of cotton (*Gossypium hirsutum)* were sown after 2 weeks at 50 seeds per two meter row. For the infestation of nematode, after germination of seedlings each row was inoculated with aqueous suspension of *Meloidogyne javanica* eggs/ juveniles at 2000/two meter row. Plants grown in unamended soil served as control, while topsin-m (200 ppm at 200 mL/m) and carbofuran (1 g per meter) served as positive control against fungi and nematode respectively. The experiment was conducted with complete randomized block design with 4 replicates. Observations on plant growth and soilborne pathogens were recorded after 6 weeks of nematode inoculation. Whereas yield data (balls per plant) was recorded after 10 weeks.

Data analysis: Analysis of variance (ANOVA) was used to analyze the data and means were compared with least significant difference (LSD) at $p<0.05$ (Gomez & Gomez, 1984).

Results

Screen house experiment: All the test seaweeds caused a significant ($p<0.05$) suppressive effect on *M. phaseolina* and *R. solani*. Whereas *Solieria robusta*, *Stokeyia indica*, *Stoechospermum marginatum*, *Sargassum binderi, Rhizoclonium implexum* and *Spatoglossum variabile* were also effective against *F. solani* (Table 1). Seaweed soil amendment also showed a suppressive effect on nematode by reducing the nematodes population in soil (Table 1). Increased plant height and fresh shoot weight was produced by *Spatoglossum variabile* followed by *Sargassum binderi*. Brown seaweed *Spatoglossum variabile* also significantly increased fresh root weight (Table 2).

Table 1. Effect of seaweeds on the infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* on cotton roots in screen house experiment.

Treatments	*M. phaseolina*	*R. solani*	*F. solani*
	Infection %		
Control	43.7	37.5	31.2
Topsin-M	12.5	6.2	6.2
Carbofuran	18.7	12.5	18.7
Solieria robusta	18.7	12.5	12.5
Stokeyia indica	25	12.5	6.2
Stoechospermum marginatum	31.2	25	18.7
Sargassum binderi	12.5	6.2	6.2
Rhizoclonium implexum	6.2	25	18.7
Halimeda tuna	18.7	12.5	12.5
Melanothamnus afaqhusainii	12.5	18.7	25
Spatoglossum variabile	6.2	18.7	18.7

$LSD_{0.05}$ Treatments=10.3[1], Pathogens=5.6[2]

Table 2. Effect of seaweeds on *Meloidogyne javanica* and growth of cotton plants in screen house experiment.

Treatments	Shoot length (cm)	Fresh shoot weight (g)	Root length (cm)	Root weight (g)	No. of knots/ root system	Nematodes/100 g soil
Control	23.68	4.6	15.3	0.94	6.3	690
Topsin-M	25.3	5.59	16.04	0.96	0.0	925
Carbofuran	25.9	7.0	15.6	1.04	0.82	435
Solieria robusta	29.05	2.3	13.17	1.01	2.85	450
Stokeyia indica	30.5	8.0	12.75	1.08	2.81	340
Stoechospermum marginatum	30.78	7.4	13.6	1.01	0.0	95
Sargassum binderi	33.12	7.9	15.12	1.03	0.0	50
Rhizoclonium implexum	32.95	6.92	12.62	0.96	0.57	65
Halimeda tuna	29.5	5.3	14.7	1.12	1.5	354
Melanothamnus afaqhusainii	32.85	7.5	15.45	1.13	4.6	275
Spatoglossum variabile	34.75	9.05	13.8	1.37	3.6	250
$LDS_{0.05}$	4.01[1]	1.25[1]	2.56[1]	0.37[1]	3.46[1]	337[1]

Field experiment: Of the seaweeds used *Solieria robusta, Stokeyia indica, Stoechospermum marginatum, Melanothamnus afaqhusanii* and *Spatoglossum variabile* significantly (p<0.05) suppressed *M. phaseolina.* Whereas against *R. solani*, *Solieria robusta* and *Stokeyia indica* were effective (Table 3). Seaweeds also caused a suppressive effect on *M. javanica* by reducing the numbers of galls on roots. Some seaweeds like *S. indica, Rhizoclonium implexum* and *Melanothamnus afaqhusanii* also significantly (p<0.05) reduced nematodes population in soil (Table 4). Seaweeds *M. afaqhusainii* and *Spatoglossum variabile* showed a positive effect on plant growth by increasing plants height, fresh shoot and root weight (Table 4). Larger numbers of balls per plant were achieved by the application of *Melanothamnus afaqhusainii* and *Spatoglossum variabile* (Table 4).

Table 3. Effect of seaweeds on the infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* on cotton roots in field experiment.

Treatments	*M. phaseolina*	*R. solani*	*F. solani*
	Infection %		
Control	25	31.2	25
Topsin-M	0.0	18.7	6.2
Carbofuran	6.2	12.5	6.2
Solieria robusta	0.0	0.0	18.7
Stokeyia indica	6.2	6.2	12.5
Stoechospermum marginatum	0.0	18.7	25
Sargassum binderi	12.5	18.7	25
Rhizoclonium implexum	12.5	25	18.7
Melanothamnus afaqhusainii	6.2	18.7	25
Spatoglossum variabile	6.2	25	25
$LSD_{0.05}$ Treatments=10.3[1], Pathogens=5.6[2]			

[1]Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.
[2]Mean values in rows showing differences greater than LSD values are significantly different at p< 0.05

Table 4. Effect of seaweeds on the growth and yield of cotton plants in field experiment.

Treatments	Shoot length (cm)	Fresh shoot weight(g)	Root length (cm)	Root weight (g)	No. of knots/ root system	Nematode/ 100 g soil	Balls/4 plants
Control	9.9	2.52	20.22	1.26	4.5	750	29.45
Topsin-M	11.15	2.6	15.57	1.55	0.0	460	24.47
Carbofuran	10.02	2.05	19.77	1.21	0.0	510	20.3
Solieria robusta	11.2	1.86	18.47	1.27	0.06	213	23.77
Stokeyia indica	15.12	2.35	18.92	1.32	0.06	77.5	27.15
Stoechospermum marginatum	12.95	2.55	22.67	1.37	0.0	152.5	27.4
Sargassum binderi	14.8	2.97	18.42	1.27	0.18	112.5	28.35
Rhizoclonium implexum	10.12	1.97	19.55	1.1	0.0	80	25.8
Melanothamnus afaqhusainii	27.85	5.6	23.92	2.17	0.12	40	36.72
Spatoglossum variabile	27.77	4.1	20.9	1.67	0.18	182.5	38.22
$LSD_{0.05}$	6.79[1]	1.14[1]	4.14[1]	0.42[1]	0.5[1]	258.5[1]	5.40[1]

[1]Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.

Discussion

Biostimulants are organic matters, when applied in small amount can enhance plant growth, while efficacy of traditional plant nutrients are unable to produce similar benefits (Hamed *et al.,* 2017). Interest in the application of seaweed extract has been increased recently due to their plant growth promoting properties, triggering diseases resistance pathway and increasing stress tolerance (Arioli *et al.,* 2015). This study revealed that some seaweed can suppressed parasitic fungi and nematodes as do the commercial fungicides and nematicide like topsin-M and carbofuran. Suppression of plant root pathogens and improvement of plant growth have been reported by the application of seaweed (Ara *et al.,* 1997; Pardee *et al.,* 2004; Sultana *et al.,* 2007, 2008, 2009). Increased in tomato yield has also been reported by the seaweed soil amendment under field condition (Sultana *et al.,* 2011). Seaweed induced systemic resistance in cotton against charcoal rot and also improved plant growth as reported by Rahman *et al.,* (2017). Seaweed contains alginate which has been reported to inhibit the virus infectivity in plants (Pardee *et al.,* 2004). In this study, some seaweed besides suppressing root rotting fungi and root knot nematode also improved plant growth.

Seaweed have been used as biostimulant in agriculture that enhanced plant growth and yield (Hamed *et al*., 2017; Mooney & van Staden,1986; Rathore *et al.,* 2009). They are now gaining popularity as an important source for plant improvement due to presence of mineral contents, amino acids and growth regulator like auxins, gibberellins and cytokinin (Stikk & Van Staden, 1997ab). Application of seaweed have produced healthy plants, resulting in increased in number and weight of fruits (Baloch *et al.,* 2013). They also found that plant grown in seaweed amended soil exhibited earlier fruiting than control or chemical fungicide treated plants. Marine macro-algae have been reported to influence respiration, photosynthesis, nucleic acid synthesis and ion uptake (Craigie, 2011, Khan *et al.,* 2009) which enhanced nutrient availability, increased chlorophyll production and increased antioxidants (Khan *et al.,* 2009, Rahman *et al.,* 2017). It is seems that suppression of plant root pathogens, enhanced availability of nutrients and presence of growth regulators in seaweed improve the plant growth than non-seaweed treated plants.

Acknowledgement

Authors are thankful to Pakistan Agricultural Research Council, Islamabad for providing financial assistance under Agricultural Linkage Program (ALP) with USA.

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(Received for publication 22 March 2017)