

Impact of seaweeds on fluorescent *Pseudomonas* and their role in suppressing the root diseases of soybean and pepper

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Summary

Incorporation of organic matter in soil before sowing of seeds or transplanting the seedlings has been reported to increase microbial activity in soil, especially fluorescent *Pseudomonas* that play a vital role in suppressing the root rotting fungi and parasitic nematodes invading plant roots. In this study, dry powder of seaweeds *Sargassum binderi*, *S. tenerrimum*, *Halimeda tuna*, *Stoechospermum marginatum*, *Padina tetrastromatica*, *Stokeyia indica* and *Solieria robusta* were applied as soil amendment two weeks before sowing of soybean seeds or transplanting of pepper seedlings in screen house and in field experiments. Application of some seaweeds and topsin-M (fungicide) and carbofuran (nematicide) showed more or less similar suppressive effects on root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and root knot nematode *Meloidogyne javanica* on soybean and pepper plants. Seaweed also showed a positive effect on plant growth by enhancing fresh shoot weight and plant height. Incorporation of seaweeds in soil increased the population of fluorescent *Pseudomonas* around the roots of soybean and pepper compared to a non-seaweed control. However, the increased population of fluorescent *Pseudomonas* around the roots did not correlate with disease suppression.

Introduction

Management and manipulation of natural communities of antagonistic microorganisms through organic amendments have received less attention, in spite of the fact that these strategies have resulted in highly effective forms of biological control (HOITINK and BOEHM, 1999). Soil amendments have the potential to provide disease control through a variety of mechanisms, including chemicals which produce antimicrobial compounds during decomposition (BROWN and MORRA, 1997; TENUTA and LAZAROVITS, 2002), and biological (CHEN et al., 1988; MAZZOLA, 2004). Plants with therapeutic effects have received the attention of scientists as an alternate method of disease control, which would also protect our environment from the use of hazardous chemicals.

The direct application of seaweeds in farming is a practice that extends over hundreds of years, and it is often more successful than using chemical fertilizers. Seaweeds contain elaborated secondary metabolites that play a significant role in the defense of the host against predators and parasites (PARACER et al., 1987; SULTANA et al., 2008; 2009). The use of brown algae, *Sargassum* spp., showed significant ($p < 0.05$) control of root infecting fungi on sunflower (ARA et al., 1996) and root knot nematode in okra (ARA et al., 1997). There are reports that the delivery of microbial antagonists with urban and agricultural wastes as mulches was found to be very effective in suppressing root pathogens of avocado and citrus (CASALE et al., 1995). Similarly, addition of some compost to soil increased the population of plant growth promoting rhizobacteria (PGPR) in the tomato rhizosphere exhibiting antagonism towards *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Pythium ultimum*

and *Rhizoctonia solani* (ALVAREZ et al., 1995). The present report describes the effect of soil amendment with seaweeds on root diseases of soybean in screen house experiments. The impact of soil amendments on the population of fluorescent *Pseudomonas* and their role in protecting soybean and pepper from root rotting fungi and root knot nematode under field condition has also been reported.

Materials and methods

Seaweeds *Dictyota indica*, *Padina tetrastromatica*, *Stoechospermum marginatum*, *Stokeyia indica*, *Sargassum swartzii*, *Solieria robusta* and *Halimeda tuna* collected from Buleji Beach, Karachi, were used in these experiments, topsin-M (fungicide) and carbofuran (nematicide) were used to compare the efficacy of these seaweeds with common commercial pesticides. The experiments were conducted both under screen house and field conditions on soybean (*Glycine max* (L.) Merr.), and under field condition on pepper (*Capsicum annum* L.).

Screen house experiment

Dry powder of seaweeds was mixed with sandy loam soil (pH 8.1) to give a concentration of 1.0% w/w. The soil was naturally infested with 3-7 sclerotia of *Macrophomina phaseolina* g⁻¹ of soil as determined by wet sieving and dilution plating (SHEIKH and GHAFFAR, 1975), 2-6% surface colonization of *Rhizoctonia solani* on sorghum seeds (10 sterilized seeds were spread on 100 g wet soil in a Petri dish with 5 replicates of each sample) used as baits (WILHELM, 1955) and 3,000 cfu g⁻¹ of soil of a mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution (NASH and SNYDER, 1962). One kg of amended soil was transferred to 12 cm diam. clay pots. The pots were watered daily to allow the decomposition of the organic substrate. After three weeks, 6 seeds of soybean were sown in each pot and pots were kept randomized on a screen house bench of Department of Botany at 50% WHC (KEEN and RACZKOWSKI, 1921) with four replicates of each treatment. After germination, only four seedlings were kept and excess were removed. The seedlings were inoculated with *Meloidogyne javanica* eggs/juveniles at 2000 eggs/J₂ (2nd stage juveniles) per pot. Topsin-M (20 ml/pot of 200 ppm) served as positive control against root infecting fungi, whereas carbofuran at 0.1 g per pot served as positive control against nematode.

To determine the efficacy of seaweeds and pesticides on the root pathogens and plant growth, plants were uprooted after six weeks of nematode inoculation. Observations were made on plant height, fresh shoot weight, root length and root weight. Nematode infection was determined by counting the numbers of galls per root system (TAYLOR and SASSER, 1978). To determine nematode penetration and infection by root-infecting fungi, roots from each plant were cut into 1cm long pieces and five pieces of tap roots from each plant were used for assessment of fungal infection. The remaining roots were mixed thoroughly, and 1 g sub-sample was wrapped in muslin cloth and dipped in boiling 0.25% acid fuchsin stain for 3-5 minutes.

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Roots were left in the stain to cool, and then washed under tap water to remove excess stain. Roots were then transferred to vials containing glycerol and water (1:1 v:v) with a few drops of lactic acid. Roots were macerated in an electric blender for 45 seconds and the resulting slurry was suspended in 50 ml water. Numbers of juveniles and females in 5 ml sub samples were counted with the aid of a dissecting microscope and numbers of nematode/g root were calculated (SIDDIQUI and EHTESHAMUL-HAQUE, 2001). To determine the incidence of fungal infection, 1 cm long root pieces from tap roots (five pieces from each plant) were surface disinfested with 1% Ca(OCl)₂ and plated onto potato dextrose agar amended with penicillin (100,000 units/litre) and streptomycin (0.2 g/litre). Colonies of *Macrophomina phaseolina*, *Rhizoctonia solani* and species of *Fusarium* were recorded after incubation for 5 days at 28°C. The experiment was conducted twice. Data were subjected to analysis of variance (ANOVA) and means were separated using the least significant difference (LSD) according to GOMEZ and GOMEZ (1984).

Field Experiments

The efficacy of seaweeds were also examined in 2 x 2 m field plots at the Crop Diseases Research Institute, Pakistan Agricultural Research Council, Karachi University Campus, using soybean and pepper as test crops. Dry powder of seaweeds: *Padina tetrastomatica*, *Stoechospermum marginatum*, *Stokeyia indica*, *Sargassum swartzii*, *Solieria robusta*, *Halimeda tuna*, and *Dictyota indica* were mixed in sandy loam soil at 70 g per two meter rows and watered 2-3 days interval to allow the organic matters to decompose. The soil had a natural infestation of 6-18 sclerotia/g of soil of *Macrophomina phaseolina*, 5-12% colonization of *Rhizoctonia solani* on sorghum seeds used as baits and 2800 cfu/gm of soil of mixed population of *Fusarium oxysporum* and *F. solani*. After two weeks, seeds of soybean (*Glycine max*) were sown at 50 seeds per two meter row. After germination, each row was inoculated with aqueous suspension of *Meloidogyne javanica* eggs/juveniles at 2000/two meter row. Seeds treated with topsin-M served as positive control against root infecting fungi while carbofuran at 0.5 g per meter served as positive control against nematode. Each treatment was randomized and replicated four times. Plants were watered

2-3 days intervals depending upon requirement of plants.

To assess the effect of seaweeds on infection of root infecting fungi and root knot nematode, plants were uprooted after 30 and 60 days (at 4 plants per replicate; a total of 16 plants per treatment) of nematode inoculation. Roots were washed under running tap water and incidence of root infecting fungi and root knot nematode on roots were determined as described above. Population of fluorescent *Pseudomonas* around the roots was determined using S-1 selective medium for fluorescent *Pseudomonas* (GOULD et al., 1985). Data on plant growth were also recorded, where plant heights, root lengths fresh shoot weights and root weights were recorded for 4 plants per replicate and averaged.

In case of pepper, three-week-old seedlings of equal size, raised in steam sterilized soil were transplanted in each row at 12 seedlings per row. After one week of seedling transplantation, each row was inoculated with aqueous suspension of *Meloidogyne javanica* eggs/juveniles at 2000/ two meter row. Other details were similar except observations were recorded after 45 and 90 days of nematode inoculation.

Results

Screen House Experiment

Application of seaweeds *Stokeyia indica*, *Solieria robusta* and *Halimeda tuna* caused a complete suppression of *Macrophomina phaseolina* infection on soybean roots (Tab. 1), whereas 6.2% *Rhizoctonia solani* infection was observed only in control plants. Of the seaweeds used, *S. robusta* also caused complete suppression of *Fusarium solani* infection. Other seaweeds *P. tetrastomatica*, *Stoechospermum marginatum*, *Sargassum swartzii*, *Halimeda tuna* and *Dictyota indica* also significantly ($p < 0.05$) reduced *F. solani* infection (Tab. 1). Seaweeds also caused significant suppressive effect on nematode infection by reducing the numbers of galls per root system and nematode's penetration in roots. Maximum reduction in gall formation was achieved by the application of *S. swartzii*, whereas *H. tuna* caused maximum reduction in nematode penetration in roots followed by *Solieria robusta* and *Stoechospermum marginatum* (Tab. 1). Seaweeds also showed significant beneficial effect on plant growth. Maximum increase in root length was produced by *Solieria robusta* followed by

Tab. 1: Effect of seaweeds on the infection of root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum* on soybean roots after 45 days of nematode inoculation in screen house experiment.

| Treatments | <i>M. phaseolina</i> | <i>R. solani</i> Infection % | <i>F. solani</i> | No. of knots | Females/Juveniles per gram roots |
|--|-------------------------------|---------------------------------|------------------|-------------------|-------------------------------------|
| Control | 18.7 | 6.2 | 25 | 52.6 | 337.5 |
| Topsin-M | 18.7 | 0 | 12.5 | 50.9 | 420 |
| Carbofuran | 0 | 0 | 6.2 | 45.2 | 510 |
| <i>Stokeyia indica</i> | 0 | 0 | 18.7 | 28.2 | 147 |
| <i>Halimeda tuna</i> | 0 | 0 | 12.5 | 26.5 | 40 |
| <i>Dictyota indica</i> | 6.2 | 0 | 6.2 | 43.8 | 182 |
| <i>Padina tetrastomatica</i> | 6.2 | 0 | 12.5 | 49.6 | 270 |
| <i>Sargassum swartzii</i> | 12.5 | 0 | 6.2 | 19.0 | 112 |
| <i>Stoechospermum marginatum</i> | 6.2 | 0 | 12.5 | 46.1 | 77 |
| <i>Solieria robusta</i> | 0 | 0 | 0 | 28.9 | 76 |
| LSD _{0.05} for fungal pathogens | Treatments= 10.6 ¹ | pathogens= 5.8 ² | | 37.6 ¹ | 166 ¹ |

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

²Mean values in rows for fungi showing differences greater than LSD values are significantly different at $p < 0.05$.

Stokeyia indica, *Solieria robusta* and *Stokeyia indica* also caused maximum fresh root weight while maximum shoot length was achieved by the application of *S. indica* (Tab. 2).

Field Experiment

Soybean

After 30 days, no infection of *Macrophomina phaseolina* was found on any plant. All the test seaweeds caused a significant ($p < 0.05$) reduction in *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum* infection (Tab. 3). Application of *Dictyota indica*, *Padina tetrastromatica*, *Sargassum swartzii*, and *Stoehospermum marginatum* resulted in complete suppression of *F. solani*. *Padina tetrastromatica*, *S. marginatum* and *Solieria robusta* also caused complete inhibition of *F. oxysporum* on soybean roots (Tab. 3).

Effectiveness of *S. marginatum* and *S. robusta* were also found after 60 days. Seaweeds caused a significant increase in PGPR population in the rhizosphere of soybean. *Dictyota indica* caused maximum increase in PGPR population in soil around the roots (Tab. 4). Greater plant height was produced by *S. robusta*, whereas maximum fresh shoot weight was achieved by the application of *S. marginatum* (Tab. 4).

After 60 days, *Halimeda tuna* and *Solieria robusta* completely suppressed *Macrophomina phaseolina* infection. While other seaweeds *Stokeyia indica*, *Dictyota indica* and *Stoehospermum marginatum* also significantly ($p < 0.05$) reduced *M. phaseolina* (Tab. 3). All the test seaweeds also significantly ($p < 0.05$) prevented *Rhizoctonia solani* and *Fusarium solani* infection. Complete suppression of *F. oxysporum* was also achieved by the application of *H. tuna*, *S. marginatum* and *Solieria robusta* (Tab. 3).

Tab. 2: Effect of seaweeds on the growth of soybean after 45 days of nematode inoculation in screen house experiment.

| Treatments | Plant height (cm) | Fresh shoot weight (g) | Root length (cm) | Fresh root weight (g) |
|---------------------------------|-------------------|------------------------|-------------------|-----------------------|
| Control | 32.8 | 4.0 | 32.6 | 3.05 |
| Topsin-M | 35.6 | 3.5 | 35.6 | 3.1 |
| Carbofuran | 37.6 | 3.5 | 27.4 | 3.8 |
| <i>Stokeyia indica</i> | 46.3 | 4.5 | 41.2 | 4.6 |
| <i>Halimeda tuna</i> | 42.8 | 4.1 | 35.5 | 3.6 |
| <i>Dictyota indica</i> | 44.9 | 3.8 | 35.9 | 3.9 |
| <i>Padina tetrastromatica</i> | 40.4 | 4.3 | 34.6 | 3.5 |
| <i>Sargassum swartzii</i> | 41.7 | 4.3 | 33.2 | 4.3 |
| <i>Stoehospermum marginatum</i> | 39.9 | 4.0 | 42.9 | 4.0 |
| <i>Solieria robusta</i> | 43.4 | 4.4 | 33.1 | 4.6 |
| LSD _{0.05} | 14.7 ¹ | 0.8 ¹ | 10.1 ¹ | 1.0 ¹ |

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

Tab. 3: Effect of seaweeds on the infection of root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum* and *Meloidogyne javanica* on soybean roots in field experiment.

| Treatments | <i>M. phaseolina</i> | | <i>R. solani</i> | | <i>F. solani</i> | | <i>F. oxysporum</i> | | No. of knots | | Females/Juveniles per gram roots | |
|---------------------------------|---|---------|------------------|---------|------------------|---------|---------------------|---------|--------------|---------|----------------------------------|-----------------|
| | 30 Days | 60 Days | 30 Days | 60 Days | 30 days | 60 Days | 30 days | 60 Days | 30 Days | 60 Days | 30 Days | 60 Days |
| Control | 0 | 25 | 62.5 | 87.5 | 25 | 75 | 43.7 | 18.7 | 0.81 | 18 | 6.8 | 165 |
| Topsin-M | 0 | 0 | 12.5 | 62.5 | 0 | 25 | 0 | 6.2 | 1.1 | 11 | 0.5 | 77 |
| Carbofuran | 0 | 0 | 0 | 18.7 | 0 | 6.2 | 0 | 0 | 1.8 | 17 | 1.1 | 87 |
| <i>Stokeyia indica</i> | 0 | 12.5 | 31.2 | 56.2 | 12.5 | 25 | 25 | 6.2 | 0.62 | 12 | 6.7 | 30 |
| <i>Halimeda tuna</i> | 0 | 0 | 18.7 | 50 | 18.7 | 12.5 | 6.2 | 0 | 0.43 | 13 | 4.7 | 70 |
| <i>Dictyota indica</i> | 0 | 6.2 | 6.2 | 62.5 | 0 | 18.7 | 6.2 | 6.2 | 1.1 | 11 | 6.7 | 35 |
| <i>Padina tetrastromatica</i> | 0 | 50 | 12.5 | 56.2 | 0 | 56.2 | 0 | 12.5 | 1.0 | 10 | 3.8 | 120 |
| <i>Sargassum swartzii</i> | 0 | 31.2 | 25 | 50 | 0 | 43.7 | 18.7 | 12.5 | 0.43 | 7 | 1.3 | 37 |
| <i>Stoehospermum marginatum</i> | 0 | 12.5 | 18.7 | 18.7 | 0 | 31.2 | 0 | 0 | 0.72 | 9 | 1.3 | 25 |
| <i>Solieria robusta</i> | 0 | 0 | 18.7 | 25 | 6.2 | 31.2 | 0 | 0 | 0.48 | 8 | 0.5 | 42 |
| | Treatments= 14.4 ¹ Pathogens= 9.1 ¹ | | | | | | | | ns | ns | ns | 93 ¹ |

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

²Mean values in rows for fungi showing differences greater than LSD values are significantly different at $p < 0.05$.

Stoechospermum marginatum caused maximum reduction in nematode infection by reducing nematodes penetration in roots followed by *S. indica* (Tab. 3). Greater root weight was produced by *S. indica*. Highest increase in PGPR population was achieved by the application of *Padina tetrastromatica* (Tab. 4). Greater plant height and fresh shoot weight were produced by *H. tuna*, whereas maximum root length was resulted by the application of *S. robusta* followed by *P. tetrastromatica* (Tab. 4).

Pepper

After 45 days, application of seaweeds *Sargassum binderi*, *S. tenerrimum*, *Halimeda tuna*, *Stoechospermum marginatum*, *Padina tetrastromatica* and *Stoekyia indica* caused complete suppression

of root rotting fungi *M. phaseolina* and *R. solani*. Highest reduction in *F. solani* infection was achieved by the application of *Stoekyia indica* and *Stoechospermum marginatum* (Tab. 5). Seaweeds also caused significant suppressive effect on root knot nematode. Highest reduction in root galls was caused by *Solieria robusta* followed by topsin-M (Tab. 5). *Halimeda tuna* also significantly reduced gall formation on roots as compared to control. Incidence of roots associated fluorescent pseudomonads did not show any correlation with diseases suppression (Tab. 6). In many cases, increased population of fluorescent *Pseudomonas* in the rhizosphere did not cause greater reduction of root rotting fungi and root knot nematode as compared to plants having less population of fluorescent *Pseudomonas* in the rhizosphere. Greater plant height was achieved by the application of *Stoekyia indica*, while *S. robusta*

Tab. 4: Effect of seaweeds on the growth of soybean and population of rhizospheric fluorescent *Pseudomonas* (PGPR) in field experiment.

| Treatments | Plant height (cm) | | Fresh shoot weight (g) | | Root length (cm) | | Fresh root weight (g) | | PGPR (X1000) | |
|----------------------------------|-------------------|-------------------|------------------------|---------|------------------|------------------|-----------------------|------------------|------------------|------------------|
| | 30 Days | 60 Days | 30 Days | 60 Days | 30 days | 60 days | 30 days | 60 Days | 30 Days | 60 Days |
| Control | 12.6 | 26.4 | 2.0 | 11.1 | 6.8 | 6.7 | 0.39 | 1.06 | 41 | 497 |
| Topsin-M | 13.9 | 34.4 | 2.4 | 14.7 | 7.3 | 12.9 | 0.44 | 1.2 | 50 | 256 |
| Carbofuran | 14.4 | 38.0 | 3.0 | 18.0 | 6.5 | 13.0 | 0.40 | 1.6 | 93 | 111 |
| <i>Stoekyia indica</i> | 15.4 | 30.1 | 3.1 | 14.4 | 6.3 | 14.3 | 0.38 | 3.5 | 49 | 627 |
| <i>Malimeda tuna</i> | 12.0 | 49.7 | 2.9 | 23.4 | 7.5 | 12.9 | 0.57 | 1.3 | 250 | 667 |
| <i>Dictyota indica</i> | 14.2 | 36.2 | 2.7 | 13.5 | 7.1 | 13.2 | 0.38 | 1.1 | 306 | 496 |
| <i>Padina tetrastromatica</i> | 14.1 | 31.8 | 2.9 | 11.2 | 6.5 | 14.4 | 0.37 | 1.0 | 200 | 687 |
| <i>Sargassum swartzii</i> | 16.0 | 31.6 | 2.5 | 11.0 | 6.7 | 10.6 | 0.39 | 0.9 | 187 | 455 |
| <i>Stoechospermum marginatum</i> | 15.9 | 35.5 | 3.2 | 12.1 | 7.1 | 12.9 | 0.40 | 0.9 | 113 | 647 |
| <i>Solieria robusta</i> | 17.6 | 35.9 | 2.9 | 13.9 | 6.8 | 15.8 | 0.8 | 1.2 | 82 | 377 |
| LSD _{0.05} | 4.6 ¹ | 10.3 ¹ | 1.3 ¹ | ns | 1.4 ¹ | 3.9 ¹ | 0.7 ¹ | 2.6 ¹ | 195 ¹ | 365 ¹ |

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

Tab. 5: Effect of seaweeds on the infection of root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and root knot nematode *Meloidogyne javanica* on pepper roots in field experiment.

| Treatments | <i>M. phaseolina</i> | | <i>R. solani</i> | | <i>F. solani</i> | | No. of knots | | Females/Juveniles per gram roots | |
|----------------------------------|-------------------------------|---------|-------------------------------------|---------|------------------|---------|-------------------|-------------------|----------------------------------|-------------------|
| | 45 Days | 90 Days | 45 Days | 90 Days | 45 days | 90 Days | 45Days | 90 Days | 45 Days | 90 Days |
| Control | 45 | 25 | 31.2 | 25 | 62.5 | 31.2 | 38.5 | 18 | 23 | 77.5 |
| Topsin-M | 0 | 0 | 6.2 | 0 | 37.5 | 0 | 9.0 | 11 | 8.5 | 52.5 |
| Carbofuran | 0 | 6.2 | 0 | 0 | 12.5 | 6.2 | 17.3 | 17 | 15.2 | 24.5 |
| <i>Sargassum binnderi</i> | 0 | 6.2 | 0 | 0 | 18.7 | 6.2 | 26.1 | 12 | 16.7 | 45.0 |
| <i>S.tenerrimum</i> | 0 | 6.2 | 0 | 6.2 | 18.7 | 6.2 | 29.4 | 13 | 13.7 | 49.2 |
| <i>Halimeda tuna</i> | 0 | 12.5 | 0 | 6.2 | 25 | 0 | 13.0 | 11 | 15.7 | 32.0 |
| <i>Stoechospermum marginatum</i> | 0 | 0 | 0 | 0 | 6.2 | 0 | 26.7 | 10 | 13.2 | 24.2 |
| <i>Padina tetrastromaticai</i> | 0 | 0 | 0 | 0 | 25 | 0 | 13.6 | 7 | 17.5 | 39.5 |
| <i>Stoekyia indica</i> | 0 | 6.2 | 0 | 0 | 6.2 | 6.2 | 18.4 | 9 | 11.2 | 73.2 |
| <i>Solieria robusta</i> | 6.2 | 0 | 0 | 0 | 18.7 | 0 | 8.4 | 8 | 6.5 | 22.2 |
| LSD _{0.05} | Treatments = 6.7 ¹ | | Fungal pathogens = 3.7 ² | | | | 23.1 ¹ | 14.3 ¹ | 11.3 ¹ | 41.8 ¹ |

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

²Mean values in rows for fungi showing differences greater than LSD values are significantly different at p< 0.05.

Tab. 6: Effect of seaweeds on the growth of pepper and population of rhizospheric fluorescent *Pseudomonas* (PGPR) in field experiment.

| Treatments | Plant height (cm) | | Fresh shoot weight (g) | | Root length (cm) | | Fresh root weight (g) | | PGPR (X1000) | |
|----------------------------------|-------------------|-------------------|------------------------|-------------------|------------------|------------------|-----------------------|------------------|-------------------|---------|
| | 45 Days | 90 Days | 45 Days | 90 Days | 45 days | 90 days | 45 days | 90 Days | 45 Days | 90 Days |
| Control | 27.5 | 43.9 | 12.9 | 32.0 | 9.4 | 20.2 | 4.0 | 4.8 | 29.5 | 3.0 |
| Topsin-M | 30.5 | 51.0 | 16.2 | 43.4 | 12.4 | 20.5 | 2.8 | 5.8 | 46.4 | 4.2 |
| Carbofuran | 28.8 | 48.0 | 15.5 | 35.6 | 21.5 | 20.2 | 2.8 | 4.5 | 18.9 | 5.7 |
| <i>Sargassum binnderi</i> | 27.5 | 53.1 | 14.6 | 36.5 | 10.9 | 19.2 | 2.2 | 5.1 | 44.6 | 53.6 |
| <i>S.tenerrimum</i> | 31.4 | 45.3 | 18.0 | 23.7 | 36.3 | 17.9 | 3.1 | 3.9 | 61.7 | 37.0 |
| <i>Halimeda tuna</i> | 25.8 | 44.8 | 14.1 | 30.3 | 13.4 | 18.3 | 2.1 | 2.9 | 36.1 | 21.6 |
| <i>Stoechospermum marginatum</i> | 29.4 | 39.2 | 11.6 | 21.6 | 33.9 | 35.9 | 2.5 | 2.7 | 12.9 | 3.0 |
| <i>Padina tetrastromaticai</i> | 29.5 | 45.3 | 13.8 | 42.1 | 17.7 | 19.6 | 3.0 | 4.5 | 87.0 | 4.5 |
| <i>Stoekyia indica</i> | 32.3 | 59.5 | 21.6 | 57.4 | 12.6 | 22.1 | 3.7 | 6.8 | 59.0 | 8.3 |
| <i>Solieria robusta</i> | 29.6 | 61.6 | 25.5 | 52.2 | 14.2 | 19.5 | 2.7 | 4.3 | 95.2 | 15.4 |
| LSD _{0.05} | 6.3 ¹ | 15.0 ¹ | 10.5 ¹ | 24.8 ¹ | 5.1 ¹ | 5.6 ¹ | 1.6 ¹ | 3.3 ¹ | 51.7 ¹ | ns |

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

produced maximum fresh shoot weight while *Stoechospermum marginatum* produced maximum root length (Tab. 6).

Seaweed amendment application was still found effective after 90 days in suppressing the *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* infection (Tab. 5). *Solieria robusta*, *Padina tetrastromatica* and *Stoechospermum marginatum* completely controlled the *M. phaseolina*, *R. solani* and *F. solani* infection on pepper roots (Tab. 5). *Solieria robusta* and *S. marginatum* significantly ($p < 0.05$) reduced gall formation and penetration of nematodes in roots (Tab. 5). Population of fluorescent pseudomonads in the pepper rhizosphere did not correlate with disease suppression (Tab. 6). Greater plant height was observed for *S. robusta* followed by *S. indica*. The highest fresh shoot weight was produced by using *Stoekyia indica* followed by *Solieria robusta*. Greater root length was found in plants grown in *S. marginatum* amended soil (Tab. 6).

Discussion

Control of root-infecting fungi using antagonistic plants and phytochemicals offers an alternate strategy to the prevalent use of synthetic pesticides. A wide variety of plant materials have been found effective against plant parasitic fungi associated with crop plants (EHTESHAMUL-HAQUE et al., 1995; 1996; MANSOOR et al., 2007; MAZZOLA, 2004). Plant pathogenic fungi and plant parasitic nematodes are serious threat to modern agriculture and important limiting factor of low yield of most of the field crops. Marine biosphere is an untapped reservoir of agrochemically potent compounds. In this study, seaweeds like *Sargassum swartzii*, *Solieria robusta*, *Stoechospermum marginatum*, *Halimeda tuna*, and *Stoekyia indica* showed more or less similar suppressive effect on root rotting fungi and root knot nematode like chemical fungicides (topsin-M) and nematicide (carbofuran). Considerable evidence has been accumulated in recent years to support and identify the benefits associated with the use of seaweed in crop production systems. Seaweed extracts have been reported to increase plant resistant to pests and diseases, plant growth, yield and quality (YVIN et al., 1989; JOLVET et al., 1991; VERKLEIJ, 1992). Application of seaweed to plants can result in decreased levels of

nematode attack (ARA et al., 1997; WU et al., 1997; 1998). Seaweeds contain elaborate secondary metabolites that play a significant role in the defense of the host against predators and parasites which offer a potential novel approach to control population of plant parasitic nematodes (JACOBS et al., 2003; PARACER et al., 1987).

In this study, application of some seaweed significantly increased rhizosphere population of fluorescent *Pseudomonas* (PGPR) on soybean and pepper. PGPR have been reported to improve plant growth either through direct stimulation or by suppression of pathogens (RAAJMAKERS and WELLER, 1998; WELLER et al., 2002). Of the various rhizosphere bacteria, fluorescent Pseudomonads are aggressive colonizers of the rhizosphere of various crop plants and have broad spectrum antagonistic activity against plant pathogens (PARVEEN et al., 1998; WELLER et al. 2002). Species of *Pseudomonas* are also reported to induce systemic resistance in plants against invading pathogens (DE MEYER et al., 1999; ZHOU and PAULITZ, 1994). These characteristics make these species good candidates to use for biocontrol against soil-borne plant pathogens (DE MEYER et al., 1999; PARVEEN et al., 1998; WELLER et al., 2002). *Pseudomonas aeruginosa* has been reported to suppress root knot infection on pepper, water melon, guar, pumpkin (PARVEEN et al., 1998), okra (ARA et al., 1997) and tomato (SIDDIQUI and EHTESHAMUL-HAQUE, 2001).

The increased population of PGPR in this study however did not correlate with disease suppression. The seaweed could affect cell metabolism through the induction of the synthesis of antioxidant molecules which could favor plant growth and plant resistant to stress (ZHANG and SCHMIDT, 2000). Anti-oxidants enzymes provide a degree of crop protection from free radical oxidants arising from normal metabolism and any number of biotic and abiotic stresses. WU et al., (1997) demonstrated that the betaines present in different extract decreased the nematode infestation. Presumably, besides stimulating the population of PGPR in the rhizosphere, seaweeds suppressed the root rotting fungi and root knot nematode via producing antimicrobial compounds or synthesis of antioxidant molecules. Seaweeds contain 1-aminocyclopropane-1-carboxylic acid (ACC) which has antimicrobial activity (NELSON and VAN STANDEN, 1985). Similarly polyphenols are well known for their antioxidant activity are widely distributed in seaweeds (TARIQ et al., 2011).

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