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EVALUATION OF SYSTEMIC DEFENSE RESPONSES IN SOYBEAN INDUCED BY *SARGASSUM ILICIFOLIUM* AND ENDOPHYTIC *PSEUDOMONAS AERUGINOSA* AGAINST ROOT KNOT NEMATODE

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ABSTRACT

The impact of brown seaweed S*argassum ilicifolium* with or without endophytic biocontrol agent *Pseudomonas aeruginosa* were tested for assessing induced systemic resistance in soybean against the root knot nematode (*Meloidogyne javanica)*in screen house experiment. Combination of *S. ilicifolium*with endophytic *P. aeruginosa* effectively reduced the nematode populations, gall formation and also promoted growth parameters (shoot length, root length, shoot weight and root weight) of *M. javanica* infected plants. Alone and combined treatment of biocontrol agent *P. aeruginosa* with amendment of seaweed led to induced resistance reactions against root knot nematode as a result of boost up the antioxidant DPPH free radical scavenging activity, scavenging % of H_2O_2 with enhanced production of signal transduction molecules like phenolic contents and salicylic acid. However highest ABTS radical scavenging activity of soybean plants were observed in mixed treatment of both with excluding of standard BHT. It is supposed that the enhanced enzymatic (DPPH scavenging activity, H_2O_2 scavenging %, ABTS scavenging activity) and non-enzymatic antioxidant (phenolic contents and salicylic acid) activities may be either directly or indirectly involved in the induction of systemic resistance through reduction of root knot nematode in soybean by organic amendment and endophytic biocontrol *P. aeruginosa.*

Keywords: *Pseudomonas aeruginosa*, induced systemic resistance, *Sargassum ilicifolium*, root knot nematode, soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is considered the most economically important and nutritionally significant oilseed crop which greatly cultivated in all over the world (Bruinsma and Antoniolli, 2015)**.** *Meloidogyne incognita* and *Meloidogyne javanica* are major threat to soybean which reduces the production of crops. Worldwide annually 12% severe reduction of various cash crops observed by most destructive group of parasitic root knot nematodes i.e. *Meloidogyne*s pecies (Barker and Koening, 1998). The control of root-knot nematode pest is more complicated because of unavailability of registered nematicides and lacking of genetic host plant resistance (Fourie *et al.,* 2015). The excessive and unsystematic apply of commercial fertilizers and chemical pesticides to control the parasitic root-knot nematodes have revealed harmful ecological consequences and produce direct detrimental effects on living organisms (Rizvi *et al.,* 2012). Globally

numerous investigators are in the pursuit of developing standards pertaining to the root-knot nematode approach by nonchemical and eco-friendly substitutes like soil management, organic amendments, fertilization, biological control agent to stabilize crop production (Collange *et al.,* 2011). Recent studies emphasized the use of safe environmental and easily biodegradable pest control agents through plant derivation. In instance, a number of plant organic compost i.e. different plant parts, oily seed cakes, seaweeds, etc. have been utilized to advance the growth, yield and quality of crops (Rahman *et al.,* 2016 and 2017; Pise and Sabale, 2010) and as well as to induce nematode control strategy (Zaki *et al*., 2005; Ibrahim *et al*., 2007).

Marine algae particularly brown seaweeds are acknowledged to contain a wide range of unique biocidal compounds which act as a fungicidal, insecticidal and nematicidal agent with addition to high nutritional product. Ganapathi *et al.,* (2013) acknowledged that *Sargassum ilicifolium* is good source of carbohydrates, proteins, lipids, vitamins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), amino acids and important potential elicitors which induced resistance against biotic and abiotic stress. Sbaihat*et al.* (2015) confirmed that brown algae occurring as a source of potential elicitors to induce defense resistance and serve as natural plant protectants. Extract of brown algae like *Laminaria digitata, Sargassum fusiforme* was exposed to inducing several defense responses due to the presence of phytochemical β-1,3-glucan laminar in (Klarzynski *et al.,* 2000). Fernando *et al.,* (2016) isolated the universal phenolic compounds from marine algae which exhibited beneficial biological properties, including antioxidant, anticancer, antimicrobial and anti-inflammatory along with several other bioactivities centered on their antioxidant properties.

Nowadays, research on the employ of endophytic antagonistic microbes to manage plant parasitic nematodes is getting gradually greater attention (Hallman *et al.,* 2009). Several workers demonstrated that there are numbers of beneficial plant growth promoting rhizobacteria specially *Pseudomonas* species that enhanced plant productivity as hostile invaders and have a wide range of antagonistic activity against fungal diseases (Siddiqui *et al.,* 2001) along with a nematode infestation (Siddiqui and Mahmood, 1999).

This research reports the effect of seaweed usage as an amendment with biocontrol activity of endophytic bacteria improves plant growth by elaborating key metabolites of soybean to induce defense responses against root rot nematode.

MATERIAL AND METHODS

BACTERIAL STRAIN

Bacterial culture of *Pseudomonas aeruginosa* (ABPL-251) was received from Karachi University Culture Collection (KUCC) and multiplied on Potato Dextrose Agar Medium. Ehteshamul-Haque *et al.* (2007) had formerly examined endophytic *P. aeruginosa* which exposed as a potent nematicidal activity.

NEMATODE CULTURE AND INOCULATION

Susceptible egg plants were grown in earthen pots to achieve egg masses of parasitic root knot nematode (*Meloidogyne javanica*) for inoculation of soybean plant to generate a biotic stress. After 7-8 weeks of infection, some infected roots of eggplant were collected, washed with tap water, and then dissected under the stereomicroscope to verify the presence of nematode population. The infected roots were sliced into small pieces and put into 1L measuring cylinder containing 200 mL (0.5%) sodium hypochlorite solution

(Robin bleach) at the ratio of 1:40. Capped the mouth of cylinder tight and was shaken vigorously up to 10 min until to dissolve the gelatinous matrix. Nematode eggs were collected in water after sieving through a 400 mesh and then washed with distilled water (Bem *et al.,* 2014). Eggs were calculated by observing serial dilutions with a light microscope (Corbett *et al.,* 2011).

Screen House Experiment

Soybean plants assigned to the *M. javanica* stress. Dry powder of *S. ilicifolium* (1% w/w) was added in 1 kg sandy loam soil per pot for soil amendment. Six seeds per pot of soybean were sown and randomized on screen house with four replicates.

P. aeruginosa strain maintained on Kings's B medium at 28 ºC and 25 mL aqueous suspension contained $8x10^8$ cells mL^{-1} was poured in each pot for inoculation. Population of root knot nematode *M. javanica* was obtained from infected eggplant and 5,000 eggs per pot were inoculated around the root zone by making holes in a soil after 3-5 leave stage. The clay pots were kept on bench in screen house and watered regularly to uphold 50% W.H.C. Pots without organic amendment, antagonistic agent and nematode inoculation considered as control. The shoot length, shoot weight, root length and root weight of fresh plant were measured following 30 days of treatments.

ANALYSIS OF DATA

The screen house experiment was finished after 30 days at Agricultural Biotechnology and Phytopathology Laboratory, Department of Botany, University of Karachi. Observation on plant growth parameters and secondary metabolites specifically phenolic contents and salicylic acid were scrutinized with one-way analysis of variance and the group means were assessed by Duncan's Multiple Range Test at probability level of $P<0.05$. Antioxidant activities were examined by Two-way analysis of variance to sort out effective treatments (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The present result showed that the combined treatment of *S. ilicifolium* and *P. aeruginosa* significantly ($p<0.05$) encouraged the vegetative growth of soybean plant. The infection of root knot nematode *M. javanica* drastically reduced the growth parameters in plant comparison to untreated control. The maximum improvement in shoot length, shoot weight, root length and root weight was observed with the combination of *S. ilicifolium* and *P. aeruginosa* under nematode infection of *M. javanica.* Santoyo *et al.* (2016) and Rahman *et al*, (2017) reported that when plants were inoculated with bacterial endophytes capable of producing IAA which are responsible for increasing plant height and biomass. According to Ehteshamul-Haque *et al.* (2013) and Bokhari *et al.* (2014) the fluorescent *Pseudomonas, P. aeruginosa,* was found to reduce several soilborne pathogens on several crops and improved plant growth by a number of antimicrobial by-products i.e. organic acids, hydrogen sulfide, phenols, tannins and nitrogenous compounds are discharged during the decomposition of organic amendments, or produced through micro-organisms that engaged in such degradation. In previous research, scientists reported the suppression of root knot nematode and root rotting fungi on chili, sunflower, tomato, soybean (Sultana *et al.,* 2007; 2008; 2011a,b) eggplant and watermelon (Baloch *et al.,* 2013) by the utilization of seaweeds as soil organic amendment. Khan *et al.* (2015) examined the nematicidal activity of 32 seaweeds and

recorded the highest larval mortality (99%) by using the extract of *Sargasssum tenerrimum.*

Table 1. Effect of *Sargassum ilicifolium* amendment and *Pseudomonas aeruginosa* on growth parameters of soybean plant.

Treatments	**Growth Parameters**				
	Shoot length (cm)	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**	**No. of Knots /root**
Control	15.4	2.92	15.3	0.56	15
Sargassum ilicifolium	19.5	4.32	17.8	1.08	10
Pseudomonas aeruginosa	19.1	4.52	18.6	0.60	9
S. ilicifolium + P. aeruginosa	21.9	4.66	19.9	0.96	6
Meloidogynejavanica	14.8	1.95	11.9	0.69	23
S. ilicifolium + M. javanica	15.6	2.87	13.1	0.66	12
P. aeruginosa + M. javanica	16.0	3.06	14.5	0.62	10
S. ilicifolium + P. aeruginosa + M. javanica	16.7	3.06	15.5	0.66	7
$LSD_{0.05}$	**1.33[1]**	**1.18[1]**	**1.64[1]**	**0.25[1]**	**2.63[1]**

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

Table 2. Effect of *Sargassum ilicifolium* amendment and *Pseudomonas aeruginosa* on DPPH free radical scavenging activity, scavenging % of H_2O_2 and phenolic contents.

Treatments	**Antioxidant activity Inhibition %**		**Scavenging % of H_2O_2**	**Phenolic contents mg mL^{-1}gallic acid**
	0 min	**30 min**		
Standard (BHT)	70.4	77.2	—	—
Control	20	15.6	21.3	3.25
Sargassum ilicifolium	25	19	25.3	3.50
Pseudomonas aeruginosa	23	19.8	27.4	3.65
S. ilicifolium + P. aeruginosa	28	18	29.2	4.37
Meloidogyne javanica	18.6	13	13.6	3.38
S. ilicifolium + M. javanica	25	17.6	21.4	2.90
P. aeruginosa + M. javanica	20.3	19.6	18.7	3.36
S. ilicifolium + P. aeruginosa + M. javanica	21.3	19	22.2	3.30
$LSD_{0.05}$				
Treatment[1]	**2.43[1]**		**3.81[1]**	**0.54[1]**
Time[2]	**1.14[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 3. ABTS radical scavenging activity of soybean plant with combined effect of *Sargassum ilicifolium* and *Pseudomonas aeruginosa.*

Treatments	ABTS Assay %					
	0 min	**1 min**	**2 min**	**3 min**	**4 min**	**5 min**
Standard (BHT)	70.4	72.4	73	73.6	74.4	74.7
Control	49.6	52	54.6	57	59	61.6
Sargassum ilicifolium	55	56.6	57.3	63.6	64	69
Pseudomonas aeruginosa	48.3	50	52	56.6	59	60.3
S. ilicifolium + P. aeruginosa	55	58.3	62.3	65.6	68.3	72.6
Meloidogyne javanica	47	50.3	51.3	56.3	59	63
S. ilicifolium + M. javanica	49.6	51.6	54.6	59	61.3	65.6
P. aeruginosa + M. javanica	53.6	54.6	55	59.3	62	67
S. ilicifolium + P. aeruginosa + M. javanica	51.6	56	58.3	62.6	66.3	69.3
$LSD_{0.05}$	**Treatments[1] = 0.90[1]**				**Time[2] = 0.74[2]**	

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

Table 4. Effect of *Sargassumilicifolium*along with *Pseudomonas aeruginosa* on the salicylic acid and proline contents of soybean plants under screen house.

Treatments	Salicylic acid	Proline contents
	µg g^{-1}	**µmoles g^{-1} DM**
Control	2.8	4.44
Sargassumilicifolium	4.3	2.59
Pseudomonas aeruginosa	5.2	2.92
S. ilicifolium+ P. aeruginosa	6.1	2.34
Meloidogynejavanica	2.5	5.54
S. ilicifolium+ M. javanica	3.7	3.87
P. aeruginosa + M. javanica	5.2	3.39
S. ilicifolium+ P. aeruginosa + M. javanica	7.3	3.23
$LSD_{0.05}$	**1.13[1]**	**0.81[1]**

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

In this research application of *S. ilicifolium* and *P. aeruginosa* significantly ($p<0.05$) suppressed nematode infectivity with reducing the no. of knots / root in comparison to healthy control. Individual treatment of *S. ilicifolium* and *P. aeruginosa* also found effective to decrease no. of knots in the existence of parasitic root knot nematodes *M. javanica* when compared to untreated control (Table 1). Seaweeds contain complicated secondary metabolites that play an important role in the defense mechanism of the host plant against predators and parasites (Ara *et al.,* 2005). Ashoub and Amara (2010) demonstrated that fluorescent pseudomonads have ability to produce a lot of toxic secondary metabolites (indoles compounds, phenazines, phenylpyrroles and pterines) to kill *M. incognita* juveniles. Siddiqui and Shaukat (2002) also concluded that *P. aeruginosa* and seaweed application result in decreased penetration of *M. javanica* in tomato and okra plants.

Phenolic compounds including salicylic acid play significant roles in lignin biosynthesis, proceed as allelopathic compounds and control plant responses to abiotic and pathogen attacks (Vlot *et al.,* 2009). In the present study, the total phenolic contents were significantly ($p<0.05$) enhanced by provided the treatment of *S. ilicifolium* and *P. aeruginosa* to inhibit the infection of parasitic root knot nematode *M. javanica*. Comparable to *M. javanica*, separate application of *S. ilicifolium* and *P. aeruginosa* was also responsible for accumulation of higher phenolic contents to provide defense under infection of root knot nematode (Table 2). Our results are supported by Akram *et al.,* (2013) who found that a significant increase in total phenolic contents was observed in bacterial treated plants. Salicylic acid (SA) is considered to be a plant signal molecule and involved in induction of SAR, which activates many defense compounds (Serghini *et al.,* 2001). Result illustrated that the dual application of *S. ilicifolium* and *P. aeruginosa* significantly ($p<0.05$) improved the synthesis of salicylic acid in *M. javanica* infected plant and (uninfected) healthy plant. Salicylic acid accumulation more than on the healthy plants, indicating induction of physiological immunity in infected plants (Table 4). In this regard Ueno *et al.,* (2011) accounted that exogenous application of SA and IAA has been exhibited to improved plant resistance against pathogens by performing as potent inducer of systemic resistance.

Phenols act as free radical scavengers as well as substrates for many antioxidant enzymes (Martin-Tanguy, 2001). Plant amended with *S. ilicifolium* and inoculated with *P. aeruginosa* showed highest scavenging activity of H_2O_2 as followed by separate treatment of *P. aeruginosa* and *S. ilicifolium* in comparison to healthy and unhealthy control (Table 2). Borden and Higgins, (2002); Mellersh *et al.,* (2002) suggested that the high generation of ROS, especially H_2O_2 as a result of pathogens infection appears to be an important element of disease-resistant mechanisms. The reduction of DPPH-free radicals was achieved with combined use of algal amendment of *S. ilicifolium* and *P. aeruginosa* initially at 0 minute and decreased at 30 minutes (Table 2). However, the best results of ABTS scavenging activity was obtained at 0 to 5 minute in *S. ilicifolium* and *P. aeruginosa*treatment contrasted to untreated control or infected control. Moreover, all treated plant showed increasing significant inhibition % as compared to control (Table 3). These antioxidant enzymes scavenged the free radical produced by nematode invasion and protecting the plant against the stress of the nematode. Thompson, (2004) reported the same finding that the seaweed boosted the plant immune system and enhances plant's capability to resist the abiotic and biotic stresses (Santaniello *et al.,* 2017). These consequences are in agreement of Baxter *et al.* (2014) who found that seaweed application might assist in stimulating the enzymatic and non-enzymatic antioxidative

systems of plant which play a vital role in plant defense to aggravate reactive oxygen species (ROS) leading nematode infection. Furthermore, Kella *et al*. (2017) stated that the assay of defensive enzymes in the plants treated with endophytic PGPR as bio-fertilizations encouraged a greater amount of catalase, peroxidase and polyphenol oxidase in comparison with the healthy plants.

Proline accumulation is a common metabolic response to both abiotic and biotic stress and when higher plants are exposed to stress; many plants accumulate high amounts of proline in tissues (Mazid *et al.,* 2011). Our investigation demonstrated that under infection of soil parasitic nematode *M. javanica*, the amount of total proline contents were significantly increased. It was revealed that to fight against soil parasitic nematode *S. ilicifolium* and *P. aeruginosa* alone and together notably reduced proline contents as contrasted to the infected plant (Table 4). Al-Wakeel *et al.* (2013) also found the same results that nematode infected plants illustrated a pronounced increase in the levels of proline as compared with the non-infected healthy plants.

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