BIOCONTROL POTENTIAL OF ENDOPHYTIC *PSEUDOMONAS AERUGINOSA* AND BROWN SEAWEED ENHANCES THE PLANT GROWTH AND ACTIVITY OF ANTIOXIDANT DEFENSIVE ENZYMES IN *GLYCINE MAX* AGAINST *MACROPHOMINA PHASEOLINA*

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ABSTRACT

The efficacy of *Pseudomonas aeruginosa* and *Sargassum ilicifolium* alone or in combination was examined to induce systemic resistance against a pathogenic strain *of Macrophomina phaseolina* in soybean under screen house condition. Incorporation of *S. ilicifolium* better accomplish the nutrient of host plant and significantly ($p < 0.05$) improved the plant growth parameters as well as in combination with *P. aeruginosa* the pathogenicity of root rotting fungi was significantly ($p<0.05$) suppressed when compared with control treatment. Our results showed that salicylic acid is interrelated signaling molecule that induced defense mechanism when accumulated (7.6 µg g^{-1})in disease plants by application of both treatments. Similar results were also obtained in total phenolic content. DPPH assay was evaluated at 0 min and 30 min., initially antiradical scavenging activity of plant samples boost up at 0 min but gradually declined after 30 min. Highest inhibition % of DPPH radical was noticed in healthy plant by combined treatment (31.6 %) but *S. ilicifolium* amendment also significantly ($p < 0.05$) scavenged free radical (30.3%) produced in disease plant. A potential scavenging activity evaluated by H_2O_2 inhibition is 25.4% while, in ABTS assay 74% was recorded in the same treatment of *S. ilicifolium* + *P. aeruginosa* + *M. phaseolina* with respect to infected plant of *M. phaseolina* (12.2 % inhibition and 61.6 %). Both combinations were able to protect soybean plants against charcoal rot disease compared with check treatment. Indeed, *S. ilicifolium* and *P. aeruginosa* have been shown to induce resistance mechanism in plant by enhanced antioxidants scavenging potential of ROS outburst and detoxify the pathogenicity of *M. phaseolina* by accumulation of secondary metabolites.

Keywords: Endophytic bacteria, *Pseudomonas aeruginosa*, brown seaweed, plant growth, antioxidant defensive enzyme, *Glycine max*, *Macrophomina phaseolina*.

INTRODUCTION

Biological control is a chemical-less potential method for management of plant disease via suppressing the destructive consequences of pathogen by the use of beneficial microbes (Vanitha and Ramjegathesh, 2014).Plant growth promoting bacteria(PGPR) act as biocontrol agent by improving plant performance under stress environments directly by facilitating resource acquisition, production of siderophore (Jahanian *et al*., 2012; Tian *et al*., 2009)improving nitrogen uptake and solubilization of phosphate (Ahemad and Khan, 2012a), modulating phytohormones levels (Tank and Saraf, 2010) and obliquely via suppressing the inhibitory effects of different pathogenic agents on plant growth and development (Ahemad and Khan, 2012b). Different strains of *Pseudomonas aeruginosa* have potential to advance plant growth and development in several crops including soybean (Ehteshamul-Haque *et al*., 1996). Numerous studies have been approved to elicit induced systemic resistance (ISR) by rhizobacterial components such as cyclic lipopeptides 2,4-diacetylphloroglucinol, lipopolysaccharides (LPS), siderophores, flagella, homoserine lactones, volatiles like 2,3-butanediol (2,3-BD) and acetoin (Lugtenberg and Kamilova, 2009) in plants. It was reported that inoculation of *Pseudomonas* spp. systemically restricted plants against *Fusarium* wilt caused by *Fusarium oxysporum* (Ramamoorthy *et al.*, 2001; Van Peer *et al.*, 1991).

Phenolic compounds were documented to put forth their control on physiological and biochemical processes (Hayat *et al.*, 2007). Enhanced polyphenol synthesis and accumulation is the universal comeback of the plant toward different biotic/abiotic stresses (Naczk and Shahidi, 2004). Plants developed defense antioxidative mechanisms to combat the danger posed by reactive oxygen species (ROS) (Asada, 2006;Halliwell, 2006).Salicylic acid frequently enhanced and stimulates the expression of pathogenesis related proteins and begins the advancement of systemic acquired resistance and hypersensitive response (Gharib, 2007). In tobacco plants, linear β1,3-glucan laminarin derived from brown algae elicits a variety of defense reactions such as the stimulation of phenylalanine ammonia

lyase, caffeic acid O-methyl transferase, and lipoxygenase activities, as well as the accumulation of salicylic acid and PR proteins (Klarzynski *et al.,* 2000).Soybean occupies a leading role at the worldwide scale among different oil crops. Soybean plants are exposed to assault by numerous viral diseases, fungal infections and bacterial growth by the invasion of the different pathogens phytotoxins are synthesized in plant and about 20-46% production of soybean yield losses **(**Hamid *et al.,* 2014; Fawzy*et al.,* 2011). *Macrophomina phaseolina* (Tassi) Goid. is one of most wide spread soilborne fungus that causes charcoal rot on soybean leads to early maturation, chlorosis and incomplete pod filling finally reduced the crop production and yield (Partridge, 2003).

Seaweeds are widely recognized as a food ingredient, good biofertilizer with an extensive beneficial effect like, enhanced plant productivity, and improved resistance capability to biotic or abiotic stress. Rahman *et al.*(2017) experimented that amended soil with *Sargassum ilicifolium* and *P. aeruginosa* enhanced plant growth and had higher accumulation of plant resistance markers like phenolic contents, salicylic acid and enhanced activity of antioxidant enzymes are associated with induced systemic resistance (ISR).Among brown seaweeds, *Sargassum* spp. had been extensively researched for its unique biochemical compounds, enhanced antioxidant prospective (Ganapathi *et al.,*2013)but the literature survey illustrates there is no evidence of using *Sargassum ilicifolium* against the control of root-rot disease in soybean and evaluation of defense enzymes involved in induced systemic resistance. The objective of this research was to study the effects of seaweed amendment with /without *P. aeruginosa* on root rooting fungi, plant growth parameters and activation of defensive markers in soybean plant.

MATERIALS AND METHODS

Preparation of bacterial inoculum

Potential strain of plant growth promoting rhizobacteria (PGPR) *Pseudomonas aeruginosa* (ABPL-251) was received from Karachi University Culture Collection (KUCC) and multiplied on King's B (KB) Broth.

Fungal isolate and inoculum

M. phaseolina was isolated from diseased plant samples and cultured on Potato Dextrose Agar Medium.

Experiment layout

The screen house experiments were conducted at the Department of Botany in Agricultural Biotechnology and Phytopathology Laboratory. All experiments were designed as Randomized Complete Block design. This experiment of soybean was terminated following 30 days of treatment afterward shoot length, shoot weight, root length and root weight of fresh plant were measured.

Plant growth and disease induction treatment

Brown seaweed *S. ilicifolium* was collected from Buleji a coastal area of Karachi coast and 1 % w/w dry powder was combined with sandy loam soil (pH 8.0). One kg soil was filled in 15 cm diameter earthen pots and be reserved at 50% water holding capacity (W.H.C.) with the daily adaptation of water. The pots were watered 2-3 days for complete decomposition of seaweed. Six sterilized soybean seeds were sown per pot and 25 mL *P. aeruginosa* ($8x10^8$cfu mL^{-1}) aqueous suspension was drenched onto each pot. Pots were irrigated with distilled water until germination occurred. Subsequent to germination four plantlets were kept in each pot and surplus were eliminated. The soil was naturally infested with 3000 cfu g^{-1} population of *Fusarium oxysporum* and *Fusarium solani*, *Rhizoctonia solani* colonization 3-10% and 2-8 sclerotia g^{-1} soil of *M. phaseolina*. For biotic stress, five day old culture of *M. phaseolina* was grind in 1000 mL^{-1} distilled water and 25 mL of this suspension (mycelial/sclerotial) containing $3.0x10^2$sclerotia $mL^{-1}$was inoculated around the roots by carefully removing the upper layer of soil and after inoculation the soil was spread over inoculum. Plants grown in unamended, un-inoculated or uninfested soil served as control.

Disease assessment

Same procedure was followed in determination of infection percentage of root tissues as described in our previous research article (Rahman *et al*., 2016).

Sample preparation for biochemical analysis

Soybean (*Glycine max*) leaves were collected in small paper bags and oven dried at 70°C. Samples were extracted within Ethanol (96% v/v) at the dilution of 10 mg mL^{-1} at room temperature. The leaf extract were homogenized at 1600×g for 15 minutes and the supernatant was separated for biochemical analysis. All investigations were completed in triplicate and results were based on the dry weight (DW) of samples.

ANTIOXIDANT ENZYMES ACTIVITIES

DPPH Radical Assay

The free radical scavenging activity of the treated plant sample was measured by2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described by Tariq *et al*.,(2011). The stock solution of DPPH (30 µM) was prepared in DMSO and working solution was diluted by mixing ethanol to achieve an absorbance about 0.9±0.02 at 517 nm by means of spectrophotometer. A 0.8 mL Tris-HCl buffer solution (100 mM, pH 7.4) was mixed with 200 µL of the sample aliquot in the test tube and vortex carefully for about 2-3 sec. First absorbance of reaction mixture was taken immediately after vortex against blank (aqueous ethanol) then kept in the dark for 30 min at room temperature and records the second absorbance at 517 nm. Control was prepared with 1mL DPPH and 1mL aqueous ethanol. Results of antioxidant activity were based on the percentage of DPPH radical scavenged as the following equation.

$$\text{Antioxidant activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS Assay

ABTS (2, 2-azinobis (3-ethylbenzthiazoline-6-sulphonicacid) radical scavenging activity was determined by Re *et al*. (1999) with some modifications. The cation radical ($ABTS^{\bullet+}$) of ABTS was generated by allowing to reserve the mixture of ABTS (7 mM) with potassium persulphate (140 mM) in dark for 24 h at room temperature. Before use the working $ABTS^{\bullet+}$ solution was diluted with 50% ethanol to give an absorbance of 0.7 ± 0.01 at415 nm. An aliquot of 1 µL of the sample solution (10mg/mL) was combined with 100 µL the ABTS solution and the absorbance was examined on micro plate reader (BioRad Model 680) at 0, 1, 2, 3, 4 and 5 min reaction time intervals. Control test tube consists of 100 µL ABTS reagent and 1 µL ethanol solvent without any plant extract. Absolute ethanol was used as a blank. Butylated Hydroxy Toluene (BHT) was used as a reference compound and results were expressed as inhibition percentage of ABTS and calculated by formula

$$\text{Scavenging activity (\%)} = \{1\text{-abs of sample / abs of control}\} \times 100$$

Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity of leaf sample was evaluated by applying the technique of Ruch *et al.*, (1989). Phosphate buffer solution (0.1M) was Prepared with 7.4 pH. Hydrogen peroxide (40 mM) solution was prepared in phosphate buffer. At a dilution of 10 mg mL^{-1} leaf aliquots were mixed with 0.6 mL H_2O_2 solution (40 mM) then make up the final volume up to 3 mL. The absorbance of the reaction mixture was recorded at 230 nm on spectrophotometer. Phosphate buffer without H_2O_2 served as a blank. The scavenging activity of H_2O_2 was evaluated with a formula.

$$\text{\% scavenging activity of } H_2O_2 = \frac{A_0 - A_1}{A_0} \times 100$$

ASSESSMENT OF BIOCHEMICAL DEFENSE MARKERS

Phenolic compounds

Accumulation of phenolic contents was calculated by the technique of Chandin *et al*. (2008). In this assay, 100 µL (0.1 mL) plant extract was mixed with 2 mL of freshly prepared Na_2CO_3 (2% w/v) then kept at room temperature. After 2 min incubation, 0.1mL of Folin-Ciocalteau Phenol reagent (50%) was added in test tube, shakes thoroughly and incubated in dark for 30 minutes at room temperature. Finally, absorbance of the reaction mixture was recorded at 720 nm against gallic acid as standard curve.

Salicylic acid

Warrier *et al.* (2013) method was followed for the estimation of salicylic acid (SA). Briefly, cooled aliquot of each plant (0.1 mL with concentration 10mg/mL) was mixed with 3.0 mL freshly prepared ferric chloride (0.1%). The absorbance of the reaction mixture was determined by spectrophotometer at 540 nm. The data was estimated using calibration curve of SA (µg mL^{-1}) and expressed in µg g^{-1}dried weight of sample.

Statistical Analysis

Plant growth parameters were investigated with one-way analysis of variance and the group means were evaluated by Duncan's Multiple Range Test at probability of $P<0.05$. Fungal infection and antioxidant activities

were examined by Two-way analysis of variance to sort out treatments producing significant effects (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Plant growth promotion and induced systemic resistance

Data present in Table 1 reveal that greater shoot length, shoot weight, root length and root weight were produced by *S. ilicifolium* inoculated with *P. aeruginosa* when distinguished to control plant. In the presence of charcoal root rot fungi *M. phaseolina*, maximum growth parameters were significantly ($p < 0.05$) enhanced by the mixed use of *S. ilicifolium* and *P. aeruginosa*. While, *M. phaseolina* treated disease plant showed reduced in plant growth parameters. According to Bhattacharya *et al*. (1994) growth of plant inhibits due to *M. phaseolina* by production of a toxin known as phaseolinon. Single application of *S. ilicifolium* and *P. aeruginosa* also significantly ($p < 0.05$) improved plant growth in healthy plant or infected plant in comparison to untreated/treated control. Several reports showed that significant increase in plant growth by *Sargassum ilicifolium* and plant growth promoting *P. aeruginosa* due to its high nutritional content such as carbohydrates, proteins, lipids, vitamins; they act as soil conditioner and assists retention of moisture, source of trace element and contain bio-control properties, phytohormones for example auxins, gibberellins and precursor of ethylene and glycine betaine which have an effect on overall plant growth under stress condition (Rahman *et al*., 2016; Pise and Sabale 2010; Mat-Atko, 1992). *Pseudomonas* spp. completely reduce the growth of *M. phaseolina*, *Fusarium* spp. and *Rhizoctonia* spp. in soybean (El-Barougy *et al*., 2009) by producing antifungal metabolites and increase plant biomass production through IAA synthesis.

Pathogen and disease suppression

All treatments showed significant ($p < 0.05$) effect over control against soil borne plant pathogens such as *Macrophomina phaseolina, Rhizoctonia solani*, *Fusarium solani* and *Fusarium oxysporum.* Separate and combined application of *S. ilicifolium* or antagonistic agent *P. aeruginosa* showed maximum reduction of soil borne plant pathogens in healthy plant as well as in infected plants. Significant ($p<0.05$) reduction of *M. phaseolina, R. solani, F. solani* was achieved by combined treatment of *S. ilicifolium* when compared to control. In diseased plants, infection % of *M. phaseolina* and other pathogens was significantly ($p<0.05$) suppressed by the application of organic amendment *S. ilicifolium* and *P. aeruginosa* alone or combined (Table 2).Similar result were found by Raghavendra *et al.* (2007) and Aziz *et al.* (2003**)** that brown seaweed secretes several potential elicitors which actively encouraged resistance against *Xanthomonas campestris* pv. *Malvacearum* in cotton plant, *Botrytis cinerea* in grapevine and *Plasmopara viticola*. This suppression of diseases may be attributed by synthesis of antibiotics (such as 2, 4-diacetylphloroglucinol, oomycin, phenazine-1-carboxyclic acid, pyrrolnitrin, pyoluteorin) (Ayyadurai *et al*., 2006; Thomashow *et al*., 1997) and chitinase (lytic enzymes) to destroy the cell wall of pathogen (Radjacommare *et al*., 2004) which are released by PGPR.

Effect of resistance inducers

Salicylic acid

The amount of salicylic acid was significantly ($p < 0.05$) enhanced with the treatment of *S. ilicifolium* and *P. aeruginosa* alone or in combination (8.23 $\mu g g^{-1}$). The least amount of salicylic acid was found in control (3.1$\mu g\ g^{-1}$) and *M. phaseolina* (1.9$\mu g\ g^{-1}$) infected plant. In the presence of *M. phaseolina*, combined treatment of both *S. ilicifolium* and *P. aeruginosa* significantly enhanced (7.6$\mu g\ g^{-1}$) synthesis of salicylic acid when compared to untreated control and the infected plant. Individually, *S. ilicifolium* or *P. aeruginosa* also showed to be best for accumulation of key molecule salicylic acid in diseased and healthy plant (Table 3). Rahman *et al*. (2017) found that salicylic acid (SA) concerned with physiological and biochemical changes in plant linked with induction of disease resistance subsequent to an early pathogen attack. Many researchers revealed that charcoal root rot pathogen successfully reduced by the production of salicylic acid, siderophores, hydrogen cyanide and lytic enzymes which involve in resistance mechanism with inoculation of different *Pseudomonas* strains (Klessig and Malamy, 1994; Scher and Baker, 1982; Bakker and Schippers, 1987; Velazhahan *et al*., 1999). Farkas and Kiraly (1962) described that many defensive enzymes have the capacity to discharge phenolic compounds at the place of parasitic assault and these build up fungi-toxic compounds.

Total Phenolic Contents

Significant ($p < 0.05$) amount of phenolic contents (3.28 $mgmL^{-1}$ gallic acid) was calculated in the plant when treated with *S. ilicifolium* along with *P. aeruginosa.* Individual or combined application of *S. ilicifolium* with *P. aeruginosa* found to be effective for maximum accumulation of phenolic contents in healthy and unhealthy plant.

Similarly, result indicated that significantly high amount of phenolic contents provides protection against various pathogens by inhibiting peroxidation of lipid, hydroperoxides (Pokorny *et al*., 2001; Pitchaon *et al*., 2007) and preventing decomposition of chelate metal ions (Balasundram *et al.,* 2006). Plant amended by *S. ilicifolium* showed a prominent stimulation of phenolic contents (3.17mg mL^{-1} gallic acid) as correlated with *P. aeruginosa* (3.04mgmL^{-1} gallic acid) treatment. Whereas, in the presence of charcoal pathogen *M. phaseolina*, the production of phenolic contents were significantly ($p < 0.05$) enhanced for the activation of resistance mechanism by individual or combined treatment of *S. ilicifolium* and *P. aeruginosa* comparatively to control (Table 3). Raghavendra *et al*. (2007) demonstrated that elevated amount of phenolic contents by apply of seaweed extract is a key strategy to protect plants against deleterious effect challenged by *Xanthomonas campestris* pathogen in cotton crop.

Table 1. Effect of *S. ilicifolium amendment* and *P. aeruginosa* on growth parameters of soybean.

Treatments	Growth Parameters			
	Shoot length (cm)	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**
Control	14.1	1.67	13.6	0.42
Sargassum ilicifolium	18.0	2.87	17.5	0.50
Pseudomonas aeruginosa	17.3	3.06	15.7	0.36
S. ilicifolium + P. aeruginosa	21	3.50	18.1	0.52
Macrophomina phaseolina	13.6	2.21	12.8	0.28
S. ilicifolium + M. phaseolina	15.6	2.33	13.4	0.35
P. aeruginosa + M. phaseolina	15.9	2.65	15.1	0.37
S. ilicifolium + P. aeruginosa+ M. phaseolina	17.9	2.67	17.1	0.4
$LSD_{0.05}$	**2.04[1]**	**0.80[1]**	**1.35[1]**	**0.14[1]**

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

Table 2.Effectof *S. ilicifolium* and *P. aeruginosa* on infection % of *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *Fusarium oxysporum* on soybean plant.

Treatments	Infection%			
	M. phaseolina	***R. solani***	***F. solani***	***F. oxysporum***
Control	87.5	75	62.5	62.5
Sargassum ilicifolium	62.5	50	25	31.25
Pseudomonas aeruginosa	56.25	43.75	31.25	25
S. ilicifolium + P. aeruginosa	37.5	25	25	18.75
Macrophomina phaseolina	93.75	68.75	75	62.5
S. ilicifolium + M. phaseolina	75	43.75	62.5	50
P. aeruginosa + M. phaseolina	62.5	31.25	50	31.25
S. ilicifolium + P. aeruginosa+ M. phaseolina	50	31.25	37.5	25
$LSD_{0.05}$	**Treatments =16.7 Pathogens = 11.4**			

[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. Effectof *S. ilicifolium* and *P. aeruginosa* on DPPH free radical scavenging activity, scavenging % of H_2O_2, and phenolic contents of soybean plant.

Treatments	Antioxidant activity Inhibition %		Scavenging % of H_2O_2	Salicylic acid µg g^{-1}	Phenolic contents mg mL^{-1} gallic acid
	0 min	30 min	µg g^{-1}		
Standard (BHT)	70.4	77.2	_	_	_
Control	21	9.6	32.4	3.1	2.32
Sargassum ilicifolium	29	12.3	37.8	6.1	3.17
Pseudomonas aeruginosa	22	13.6	38.6	7.6	3.04
S. ilicifolium + P. aeruginosa	31.6	22.3	30.6	8.23	3.28
Macrophomina phaseolina	25	12	12.2	1.9	2.50
S. ilicifolium + M. phaseolina	30.3	17	16.6	4.0	2.74
P. aeruginosa + M. phaseolina	24	20	20.2	5.5	2.26
S. ilicifolium + P. aeruginosa+ M. phaseolina	22.3	18	25.4	7.6	2.33
$LSD_{0.05}$					
Treatments	**2.66**		**3.70**	**0.87**	**0.48**
Time	**1.25**		**---**	**---**	**---**

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

Table 4.ABTS radical scavenging activity of soybean plant with combined effect of *S. ilicifolium* and *P. 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	46	50.3	54.3	56.6	59.3	63.3
Sargassum ilicifolium	54.3	56.6	58.6	66.3	71.3	72.6
Pseudomonas aeruginosa	51	55.3	57.3	59	63.3	69.6
S. ilicifolium + P. aeruginosa	57.6	58.6	65.6	69.6	72.6	78.3
Macrophomina phaseolina	45.6	49.3	53.3	56.6	58	61.6
S. ilicifolium + M. phaseolina	50.6	54.3	56	56	62	67
P. aeruginosa + M. phaseolina	56	57.6	62.3	58	70.3	72
S. ilicifolium + P. aeruginosa+ M. phaseolina	56.6	57.6	61	68.6	70.3	74

$LSD_{0.05}$ Treatments = 0.83 Time = 0.68

[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 $p < 0.05$.

DPPH Free Radical Scavenging Activity

The DPPH scavenging activity of free radical was calculated at initial 0 minute and after 30 minutes incubation. Initially at 0 minute, scavenging activity of all samples was higher than after 30 minutes it showed declined. According to Meir *et al.* (1995) decrease in the absorbance of DPPH was attributed by the reaction of antioxidant molecules and radicals which was observed because of discoloration of DPPH from purplish to yellow. Mixed application of *S. ilicifolium +P. aeruginosa* shown significant ($p < 0.05$) scavenging activity at 0 minute (31.6%) and after 30 minutes (22.3%) while lowest activity was observed in control at 0 minute (21%) and after 30 minutes (9.6%). Individual and combined treatment of *S. ilicifolium* or *P. aeruginosa* significantly ($p < 0.05$) scavenged free

radicals in the existence of *M. phaseolina,* when compared treated and untreated control (Table 3). However, scavenging activity of standard BHT antioxidant was comparatively higher than all treated plants. It has been previously described that brown algae *S. ilicifolium* contain a number of dynamic compounds such as sulfated polysaccharides, terpenoids, polyphenols, phlorotannins, plastoquinones, sargaquinoic acids, sargachromenol, steroids, glycerides, fucoidan, flucoxanthin and flavonoids which are responsible for efficient antioxidant defense system (Kuda and Ikemori, 2009; Pavia *et al*., 1997).

Scavenging % of H_2O_2

Plant inoculated with *P. aeruginosa* (38.6%) showed highest scavenging activity of H_2O_2 as followed by *S. ilicifolium* (37.8%) and *S. ilicifolium* + *P. aeruginosa* (30.6%) in comparison to healthy (32.4%) and unhealthy control (12.2%). *M. phaseolina* infected plant showed lower (12.2%) scavenging activity of H_2O_2 although given the treatment of *S. ilicifolium* +*P. aeruginosa* significantly ($p < 0.05$) increased (25.4%) scavenging activity of disease plant. Single treatment of *P. aeruginosa* is enlighten to be best for improving scavenging activity of plant under healthy and disease environment when compared to *S. ilicifolium* alone (Table 3). ROS production is noticeably increased under stress conditions. Hydrogen peroxide is not very reactive itself, but it may cause toxicity in the cells if it is produced hydroxyl radical. Therefore, the removal of H_2O_2 is very essential for antioxidant defensive mechanism of cell (Halliwell, 1991). Whereas, usually a rapid increase in ROS production was associated with plant responses to pathogens (Delledonne *et al.,* 1998).

ABTS Radical Scavenging Activity

ABTS scavenging activity of all treated plants was recorded at 0, 1, 2, 3, 4, 5 minute time interval which was increased with respect to time. All treated plants showed significant ($p < 0.05$) scavenging activity. Shao *et al.* (2007) and Mittler, (2002) found that increased levels of antioxidants, engaged in the detoxification process of ROS in resistant plants against various types of environmental stresses. Combined use of *S. ilicifolium* and *P. aeruginosa* successfully scavenged free radical which was produced by *M. phaseolina* infestation comparatively to healthy control. Scavenging activity of plants was enhanced by incorporation of seaweed *S. ilicifolium* with inoculation of biocontrol agent *P. aeruginosa* including standard BHT treatment. Significant ($p < 0.05$) increase in scavenging activity of plants was also noticed when *S. ilicifolium* and *P. aeruginosa* applied separately. Plant infected with charcoal pathogen exhibited lowest antioxidant activity (Table 4). Cote and Hahn (1994); Ebel and Cosio (1994) demonstrated that carbohydrate polymers, lipids, (glyco) peptides, (glyco) proteins and various types of elicitors are secreted by microorganisms and seaweed (Bouarab *et al.,* 1999; Klarzynski *et al.,* 2000**;** Potin *et al.,* 1999) which have been involved to induced defense mechanism in plant.

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