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ENHANCEMENT OF DEFENSIVE CAPABILITY OF OKRA PLANT (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH) AGAINST ROOT ROT DISEASE BY THE ENDOPHYTIC FLUORESCENT *PSEUDOMONAS* AND SEAWEED SOIL AMENDMENT

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Abstract

Management of plant root diseases by the application of seaweed and endophytic bacteria, particularly fluorescent *Pseudomonas*, is capturing the interest of plant scientists. In this study, the effect of seaweed soil amendment alone or mixed with endophytic fluorescent *Pseudomonas* (EFP-47) in reducing the root infecting fungi of okra was evaluated in pots and field plot experiments. The experiments conducted in 2019 and repeated in 2020, showed that soil amendment with seaweed, *Stokeyia indica*, and *Ulva fasciata* alone or mixed with fluorescent *Pseudomonas* significantly suppressed *Macrophomina phaseolina, Fusarium solani*, and *Rhizoctonia solani* on okra roots compared to untreated control plants. In general *U. fasciata* + EFP-47 treated plants showed maximum inhibition of root rotting fungi. In addition, seaweed and *Pseudomonas* (EFP-47) applications increased the plant height and fresh weight in pots as well as in field plot experiments. Seaweed used alone or mixed with *Pseudomonas* (EFP-47) ameliorated the activity of plant resistance markers like salicylic acid and polyphenolic contents, improved antioxidant activity and phosphorus uptake in plants. It is suggested that endophytic fluorescent *Pseudomonas* and seaweed could be used for the management of root diseases of okra.

Key words: Root rot; Okra; Seaweed; Endophytic *Pseudomonas*, Disease management, Antioxidant activity, Systemic resistance.

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench], a vegetable crop is highly nutritious and contains a major source of vitamins, minerals, carbohydrates, and fats (Emuh *et al*., 2006). Pakistan is one of the largest okra-producing countries where several varieties are cultivated (Tiendrébéogo *et al*., 2010). However, several pathogenic fungi including, *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina*, *Verticillium*, and *Sclerotinia* cause huge losses to the okra crop. Most of the soil-borne fungi remain in the soil by producing various survival structures such as chlamydospore, oospore, and sclerotia (Baysal-Gurel *et al*., 2012). Nowadays research work for finding alternate methods in crop protection and production has increased tremendously since chemical fertilizers and pesticides pollute the environment (Sultana *et al*., 2018; Moin *et al*., 2021; Urooj *et al*., 2021).

Useful bacteria associated with plants are recognized to reduce plant disease either directly through microbial antagonism or indirectly by inducing systemic resistance (ISR) in plants (Van Wees *et al*., 2008, Lugtenberg & Kamilova, 2009; Moin *et al*., 2020). Among beneficial soil bacteria, fluorescent pseudomonads are known to have a positive effect on plant growth and development (Moin *et al*., 2020). These bacteria directly suppress plant pathogens and also induce systemic resistance (Noreen *et al*., 2019; Chen *et al*., 2000; DeMyer & Hofte, 1997). Fluorescent pseudomonads are generally associated with plant roots, but they also occur as endophytes and play a vital role in improving the plant's immune system (Korejo *et al*., 2019; Moin *et al*., 2020).

Incorporation of organic materials in soil like neem cake, cotton cake, and mustard cake not only improve soil fertility but also suppress soil borne plant pathogens and induce systemic resistance (Shafique *et al*., 2015; 2016; Rahman *et al*., 2016). Similarly, seaweeds have been recognized as a rich source of a bioactive compound, when applied in soil trigger plant defensive capacity against several pathogens (Leandro *et al*., 2020) and plant pathogens (Sultana *et al*., 2011a,b; 2018). Incorporation of seaweed in soil was found to reduce root diseases of soybean and pepper under field conditions and their efficacy was comparable with Topsin-M a fungicide and carbofuran, a nematicide (Ehteshamul-Haque *et al*., 2013) in tomato and sunflower (Sultana *et al*., 2011a). In this study, we have evaluated the potential of endophytic fluorescent *Pseudomonas* in suppressing the root rot fungi, induction of systemic resistance, and phosphorus uptake in okra plants, used alone or in soil amended with seaweed in clay pots and field plots. The impact of these treatments on yield and fruit quality was also determined.

Material and Method

Collection of seaweed: Seaweeds were collected from the coastal region of Gwadar (Padizer) and Karachi (Buleji) in the months of March-April, 2019 and 2020. The algae were identified by a Phycologist and voucher specimens were deposited in the Seaweed Herbarium of M.A.H. Qadri Biological Research Centre, University of Karachi. Seaweeds were washed with tap water and spread under shade for air drying. After drying, they were ground in an electric miller to coarse powder for further use.

Bacterial culture (Endophytic fluorescent *Pseudomonas*): Endophytic fluorescent *Pseudomonas* (EFP-47), which has shown significant activity in our previous study (Moin *et al*., 2020) was used in this study. It was grown in King's B broth in 500 mL flasks at 28^{o}C ± 2 for 7 days.

Evaluation of efficacy of endophytic fluorescent *Pseudomonas* (EFP) and seaweed in suppressing the root rot, amelioration of systemic resistance, and growth of okra in pots experiments: The experiment was conducted in March 2019, in clay pots, with 4 replicates in block design. Powdered seaweed, *Stokeyia indica* and *Ulva fasciata* at 1% w/w were mixed with sandy loam soil and poured into pots at 1Kg per pot. A natural infestation of *Macrophomina phaseolina* (3-7 sclerotia g^{-1} soil), 4-9% colonization of *Rhizoctonia solani* on sorghum seeds and 3000 cfu g^{-1} soil of *Fusarium* spp., was found as determined by using the method of Sheikh & Ghaffar, (1975), Wilhelm (1955) and Nash & Snyder (1962) respectively. The pots were watered daily for 2 weeks, then aqueous suspension of *Pseudomonas* (EFP-47) (10^8 cfu mL^{-1}) was drenched in each pot and 6 seeds of okra were sown. Topsin-M (200 ppm), 25 mL per pot served as a positive control, while seeds sown in un-amended pots without any treatment served as control. In another set of pots (containing amended and un-amended soil), not inoculated with bacterial culture were also kept. The observation was recorded after 60 days of growth. The experiment was repeated in 2020.

Plant growth parameter and determination of root infection: Plants (4 from each replicate) were uprooted; length of plant and fresh weight were recorded. Roots were cut into one long piece after washing with water, then surface sterilized for 3 min with 1% bleach (sodium hypochloride) and transferred onto potato dextrose agar (PDA) plates, supplemented with penicillin (100,000 unit/L) and streptomycin (0.2g/L). Fungi emerged from each piece after 5 days of incubation at $28^oC \pm 2$ were identified and the percentage of each pathogenic fungus was calculated as described by Noreen *et al*., (2015).

Biochemical parameters

Preparation of plant sample for biochemical test: The leaves of okra were oven-dried at 80^oC for 48hours. Dried leaves sample was extracted in ethanol (96%v/v) at 0.01g/mL and centrifuged at 4000 rpm for 20min. The supernatant was collected for biochemical analysis.

Analysis of salicylic acid (SA): Cooled aliquot of leaves (0.1mL) was added to 2.9mL of $FeCl_3$ (0.1%, freshly prepared). After mixing, absorbance was taken at 540 nm on a spectrophotometer (Shimadzu, UV-1800, Japan). SA (1mg mL^{-1} in ethanol) was used for preparing standard curves (Warrier *et al*., 2013).

Quantification of polyphenol content: For the estimation of polyphenol, an aliquot (100µL) was mixed with 2mL of 2% Na_2CO_3 and incubated for 2min. at room temperature, then 0.1mL of 50% folin-ciocalteuphenol reagent was added. After mixing thoroughly, the mixture was kept for incubation at room temperature in the dark for 30min. Absorbance was recorded at 720nm. The standard curve was prepared by using gallic acid (Chandini *et al*., 2008).

Phosphorus estimation: Total phosphorus was estimated by using the dry ashing method as described by Rayan *et al*., (2001) with slight modification. Where 10mL, 2N HCl was added in 0.25g ground dry leaves, mixed, and allowed to digest for 1hour. Then filtered over Watman no.1 filter paper and 2.5mL filtrate was added to 2.5mL Barton reagent, and volume was made up to 25mL by adding distilled water. The mixture was incubated for 30min at room temperature and absorbance was recorded at 410 nm against the reagent blank. The standard curve was prepared; using potassium dihydrogen phosphate (KH_2PO_4).

DPPH- free radical scavenging activity: Antioxidant activity of leaves was determined as described by Tariq *et al*., (2011), where 0.2mL aliquots of leaf extract were mixed with 0.8mL of 10mM tris HCl buffer (7.4PH). 01mL of 30µM DPPH (prepared in DMSO) was added to the reaction mixture and vortex. A solution containing 1mL ethanol with 1mL 30µM DPPH served as control. Absorbance was recorded at 517 at 01 min and 30 min after incubating samples in dark at room temperature and free radical scavenging activity (% inhibition) was calculated.

Evaluation of efficacy of endophytic fluorescent *Pseudomonas* (EFP-47) and seaweed in suppressing the root rot, amelioration of systemic resistance and growth of okra in field plot experiments: The planting rows of field plots (2x2 m) were amended with seaweed *S. indica* and *U. fasciata* at 70g per 2 m planting row and watered for 2 weeks at three days interval for the decomposition of seaweed. The bacterial *Pseudomonas* (EFP-47) suspension was drenched in each row at 200 mL per row and okra at 20 seeds per row was sown. Topsin-M (200 ppm), 200 mL per 2 meters row served as the positive control, and plots without seaweed and bacterial suspension served as a negative control. The experiment was designed in a randomized complete block with 4 replicates. The experiment was conducted in 2019 and repeated in 2020 in similar conditions.

Statistical Analysis

Statistical software SPSS, version (16) was used for the analysis of variance (ANOVA), and means were separated and significant level at $p<0.05$ was determined using Duncan's Multiple range test for growth parameters, while two-way ANOVA was applied for fungal infection.

Results

Efficacy of endophytic fluorescent *Pseudomonas* (EFP-47) and seaweed in suppressing the root rot fungi, amelioration of systemic resistance, and growth of okra in pots experiments-2019

Effect on plant growth: Okra plants grown in soil amended with *U. fasciata* showed maximum improvement in shoot growth (36.19cm) compared to control (27.25cm), whereas maximum root growth was observed by the application of *Pseudomonas* EFP-47 (12.06 cm) compared to control plants (10.44cm) (Table 1). Moreover, maximum shoot weight and root weight were achieved in the soil amended with *U. fasciata* (12.99g) and 1.59g compared to control (Table 1).

Table 1. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on the growth of okra plant under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in pots experiment.

Treatments	2019				2020			
	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Control	27.25 ± 2.59c	10.44 ± 0.82ab	7.04 ± 1.50c	1.06 ± 0.30b	26.56 ± 1.53d	10.12 ± 0.59d	2.31 ± 0.28d	0.23 ± 0.02c
Topsin-M (200 ppm)	31.13 ± 2.50bc	10.81 ± 1.08ab	7.1 ± 0.40c	0.94 ± 0.26b	32.99 ± 1.18c	12.18 ± 0.42bc	3.12 ± 0.25c	0.34 ± 0.06b
Stockia indica	32.19 ± 1.28bc	11.44 ± 1.26ab	8.69 ± 2.34bc	1.22 ± 0.33ab	39.62 ± 2.89b	12.87 ± 0.52abc	5.25 ± 0.35ab	0.48 ± 0.01a
Ulva fasciata	36.19 ± 2.72a	10.25 ± 1.92ab	12.99 ± 3.19a	1.59 ± 0.42a	44.18 ± 1.80a	13.63 ± 0.77a	5.54 ± 0.40a	0.49 ± 0.06a
Pseudomonas (EFP-47)	34.06 ± 3.23ab	12.06 ± 1.06a	8.73 ± 1.01bc	1.3 ± 0.22ab	31 ± 2.27c	11.62 ± 1.31c	2.49 ± 0.19d	0.25 ± 0.02c
S.indica +*Pseudomonas* EFP-47	35.43 ± 3.46ab	10.31 ± 1.14ab	11.13 ± 2.56ab	1.18 ± 0.32ab	43.75 ± 3.58a	13.18 ± 1.08ab	4.67 ± 0.47b	0.46 ± 0.03a
U. fasciata+ *Pseudomonas* EFP-47	32.69 ± 2.97ab	10 ± 0.40b	12.32 ± 3.69a	1.60 ± 0.62a	40.5 ± 3.42ab	13.25 ± 0.88ab	5.22 ± 0.77ab	0.47 ± 0.03a
LSD=0.05	4.4[1]	1.75[1]	3.34[1]	0.53[1]	3.77[1]	1.29[1]	0.6[1]	0.05[1]

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

Suppression of root rotting fungi: *F. solani* was suppressed maximum in the combined treatment of *U. fasciata* + EFP-47 (68.7%) as compared to control plants (93.7%). Combined treatment of *S. indica* with endophytic fluorescent *Pseudomonas* (EFP-47) reduced infection (6.2%) *of Rhizoctonia solani* significantly ($p<0.05$) however *S. indica* reduced *M. phaseolina* infection (Table 2).

Polyphenolic contents, salicylic acid (SA), and phosphorus uptake: The salicylic acid in leaves was found significantly ($p<0.05$) highest in plants treated with combined application of *U. fasciata* + EFP47 (0.134mg/g) as compared to control (0.096mg/g), whereas plants grown in *S. indica* + EFP47 amended soil showed maximum polyphenol content (0.145mg/g) compared to untreated control (0.048mg/g) (Table 3).

Maximum phosphorus uptake (17.3ppm) was found in plants grown in *S. indica* amended soil in contrast to control plants (15.49ppm) (Table 3).

Free radical scavenging activity: All treated plants exhibited a higher percentage of antioxidant activity/ free radical scavenging activity at 01 min and 30min compared to untreated control plants (Fig. 1). The highest antioxidant activity was found in EFP-47 (33.16%) treated plants at 01 min whereas after 30min, application of *U. fasciata* exhibited the highest antioxidant activity (56.14%) than control plants (49.29%).

Efficacy of endophytic fluorescent *Pseudomonas* (EFP) and seaweed in suppressing the root rot, amelioration of systemic resistance, and growth of okra in pots experiments-2020

Effect on plant growth: Application of *U. fasciata* produced the highest shoot and root length (44.18 and 13.63cm) in comparison to control (26.56 and 10.12 cm) respectively (Table 1). Similarly, shoot weight and root weight significantly increased in plants treated with *U. fasciata* (5.54g and 0.49g) compared to un-amended control plants (2.31g and 0.23g) respectively (Table 1).

R**oot rotting fungi:** *S. indica* and *U. fasciata* alone or mixed with fluorescent *Pseudomonas* EFP-47 significantly ($p<0.05$) suppressed the infection of *F. solani*, *R. solani*, and *M. phaseolina* (62.5%, 12.5 and 18.7%), respectively compared to control plants (87.5%, 43.7% and 25%) respectively (Table 2).

Polyphenolic contents, salicylic acid (SA), and phosphorus uptake: *S. indica* induced maximum amount of polyphenol (0.365mg/g) and salicylic acid (0.125mg/g) in treated plants compared to untreated control (0.161 mg/g and 0.095 mg/g) respectively (Table 3).

Similarly, the application of *U. fasciata* enhanced phosphorus uptake (18.0ppm) in treated plants than control plants (13.55ppm) (Table 3).

Free radical scavenging activity: Combined application of *U. fasciata* + EFP-47 showed maximum free radical scavenging activity (32.03%) at 01 min and 60.42% at 30min, compared to untreated plants 23.31% and 57.82%, respectively (Fig. 1).

Table. 2. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina*) infecting okra roots under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in pots experiment.

Treatments	2019			2020		
	Infection %					
	F. solani	***R. solani***	***M. phaseolina***	***F. solani***	***R. solani***	***M. phaseolina***
Control	93.75	56.25	37.5	87.5	43.75	25
Topsin-M (200 ppm)	93.75	31.25	18.75	68.75	25	37.5
S. indica	81.25	31.25	6.25	62.5	12.5	18.75
U. fasciata	87.5	43.75	12.5	68.75	6.25	25
Pseudomonas EFP-47	87.5	31.25	12.5	68.75	12.5	43.75
S. indica +*Pseudomonas* EFP-47	87.5	6.25	18.75	75	25	18.75
U. fasciata + *Pseudomonas* EFP-47	68.75	25	12.5	62.5	12.5	25
LSD=0.05	Treatment = 12.31[1], Pathogen = 8.06[2], Time = 6.58[3]					

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

[2]Mean values in rows for pathogen showing differences greater than LSD values are significantly different at $p< 0.05$

[3]In row, mean values showing differences more than LSD value for days are significantly different at $p<0.05$

Table 3. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on resistance markers and phosphorus uptake by okra plants under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in pots experiment.

Treatment	2019			2020		
	Salicylic acid (mg/g)	**Polyphenol (mg/g)**	**Phosphorous (ppm)**	**Salicylic acid (mg/g)**	**Polyphenol (mg/g)**	**Phosphorous (ppm)**
Control	0.096±002c	0.048±025d	15.49±0.22b	0.095±002c	0.161±007d	13.55±1.20d
Topsisn-M (0.1%)	0.116±011b	0.070±015cd	16.78±0.36ab	0.107±009b	0.223±020c	15.97±1.85bc
S. indica	0.118±006b	0.090±017bc	17.30±0.87a	0.125±001a	0.365±001a	16.54±0.29bc
U. fasciata	0.114±006b	0.131±002a	16.51±.14ab	0.122±010a	0.264±055bc	18.00±0.29a
Pseudomonas EFP-47	0.116±006b	0.079±024bc	16.97±1.47a	0.118±007a	0.233±016bc	15.30±0.40cd
S. indica+ *Pseudomonas* EFP-47	0.114±002b	0.145±002a	16.78±0.91ab	0.107±007b	0.267±028b	16.30±1.81abc
U. fasciata +*Pseudomonas* EFP-47	0.134±006a	0.099±007b	17.05±1.02a	0.106±001bc	0.251±017bc	17.46±0.43ab
LSD=0.05	0.009[1]	0.02[1]	1.2[1]	0.01[1]	0.03[1]	1.77[1]

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

Efficacy of endophytic fluorescent *Pseudomonas* (EFP-47) and seaweed in suppressing the root rot, amelioration of systemic resistance, and growth of okra in field plot experiments-2019.

Plant growth parameter and yield: Plant height and shoot weight significantly ($p<0.05$) increased in treated plants compared with control plants. Plants grown in soil amended with *U. fasciata* showed maximum plant height (71.87 cm), shoot weight (115.1g) respectively compared to control plants 47.8 cm and 47.7g respectively (Table 4).

The highest fruit yield was recorded in seaweed treated plants (Table 6). It was found that the plants grown in seaweed (*U. fasciata*) amended soil produced early and a maximum number of fruits 4.5 and 6.75 than control 2.5 and 1.5 (per plant) respectively at first and second harvest. Plants grown in soil amended with *S.indica* showed maximum fruit yield in the first and second harvest (46.1g and 61.57g) followed by *U. fasciata*, 34.69 g, and 50.94g respectively. In the third harvest, fruit yield was further increased and *U. fasciata* treated plants showed maximum fruit yield as (78.2g) followed by combined treatment of *S. indica*+EFP-47 (72.02g) as compared to control plants (21.3 g) (Table 6).

Suppression of root rotting fungi: Significant suppression of *R. solani* and *M. phaseolina* was found where *S. indica* and *U. fasciata* were used alone or mixed with EFP-47 compared to control plants (Table 5), however, *F.solani* was found significantly ($p<0.05$) reduced by *U. fasciata* + EFP-47 followed by *S. indica* seaweed used alone or mixed with EFP-47 (Table 5).

Polyphenolic contents, salicylic acid (SA), and phosphorus uptake: All treatments were found significantly ($p<0.05$) ameliorated the concentration of salicylic acid and polyphenolic content compared to control plants (Table 7), where *S. indica* + EFP-47 and *U. fasciata* + EFP-47 were found better than other treatments for SA (0.141mg/g) and polyphenolic contents (0.736mg/g) compared to untreated control as SA (0.123mg/g) and polyphenol (0.338mg/g).

All treated plants significantly increased phosphorus uptake compared to untreated control plants. Phosphorus uptake was 18.24ppm in the *U. fasciata* +EFP47 treatment whereas in untreated control plants it was found 14.31ppm (Table 7).

Free radical scavenging activity: The highest antioxidant activity in plants at 01min (43.67%) was noted in the combined treatment of *U. fasciata* + EFP-47, whereas at 30min, plants grown in *S. indica* amended soil showed the highest antioxidant activity (51.16%) followed by *U. fasciata* + EFP-47 compared to control plants at 01 min (22.84%) and 30 min (44.35%) (Fig. 2).

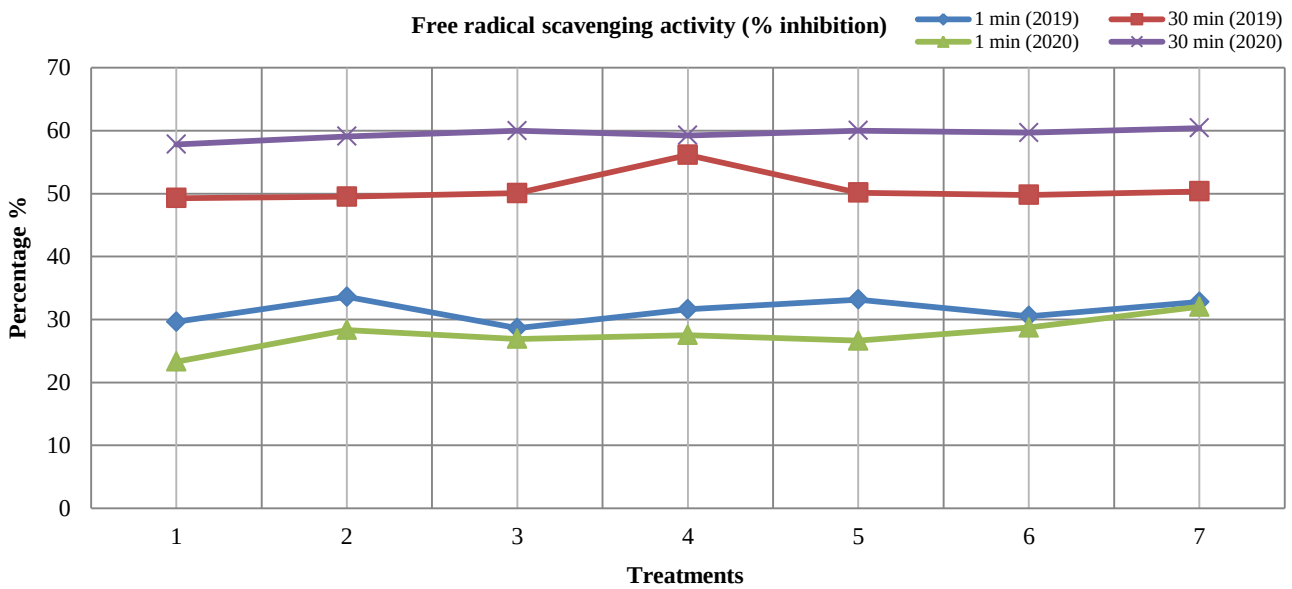

Fig. 1. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on free radical scavenging activity of okra plant under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in pots experiment.
1= Control, 2 = Topsin-M, 3 = *S. indica*, 4 = *U. fasciata*, 5 = EFP-47, 6 = *S. indica* + EFP-47, 7 = *U. fasciata* + EFP-47
$LSD_{0.05}$ 2019 at 1 min= 3.85, at 30 min= 3.35; 2020 at 1 min = 12.35, at 30 min = 3.29

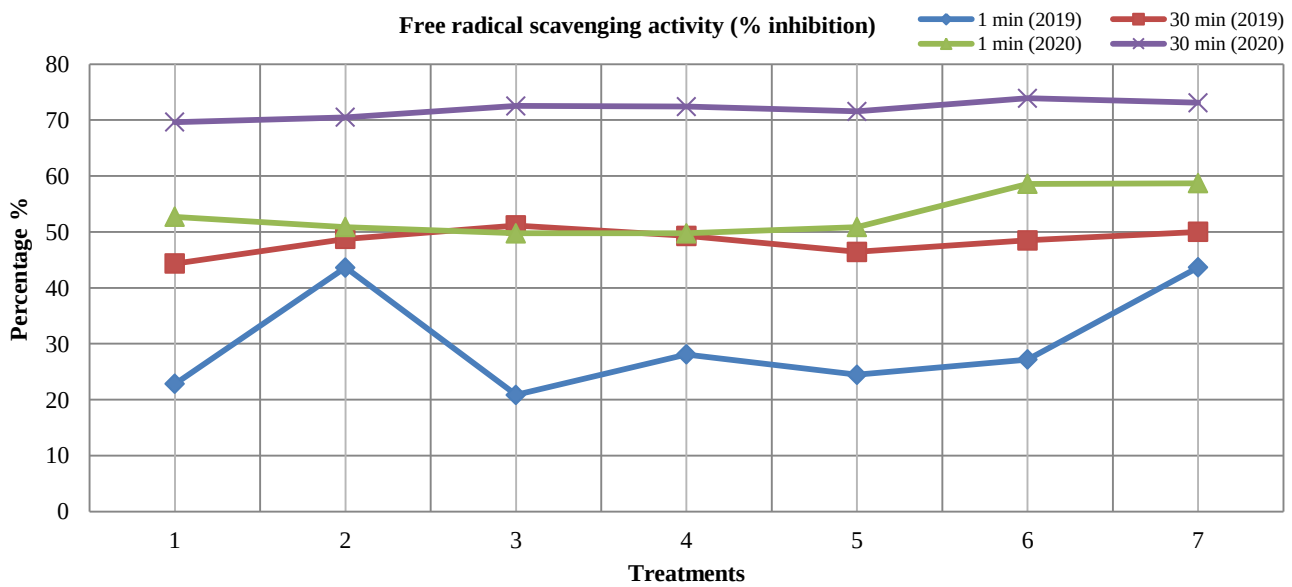

Fig. 2. Effect of seaweed and endophytic fluorescent pseudomonas on the antioxidant activity in field plot experiments.
1 = Control, 2 = Topsin-M, 3 = *S. indica*, 4 = *U. fasciata*, 5 = EFP-47, 6 = *S. indica* + EFP-47, 7 = *U. fasciata* + EFP-47
$LSD_{0.05}$ 2019 at 1 min = 15.16, at 30 min = 9.55; 2020 at 1 min= 4.03, at 30 min= 2.58

Efficacy of endophytic fluorescent *Pseudomonas* (EFP-47) and seaweed in suppressing the root rot, amelioration of systemic resistance, and growth of okra in field plot experiments-2020

Plant growth parameter and yield: *U. fasciata* produced maximum plant height (84.2 cm) and shoot weight (96.3 g) followed by combined application of *U. fasciata* + EFP-47 as (84.2cm and 85.8 g) and EFP-47 used alone (83.8 cm and 87.3 g) in contrast to 74.8 cm plant height and 74.6 g of fresh shoot weight in control plants, respectively (Table 4).

Plants were grown in seaweed amended soil and treated with EFP-47 significantly ($p<0.05$) enhanced fruit yield compared to control plants (Table 6). The combined application of *U. fasciata*+EFP-47 exhibited the highest fruit numbers and yield as 3.75 and 24.7 g (per plant) in contrast to control plants 2.75 and 12.6 g (per plant). Lesser fruit yield was also noted in Topsin-M treated plants 1.75 and 10.13g (per plant) in the first harvest. However, in the second harvest, the number of fruit and weight was increased where plants grown in combined treatment of *S. indica*+EFP-47 produced the highest yield. At the third harvest, *U. fasciata* amended plants showed the maximum number of fruits (7.75) and yield (112.1 g) (per plant) than control plants 4 and 55.48 g (per plant), respectively (Table 6).

Table 4. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on the growth of okra plant under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in field plot experiments.

Treatment	2019				2020			
	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Control	47.8 ± 1.26b	19.3 ± 5.34ab	47.7 ± 1.64f	6.6 ± 0.70b	74.8 ± 1.55d	22.5 ± 1.69a	74.6 ± 1.53d	11.6 ± 0.29f
Topsisn-M (200 ppm)	48.5 ± 1.55b	20.3 ± 4.65ab	60.9 ± 3.760d	6.7 ± 1.03b	81.3 ± 1.56b	22.1 ± 1.56a	86.0 ± 1.41b	15.1 ± 0.25c
S. indica	68.3 ± 8.32ab	22.6 ± 3.60a	102.0 ± 4.28b	12.7 ± 1.35ab	80.3 ± 1.88bc	22.4 ± 0.77a	82.5 ± 2.10c	14.4 ± 0.25d
U .fasciata	71.8 ± 1.35a	23.7 ± 2.20a	115.1 ± 1.76a	18.1 ± 1.77a	84.2 ± 2.65a	23.2 ± 2.08a	96.3 ± 0.81a	17.3 ± 0.17b
Pseudomonas EFP-47	52.1 ± 9.63ab	20.3 ± 3.12ab	52.7 ± 3.30e	10.8 ± 0.89b	83.8 ± 2.30a	23.9 ± 1.01a	87.3 ± 1.94c	14.4 ± 1.27d
S.indica+*Pseudomonas*EFP-47	49.8 ± 1.29b	16.3 ± 4.75b	74.8 ± 1.77c	9.4 ± 1.66b	78.4 ± 1.76c	22.8 ± 1.18a	82.5 ± 0.73c	13.1 ± 0.54e
U. fasciata+ *Pseudomonas* EFP-47	54.1 ± 1.62ab	21.4 ± 3.43ab	51.9 ± 4.71ef	5.9 ± 1.36b	84.2 ± 2.87a	24.1 ± 1.03a	85.8 ± 1.35b	17.9 ± 2.14a
LSD=0.05	19.1[1]	5.4[1]	4.4[1]	6.8[1]	2.4[1]	3.5[1]	2.22[1]	2.7[1]

[1]Mean values in a column for each parameter showing differences greater than LSD values are significantly different at p<0.05

Suppression of root rotting fungi: Fluorescent *Pseudomonas* + seaweed reduced root rot fungal infection compared to untreated plants. Combined application of *U. fasciata* + EFP-47 suppressed *F.solani* infection maximum (37.5%) followed by Topsin-M (43.75%) compared to control plants (75%). Moreover, minimum infection of *R. solani* and *M. phaseolina* was found in Topsin-M treated plants (6.2% and 0%) followed by *U. fasciata* mixed with EFP-47 (12.5% and 18.7%) in contrast to control plants (43.7% and 37.5%) (Table 5).

Polyphenolic contents, salicylic acid (SA), and phosphorus uptake: Plants treated with *S. indica* significantly (p<0.05) produced higher amount of resistance marker such as salicylic acid (0.211mg/g), whereas *Pseudomonas* (EFP-47) showed maximum phenolic content (0.950mg/g) compared to control plants as 0.189mg/g and 0.510mg/g, respectively (Table 7).

Moreover, seaweed and fluorescent *Pseudomonas*-treated plants showed higher amounts of phosphorus uptake than untreated control plants. Plants grown in *S. indica* amended soil showed higher amounts of phosphorus uptake (25.60ppm) compared to control plants (20.81ppm) (Table 7).

Free radical scavenging activity: The combined application of *U. fasciata*+EFP47 exhibited the highest antioxidant activity at 01 min (58.72%) and at 30 min (73.89%) than control plants in which antioxidant activity was found 52.70% at 01 min and 69.69% at 30min, respectively (Fig. 2).

Discussion

Numerous microorganisms with biocontrol activity against root rotting fungi are discovered each year but most of them showed either inconsistent performance under field conditions or completely failed. The emergence of endophytic bacteria and fungi as potential biocontrol agents has opened a new window of research. Among endophytic bacteria, fluorescent *Pseudomonas* is known to promote plant growth and ameliorate the systemic resistance of plants against plant pathogens (Moin *et al*., 2020, Korejo *et al*., 2019; Urooj *et al*., 2021). In the present study, the application of endophytic *Pseudomonas* applied alone or in soil amended with seaweed enhanced growth of the okra plant and significantly reduced *F.solani, R. solani*, and *M. phaseolina* in okra roots. Combined treatment of seaweed, *U. fasciata***,** or *S. indica* with *Pseudomonas* EFP-47 significantly reduced root rot fungi on okra in both seasons (2019 and 2020) in pot trials as well as in field plot experiments as compared to control plants. Afzal *et al*., (2013); Siddiqui & Ehteshamul-Haque, (2001) and Ehteshamul-Haque *et al*., (2007) reported that endophytic bacteria particularly bacteria belonging to fluorescent *Pseudomonas* possessed broad-spectrum antagonistic activity against root rot pathogens of crop plants. Endophytic fluorescent *Pseudomonas* play a vital role in reducing the root disease via direct suppression of pathogens or inducing systemic resistance in plants (Moin *et al*., 2020; Korejo *et al*., 2019).

Table 5. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* infecting okra roots under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in field plot experiments.

Treatments	2019			2020		
	Infection %					
	F. solani	***R. solani***	***M. phaseolina***	***F. solani***	***R. solani***	***M. phaseolina***
Control	50	50	25	75	43.75	37.5
Topsisn-M (200 ppm)	37.5	18.75	12.5	43.75	6.25	0
S. indica	31.2	18.75	12.5	62.5	18.75	6.25
U. fasciata	43.75	6.25	6.2	62.5	12.5	62.5
Pseudomonas EFP-47	56.2	12.5	0b	62.5	18.75	18.75
S. indica +*Pseudomonas* EFP-47	31.2	25	0	56.25	18.75	6.25
U. fasciata +*Pseudomonas* EFP-47	25	6.2	6.2	37.5	12.5	18.75
LSD=0.05	Treatments= 11.9[1]	Pathogens= 7.8[2]	Time =6.3[3]			

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

[2]Mean values in rows for pathogen showing differences greater than LSD values are significantly different at $p< 0.05$

[3]In row, mean values showing differences more than LSD value for days are significantly different at $p<0.05$

In this study, seaweed *S. indica* and *U. fasciata* alone or mixed with fluorescent *Pseudomonas* (EFP-47) significantly ($p<0.05$) suppressed *F. solani*, *R. solani*, *M. phaseolina* and improved plant growth in field plot experiments in both seasons compared to untreated control plants. Their efficacy was found more or less similar to Topsin-M, a commercial fungicide. Several reports indicate that seaweed could suppress root diseases of crop plants when applied as a soil amendment (Sultana *et al*., 2011a; 2018) and their efficacy is found comparable to commercial fungicides and fertilizers (Sultana *et al*., 2011b). Ehteshamul-Haque *et al*., (1996) reported better control of root diseases of okra with combined application of seaweed and rhizobia. Seaweed soil amendment has been reported to stimulate the activity of fluorescent *Pseudomonas* in the rhizosphere of soybean and pepper (Ehteshamul-Haque *et al*., 2013).

In this study, the application of seaweeds such as *U. fasciata* and S.*indica* alone or mixed with fluorescent *Pseudomonas* ameliorated the plant resistance markers like salicylic acid and polyphenolic contents with improvement in the antioxidant capability of treated plants. Fluorescent *Pseudomonas* has been reported to induce systemic resistance in plants (De Myer & Hofte, 1997; Rahman *et al*., 2016; Moin *et al*., 2020). Accumulation of phenolic compounds in pea plants treated with *P. fluorescens* against *P. ultimum* and *F. oxysporum* f. sp pisi has been reported (Benhamou *et al*., 1996). Similarly, soil amendment with seaweed and other organic matter can suppress root diseases of crop plants besides other mechanisms, also via improving the systemic resistance of host plants (Rahman *et al*., 2017; Shafique *et al*., 2015). In this study, soil amendment with *S. indica* and *U. fasciata* alone or mixed with fluorescent *Pseudomonas* not only induced defense markers like salicylic acid but also enhance the antioxidant activity of the host plant, and maximum activity was found in *U. fasciata* + EFP-47 treated plants. Seaweed can affect cell metabolism by inducing the synthesis of antioxidant molecules which promote plant growth and resistance under stress (Zhang & Schmidt, 2000; Rahman *et al*., 2017).

Martinez *et al*., (2019) reported that Mn, N, Zn, and P absorption increased in *Pseudomonas fluorescens* amended melon plants and caused competition among nutrients and limited Cu, Na, Ca, and K absorption. In this study, phosphorus uptake was increased in seaweed treated plants particularly *U. fasciata* and *S. indica* followed by combined application of *U. fasciata* with fluorescent *Pseudomonas* in pot experiment in both seasons (2019 and 2020). However, in the field plot combined application of *U. fasciata*+EFP-47 enhanced phosphorus uptake in season (2019) whereas *S. indica* alone or mixed with *Pseudomonas* amended increased phosphorus absorption in repeated season (2020) followed by *U. fasciata* in contrast to untreated control. Similar results were also reported by Halpern *et al*., (2015) that seaweed extract substantially increased N, P, and K absorption.

In this study, the application of *Pseudomonas* EFP-47 and seaweed amendment soil resulted in improvement in the yield of okra fruit. Baloch *et al*., (2013) reported that seaweed's treatment produced healthy plants, increasing in number and weight of fruits. Moreover, it has also been reported that seaweed amended plants showed an increase in length, diameter, and yield of okra than untreated control (Zodape *et al*., 2008). Xavier *et al*., (2007) applied *U. fasciata* and *Caulerpa racemosa* extracts on beans that enhanced the crop yield. Similarly, liquid extract of *Kappaphycus alvarezii* enhanced the yield potency of tomato (Zodape *et al*., 2011). In the present study plants grown in *U. fasciata* amended soil, alone or mixed with fluorescent *Pseudomonas* increased fruit yield followed by *S. indica* alone or mixed with *Pseudomonas* in both seasons.

Table 6. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on the yield of okra under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in field plot experiments.

Treatments	2019						2020					
	1st Harvest		2nd Harvest		3rd harvest		1st Harvest		2nd Harvest		3rd harvest	
	No. of fruits/Plant	Fruit wt.(g)/Plant	No. of fruits/ Plant	Fruit Wt (g) /Plant	No. of fruits/Plant	Fruit wt. (g) /Plant	No. of fruits/Plant	Fruit wt. (g) /Plant	No. of fruits/Plant	Fruit wt. (g) /Plant	No. of fruits/Plant	Fruit wit. (g) /Plant
Control	2.5 ± 0.57c	10.1 ± 5.8d	1.5 ± 0.57e	18.85 ± 5.0e	1.7 ± 0.95e	21.3 ± 4.38f	2.75 ± 0.50ab	12.6 ± 5.9ab	5 ± 1.41a	42.7 ± 6.21c	4 ± 0.81c	55.4 ± 7.18c
Topsin-M (200 ppm	4b ± 0.81c	20.9 ± 3.1c	2.7± 0.95d	27.85 ± 1.9d	3 ± 0.81de	41.3 ± 2.85e	1.75 ± 0.95b	10.13 ± 7.2b	5.7 ± 1.50a	49.4 ± 7.65bc	5.7±.95b	68.3 ± 4.18c
S. indica	3.7 ± 0.95ab	46.1 ± 8.4a	5 ± 0.81b	61.57 ± 8.3a	4.7 ± 0.95bc	61.5 ± 3.77c	2 ± 0.81b	21.5 ± 1.08ab	6.5 ± 2.38a	53.4 ± 4.48b	5.5 ± 0.57b	61.8 ± 5.21c
U. fasciata	4.5 ± 1a	34.6 ± 4.3b	6.7 ± 0.95a	50.94 ± 6.8b	6.2 ± 0.50a	78.2 ± 2.72a	2.5 ± 1ab	19.1 ± 6.73ab	7.5 ± 0.57a	55.1 ± 4.47ab	7.7 ± 0.95a	112.1 ± 1.02a
Pseudomonas EFP-47	2.2 ± 0.50c	10.5 ± 3.5d	3.7 ± 0.95cd	19.61 ± 2.9e	4 ± 1.15cd	53.8 ± 6.08d	1.75 ± 0.95b	18.5 ± 8.00ab	7 ± 1.82a	57.6 ± 4.38ab	5.7 ± 1.70b	85.4 ± 1.92b
S. indica+ EFP-47	3 ±.00bc	26.3 ± 2.0c	4.2 ± 0.50bc	33.22 ± 3.6cd	5.7 ± 0.95ab	72.0 ± 6.31ab	2 ± 1.15b	18.5 ± 7.96ab	6.5 ± 1a	63.4 ± 3.28a	5 ± 0.81bc	61.7 ± 0.76c
U. fasciata+EFP-47	4 ± 0.00a	25.4 ± 0.4c	3.5 ± 0.57cd	39.48 ± 1.2c	5.7 ± 0.95ab	67.1 ± 4.06bc	3.75 ± 0.50a	24.7 ± 7.45a	5.7 ± 0.95a	54.5 ± 4.12b	5.25 ± 0.50bc	69.6 ± 4.51c
LSD=0.05	0.7[1]	5.5[1]	1.1[1]	6.28[1]	1.3[1]	6.8[1]	1.28[1]	10.9[1]	2.24[1]	8.1[1]	1.3[1]	13.2[1]

[1]Mean values in a column for each parameter showing differences greater than LSD values are significantly different at p<0.05

Table 7. Effect of endophytic fluorescent *Pseudomonas* (EFP-47) on resistance markers and phosphorus uptake by okra plants under soil amended with seaweed, *Stokeyia indica* and *Ulva fasciata* in field plot experiments.

Treatment	2019			2020		
	Salicylic acid (mg/g)	Polyphenol (mg/g)	Phosphorous (ppm)	Salicylic acid (mg/g)	Polyphenol (mg/g)	Phosphorous (ppm)
Control	0.123 ± 0.004c	0.338 ± 0.244a	14.3 ± 1.13c	0.189 ± 0.005b	0.510 ± 0.029a	20.8 ± 1.37b
Topsisn-M (200 ppm)	0.131 ± 0.007abc	0.522 ± 0.194a	16.8 ± 0.98abc	0.201 ± 0.015ab	0.865 ± 0.316a	23.9 ± 1.14ab
S. indica	0.133 ± 0.006abc	0.574 ± 0.158a	16.9 ± 1.51ab	0.211 ± 0.005a	0.695 ± 0.206a	25.6 ± 3.95a
U. fasciata	0.128 ± 0.010bc	0.645 ± 0.333a	16.7 ± 1.36aabc	0.200 ± 0.008ab	0.730 ± 0.249a	24.6 ± 2.15a
Pseudomonas EFP-47	0.136 ± 0.004ab	0.528 ± 0.074a	14.5 ± 2.65bc	0.206 ± 0.005ab	0.950 ± 0.318a	23.62 ± 1.46ab
S. indica+ *Pseudomonas* EFP-47	0.141 ± 0.006a	0.707 ± 0.344a	15.1 ± 1.19bc	0.204 ± 0.012ab	0.937 ± 0.415a	24.80 ± 1.09a
U. fasciata+*Pseudomonas* EFP-47	0.138 ± 0.056ab	0.736 ± 0.148a	18.2 ± 0.85a	0.208 ± 0.005a	0.660 ± 0.195a	23.0 ± 1.19ab
LSD=0.05	0.01[1]	0.41[1]	2.3[1]	0.01[1]	0.40[1]	3.1[1]

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

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