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# EFFICACY OF PLANT GROWTH PROMOTING MICROBES IN INDUCING SYSTEMIC RESISTANCE TO ROOT ROT AND PHYSIOCHEMICAL PROPERTIES OF TOMATO FRUIT

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**Abstract**

Tomato production and quality are of great economic importance, but farmers face significant losses due to root rot infection, which affects tomato yield. Globally, a wide range of chemical pesticides are used to control plant diseases that have an impact on the environmental and nutritional content of the crop. In this scenario, plant growth-promoting microorganisms (PGPMs) are safe and effective substitutes for the control of root infections. In this study, we have investigated the effect of different plant growth promoting microorganisms (PGPMs) on the suppression of root rot disease in tomato plants. In field plot experiments, conducted in the season I and repeated in season II, the application of PGPMs improved the systemic resistance of plants against root rot pathogens via improving the functioning of antioxidant molecules. They considerably reduced the infection caused by *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani,* and *Fusarium oxysporum* compared to untreated control plants. Isolates of PGPMs significantly ($p<0.05$) enhanced the amount of total phenol, and salicylic acid with an improvement in crop yield and quality of fruit (dry matter, soluble solids, fruit acidity, and lycopene content). In season I, *Penicillium* (Pen1-R) whereas in season II, *Trichoderma* (ET-6) treated plants produced the highest quality of tomato fruit with better plant growth and suppression of root rot disease. In both seasons, *Penicillium* and *Trichoderma* were found to be effective as compared to untreated control plants and plants treated with carbendazim, a commercial fungicide. This research will help to make an impact on the production of tomatoes in a sustainable way.

**Key words:** Root rot; Biocontrol; Endophytic; Beneficial microorganisms; Salicylic acid; Antioxidant.

## Introduction

The use of biopesticides and biofertilizers is an option to maintain high food demand with minimal environmental impact (Deng *et al*., 2019). Many biological agents, including necrotizing pathogens, non-pathogens, and rhizosphere-associated bacteria and fungi, can help plants develop systemic resistance to diseases (Romera *et al*., 2019). Plant colonisation with certain plant growth promoting microbes (PGPMs) can induce systemic resistance (ISR) and stimulation of plant defence against various pathogens (Moin *et al*., 2020; Urooj *et al*., 2021). ISR, which includes growth promotion, physiological tolerance, antioxidants and antimicrobial regulation, is developed in response to an external stimulus and introduces the plant's defensive immune system capabilities (Jain & Das, 2016). According to Zehra *et al*., (2017), a new plant protection technique is believed to involve pre-treating a plant with a biological inducer to activate its defence systems. Several enzymatic and non-enzymatic antioxidants, including phenols (secondary metabolites), which have redox properties, the ability to donate hydrogen, and singlet oxygen quenchers that enable them to serve as reducing agents, and protect plants by scavenging free radicals (Bagheri *et al*., 2013). A phenolic compound such as salicylic acid (S.A) is presents in higher plants and is a fundamental molecule in the signal transduction pathway that activates the defence response against various harmful pathogens in many species. S.A is a true plant hormone that goes beyond the defence process in plant immunity and responds to abiotic stress and biotic stress in cotton (Rahman *et al*., 2016). PGPMs such as fluorescent *Pseudomonas* and *Trichoderma* can promote plant growth by suppressing plant diseases (Moin *et al*., 2021). Some of these strains have a strong antagonistic potential and can reduce the severity of plant diseases by suppressing plant pathogens, mainly in the soil or on/in plant roots (Viterbo *et al*., 2010).

Tomato (*Lycopersicon esculentum* Mill) is the world's most highly edible and nutritious vegetable crop which is consumed raw, cooked, and processed (Hossain *et al*., 2010). Tomatoes are an important source of minerals, health-promoting vitamins, and disease-fighting phytochemicals, particularly lycopene (Pinela *et al*., 2016). Qualitative characteristic of tomatoes i.e., acidity, soluble solids, and total dry matter are important quality attributes for processed and fresh market tomatoes (Powell *et al*., 2003). Tomatoes are an important vegetable crop for the tomato processing market. High soluble solids and dry matter content are desirable trait for the tomato canning industry as they enhance the quality of processed product and lower processing costs (Turhan & Seniz, 2009). Tomato yield losses due to root rot disease can account for up to 25% of the overall annual production and losses for individual growers may be higher. Over 2,000 plant species are affected by *Fusarium* spp., (Zehra *et al*., 2022). Losses (60-85%) have been recorded caused by *Fusarium oxysporum* and *Fusarium solani* in combination with root-knot nematodes (Parveen *et al*., 2020a). A wide variety of chemical pesticides are currently available for the management of plant diseases that not only affect the nutritional quality of tomatoes but also the environment.

Organic produce is viewed as a more nutritious and healthier option than conventional produce, and consumers are increasingly more concerned about environmental sustainability and food safety. According to some authors,

the use of organic methods has resulted in higher nutritional content (Carricondo-Martínez *et al*., 2022). In this context, plant growth promoting microorganisms (PGPMs) are a healthy and efficient substitute for chemical pesticides. Plant growth promoting microorganisms (PGPMs) have a high potential to prevent root rot infection by increasing plant resistance biomarkers as well as the physicochemical qualities of tomato fruit. This research aimed to explore the antagonistic potential of beneficial microorganisms to suppress root infection and provide an alternative and environmentally friendly solution to promote the biochemical and nutritional quality of tomato fruit.

## Materials and Methods

**Source of biocontrol agents and inoculum preparation:** Fluorescent *Pseudomonas* (MRFP-205, MRFP-206, MRFP-212, and EFP-47), Rhizobia (NFB-1) and endophytic fungus *Trichoderma* (ET-6, ET-9) used during the experiment were previously evaluated in different crops (Ehteshamul-Haque *et al*., 2015; Moin *et al*., 2020; 2021; Parveen *et al*., 2020b). Whereas, endophytic *Penicillium* (Pen1-R) has been isolated from potato. Fluorescent *Pseudomonas* was grown in King's B broth and rhizobia in Yeast extract mannitol (YMA) broth for 5 days at 28°C. In the case of fungi, a disc of fungus was inoculated into potato dextrose broth (PDB) and incubated for 10 days.

**Experimental design:** After growth, the population of each biocontrol agent was examined by serial dilution method, and their biological control potential activity was also examined in field plots, using tomato as a test crop. Seeds of tomato cultivar (commander-F1) were grown in earthen pots. Roots of tomato seedlings (3 weeks old) were dipped in bacterial and fungal aqueous gum Arabic (1%) suspension ($10^8$ mL $^1$) for 1 hour and transplanted in rows (10 seedlings/row) of 2x2meters field plots at the experimental field of the Biological Research Centre, University of Karachi. The sandy loam soil had a natural infestation of *Macrophomina Phaseolina* (4-8 sclerotia $g^{-1}$ soil), *Fusarium* species (3500 cfu $g^{-1}$ soil), and *Rhizoctonia solani* (7-12% colonization of sorghum seeds used as baits) as determined by using the techniques of Nash & Snyder (1962), Sheikh & Ghaffar (1975) & Wilhelm (1955) respectively. Un-inoculated field plots were served as negative control, while 200ppm Carbendazim @ 200mL/plot was served as a positive control. All treatments were replicates four times and treatments were randomized in a block design. Plants were watered as required. Observations were made at 45 and 90 days, including growth parameters, physical parameters, and biochemical parameters of tomato. The prevalence of root rot fungus was assessed using the method of Moin *et al*., (2020).

**Root rot fungal infestation:** Four tomato plants from each plot were uprooted after 45 and 90 days of replanting. The taproots were cut into 1 cm long pieces, washed with clean water and sterilized with sodium hypochlorite 1% (NaOCl) for about 3 minutes. From each treatment, four tap root sections were placed on a petri plate (four replicates) having potato dextrose agar (PDA) with the antibiotics penicillin (100,000 units/L) and streptomycin (0.2g/L). The plates were incubated at 28°C for a period of 7 days. After the identification of the fungi emerging from each piece, the following formula was used to determine the percentage of infection for each fungus: (Moin *et al*., 2020).

$$\text{Percent infection (\%)} = \frac{\text{Total number of plants infected by a fungus}}{\text{Total number of plants}} \times 100$$

**Analysis of tomato fruit quality parameters:** Physiological and biochemical parameters of tomato leaves and fruits were investigated, furthermore, fruit and leaf antioxidants i.e., lycopene, salicylic acid, polyphenols, and free radical scavenging activity were recorded.

**Percentage of moisture content:** The moisture content percentage of tomato fruit was calculated according to the standard method of Anon., (2000).

$$\text{Moisture \%} = \frac{\{W_1 - W_2\} \times 100}{W_1}$$

Whereas:
$W_1$ = Initial weight of tomato sample
$W_2$ = Oven-dried weight of tomato sample.

**Total dry matter:** The total dry matter (DM) of a sample was determined by drying the tomatoes at a temperature of 105°C till a constant mass was achieved (Anon., 2000).

**Total soluble solids:** The soluble solids present in the tomatoes were observed using a hand refractometer (Atago Co., Tokyo, Japan) ranging 0-32% (Anon., 2000).

**pH determination:** The homogenised tomato pulp and juice were used for pH determination as described of (Anon., 2000).

**Total titratable acidity:** According to the method of (Anon., 2000), 5mL of tomato juice titrated with 0.1M NaOH, using a few drops of phenolphthalein as an indicator. The percentage of citric acid calculated as follows:

$$\text{Citric acid percentage (\%)} = V \times N \times W_{meq} \times 100/Y$$

Whereas: V= NaOH volume (mL) used
N= NaOH Normality
$W_{meq}$= Milliequivalent of citric acid (0.064)
Y= volume of the sample (mL).

**Quantitative analysis of lycopene:** To analyse lycopene, an ethanol: hexane: acetone (1:2:1) (v/v) mixture was used as described by Anthon & Barrett (2006) with slight modification. For lycopene determination, 1mg fresh sample was mixed with distilled water (1mL) and kept in a 30°C water bath for one hour. A solution (8 mL) of ethanol: hexane: acetone (1:2:1) was added, the mixture was immediately capped and vortex. Incubate the reaction mixture at room temperature for 10 min under bright light. Vortex again after adding distilled water (1mL) to each sample. The reaction mixture was incubated for a second time at room temperature for about 10 min, the absorbance/O.D. was observed at 503 nm.

Lycopene content calculated by,
Lycopene (mg/kg) = $Abs_{503} \times 537 \times 8 \times 0.55/0.10 \times 172$
Or, Absorbance $_{503}$nm ×137.4

**Preparation of sample for the determination of total phenol, flavonoids, salicylic acid, and DPPH free radical scavenging activity of fruits and leaves:** 1g of dried fruit or leaf samples were crushed in ethanol (96 % v/v), the resulting concentration was 10mg per millilitre of ethanol, the mixture was centrifuged at 1600xg for 15min and the supernatant was collected for further investigation.

**Estimation of total phenols:** Using the technique of Chandini *et al*., (2008), the total phenolic content of the extract was determined.

**Estimation of total flavonoid content:** The flavonoid content of the sample was estimated using Marinova *et al*., (2005) assay.

**Estimation of salicylic acid (S.A):** Salicylic acid was measured using the technique suggested by Warrier *et al*., (2013).

**Estimation of free radical scavenging activity by (DPPH) assay:** The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay by Tariq *et al*., (2011) was used to determine the free radical scavenging activity (%).

Free radical scavenging activity was calculated as follows:

$$\text{Free radical scavenging activity \%} = \frac{\{\text{Absorbance of control} - \text{Absorbance of sample}\}}{\text{Absorbance of control}} \times 100$$

**Statistical analysis**

Statistical significance ($p<0.05$) was examined by using one-way analysis of variance (ANOVA). The analysis was carried at least in triplicate. $p<0.05$ was used to evaluate the level of significance. Three-ways analysis of variance was also performed to examine the percentage of infection between different treatments, pathogens, and days.

**Results**

**Effect of PGPMs on root rot infection:** In both years, all PGPMs treatments significantly *($p<0.05$)* inhibited or limited the development of *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, and *Fusarium oxysporum*, compared to untreated control plants (Tables 1 and 2). In 2017 complete control of *Fusarium solani* and *Fusarium oxysporum* was observed when plants were treated with Rhizobia (NFB-1). Application of *Trichoderma* (ET-6, ET-9) significantly reduced *Macrophomina phaseolina* infection and was also effective against *Fusarium oxysporum*. However, *Rhizoctonia solani* was suppressed by *Pseudomonas* (EFP-47) after 45 days. In 2018, *Pseudomonas* isolates (MRFP-212 and EFP-47) were most effective against root rot infection. Complete control of *Rhizoctonia solani* by *Pseudomonas* (MRFP-212) was observed after 90 days, whereas; *Fusarium oxysporum* was completely controlled by *Pseudomonas* (EFP-47) after 90 days (Table 2).

In general, Rhizobia (NFB-1) and *Pseudomonas* (EFP-47) suppressed infection in both years as compared to the untreated control.

**Growth parameters:** Beneficial microbes have a positive effect on the growth of tomato plants compared to untreated control group plants. At 45 and 90 days, the maximum positive effect was observed in plants treated with *Penicillium* (Pen1-R) and *Trichoderma* (ET-9) in both years. It is interesting to note that the maximum fresh shoot weight and root length were observed at the highest value in plants treated with *Penicillium* (Pen1-R), but the maximum shoot length and root weight were observed in plants treated with *Trichoderma* (ET-9) in 2017 and 2018 (Table 3).

**Effect of PGPMs on fruit production:** In 2017, *Trichoderma* (ET-6) produced the maximum number (9 fruits /plant) fruits followed by *Trichoderma* (ET-9), whereas in 2018, *Pseudomonas* (MRFP-206) produced the maximum number of fruits followed by *Trichoderma* (ET-6) (Fig. 1). Furthermore, the maximum weight of the fruits was observed in carbendazim treatment in 2017, but it was dropped in the next season, whereas in 2018 *Trichoderma* (ET-6) produced the highest average weight of fruits followed by *Trichoderma* (ET-9) (Figs. 2 and 4).

**Biochemical composition and fruit quality:** A wide variation was observed in the basic physiochemical composition among the treatments (Table 3). Minimum moisture content (91.6% - 92.7%) and maximum dry matter (8.3% and 7.2%) of fruits were found in plants cultivated with *Trichoderma* (ET-6) as compared to untreated control in 2017 and 2018, respectively. In this study the maximum acidity of tomatoes was recorded in plants treated with *Penicillium* (Pen1-R) (0.14% and 0.12%) during the years 2017 and 2018 respectively, followed by Rhizobia (NFB-1) which showed a slight decrease value (0.13% and 0.12%). However, the pH value showed the opposite trend. Total soluble solids (TSS) varied from 4.3 to 5.3 percent (Table 4). Our findings indicated that the proportion of solids varied between different treatments; *Pseudomonas* (MRPF-212) had much higher proportion of soluble solids compared to other treatments during both years (Fig. 3).

**Lycopene and antioxidants of mature fruit:** The application of *Penicillium* (Pen1-R) resulted significantly ($p<0.05$) higher lycopene content in tomatoes (101.1-74.81mg/kg) as compared to the untreated control in 2017 and 2018 respectively. The second highest amount of lycopene was observed in the treatment with *Pseudomonas* (MRFP-205) followed by *Trichoderma* (ET-6) in both years (Table 5).

**Table 1. Effect of endophytic microorganisms on infection of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani,* and *Fusarium oxysporum* in tomato plant in field experiment 2017.**

| Treatments | Infection % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *M. phaseolina* | | *R. solani* | | *F. solani* | | *F. oxysporum* | |
| | 45 Days | 90 Days | 45 Days | 90 Days | 45 Days | 90 Days | 45 Days | 90 Days |
| Control | 37.5 | 43.7 | 37.5 | 25.0 | 50.0 | 75.0 | 31.2 | 31.2 |
| Carbendazim (0.1%) | 18.7 | 37.5 | 37.5 | 62.5 | 43.7 | 31.2 | 25.0 | 50.0 |
| *Pseudomonas (*MRFP-205) | 25 | 18.7 | 18.7 | 43.7 | 62.5 | 0.0 | 12.5 | 6.2 |
| *Pseudomonas* (MRFP-206) | 18.7 | 37.5 | 25 | 56.2 | 43.7 | 43.7 | 12.5 | 12.5 |
| *Pseudomonas* (MRFP-212) | 18.7 | 18.7 | 12.5 | 43.7 | 31.2 | 37.5 | 12.5 | 0.0 |
| *Pseudomonas* (EFP-47) | 18.7 | 25 | 6.2 | 37.5 | 25.0 | 43.7 | 18.7 | 6.2 |
| *Penicillium* (Pen1-R) | 31.2 | 25 | 18.7 | 37.5 | 31.2 | 31.2 | 6.2 | 12..5 |
| *Trichoderma* (ET-9) | 12.5 | 12.5 | 12.5 | 56.2 | 31.2 | 25.0 | 12.5 | 12.5 |
| *Trichoderma* (ET-6) | 2 | 6.2 | 18.7 | 43.7 | 31.2 | 18.7 | 12.5 | 6.2 |
| Rhizobia (NFB-1) | 31.2 | 25 | 37.5 | 31.2 | 37.5 | 0.0 | 0.0 | 0.0 |
| $LSD_{0.05}$ | Treatments = 9.9[1], Pathogens= 6.2[2], Days = 4.4[3] | | | | | | | |

**Table 2. Effect of endophytic microorganism on infection of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani,* and *Fusarium oxysporum* in tomato plant in field experiment 2018.**

| Treatments | Infection % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *M. phaseolina* | | *R. solani* | | *F. solani* | | *F. oxysporum* | |
| | 45 Days | 90 Days | 45 Days | 90 Days | 45 Days | 90 Days | 45 Days | 90 Days |
| Control | 50 | 56.2 | 31.2 | 18.7 | 56.2 | 43.7 | 18.7 | 37.5 |
| Carbendazim (0.1%) | 25 | 31.2 | 25 | 31.2 | 56.2 | 62.5 | 25 | 6.2 |
| *Pseudomonas* (MRFP-205) | 43.7 | 12.5 | 6.2 | 31.2 | 75 | 50 | 18.7 | 25 |
| *Pseudomonas* (MRFP-206) | 6.2 | 50 | 18.7 | 6.2 | 62.5 | 31.2 | 18.7 | 43.7 |
| *Pseudomonas* (MRFP-212) | 25 | 18.7 | 18.7 | 0 | 75 | 62.5 | 12.5 | 37.5 |
| *Pseudomonas* (EFP-47) | 6.2 | 25 | 6.2 | 25 | 25 | 43.7 | 12.5 | 0.0 |
| *Penicillium* (Pen1-R) | 25 | 37.5 | 6.2 | 18.7 | 56.2 | 43.7 | 12.5 | 31.2 |
| *Trichoderma* (ET-9) | 43.7 | 31.2 | 18.7 | 62.5 | 50 | 37.5 | 18.7 | 31.2 |
| *Trichoderma* (ET-6) | 25 | 37.5 | 6.2 | 56.2 | 50 | 50 | 6.2 | 12.5 |
| Rhizobia (NFB-1) | 12.5 | 75 | 37.5 | 0.0 | 62.5 | 31.2 | 6.2 | 37.5 |
| $LSD_{0.05}$ | Treatments = 27.5[1], Pathogens= 17.3[2], Days= 12.2[3] | | | | | | | |

**Table 3. Effect of endophytic microorganism on the growth of tomato plants in field experiments.**

| Treatments | Growth parameters of tomato plants | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot length (cm) 2017 | | Shoot length (cm) 2018 | | Shoot weight (g) 2017 | | Shoot weight (g) 2018 | | Root length (cm) 2017 | | Root length (cm) 2018 | | Root weight (g) 2017 | | Root weight (g) 2018 | |
| | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days |
| Control | 14.6 | 37.5 | 10 | 65.9 | 21.85 | 50.1 | 13.6 | 88.2 | 6.5 | 9 | 5.69 | 25.7 | 2.5 | 5.6 | 1.2 | 9 |
| Carbendazim (0.1%) | 14.2 | 38.1 | 9.6 | 67.3 | 22.2 | 56 | 13.1 | 89.2 | 7.6 | 8.7 | 5.1 | 29.2 | 2.3 | 5.6 | 1.2 | 8.1 |
| *Pseudomonas* (MRFP-205) | 14.3 | 39.1 | 10.3 | 65.7 | 23.25 | 50.9 | 11.0 | 91.2 | 7.7 | 10.1 | 5.3 | 22.2 | 2.8 | 6.6 | 1 | 8.1 |
| *Pseudomonas* (MRFP-206) | 13.5 | 39.1 | 10.3 | 66 | 21.5 | 55.4 | 12.4 | 88.2 | 6.6 | 9 | 5.6 | 28.7 | 2.7 | 6.9 | 1.3 | 9.2 |
| *Pseudomonas* (MRFP-212) | 15.3 | 39.1 | 10.1 | 55.8 | 20.6 | 53.5 | 10.8 | 92 | 9.4 | 11.4 | 6.6 | 20.5 | 2.7 | 5.7 | 1.1 | 6.9 |
| *Pseudomonas* (EFP-47) | 15.0 | 36.8 | 9.1 | 64.9 | 19.4 | 52.3 | 8.8 | 92.8 | 6.5 | 9.4 | 6.1 | 28.2 | 2.5 | 5.5 | 1 | 6.9 |
| *Penicillium* (Pen1-R) | 15.1 | 43.1 | 12.6 | 71.9 | 26.9 | 57.9 | 14.0 | 94.8 | 8 | 8.7 | 5.8 | 30 | 3 | 5.3 | 1.1 | 7.9 |
| *Trichoderma* (ET-9) | 16.1 | 41.4 | 14.2 | 76.1 | 25.3 | 53.3 | 12.2 | 92.1 | 8.2 | 8.7 | 4.9 | 28.5 | 3.1 | 8.1 | 1.6 | 10.5 |
| *Trichoderma* (ET-6) | 15.8 | 31 | 13.2 | 66.2 | 22.2 | 56.4 | 14.9 | 91.1 | 7.3 | 9.3 | 6.1 | 24.4 | 2.8 | 5.1 | 1.1 | 8.9 |
| Rhizobia (NFB-1) | 16.0 | 39.9 | 9.2 | 74.9 | 23.5 | 55 | 11.1 | 90 | 8.1 | 9.5 | 4.9 | 28.5 | 2.5 | 5.8 | 1.2 | 6.5 |
| LSD $_{0.05}$ | 0.79[1] | 1.9[1] | 1.2[1] | 6.4[1] | 2.7[1] | 1.4[1] | 0.4[1] | 1.9[1] | 1.2[1] | 1.7[1] | 0.3[1] | 4.9[1] | 0.8[1] | 0.9[1] | 0.1[1] | 1.1[1] |

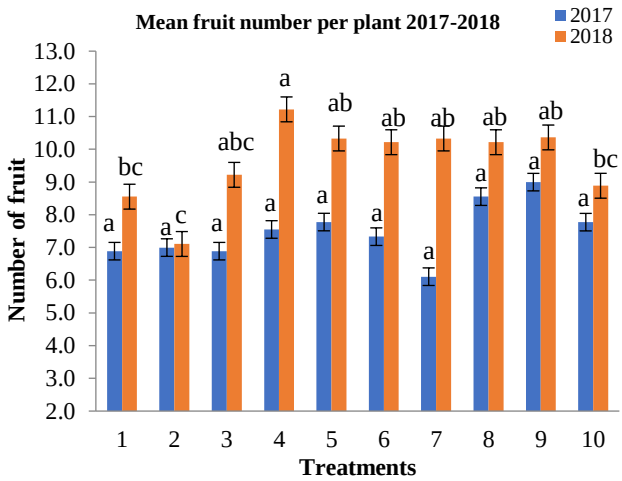


Fig. 1. Effect of plant growth promoting microbes on mean tomato fruit numbers in 2017 and 2018.

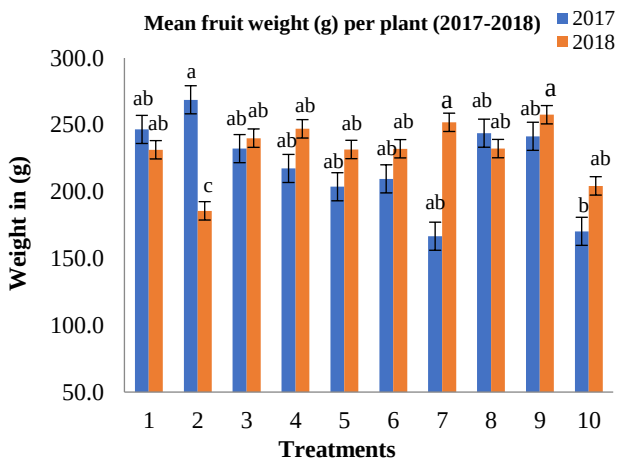


Fig. 2. Effect of plant growth promoting microbes on mean tomato fruit weight in 2017 and 2018.

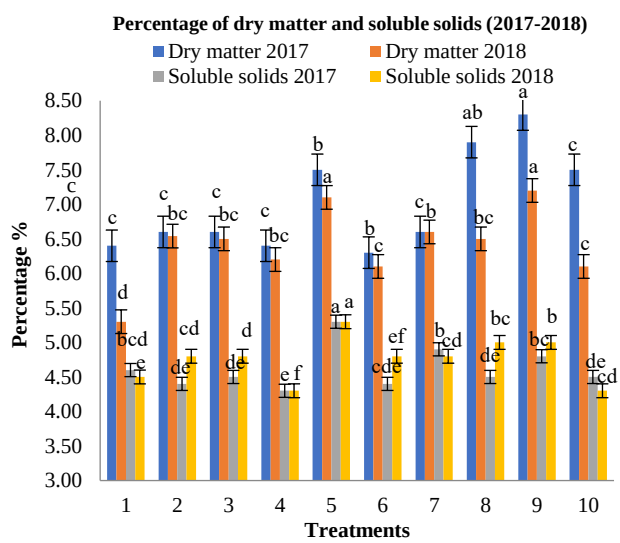


Fig. 3. Effect of plant growth promoting microbes on tomatoes dry matter and soluble solids in 2017 and 2018.
1- Control; 2- Carbendazim; 3- *Pseudomonas* (MRFP-205); 4- *Pseudomonas* (MRFP-206); 5- *Pseudomonas* (MRFP-212)
6- *Pseudomonas* (EFP-47); 7- *Penicillium* (Pen1-R); 8- *Trichoderma* (ET-9); 9- *Trichoderma* (ET-6); 10- Rhizobia (NFB-1)
Bars bearing different superscript letters are showing significant difference at $p<0.05$ with their respective control or among them, according to Duncan's multiple range test.

**Table 4. Effect of endophytic microorganism on the Biochemical Parameters of tomato fruits in field experiments.**

| Treatments | Biochemical parameter | | | | | |
|---|---|---|---|---|---|---|
| | Moisture % | | % Citric acid | | pH | |
| | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| Control | 93.5 | 94.6 | 0.10 | 0.11 | 4.14 | 4.27 |
| Carbendazim (0.1%) | 93.3 | 93.4 | 0.12 | 0.12 | 4.15 | 4.37 |
| *Pseudomonas* (MRFP-205) | 93.3 | 93.4 | 0.11 | 0.10 | 4.08 | 4.36 |
| *Pseudomonas* (MRFP-206) | 93.5 | 93.7 | 0.09 | 0.08 | 4.15 | 4.37 |
| *Pseudomonas* (MRFP-212) | 92.4 | 92.8 | 0.11 | 0.12 | 4.02 | 4.34 |
| *Pseudomonas* (EFP-47) | 93.6 | 93.8 | 0.08 | 0.07 | 4.10 | 4.34 |
| *Penicillium* (Pen1-R) | 93.3 | 93.3 | 0.14 | 0.12 | 4.05 | 4.12 |
| *Trichoderma* (ET-9) | 92.0 | 93.4 | 0.01 | 0.10 | 4.13 | 4.35 |
| *Trichoderma* (ET-6) | 91.6 | 92.7 | 0.09 | 0.09 | 4.17 | 4.33 |
| Rhizobia (NFB-1) | 92.4 | 93.8 | 0.13 | 0.12 | 4.02 | 4.10 |
| $LSD_{0.05}$ | 0.64[1] | 0.39[1] | 0.05[1] | 0.06[1] | 0.14[1] | 0.1[1] |

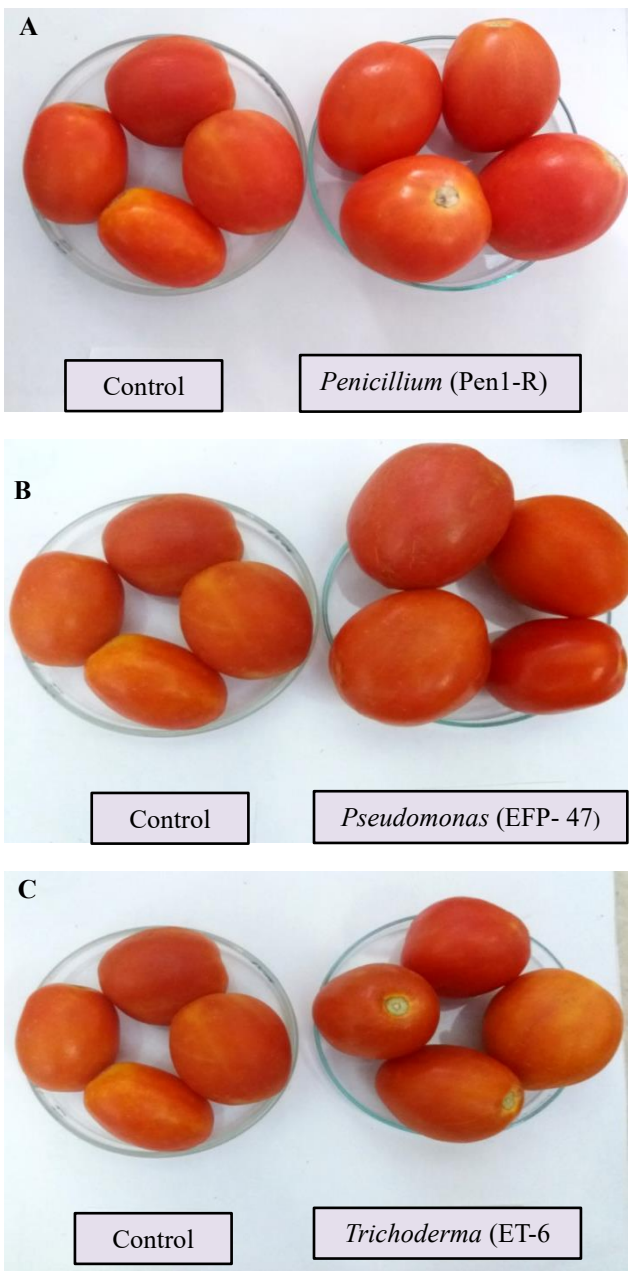


Fig. 4. Effect of plant growth promoting microbes on tomato fruit quality.

The amount of polyphenols was highest in fruits treated with *Trichoderma* (ET-9) followed by *Pseudomonas* (EFP-47) in 2017. However, in 2018 the highest amount of polyphenols was recorded in *Pseudomonas* (MRFP-206 and EFP-47). The results of this study found the highest amount of salicylic acid in *Pseudomonas* (EFP-47) in both seasons compared to untreated control fruits. In addition, the maximum flavonoid content was observed in *Trichoderma* (ET-9) in 2017, whereas in 2018 *Pseudomonas* (MRFP-206 and 212) treatments showed the maximum value of flavonoids (Table.5). In this study, the value of free radical scavenging activity was recorded highest in *Trichoderma* (ET-6) in 2017 and 2018 (Table 5). In general, the highest percentage of free radical scavenging activity was observed in fruits grown with beneficial microorganisms as compared to untreated control plants.

**Antioxidant activity of leaves:** The amount of salicylic acid and polyphenol in leaves was found highest in the *Penicillium* (Pen1-R) treatment after both time intervals in both seasons. In 2017, the highest antioxidant activity of leaves was found after 45 days at 30 min in Rhizobia (NFB-1) treated plants. However, after 90 days, *Pseudomonas* (MRFP-205 and 206) produced the highest antioxidants at 30 min compared to the untreated control. Furthermore, in 2018, *Pseudomonas* (MRFP-206 and MRFP-212) produced the highest percentage of free radical scavenging activity at 30 min followed by *Trichoderma* (ET-9) as compared to untreated control at 90 days (Table 6).

## Discussion

Plants live in versatile environments where they interact with multiple organisms, including detrimental and pathogenic, but also beneficial microbes, (Pieterse *et al*., 2007; Pineda *et al*., 2010). The diversity of beneficial microbes is enormous and different microbes have different effects on plants. Although there is a vast amount of research regarding on the potential of PGPMs as a biocontrol agents against pathogenic fungus, particularly root rotting fungi, their practical usage in agriculture is minimal. In this study, we used the two most common fungal antagonists (*Trichoderma* and *Penicillium*) that were associated with the roots of different plants in agricultural soils (Nallanchakravarthula *et al*., 2014).

**Table 5. Effect of endophytic microorganism on the antioxidants of tomato fruit in field experiments.**

| Treatments | Antioxidants of tomato fruit | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lycopene (mg/kg) | | Flavonoids ($\mu$g/mL) | | Salicylic acid ($\mu$g/mL) | | Polyphenol (mg% GAE) | | Free radical scavenging activity (%) | | | |
| | | | | | | | | | 2017 | | 2018 | |
| | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 01 (min) | 30 (min) | 01 (min) | 30 (min) |
| Control | 60 | 66.0 | 0.018 | 0.017 | 0.008 | 0.005 | 44.8 | 61.4 | 24.6 | 39.8 | 57.2 | 58.8 |
| Carbendazim (0.1%) | 67.5 | 63.2 | 0.014 | 0.018 | 0.009 | 0.007 | 41.9 | 77.1 | 32.8 | 46.9 | 54.4 | 65.6 |
| *Pseudomonas* (MRFP-205) | 87.8 | 66.4 | 0.027 | 0.012 | 0.008 | 0.004 | 52.6 | 77.3 | 42.0 | 28.8 | 55.1 | 59.8 |
| *Pseudomonas* (MRFP-206) | 60.9 | 60.9 | 0.018 | 0.02 | 0.010 | 0.003 | 40.7 | 80.3 | 37.7 | 39.3 | 51.4 | 66.3 |
| *Pseudomonas* (MRFP-212) | 61.66 | 65.8 | 0.019 | 0.02 | 0.003 | 0.002 | 48.3 | 75.6 | 18.8 | 56.4 | 48.6 | 66.7 |
| *Pseudomonas* (EFP-47) | 59.9 | 62.1 | 0.027 | 0.012 | 0.010 | 0.006 | 58.7 | 77.9 | 34.1 | 31.9 | 59.7 | 71.5 |
| *Penicillium* (Pen1-R) | 101.1 | 74.8 | 0.015 | 0.017 | 0.007 | 0.006 | 48.2 | 75.7 | 19.0 | 62.1 | 37.8 | 60.2 |
| *Trichoderma* (ET-9) | 63.7 | 65.1 | 0.03 | 0.01 | 0.007 | 0.004 | 64.2 | 76.4 | 19.9 | 42.1 | 60.6 | 67.2 |
| *Trichoderma* (ET-6) | 88.4 | 64.0 | 0.017 | 0.007 | 0.007 | 0.005 | 47.4 | 76.8 | 39.7 | 41.9 | 63.6 | 77.2 |
| Rhizobia (NFB-1) | 81.3 | 66.7 | 0.015 | 0.016 | 0.007 | 0.004 | 48.7 | 76.0 | 31.4 | 40.3 | 60.6 | 68.0 |
| LSD $_{0.05}$ | 2.9[1] | 1.2[1] | 0.007[1] | 0.002[1] | 0.008[1] | 0.004[1] | 2.5[1] | 3.2[1] | 9.8[1] | 13.6[1] | 3.3[1] | 8.2[1] |

**Table 6. Effect of endophytic microorganism on the antioxidants of tomato leaves in field experiments.**

| Treatments | Antioxidants of tomato leaves | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salicylic acid ($\mu$g/mL) 2017 | | Salicylic acid ($\mu$g/mL) 2018 | | Polyphenol (mg% GAE) 2017 | | Polyphenol (mg% GAE) 2018 | | Free radical scavenging activity (%) 2017 | | | | Free radical scavenging activity (%) 2018 | | | |
| | | | | | | | | | 01 (min) | 30 (mins) | 01 (min) | 30 (mins) | 01 (min) | 30 (mins) | 01 (min) | 30 (mins) |
| | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | 90 days | 45 days | | 90 days | | 45 days | | 90 days | |
| Control | 19.0 | 16.7 | 28.3 | 30.8 | 51.5 | 44.4 | 47.7 | 101.3 | 39.8 | 33.3 | 13.5 | 71.9 | 35.3 | 37.4 | 55.5 | 71.5 |
| Carbendazim (0.1%) | 20.0 | 16.0 | 28.7 | 32.4 | 48.2 | 54.2 | 37.9 | 97.0 | 35.6 | 42.0 | 34.2 | 73.1 | 34.2 | 37.5 | 59.1 | 71.5 |
| *Pseudomonas* (MRFP-205) | 24.0 | 17.3 | 28.9 | 32.2 | 50.3 | 52.9 | 46.5 | 101.3 | 51.9 | 42.1 | 36.2 | 78.0 | 20.7 | 34.2 | 54.0 | 67.8 |
| *Pseudomonas* (MRFP-206) | 19.4 | 17.5 | 30.6 | 31.4 | 65.1 | 57.8 | 48.7 | 110.3 | 45.3 | 34.8 | 30.1 | 78.3 | 35.3 | 60.5 | 56.7 | 75.4 |
| *Pseudomonas* (MRFP-212) | 22.6 | 15.8 | 29.1 | 32.4 | 61.8 | 46.5 | 43.2 | 68.3 | 31.7 | 30.3 | 12.5 | 73.2 | 37.5 | 63.4 | 52.7 | 74.3 |
| *Pseudomonas* (EFP-47) | 25.5 | 19.1 | 31.0 | 24.9 | 78.1 | 49.0 | 38.9 | 72.9 | 12.5 | 36.9 | 30.4 | 73.4 | 29.9 | 42.3 | 54.7 | 73.4 |
| *Penicillium* (Pen1-R) | 32.7 | 24.4 | 31.2 | 34.5 | 76.7 | 52.8 | 56.7 | 147.5 | 43.9 | 48.0 | 20.7 | 73.7 | 30.8 | 34.5 | 47.0 | 69.3 |
| *Trichoderma* (ET-9) | 23.2 | 17.2 | 25.7 | 26.6 | 69.4 | 54.8 | 36.3 | 76.5 | 22.7 | 50.6 | 47.8 | 74.3 | 29.7 | 63.8 | 55.4 | 68.6 |
| *Trichoderma* (ET-6) | 30.6 | 17.4 | 30.7 | 24.2 | 55.8 | 45.9 | 41.3 | 79.3 | 42.5 | 78.7 | 35.2 | 74.0 | 24.8 | 54.5 | 47.6 | 71.5 |
| Rhizobia (NFB-1) | 28.8 | 18.9 | 27.9 | 32.9 | 53.5 | 45.3 | 36.0 | 75.1 | 25.4 | 79.4 | 15.5 | 73.5 | 30.1 | 36.2 | 60.6 | 70.4 |
| LSD $_{0.05}$ | 8[1] | 3[1] | 3[1] | 9[1] | 4.2[1] | 1.1[1] | 3.6[1] | 1.6[1] | 10.8[1] | 13.9[1] | 8.6[1] | 4.4[1] | 6.1[1] | 6.6[1] | 6.6[1] | 2.5[1] |

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

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

[3]Mean values in row for days showing differences greater than LSD values are significantly different at p<0.05

We also used three fluorescent *Pseudomonas* strains and one Rhizobial isolate that have been previously tested on different crops, individually or in combination (Ehteshamul-Haque *et al*., 2007; Moin *et al*., 2020; 2021; Parveen *et al*., 2020b). In both years among other treatments *Penicillium* and *Trichoderma* isolates were found to be effective as compared to untreated control plants and plants treated with carbendazim, a commercial fungicide (Fig. 4). Trichoderma, free-living fungi that are typically found in the roots of plants, are known for their ability to act as a biocontrol agents (Ehteshamul-Haque & Ghaffar 1992; Moin *et al*., 2021). Our results show a decreasing trend in root infection when treated with biological treatments, particularly the isolates of rhizobia (NFB-1) and *Pseudomonas* (EFP-47) isolates suppressed the infection during in both years compared to the control. Suppression of root rot disease and improvement in plant growth were also reported previously by Moin *et al*., 2021 & Parveen *et al*., 2020b.

Plants contain many phenolic compounds that are important for the growth and reproduction of plants. Plant phenols are natural antioxidants that act as antibiotics and natural pesticides; antioxidants can slow or inhibit the oxidation process of cellular compounds for instance lipids, carbohydrates, proteins and DNA (Kaurinovic & Vastag, 2019). The current results demonstrate that the phenolic compounds of fruits and leaves contribute positively to their antioxidant capacity, in addition to reducing the levels of free radicals, as similarly reported by Shafique *et al*., (2015).

The lycopene content in tomatoes is responsible for the red colour and is an important quality indicator as well as considered an antioxidant with high biological activity (Przybylska, 2020). Our investigation reveals that among all treatments, *Penicillium* (Pen1-R) treatment enhances the lycopene content as compared to untreated plants. Furthermore, other antioxidant compounds examined in tomato fruit were better influenced by the beneficial microorganism application.

The significance of plant growth-promoting beneficial microbes in improving plant fitness via stimulating the tolerance to biotic and abiotic stresses has been revealed, however very few experiments have been conducted in real field conditions to improve the fruit quality and healthiness for human consumption (Zhang *et al*., 2008). Beneficial microbes such as *P. fluorescens* not only help plants 'deal' with root rot diseases and improve plant resistance but also improve the crop yield and fruit quality (Ramos-Solano *et al*., 2014). These microorganisms colonise plant roots and produce root systems that directly improve crop yield by increasing root hair biomass production and root hair growth for better water absorption and conduction (Harman *et al*., 2004). Beneficial microorganisms could be used as biocontrol agents against root rotting fungi; they produce ammonia, HCN (hydrogen cyanide) and also volatile antifungal compounds. Microorganisms that promote development of plant are gaining attention as biocontrol agents because they suppress plant diseases and have a beneficial effect on plant growth (Gómez-Lama Cabanás *et al*., 2014; Prieto *et al*., 2011).

This study has explored an environmentally friendly option to improve tomato plant growth, fruit yield, and some physical and chemical characteristics of its fruit through the application of plant growth promoting microorganisms. It would suggest that beneficial bacteria and fungi present in plant roots may serve as a possible substitute for chemical pesticides.

## Conclusions

This study provides evidence that PGPMs have exceptional potential against several root rot infections. These species have the potential to have significantly impact on fruit production and quality. This study will enables the selection of the best plant-associated bacteria for field inoculation, resulting in potential biocontrol, improved systemic resistant markers, decreasing chemical inputs, natural soil sustainability, and improved fruit productivity and quality.

### References

Anthon, G. and D.M. Barrett. 2006. Standardisation of a rapid spectrophotometric method for lycopene analysis. *Acta Hort.*, 758: 111-128. DOI: 10.17660/ActaHortic. 2007.758.12

Anonymous. 2000. *Official Methods of Analysis*. 17th Edition. The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bagheri, S., H. Ahmadvand, A. Khosrowbeygi, F. Ghazanfari, N. Jafari, H. Nazem and R.H. Hosseini. 2013. Antioxidant properties and inhibitory effects of *Satureja khuzestanica* essential oil on LDL oxidation induced–CuSO4 *In vitro*. *Asian Pac. J. Trop. Biomed.*, 3: 22-27.

Carricondo-Martínez, I., F. Berti and M.D.C. Salas-Sanjuán. 2022. Different Organic Fertilisation Systems Modify Tomato Quality: An Opportunity for Circular Fertilisation in Intensive Horticulture. *Agron. J*., 12(1): 174.

Chandini, S.K., P. Ganesan and N. Bhaskar. 2008. In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chem*., 107: 707-713.

Deng, S., H.M.L. Wipf, G. Pierroz, T.K. Raab, R. Khanna and D. Coleman-Derr. 2019. A plant growth-promoting microbial soil amendment dynamically alters the strawberry root bacterial microbiome. *Sci. Rep.*, 9(1): 17677.

Ehteshamul-Haque, S., G. Parveen, V. Sultana and J. Ara. 2007. Utilisation of plant growth-promoting and nodule producing bacteria for the management of root rotting fungi and root knot nematode. *Phytopath* 97: S31-S31. 3340 PILOT KNOB ROAD, ST PAUL, MN 55121 USA: AMER PHYTOPATHOLOGICAL SOC.

Ehteshamul-Haque, S., S.S. Bokhari, S.A. Ali, V. Sultana and J. Ara. 2015. Suppression of root diseases and enhancement of VA mycorrhizal population in sunflower and tomato by the mycorrhizospheric fluorescent *Pseudomonas. Phytopath*., 105(Suppl.4): S4.40

Gómez-Lama Cabanás, C., E. Schilirò, A. Valverde-Corredor and J. Mercado-Blanco. 2014. The biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7 induces systemic defence responses in aerial tissues upon colonisation of olive roots. Front *Microbiol*., 5: 427.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. Trichoderma species opportunistic, a virulent plant symbionts. *Nat. Rev. Microbiol.*, 2(1): 43-56.

Hossain, M.E., M.J. Alam, M.A. Hakim, A.S.M. Amanullah and A.S.M. Ahsanullah. 2010. An assessment of physicochemical properties of some tomato genotypes and varieties grown at Rangpur. *Bangladesh J. Bot.*, 4(3): 135-243.

Kaurinovic, B. and D. Vastag. 2019. *Flavonoids and phenolic acids as potential natural antioxidants* (pp. 1-20). London, UK: IntechOpen.

Jain, A. and S. Das. 2016. Insight into the interaction between plants and associated fluorescent Pseudomonas spp. *Int. J. Agron*. Article ID 4269010 https://doi.org/10.1155/2016/4269010.

Moin, S., S.A. Ali, K.A. Hasan, A. Tariq, V. Sultana, J. Ara and S. Ehteshamul-Haque. 2020. Managing the root rot disease of sunflower with endophytic fluorescent Pseudomonas associated with healthy plants. *Crop Prot.*, 130: 105066.

Moin, S., A. Rahman, G. Parveen, F. Korejo, H.A. Shafique, R. Zehra and S. Ehteshamul-Haque. 2021. Amelioration of systemic resistance in tomato against root rotting fungi by the endophytic Trichoderma species. *Pak. J. Bot.*, 53(1): 321-327.

Marinova, D., R. Ribarova and M. Atanassova. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Chem. Technol.*, 40: 255-260.

Nash, S.M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. *Phytopath.*, 52(6): 567-572.

Parveen, G., F. Urooj, S. Moin, H. Farhat, M.F. Fahim and S. Ehteshamul-Haque. 2020a. Estimation of losses caused by root rotting fungi and root knot nematodes infecting some important crops in Lower Sindh and Hub, Balochistan of Pakistan. *Pak. J. Bot.*, 52: 673-678.

Parveen, G., F. Urooj, H. A.Shafique, A. Rahman and S. Eteshamul-Haque. 2020b. Role of rhizobia in suppressing the root rot and root knot disease of chilli used alone or with *Pseudomonas aeruginosa. Pak. J. Bot.*, 52(3): 1097-1104.

Pieterse, C.M. and M. Dicke. 2007. Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends Plant Sci.*, 12(12): 564-569.

Pineda, A., S.J. Zheng., J.J. Van Loon, C.M. Pieterse and M. Dicke. 2010. Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends Plant Sci.*, 15(9): 507-514.

Pinela, J., M.B.P.P. Oliveira and I.C.F.R. Ferreira. 2016. Bioactive compounds of tomatoes as health promoters. *Nat. Bioact. Comp. Fruits & Veg.*, 2(3): 48-91.

Powell, A.L., M.S. Kalamaki, P.A. Kurien, S. Gurrieri and A.B. Bennett. 2003. Simultaneous transgenic suppression of LePG and LeExp1 influences fruit texture and juice viscosity in a fresh market tomato variety. *J. Agric. Food Chem.*, 51(25): 7450-7455.

Prieto, P., E. Schilirò, M.M. Maldonado-González, R. Valderrama, J.B. Barroso-Albarracín and J. Mercado-Blanco. 2011. Root hairs play a key role in the endophytic colonisation of olive roots by *Pseudomonas* spp. with biocontrol activity. *Microb. Ecol.*, 62(2): 435-445.

Przybylska, S. 2020. Lycopene–a bioactive carotenoid offering multiple health benefits: a review. *J. Food Sci. Technol.*, 55(1): 11-32.

Rahman, A., V. Sultana, J. Ara and S. Ehteshamul-Haque. 2016. Induction of systemic resistance in cotton by the neem cake and *Pseudomonas aeruginosa* under salinity stress and *Macrophomina phaseolina* infection. *Pak. J. Bot.*, 48: 1681-1689.

Ramos-Solano, B., A. Garcia-Villaraco, F.J. Gutierrez-Mañero, J.A. Lucas, A. Bonilla and Garcia-Second. 2014. Annual changes in bioactive contents and production infield-grown blackberry after inoculation with *Pseudomonas fluorescens*. *Plant Physiol. Bioch.*, 74: 1-8.

Romera, F.J., M.J. Lucena, C.A. Martínez-Medina, M.A. Aparicio, J. Ramos and R. Pérez-Vicente. 2019. Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. *Front. Plant Sci.*, 10, 287. DOI:10.3389/fpls.2019.00287.

Shafique, H.A., V. Sultana, J. Ara, S. Ehteshamul-Haque and M. Athar. 2015. Role of antagonistic microorganisms and organic amendment in stimulating the defence system of okra against root rotting fungi. *Pol. J. Microbiol.*, 64: 155-160.

Sheikh, A.H. and A. Ghaffar. 1975. Population study of sclerotia of Macrophomina phaseolina in cotton fields. *Pak. J. Bot.*, 7: 13-17.

Nallanchakravarthula, S., S. Mahmood, S. Alström and R.D. Finlay. 2014. Influence of soil type, cultivar and *Verticillium dahliae* on the structure of the root and rhizosphere soil fungal microbiome of strawberry. *PLoS One*, 9(10): e111455.

Tariq, A., J. Ara, V. Sultana, S. Ehteshamul-Haque and M. Athar. 2011. Antioxidant potential of seaweeds occurring at Karachi coast of Pakistan. *J. Appl. Bot. Food Qual.*, 84: 207-212.

Turhan, A. and V. Şeniz. 2009. Estimation of certain chemical constituents of fruits of selected tomato genotypes grown in Turkey. *Afr. J. Agric. Res.*, 4(10): 1086-1092.

Urooj, F., H. Farhat, A. Tariq, S. Moin, N. Sohail, V. Sultana, S.F. Hameedi, Z.I. Shams and S. Ehteshamul-Haque. 2021. Role of endophytic *Penicillium* species and *Pseudomonas monteilii* in inducing the systemic resistance in okra against root rotting fungi and their effect on some physiochemical properties of okra fruit. *J. Appl. Microbiol.*, 130(2): 604-616.

Viterbo, A., U. Landau, S. Kim, L. Chernin and I. Chet. 2010. Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. *FEMS Microbiol. Lett*., 305: 42-48.

Warrier, R.R., M. Paul and M.V. Vineetha. 2013. Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genet. Plant Physiol.*, 3(1-2): 90-97.

Wilhelm, S. 1955. Longevity of the Verticillium wilt fungus in the laboratory and field. *Phytopath.*, 45(3): 180-181.

Zehra, A., M. Meena, M.K. Dubey, M. Aamir and R.S. Upadhyay. 2017. Synergistic effects of plant defence elicitors and *Trichoderma harzianum* on enhanced induction of antioxidant defence system in tomato against Fusarium wilt disease. *Bot. Stud*., 58(1): 1-14.

Zehra, R., S. Moin, S.M. Enamullah, and S. Ehteshamul-Haque. 2022. Detection and identification of quarantine bacteria and fungi associated with imported and local potato seed tubers. *Pak. J. Bot*, 54(3): 1157-1161. DOI: 10.30848/PJB2022-3(25)

Zhang, H., X. Xie., M.S. Kim., D.A. Korneyev., S. Holaday and P.M. Paré. 2008. Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. *Plant J*., 56(2): 264-273.

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