

EFFICACY OF RHIZOBACTERIA AND HUMIC ACID FOR CONTROLLING *FUSARIUM* WILT DISEASE AND IMPROVEMENT OF PLANT GROWTH, QUANTITATIVE AND QUALITATIVE PARAMETERS IN TOMATO

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ABSTRACT

The effect of tomato seedling treated with plant growth promoting rhizobacteria (PGPR) strains viz. *Azotobacter* sp. (AZM1), *Bacillus cereus* (BCM8), *B. megaterium* (BMM5) individually or combined with humic acid were evaluated for controlling wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici*, plant growth, fruit quantitative and qualitative (cv. Super Strain-B) during 2010-2011 and 2011-2012 growing seasons. Under greenhouse conditions, all treatments significantly reduced area under disease progress curve (AUDPC) and increased plant height, fresh and dry weights of survival plants growing in pots infested with the causal pathogen compared with control. Combination treatments of humic acid with PGPR reduced significantly wilt incidence and increased plant height, fresh and dry weights of tomato plants comparing with the application of each of them alone. Under laboratory conditions, all PGPR strains and humic acid able to inhibited leaner growth of the causal pathogen with different degrees and PGPR strains were more active than humic acid in this respect. Under field conditions, all PGPR stains individually or combined with humic acid significantly reduced AUDPC and improved plant growth (plant height, number of branches plant⁻¹) quantitative (number of fruits plant⁻¹, fruit weight plant⁻¹, fruit weight, fruit yield fed. ⁻¹, Number of fruit Kg⁻¹) and qualitative (degree of fruit's color, fruit diameters, firmness, fruit height, total soluble solids) parameters of tomato fruits compared with untreated plants (control) in both growing seasons. Combination treatments of humic acid with PGPR strains increase the effectiveness of them in this respect more than used alone.

Keywords: Tomato, wilt disease, *Fusarium oxysporum*, PGPR, humic acid, *Azotobacter* sp., *Bacillus cereus*, *B. megaterium*.

INTRODUCTION

Tomato is one of the most valued vegetable crops of the world. It has a very high nutritive value and also has antioxidant and curative properties. Production of tomato is limited due to various insect pest and diseases. *Fusarium oxysporum* f. sp. *lycopersici* is known to affect tomato plants which are a crop plant of great economic importance (Suarez-Estrella *et al.*, 2007). Tomato production is significantly reduced by *Fusarium oxysporum* f. sp. *lycopersici* because it can destroy roots of tomatoes at growth stages. Many strategies to control this fungal pathogen have been investigated in the field (Khan *et al.*, 2007). Currently, the most effective method

in preventing tomato from *Fusarium* wilt is to mix the seed with chemical fungicides. The application of chemical fungicide induces other problems, such harm to other living organisms and the reduction of useful soil microorganisms (Lewis *et al.*, 1996).

Although the use of *Fusarium*-resistant tomato cultivars can provide some degree of control of these diseases, the occurrence and development of new pathogenic races is a continuing problem, and currently there are no commercially acceptable cultivars with adequate resistance to *F. oxysporum* f. sp. *lycopersici*.

Therefore, public concern is focused on alternative methods of pest control, which can play a role in integrated pest management systems to reduce our dependence on chemical pesticides (Sutton, 1996). As with other vascular plant diseases, sanitation measures are difficult to apply (Brayford, 1992). Hence, strategies

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aiming at replacement of chemical pesticides by hazardous free biological agents can be a reasonably good choice. In recent years, Plant growth promoting rhizobacteria (PGPR) has been suggested as a potentially attractive alternative disease management approach since PGPR are known for growth promotion and disease reduction in crops (Jetiyanon and Kloepper, 2002). PGPR is a mixture of beneficial microorganisms which can increase the crop yield, plant growth and also protect against plant pathogens (Seleim *et al.*, 2011). Among PGPR, *Bacillus* spp. and *Azotobacter* spp., have been reported to be effective against a broad spectrum of plant pathogens, including fungi, bacteria and viruses in many plant species (Morsy *et al.*, 2009, Abdel-Monaim, 2010 b, Mogle and Mane, 2010).

Also, Humic acid (HA) suspensions based on potassium humates have been applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases (Scheuerell and Mahaffee, 2004), stimulating plant growth through increased cell division, as well as optimizing uptake of nutrients and water and stimulating soil microorganisms (Chen *et al.*, 2004). Several reports indicated the efficiency of HA in reducing some plant diseases (Yigit and Dikilitas, 2008, Abdel-Monaim *et al.*, 2011, Abdel-Kader *et al.*, 2012).

The objective of this study was carried out to assess the efficacy of certain PGPR strains individually or combination with humic acid for the management tomato wilt disease as well as its effect on growth parameters, fruit yield and quality.

MATERIAL AND METHODS

Plant Materials: Seeds of tomato (cv. Super Strain B), were used in the present study, and obtained from Unit sale vegetable crops seeds, Horticulture Res. Institute, Agric. Res. Center, Giza, Egypt.

Source of the pathogen, PGPR agents and inoculum preparation: An aggressive isolate of *Fusarium oxysporum* f. sp. *lycopersici* (isolate FT7), obtained from Plant Pathol. Dep., New Valley Agric. Res. Station, was used in the present study. This isolate proved to be highly pathogenic to induce wilt disease on tomato plants in previous work (Abdel-Monaim, 2010 a). Inoculum of the pathogenic fungus was prepared by culturing on 50.0 mL potato dextrose broth (PDB) medium in 250 mL Erlenmeyer flasks for 10 days at 25±2°C following washing and blending in sterilized water. Colonies forming units (cfu) were adjusted to 10⁶ cfu/mL using haemocytometer slide. Soil infestation

was carried out at rate of 50 mL (10⁶ cfu/mL) / kg soil (Elad and Baker, 1985). On the other hand, three plant growth promoting rhizobacteria (PGPR, obtained from Plant Pathol. Dep., New Valley Agric. Res. Station) viz. *Azotobacter* sp (isolate AZM1), *B. cereus* (isolate BCM8) and *Bacillus megaterium* (isolate BMM5), were used in this study. These bio- control agents were previously tested against several soil born pathogens (Abdel-Monaim, 2010 b; Moubarak and Abdel-Monaim, 2011). PGPR inoculum were produced as described by Landa *et al.* (2004). Bacterial concentration in the suspension was adjusted to proximately 5 × 10⁸ cells ml⁻¹ by measuring absorbance at 600 nm in a spectrophotometer and using standard curves for each bacterial isolate.

Soil Infestation: Plastic pots, 30 cm in diameter, were filled with 5 kg formalin disinfested soil. Infestation of soil with the pathogenic fungus was done by applying the prepared inoculum, as described before, to pots at rate of 50 ml kg⁻¹, mixed thoroughly with the soil, then watered and left for one week to insure establishment and distribution of the inoculum in soil.

Effect of PGPR and humic acid on wilt disease of tomato under greenhouse conditions: The trials were carried out in the greenhouse of Plant Pathology Dep., New Valley Agric. Res. Station. A pot experiments were conducted in 2010-2011 season to investigate the influence of seedlings inoculation with each of the previously strains of PGPR as a bio-control agent individually or combination with humic acid (4 g/L) against *Fusarium* tomato wilt disease. Surface sterilized seeds of tomato, highly susceptible cultivar "Super Strain B" to *Fusarium oxysporum* f. sp. *lycopersici* (Abdel-Monaim, 2010 a) were used for all experiments. Tomato seeds were sown in trays (30×50 cm, 10 cm deep) containing sieved clay sand soil mixed with 3% peat moss, and watered twice a week (Abo-Elyousr and Mohamed, 2009). After 40 days old, healthy seedlings (15 cm in length) were dug off seedling trays and the root thoroughly washed by running water to remove any adherent particles, then treated by dipping the root at rate 100 seedlings per 100 ml of the following treatments for one hour: 1- *Azotobacter* sp, 2- *Bacillus cereus*, 3- *B. megaterium*, 4- *Azotobacter* sp+ *B. cereus* + *B. megaterium*, 5- *Azotobacter* sp. + humic acid, 6- *B. cereus* + humic acid, 7- *B. megaterium* + humic acid, 8- *Azotobacter* sp. + *B. cereus* + *B. megaterium* + humic acid, 9- Humic acid. The treated tomato seedlings were then transferred to the pathogen infested pots. Four

seedlings were transplanted in each pot and 4 replicates were planted for each treatment. In addition, untreated seedlings were transplanted in pots containing infested soil (infected control). Plants were irrigated when needed and fertilized as usual. After 8 weeks from transplanting, plants of four replicates from each treatment were uprooted, washed thoroughly with running water, blotted with tissue paper, weighed to determine fresh weights, and then oven dried at 80 °C for 24 h for dry weights.

Disease assessments: Wilt severity was estimated at 10 days interval for 60 after transplanting according to Abdou *et al.* (2001) using a rating scale of (0 – 5) on based on leaf yellowing grading, viz., 0 = healthy, 1= one leaf yellowing 2= more than one leaf yellowing, 3= one wilted leaf, 4= more than one leaf wilted, and 5= completely dead plants. Disease severity index (DSI) described by Liu *et al.* (1995) was adapted and calculated as follows:

$$DSI = \sum d / (d_{\max} \times n) \times 100$$

Where: d is the disease rating of each plant, d_{\max} the maximum disease rating and n the total number of plants/samples examined in each replicate.

The mean of area under disease progress curve (AUDPC) for each replicate was calculated as suggested by Pandey *et al.* (1989).

$$AUDPC = D [1/2 (Y_1 + Y_k) + (Y_2 + Y_3 + \dots + Y_{k-1})]$$

Where D= Time interval; Y_1 = First disease severity; Y_k = Last disease severity;

Y_2, Y_3, \dots, Y_{k-1} = Intermediate disease severity.

In vitro screening inhibitory effect of PGPR and humic acid: The tested isolates of antagonistic PGPR were streaked at one side on PDA medium in plates and incubated for 24 hours at 25°C±1, then one disc (7 mm in diameter) of *F. oxysporum* f. sp *lycopersici* was placed on the opposite side (Kaur *et al.*, 2007). On the other hand, the inhibitory effect of humic acid at concentration 4 g L⁻¹ on linear growth of *F. oxysporum* was evaluated. Tested solution of humic acid was added to conical flasks containing sterilized PDA medium before solidifying to obtain the proposed concentration and shacked gently, then dispensed into sterilized Petri dishes (9- cm- diameter). Petri dishes were inoculated with equal disks (7- mm-diam.) taken from the same culture of pathogenic fungus. Four replicates were used for each treatment. The inoculated plates with pathogenic fungus only were used as control. After 7 days incubation, linear growth of *F. oxysporum* f. sp *lycopersici* in all treatments was recorded. The decrease

of percentage that occurred in linear growth of the pathogenic fungus was determined at the end of the experiment using formula suggested by Fokemma (1973) as follows:

$$\text{Reduction in linear growth} = [(R1 - R2) / R1] \times 100$$

Where:

R1= the radius of normal growth in control plates;

R2= the radius of inhibited growth.

Field experiments: Field experiments was carried out at New Valley Agric. Res. Station Farm, New Valley governorate during 2010-2011 and 2011-2012 seasons, to evaluate the efficiency of the tested PGPR as Bio-control agents (*Azotobacter* sp., *Bacillus cereus*, *B. megaterium*) individually or combination with humic acid for controlling wilt disease of tomato plants as well as its effect on growth parameters, qualitative and quantities of fruit yield. The chosen field test area was naturally infested with *F. oxysporum*. The experimental design was a complete randomized block with four replicates. The experimental unit area was 15 m² (5 × 3 m). Each unit included three rows; each row was 5 m in length and 1 m width. Tomato seedlings cv. Super Strain B were treated by dipping the roots for one hour at rate 100 seedlings per 100 ml of the following treatments: 1- *Azotobacter* sp., 2- *Bacillus cereus*, 3- *B. megaterium*, 4- *Azotobacter* sp.+ *B. cereus* + *B. megaterium*, 5- *Azotobacter* sp+ humic acid, 6- *B. cereus* + humic Acid, 7- *B. megaterium* + humic acid, 8- *Azotobacter* sp+ *B. cereus* + *B. megaterium* + humic acid, 9- Humic acid. Seedlings transplanted into the field in 1 October in both seasons at rate 10 seedlings per row; one seedling/hill was sown with 50 cm apart between hills. Untreated seedlings were used as control. The NPK mineral fertilizers were applied at the recommended dose of Ministry of Agriculture and Land Reclamation. Disease severity was recorded every 30 days for 4 months. The mean of area under disease progress curve (AUDPC) for each replicate was calculated as above. Plant height, number of branches, number of fruits plant⁻¹, fruit weight plant⁻¹ (kg), fruit yield feddan⁻¹ (ton), Number of fruit Kg⁻¹, degree of fruit's color, fruit diameters (cm), firmness by penetration tester apparatus (kg So cm²), fruit length (cm) were calculated at the end of the growing season. Total soluble solids (T.S.S) measured by Refractometer.

Statistical Analysis: All experiments were performed twice. Analyses of variance were done using MSTAT-C program version 2.10 (1991). Least significant difference (LSD) was calculated at P ≤ 0.05 according to Gomez and Gomez (1984).

RESULTS

Effect of PGPR and humic acid on wilt disease of tomato under greenhouse conditions: A pot experiment was carried out to examine the efficiency of PGPR strains individually or combination with humic acid to antagonize wilt disease caused by *F. oxysporum* under greenhouse conditions. The obtained results in Table (1) showed the efficacy of applied PGPR strains viz. *Azotobacter* sp. *B. megaterium* and *B. cereus* and /or humic acid as seedling treatment against tested Fusarium wilt disease incidence. Presented data revealed that all applied treatments reduced significantly wilt incidence and increased plant height, fresh and dry weights of the survival plants comparing with un-treated check control. Data also showed that combination treatments of humic acid with PGPR reduced significantly wilt incidence of tomato plants comparing with the application of each of them alone. Also, applied mixed of PGPR was highly efficacy for reducing wilt incidence than applied of each of them alone. The treatment of mixed PGPR strains, *Azotobacter*

sp., *B. megaterium* and *B. cereus* recorded the highest significant reduction in AUDPC when combined with humic acid (68.02%) followed by treatment *B. megaterium* combined with humic acid (65.32%) and *B. cereus* + humic acid (63.89%). While, tomato seedling treated with *Azotobacter* sp. recoded the lowest ones (19.44%) followed by treatment *B. cereus* (28.55%). On the other hand, the effectiveness of these treatments in reducing the incidence of wilt disease is reflected on the growth of tomato plants. All treatments led to increased plant height, fresh and dry weights of survival plants compared with the control plants. The combination between mixed PGPR strains humic acid were recorded the highest plant height (25.46 cm), fresh weight (7.453 g plant⁻¹) and dry weight (2.372 g plant⁻¹) compared with 12.44 cm, 3.057 g plant⁻¹ and 0.939 g plant⁻¹ in control treatment, respectively. While tomato seedlings treated with *Azotobacter* sp gave the lowest plant height (14.62 cm), fresh weight (4.692 g plant⁻¹) and dry weight (1.520 g plant⁻¹).

Table 1. Effect of PGPR individually or combination with humic acid on area under disease progress curve caused by *F. oxysporum*, plant height, fresh and dry weights under greenhouse conditions.

Treatments	AUDPC ^a	Reduction (%)	Plant height (cm)	Fresh weight (gm plant ⁻¹)	Dry weight (gm plant ⁻¹)
<i>Azotobacter</i> sp (Az)	895.7 b	19.44	14.62 e	4.692 f	1.520 e
<i>Bacillus cereus</i> (Bc)	755.8 c	32.03	15.63 de	5.020 e	1.571 e
<i>B. megaterium</i> (Bm)	794.5 c	28.55	16.84 cd	5.264 de	1.792 c
Az + Bc + Bm	525.8 e	52.71	16.21 d	5.213 e	1.703 cd
Az + Humic acid	486.3 e	56.26	17.80 c	5.497 d	1.715 cd
Bc + Humic acid	401.5 f	63.89	22.45 b	6.284 c	2.030 b
Bm + Humic acid	385.6 fg	65.32	23.04 b	7.055 b	2.291 a
Az+ Bc+ Bm+ Humic acid	355.6 g	68.02	25.64 a	7.453 a	2.372 a
Humic acid	612.5 d	44.91	16.62 cd	5.222 e	1.613 de
Control	1111.9 a	-	12.44 f	3.057 g	0.939 f

^a Different letters indicate significant differences between tomato fungal isolates according to L.S.D. test ($P=0.05$). AUDPC= $D [1/2 (Y_1+Y_k) + (Y_2+Y_3+.....+Y_{k-1})]$; Where D= Time interval, Y_1 = First disease severity, Y_k = Last disease severity, $Y_2, Y_3,.....Y_{k-1}$ = Intermediate disease severity.

Evaluation of PGPR and humic acid for antagonistic activities against *F. oxysporum* in vitro: Plant growth promoting rhizobacteria viz. *Azotobacter* sp., *Bacillus cereus*, *B. megaterium* strains and humic acid were evaluated for antagonistic effect against *F. oxysporum* on Petri dishes containing PDA medium. Data in Fig. 1 show that all PGPR stains and humic acid succeeded in reducing the radial growth of *F. oxysporum*. PGPR strains were more active than humic acid for reducing the radial growth of pathogenic fungus. *Bacillus cereus*

recorded the highest suppressed effect for radial growth of *F. oxysporum* (52.8%) followed by *B. megaterium* and *Azotobacter* sp. (48.6 and 35.6% respectively). while humic acid recorded the lowest ones (26.3%).

Efficiency of PGPR and humic acid against *F. oxysporum* under field conditions: Effects of PGPR strains individually and/or combination with humic acid on wilt disease incidence, some growth parameters, quantity and qualitative characteristics of tomato plants in New Valley governorate was studied.

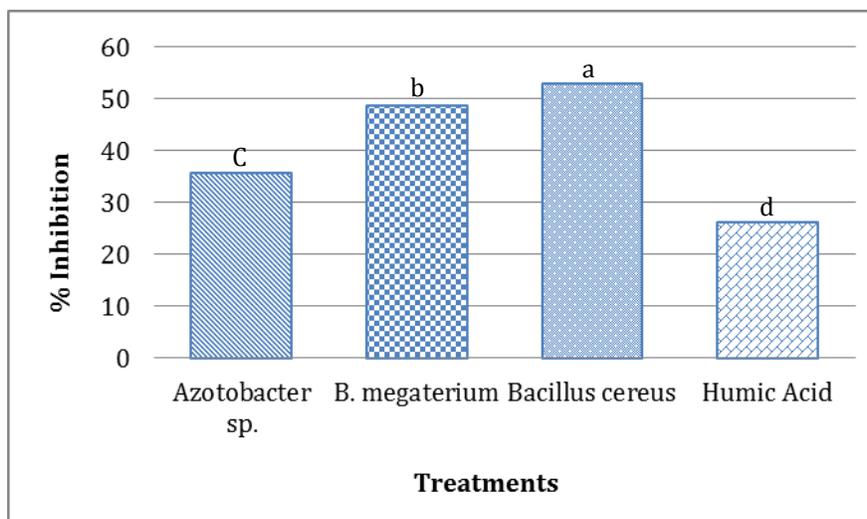


Figure 1. Effect of PGPR and humic acid on mycelia growth of *F. oxysporum* *in vitro*. Different letters indicate significant differences among treatments according to least significant difference test (LSD) ($P=0.05$).

A): Effect of PGPR and humic acid on Area under Disease Progress Curve: Data in Table (2) revealed that the high infection percentage of tomato plants with the pathogen recorded with control whereas, low infection percentage was observed in the treated seedlings with mixed PGPR strains combined with humic acid, where gave 86.49 and 87.23% reduction of AUDPC in first and second growing, respectively, followed by treatment *B. cereus*+ humic acid and *B. megaterium* +humic acid. Conversely, tomato seedlings treated with *Azotobacter* sp. showed the lowest protection against wilt disease while recorded 54.04

and 56.14 % reduction of AUDPC in first and second growing seasons, respectively. Generally, the combination between humic acid and PGPR strains individually of mixed gave highly reduction of AUDPC than used PGPR alone.

B): Growth parameters: Data presented in Table (3) revealed low values of growth parameters, (plant height and number of branches plant⁻¹) with the control treatment in comparison with other treatments. The growth parameters of tomato plants were significantly increased with the dual inoculation of PGPR strains and humic acid compared with the individual one.

Table 2. Effect of PGPR individually or combination with and humic acid on area under disease progress curve under field conditions during seasons 2010-2011 and 2011-2012.

Treatments	Season 2010-2011		Season 2011-2012	
	AUDPC *	Reduction (%)	AUDPC	Reduction (%)
<i>Azotobacter</i> sp (Az)	359.6 b	54.04	328.4 b	56.14
<i>B. cereus</i> (Bc)	305.6 cd	60.95	289.9 d	61.28
<i>B. megaterium</i> (Bm)	325.9 c	58.35	309.8 c	58.63
Az + Bc + Bm	249.6 e	68.10	230.4 f	69.23
Az + Humic acid	245 e	68.69	230.4 f	69.23
Bc + Humic acid	196.3 f	74.91	181.6 h	75.75
Bm + Humic acid	209.6 f	73.21	200 g	73.29
Az+ Bc+ Bm+ Humic acid	105.7 g	86.49	95.6 i	87.23
Humic acid	296.6 g	62.10	258.7 e	65.45
Control	782.5 a	-	748.8 a	-

Different letters indicate significant differences between tomato fungal isolates according to L.S.D. test ($P=0.05$).

* AUDPC = Area under disease progress curve.

The combination between mixed PGPR strains and humic acid was pest treatments to improve growth parameters in both growing seasons whereas recorded the highest plant height (74.565 and 76.48 cm) compared with 50.23 and 53.63 cm in control in both seasons, respectively. Also, this treatment was recorded 8.09 and 8.20 branch plant⁻¹) compared with 5.49 and 5.84 branch plant⁻¹ in control in both seasons respectively. On the other hand tomato seedling treated with *Azotobacter* sp. was lowest treatments to improve both growth parameters in both seasons.

C). Effect on chemical inducers on quantitative parameter of fruit yield: Data present in Table (4) show that all treatments were increased significantly the most quantitative parameters compared with untreated tomato seedlings (control). The dual inoculation of PGPR strains and humic acid recorded the

highest quantitative parameters i.e. No. of fruits plant⁻¹, fruit weight plant⁻¹ (kg), total yield fed.⁻¹ (ton), fruit weight (gm), No. of fruit Kg⁻¹) compared with the individual one. Also, tomato seedlings treated with humic acid gave highly fruit quantitative parameters than seedlings treated with any PGPR strains individually. Tomato seedlings treated with mixed PGPR strains + humic acid recoded highly number of fruit plant⁻¹ (82.14 and 84.25), fruit yield plant⁻¹ (6.09 and 6.02 kg), total yield fed.⁻¹ (30.14 and 30.52 ton fed⁻¹), fruit weight (74.14 and 71.45 gm) compared with 31.25 and 33.04, 1.55 and 1.60 kg, 9.05 and 9.20 ton fed⁻¹, 50.67 and 48.43 gm in control treatment in both seasons, respectively. On the other hand, tomato seedlings treated with *Azotobacter* sp. recoded the lowest proved in fruit quantitative compared the other treatments in most tested parameters.

Table 3. Effect of PGPR individually or combination with and humic acid on growth parameters of tomato cv. Super Strain B during 2010-2011 and 2011-2012 growing seasons in field.

Treatments	Season 2010-2011		Season 2011-2012	
	Plant height (cm)	No. of branches plant ⁻¹	Plant height (cm)	No. of branches plant ⁻¹
<i>Azotobacter</i> sp. (Az)	57.89 g	5.70 f	57.49 e	5.69 e
<i>Bacillus cereus</i> (Bc)	60.56 f	5.79 ef	62.81 d	5.94 e
<i>B. megaterium</i> (Bm)	58.79 g	5.71 f	59.48 e	5.96 e
Az + Bc + Bm	63.58 e	6.09 de	64.28 d	6.14 de
Az + Humic acid	66.89 d	6.65 c	67.48 c	6.81 bc
Bc + Humic acid	70.45 b	7.09 b	72.89 b	7.25 b
Bm + Humic acid	68.50 c	6.82 bc	69.58 c	6.91 b
Az+ Bc+ Bm+ Humic acid	74.56 a	8.09 a	76.48 a	8.20 a
Humic acid	62.58 e	6.25 d	64.58 d	6.42 cd
Control	50.23 h	5.49 f	53.63 f	5.84 e

Different letters indicate significant differences between tomato fungal isolates according to LSD test ($P=0.05$).

D). Effect of Fruit Yield qualitative Parameters: Data in Table (5) sowed increased significantly in qualitative tomato fruits i.e. Fruit coloring degree, Fruit height (cm), Fruit diameter (cm), Firmness (kg So cm²) and T.S.S of PGPR strains individually or combination with humic acid compared with untreated seedlings (control). The dual treatment by PGPR strains + humic acid improved of all qualitative parameters compared with the individual one. Tomato seedling treated with

mixed PGPR strains +humic acid recorded the highest fruit coloring degree (4.25 and 4.36), fruit height (6.3 and 6.39 cm), fruit diameter (5.84 and 5.84 cm), firmness (3.25 and 3.35 kg So cm²) and T.S.S. (5.48 and 5.91) compared with 3.12 and 3.19; 3.89 and 3.92 cm; 3.52 and 3.51 cm; 1.57 and 1.65 kg So cm²; 3.81 and 4.05 in control in both seasons, respectively. However, tomato seedlings treated with *Azotobacter* sp. only gave lower records ones of most fruit qualitative parameters.

DISCUSSION

There are over 120 described formae specialis and races. One of these formae specialis is *F. oxysporum* f. sp. *lycopersici*, which causes Fusarium vascular wilt in tomato plants. Control of wilt disease in tomato depends mainly on fungicides application (Amini and Sidovich, 2010). Meanwhile, fungicides always undesirable due to high cost, probability of development of resistant strains and potential hazards to the environment.

An option for reducing pollution caused by the use of synthetic agrochemical in tomato disease management is biocontrol by using of antagonist rhizobacteria belonging to the *Bacillus*, *Azotobacter* genus and/or organic substances such humic acid, because they are considered the most efficient for their inhibitory properties (El-Mohamedy, and Ahmed, 2009), stimulation of plant growth and crop yield enhancer (Wahyudi *et al.*, 2011). In this study, effective root colonization of PGPR individually or combined with humic acid is important to achieve improved plant growth and/or induced resistance. The obtained data indicate that all PGPR strains viz. *Azotobacter* sp., *B. cereus*, *B. megaterium* when used individually or combined with humic acid decreased incidence wilt disease in tomato plants as well as in greenhouse or in field, also increased fresh and dry weights of survival tomato plants growing in pots compared with control. All the PGPR strains and humic acid reduced growth of *Fusarium oxysporum* f. sp. *lycopersici* significantly.

On the other hand, these treatments significantly increased plant growth, quantitative and qualitative parameters of tomato fruits growing in both seasons (2010-2011 and 2011-2012) under field conditions. Also, the obtained data showed that the combination treatments of humic acid with PGPR reduced significantly wilt incidence of tomato plants and increased growth, quantitative and qualitative parameters comparing with the application of each of them alone.

Our study showed that tomato plants treated with PGPR and humic acid caused higher reduction in disease severity and higher fruit yield compared to the untreated control plants (Mogle and Mane, 2010, Nihorimbere *et al.*, 2010) and promote the growth of a wide range of plants (Wahyudi *et al.*, 2011). PGPR help

in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates and improve soil structure and organic matter content (Al-Taweil *et al.*, 2009). PGPR retain more soil organic N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients (Baset *et al.*, 2010). *Bacillus* have also been known to produce compounds which promote plant growth directly or indirectly viz., hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), solubilize phosphorus and antifungal activity (Shobha and Kumudin, 2012).

The mechanism of PGPR action on pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Zaghloul *et al.*, 2007), produce siderophores and hydrogen cyanide (Soleimani *et al.*, 2005), production of secondary metabolites such as Phenazine -1-Carboxylic acid (PCA), 2,4-Pyrrolnitrin, Oomycin (Knudsen, 1995) and production of antibiotics (Ehteshamul-Haque and Ghaffar, 1993)..

Humic acid is a suspension which can be applied successfully in many areas of plant production as plant growth stimulant or soil conditioner for enhancing natural resistance against diseases and pests (Scheuerell and Mahaffee, 2004), stimulation plant growth though increased cell division as well as optimized uptake of nutrients and water especially nitrogen, potassium and phosphorus which are necessary for plant growth and increases in cell permeability and soil physical conditions, enzyme activation and /or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (El-Ghamry *et al.*, 2009, Patil, 2010).

Also, humic acid could inhibit the growth and spore germination of many plant pathogenic fungi, they attributed this inhibition effect to the presence of some toxic compounds and functional properties especially COOH group content and elemental composition. Also, Loffredo *et al.*, 2007 found that humic acid substances reduced significantly the radial growth and spore germination of *Fusarium oxysporum* f. sp. *melonis* and *Fusarium oxysporum* f. sp. *lycopersic*.

Table 4. Effect of PGPR individually or combination with and humic acid on some quantity parameters of tomato crop during growing seasons 2010-2011 and 2011-2012.

Treatments	Season 2010-2011					Season 2011-2012				
	No. of fruits plant ⁻¹	Fruit weight plant ⁻¹ (kg)	Fruit weight (gm)	No. of fruit Kg ⁻¹	Total yield fed ⁻¹ (Ton)	No. of fruits plant ⁻¹	Fruit weight plant ⁻¹ (kg)	Fruit weight (gm)	No. of fruit Kg ⁻¹	Total yield fed ⁻¹ (Ton)
<i>Azotobacter</i> sp (Az)	44.25 h	2.14 h	54.01 f	18.51 b	12.36 fg	44.58 h	2.15 f	48.23 e	20.73 a	12.96 f
<i>B. cereus</i> (Bc)	56.85 f	2.49 g	59.63 e	16.77 cd	13.48 ef	56.39 f	2.85 e	50.54 cd	19.79 a	14.02 f
<i>B. megaterium</i> (Bm)	52.36 g	2.35 g	55.96 f	17.87 bc	13.09 efg	52.14 g	2.6 e	49.87 de	20.05 a	13.54 f
Az + Bc + Bm	65.48 e	3.45 e	63.38 c	15.78 d	17.25 de	67.09 e	3.52 cd	52.47 c	19.06 a	17.81 e
Az + Humic acid	69.35 d	3.64 d	62.87 cd	15.91 d	19.63 cd	70.15 d	3.69 c	52.60 c	19.01 a	19.85 d
Bc + Humic acid	75.36 b	4.96 c	70.20 b	14.24 e	23.56 bc	76.59 b	4.99 b	65.15 b	15.35 b	24.05 c
Bm + Humic acid	72.14 c	5.29 b	71.94 ab	13.90 e	26.85 ab	74.08 c	5.22 b	70.46 a	14.19 b	27.41 b
Az+ Bc+ Bm+ Humic acid	82.14 a	6.09 a	74.14 a	13.49 e	30.14 a	84.25 a	6.02 a	71.45 a	14.00 b	30.52 a
Humic acid	64.12 e	3.19 f	60.67 de	16.48 d	16.08 d ef	65.39 e	3.23 d	49.40 de	20.24 a	16.84 e
Control	31.25 i	1.55 i	49.6 g	20.16 a	9.05 g	33.04 i	1.6 g	48.43 de	20.65 a	9.56 g

Different letters indicate significant differences between treatments according to L.S.D. test (P=0.05).

Table 5. Effect of PGPR individually or combination with and humic acid on some qualitative parameters of tomato crop during growing seasons 2010-2011 and 2011-2012.

Treatments	Season 2010-2011					Season 2011-2012				
	Fruit coloring degree	Fruit height (cm)	Fruit diameter (cm)	Firmness (kg So cm ²)	T.S.S.	Fruit coloring degree	Fruit height (cm)	Fruit diameter (cm)	Firmness (kg So cm ²)	T.S.S.
<i>Azotobacter</i> sp (Az)	3.15 f	4.10 d	3.88 f	2.12 g	4.02 f	3.25 g	4.16 e	3.96 f	2.19 de	4.12 e
<i>B. cereus</i> (Bc)	3.62 e	4.29 cd	3.92 f	2.36 d	4.23 cde	3.71 de	4.32 e	3.99 f	2.42 bc	4.29 d
<i>B. megaterium</i> (Bm)	3.6 e	4.25 cd	4.12 e	2.25 ef	4.16 ef	3.65 ef	4.29 e	4.19 e	2.29 d	4.26 d
Az + Bc + Bm	3.99 b	5.26 abc	5.02 c	2.69 c	4.36 c	4.09 b	5.32 c	5.12 c	2.48 b	4.44 c
Az + Humic acid	3.65 de	4.85 bcd	4.71 d	2.34 de	4.20 cde	3.55 f	4.95 d	4.86 d	2.26 d	4.29 d
Bc + Humic acid	3.89 bc	5.25 abc	5.02 c	2.69 c	4.35 cd	3.95 bc	5.30 c	5.22 c	2.39 c	4.41 c
Bm + Humic acid	3.8 cd	5.84 ab	5.54 b	3.02 b	5.25 b	3.85 cd	5.99 b	5.63 b	3.29 a	5.29 b
Az+ Bc+ Bm+ Humic acid	4.25 a	6.3 a	5.84 a	3.25 a	5.84 a	4.36 a	6.39 a	5.92 a	3.35 a	5.91 a
Humic acid	3.25 f	4.15 cd	3.95 f	2.19 fg	4.18 def	3.14 g	4.23 e	4.05 ef	2.19 de	3.25 f
Control	3.12 f	3.89 d	3.52 g	1.57 h	3.81 g	3.19 g	3.92 f	3.51 g	1.65 e	4.05 e

Different letters indicate significant differences between treatments according to L.S.D. test (P=.05).

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