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Root Rot of Geranium transplants and Its Biological Control

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Abstract Geranium is one of the most popular greenhouses potted and bedding plants in Egypt. Root rot disease is responsible for important losses in geranium plant production. A total of 11 fungal isolates were isolated from rotted roots of geranium plants, collected from nurseries in Shoubra El-Khima and El-Qanater El-Khaireya, during winter 2012-2013. Disease plants were stunted with yellowed leaves and decayed root system with browning of the surface of the basal portion of the stem. The isolated fungi include: *Fusarium anthophilum, F. equiseti, F.proliferatum, F. semitectum, F. solani* and *Pythium ultimum,* where the highest frequency was *Fusarium semitectum* (36.36%). A total of 10 bacterial and 5 fungal isolates, were isolated from rhizospheric soil of 3 different ornamental plants, and tested, *in vitro*, in addition to a known bio-agent, *Pseudomonas fluorescens* (strain3339), for their antagonistic effect against the pathogens of root rot. *In vitro* assays indicated that *Trichoderma harizinum* (TCNu1) was highly antagonistic against *F. anthophilum, F. proliferatum* and *F. semitectum*, while it gave slight antagonistic effect against *P. ultimum*. As for *Pseudomonas fluorescens*, it showed moderate antagonistic effect against 3 tested *Fusarium spp.*, however, it slightly reduced growth *of P. ultimum*. Under greenhouse conditions, soil treatment with *Pseudomonas fluorescens* (strain3339) significantly reduced foliar disease severity, while *Trichoderma harzianum* gave the highest reduction of root rot incidence and severity. Treatment with *Trichoderma harzianum* and *Pseudomonas fluorescens* improved growth of geranium plants grown in pathogen infested soil, where main shoot length, main root length, fresh and dry weights of plants were increased. However, both bio-agents had no significant effect on number of flowers/plant. Therefore, this study confirms the potential of *Trichoderma harzianum* (TCNu1) and *Pseudomonos fluorescens* (strain3339) to be used as one component in integrated program to control root rot disease in geranium transplants in Egypt.

Keywords: Geranium, Root Rot Disease, Biological Control, *Fusarium* spp., *Pythium* sp., *Pseudomonas fluorescens* and *Trichoderma harzianum*.

Introduction

Geranium (*Pelargonium* × *hortorum* Bailey) is considered one of the most popular greenhouses potted and bedding plants worldwide (Miller, 2002). The genus *Pelargonium* includes annuals and herbaceous perennials, shrubs and subshrubs, and both evergreen and deciduous plants (Miller, 2002).

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Geranium is characterized by wide appeal and the ability to adapt to a wide range of climates, and is popular for their continuous flowering throughout the summer and early winter in most of the world (Schoellhorn, 2003). Geranium plants have many benefits within the medical and pharmaceutical fields and chemical industries (Lis-Balchin, 2002; Saraswathi *et al.*, 2011). In Egypt, geranium is popular ornamental plant and is widely cultivated in many areas.

Geranium plants are liable to attack by several soil borne pathogens, causing severe losses in geranium plant production and quality (Daughtrey *et al.* 1995; El-Gamal, 1995; Douglas, 2003). Root rot is one of the most important diseases affecting production of geranium and that reduce the production of flowering and ornamental plants (Gullino and Wardlow, 1999; Douglas, 2003; Gravel *et al.*, 2009). Geranium plants are susceptible to root diseases caused by various soil borne fungi, which include *Fusarium* spp. (Szczech, 1999; Douglas, 2003) and *Pythium* spp. (Desilets *et al.*, 1994; Moorman *et al.*, 2002; Gravel *et al.*, 2009) and *Rhizoctonia* spp. (Douglas, 2003). However, root rot must be detected and identified early to can be controlled.

Generally, fungicides that control *Pythium* diseases does not control *Rhizoctonia* and *Fusarium* root rot (Hausbeck, 2013). However, resistance to certain fungicides can be stimulated by sublethal doses of the fungicide, and that this stimulation can result in significantly higher rates of Pythium damping-off of geranium seedlings (Garzón *et al.*, 2011). Alternative approaches for root rot management aim to use biological methods to reduce use of chemical substances and to achieve effective and durable control. Several antagonists of either of the fungi *Gliocladium* and *Trichoderma* or the bacteria *Pseudomonas* and *Bacillus* have been widely used for bio-control of soil-borne pathogens (Reddy, 2016). Certain bio-agent strains of *Pseudomonas* spp. and *Trichoderma* spp. succeeded in reducing *Fusarium oxysporum* on Gladiolus (Naqvi and Ahmed, 2012) and on bean plants (Otadoh *et al.*, 2011). *Trichoderma harzianum* controlled *R. solani* in poinsettia, geraniums and periwinkles, and *Pythium* on geraniums, impatiens and petunias (Reddy, 2016). Soil treatments by beneficial rhizosphere microorganisms as *P. putida*, and *T. atroviride*, suppress Pythium root rot, caused by *Pythium ultimum* and significantly increased the fresh and dry weight of the shoot and root of organically grown geranium plants (Gravel *et al.*, 2009).

The objectives of this study aimed to identify the casual pathogens of root rot of geranium and to control the disease using different bio-agents.

Material and methods

Source of diseased plants

Geranium plants showing typical symptoms of root rot were collected from different nurseries located at Shoubra El-Khima and El-Qanater El-Khaireya, in Qaliubiya governorate, during winter 2012-2013.

Isolation, purification and identification of geranium root rot pathogens

Isolation of root rot pathogens was conducted as described by Dhingra and Sinclair (1985). Small pieces were surface-sterilized in 1 % sodium hypochlorite solution for 2 min, then washed in sterile distilled water for 4 min. Small pieces were dried between layers of sterile filter papers and were transferred to Water agar (WA) in Petri dishes, where dishes were incubated in incubator at temperature 23±2 ˚C for 2-4 days. The obtained fungal colonies were purified using hyphal tip techniques on Potato dextrose agar (PDA) medium in Petri dishes where dishes were incubated in incubator at temperature 25±2 ˚C for 10-14 days. Stock cultures were maintained on PDA slants and kept in a refrigerator at 5 ˚C till use. Identification of the isolated fungi were carried out based on microscopic and culture characteristic according to Leslie, *et al.,* (2006) for *Fusarium* spp. and Plaats-Niterink, (1981) for *Pythium* spp**.** However, pathogenicity tests of these isolates were performed and their pathogenic potentialities were proven.

Source of antagonists

Rhizosphere-colonizing bacteria and fungi were isolated from three different ornamental plant species (Carnation, Gernium and Hopbush), grown in nurseries located at Qaliubiya and Nubariya as described by El-Hadidy *et al.* (2002). From each soil sample, 10 g were suspended in 90 ml sterile water and serial dilutions to 10^6 were prepared. Dilutions from each sample were plated in triplicate on three different media: Nutrient agar (NA) and King's media B (KB) King *et al.*,1954) for isolation of bacteria, and potato dextrose agar (PDA) supplemented with 50 µg /ml Penicillin-G for isolation of fungi. Plates were incubated at 23±2 ˚C for 2-4 days, when individual colonies were picked up, purified and stored at 5 ˚C on the appropriate medium. In addition, a known bio-agent, *Pseudomonas fluorescens*(strain3339,kindly provided by Dr. Nevein A. Shehata (Potato Brown Rot Project, ARC, Giza, Egypt), was also used.

Assay of antagonism, in vitro

Antagonism between five fungal isolates and 11 bacterial isolates against the root rot pathogen (three *Fusarium* spp. isolates and an isolate of *Pythium ultimum*) were studied under *in vitro* conditions, following the dual culture technique (Skidmore and Dickinson, 1976) on PDA plates. All inoculated plates were incubated at 25±2 ˚C for 5 days. All plates were observed visually and the linear growth of the pathogens was measured. Percentages of inhibition of mycelial growth (PIMG) of pathogenic fungi colonies were calculated using the formula:

$$\mathbf{PIMG = (A1–A2) / A1 \times 100}$$

Where,

A1 : The diameter of mycelial growth of the test pathogen in control plates (cm)

A2 : The diameter of mycelial growth of the pathogen in dual culture (with the tested antagonist)

Meanwhile, an antagonism rating scale (1-5) modified from Bell *et al.*(1982) and by Otadoh *et al.,* (2011) was also used where, Class 1 = no antagonism (0 %), Class 2 =slightantagonism (1-33 %), Class 3 = moderate antagonism (34-66 %), Class 4 = high antagonism(67-99%), Class 5 = overgrowth (100 %).

Efficacy of antagonists under greenhouse conditions

Preparation of pathogen inoculum and soil infestation

Fungal mass production of four root rot fungal isolates **(***Fusarium anthophilum* GSh8, *F. proliferatum* GSh6, *F. semitectum* GQa1 and *Pythium ultimum* GSh9**)** was prepared as described by Singleton *et al.,* (1992) by growing each isolates on sorghum grain medium. Previously sterilized soil, with formalin 5%, was infested with each pathogen inoculum at a rate of 5 g/kg soil, and mixed thoroughly to ensure equal distribution of fungal inoculums and then filled in plastic pots (10 cm diameter). The infested pots were irrigated regularly day for 1 week before geranium transplanting.

Preparation of bio-agent inoculums

Inoculum of an antagonistic fungal isolate identified as *Trichoderma harzianum* (TCNu1) according to Rifai (1969), and the bacterial isolate, *Pseudomonas fluorescens* (strain3339) were selected and used in this study. Fungal mass production of *T. harzianum* (TCNu1) was obtained by growing the isolate on sorghum grain medium (Rini and Sulochana, 2007) for 2 weeks at room temperature.

Pseudomonas fluorescens (strain 3339) was grown on King's B media (KB) at (28±2ºC) for 48h (Mosa *et al.,* 1997). The bacterial cells were harvested in sterilized water and centrifuged at 10000 rpm for 5 min. Then supernatant was discarded, and the precipitate was finally suspended in sterilized distilled water. Bacterial concentrations were determined according using spectrophotometer optical density at A_{640} *nm* compared to reference curves. As compared to standard curve and the concentration was adjusted to give $1x10^9$ (cfu/ml^{-1}).

Soil treatment

Healthy geraniums transplants were planted in plastic pots (10 cm diameter) containing artificially infested soil with four selected pathogenic fungal isolates. Inoculum of *T. harzianum* (TCNu1) was added to previously pathogen infested soil 48h before transplanting, while *P. fluorescens* (strain 3339) was added as bacterial suspension (10^9cfu /ml) to infested soil at a rate of 100 ml /kg soil, at the time of transplanting.

This experiment included the following treatments:

a) Infested soil with each pathogen isolate and with adding bio-agent (Treated).

b) Infested soil with each pathogen isolate without adding bio-agent (Infested Control).

c) Non-infested and untreated soil (Control).

All treated and untreated pots were arranged in a completely randomized design, with three replicates per tested isolate. Disease symptoms were observed and disease incidence and severity were assessed 30 and 45 days after transplanting**.** The efficacy of antagonists was calculated using the following formula:

$$\textbf{Efficacy of Antagonists} = \frac{\textbf{Treatment} - \textbf{Control}}{\textbf{Treatment}} \textbf{x}\ 100$$

Disease assessment

The severity of foliar and root symptoms was assessed for each plant on a 0 to 4 scale (Sanchez-Hernandez *et al.,* 2001), according to the percentage of foliage with yellowing or necrosis or root necrosis (0 = no infection, 1 = 1-33%, 2 = 34-66%, 3 = 67-99% and 4 = dead plant) at the end of experiment. The disease indices were calculated based on the following formula (Yildiz *et al.,* 2007):

$$\textbf{Disease severity} = \frac{\Sigma(\textbf{Class rating} \times \textbf{Class frequency})}{\textbf{Total number of plants} \times \textbf{highest rating}} \textbf{x}\ 100$$

Effect on plant growth

Data of shoot and root height, number of leaves and flowers, and fresh and dry weights of plants, were recorded at the end of the experiment, after 60 days from transplanting.

Statistical analysis

All experiments were performed in triplicate. Duncan Multiple Range Test was used to evaluate the significant differences between treatments ($P \leq 0.05$). ANOVA analysis was done with the SAS statistics software (SAS Institute, Inc., 1996).

Results and discussion

Isolation, purification and identification of geranium root rot pathogens

A total of 11 fungal isolates were isolated from rotted roots of geranium plants, collected from nurseries in two locations in Egypt (Table 1). Samples from Shoubra El-Khima yielded eight fungal isolates: *i.e. Fusarium* spp. and an isolate of *Pythium* sp., while samples from El-Qanater El-Khaireya yielded two fungal isolates of *Fusarium* spp. The obtained isolates identified as *Fusarium anthophilum* (9.09%), *F. equiseti* (9.09%), *F. proliferatum* (18.18%), *F. semitectum* (36.36%), *F. solani* (18.18%) and *Pythium ultimum* (9.09%)(Table 1). These results confirm findings of Haggag and Abdel-latif (2001), Armengol *et al.* (2005) and Hassan, *et al.* (2014), whereas highest frequency of *Fusarium* spp. and *Pythium* spp. (Moorman *et al.*, 2002) were detected and are common pathogens in ornamental nurseries.

Table 1. Frequency and occurrence of fungi isolated from diseased geranium, obtained from two locations in Egypt during 2012-2013.

Pathogenic Fungal Isolate	**Shoubra El-Kheima**	**El-Qanater El-Khaireya**	**Total No. of isolates**	**Frequency (%)**
	No. of isolates	No. of isolates		
Fusarium anthophilum	1	-	**1**	**9.09**
Fusarium equiseti	1	-	**1**	**9.09**
Fusarium proliferatum	2	-	**2**	**18.18**
Fusarium semitectum	2	2	**4**	**36.36**
Fusarium solani	2	-	**2**	**18.18**
Pythium ultimum	1	-	**1**	**9.09**

Isolation, purification and identification of antagonists

Rhizosphere-colonizing bacteria and fungi were initially isolated from three different ornamental plant species (geranium, carnation and hopbush) grown in different nurseries located at Qaliubiya and Nubariya. A total of 10 bacterial and 5 fungal isolates were selected. The obtained fungal isolates were identified as *Trichoderma harzianum* according to Rifai (1969).

Assay of antagonism, in vitro

The results of the *in vitro* assay by which 15 bacterial and fungal isolates, in addition to one known bio-agent, were evaluated for their antagonistic effect against 4 fungal pathogens causing root rot of geranium and hopbush in nursery are shown in Table (2). The most antagonistic bacterial isolate was *P. fluorescens* (starin3339), which reduced mycelial growth of the four tested pathogens; meanwhile the most antagonistic fungal isolate was *T. harzianum* **(**TCNu1**)** which reduced mycelial growth of the four tested fungal pathogens isolates. In dual culture, *T. harzianum* (TCNu1) significantly reduced mycelial growth of all tested *Fusarium* isolates with varying degrees (Table 2), with slight effect against *Pythium ultimum*. However, *P. fluorescens* (strain3339) showed less effect and reduced growth of the three tested 3 *Fusarium* spp. by 39.00% to 57.08%, and slightly reduced *P. ultimum* growth (33.33%).

Table 2. Antagonistic effect of selected rhizosphere-colonizing bacterial and fungal isolates against mycelial growth of four plant pathogenic fungi, *in vitro*.

Isolate code	**Percent Inhibition of Fungus Mycelia Growth (PIMG)**			
	F. semitectum (GSh1)*	*F. solani* (HoAl10)**	*F. oxysporum* (HQa10)**	*P. ultimum* (GSh9)*
Rhizospheric Isolates:				
Fungi :				
TGSh 1	12.97	20.88	9.81	17.72
TGSh 2	17.72	9.81	9.81	12.97
THQa 1	9.81	12.97	12.97	20.88
TCNu 1	68.35	66.66	69.50	33.33
TCNu 2	20.88	9.81	9.81	12.97

Table 2. (Con.)

Isolate code	Percent Inhibition of Fungus Mycelia Growth (PIMG)			
	F. semitectum (GSh1)*	*F. solani* (HoAl10)**	*F. oxysporum* (HQa10)**	*P. ultimum* (GSh9)*
Bacteria :				
BGSh 1	8.22	7.43	9.81	4.43
BGSh 2	5.06	4.43	9.01	11.39
BGSh 3	11.39	9.01	7.43	7.43
BHQa 1	12.18	7.43	9.81	9.81
BHQa 2	9.81	5.85	12.18	5.06
BHQa 3	5.85	4.43	11.39	12.18
BHQa 4	9.81	11.39	9.01	9.81
BHQa 5	9.01	5.85	4.43	11.39
BCNu 1	4.43	5.06	11.39	7.43
BCNu 2	7.43	11.39	7.43	4.43
Known bio-agent:				
P. fluorescens (strain3339)	39.39	41.66	41.66	33.33

*****Isolated from geranium plants
******Isolated from hopbush plants

The results in Table 3 confirmed the antagonistic effect of *Trichoderma harzianum* (TCNu1) and *Pseudomonas fluorescens* (strain3339) when re-evaluated against 4 fungal pathogens causing root rot of geranium.

Table 3. Antagonistic effect, *in vitro*, of *Trichoderma harzianum*(TCNu1)and *Pseudomonas fluorescens* (strain3339) against mycelial growth of pathogenic fungal isolates, causing root rot of geranium plants.

Treatment	Isolate and code	Control (cm)	Treatment (cm)	% inhabitation	Arbitrary antagonism scale*
T. harzianum (TCNu1)	*F. anthophilum* (GSh 8)	6.00	1.50	75.00 $_{Ba}$	High
	F. proliferatum GSh 6	6.00	1.83	69.50 $_{Ca}$	High
	F. semitectum GQa 1	4.66	1.33	71.45 $_{Aa}$	High
	P. ultimum GSh 9	6.00	4.00	33.33 $_{Da}$	Slight
P. fluorescens (strain3339)	*F. anthophilum* GSh 8	6.00	3.50	41.66 $_{Bb}$	Moderate
	F. proliferatum GSh 6	6.00	3.66	39.00 $_{Cb}$	Moderate
	F. semitectum GQa 1	4.66	2.00	57.08 $_{Ab}$	Moderate
	P. ultimum GSh 9	6.00	4.00	33.33 $_{Db}$	Slight

*****Arbitrary antagonism scale (Classes 1 to 5) modified from Bell *et al.,* (1982)

These results are consistent with those obtained by several authors (Singh and Kumar, 2011; Alwathnani and Perveen, 2012; Gajera *et al*., 2012; Mokhtar and Dehimat, 2012) that *Trichoderma* spp. had the ability to antagonism various pathogenic fungi. Different mechanisms are known and include mycoparasitism, antibiotics and degrading enzymes production have been detected (Haran *et al.*, 1996; Sharma *et al.*, 2009). Also, *Pseudomonas fluorescens* had been reported as effective bio-agent against *Fusarium* spp. (Sallam *et al*., 2013; Toua *et al*., 2013), and *Pythium* spp. (Gravel *et al.*, 2009). The ability of *Pseudomonas fluorescens* to suppress plant pathogenic fungi may be due to the production of antibiotics, hydrolytic (hydrolase) enzymes that can degrade cell walls, iron-chelating siderophores, and several cyclic lipodepsipeptides (Weller, 2007; Kim *et al*., 2008).

Efficacy of antagonists under greenhouse conditions

A-Effect on root rot incidence and severity

Results presented in Table 5 indicate that treatment with *P. fluorescens* (strain3339) reduced foliar disease severity on geranium plants, grown in *Fusarium* and *Pythium* infested soil, after 45 days from transplanting. However, after 60 days, the efficacy of *T. harzianum* (TCNu1) treatment on reducing root rot severity with fungal isolates of *F. anthophilum* and *F. semitectum* was 42.85% and 100.00%,

respectively, while, it had no significant effect with *F. proliferatum* and *P. ultimum*. Meanwhile, *P. fluorescens* significantly reduced root rot severity with *F. anthophilum* (14.28%) and *F. semitectum* (60.00%), although it had no significant effect on *F. proliferatum* and *P. ultimum.* The results revealed that *T. harzianum* (TCNu1) was superior than *P. fluorescens* (strain3339) in reducing root rot severity (Table 5). These results in accordance with the findings of Haggag and Abdel-latif, (2001);Showkat *et al.,* (2012) and Akrami *et al.,* (2013), that *T. harzianum and P. fluorescens* had the ability to reduce foliar and root rot disease severity, may be due to the multiple mechanisms of action of *T. harzianum* including: co-parasitism via production of chitinases,β-1-3 glucanases and β-1-4 glucanases, production of geliotoxin, competition, solubilisation of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process according to Harman (2006); Sundaramoorthy and Balabaskar (2013). Also, the ability of *P. fluorescens* may be due to producing known secondary metabolites such as siderophores, HCN and protease and biosynthesis of these metabolites is modulated by a number of biotic and a biotic factor (Raaijmakers *et al.* 2002; Ahmadzadeh *et al.,* 2006).

Table 5. Effect and efficacy of antagonists: *Trichoderma harzianum* (TCNu1) and *Pseudomonas fluorescens* (strain3339) on incidence and severity of root rot of geranium transplants, grown in soil infested with different fungal pathogen isolates, under greenhouse conditions

Fungal Isolates	Treatment	Foliar Disease Severity (%)			Root rot severity (%) **	
		30 Days	45 Days	Efficacy (%)*	60 Days	Efficacy (%)
F. anthophilum GSh 8	*T. harzianum*	25.00 $_{Aa}$	16.66 $_{Bb}$	50.01	33.33 $_{Cc}$	42.85
	P. fluorescens	0.00 $_{Ac}$	0.00 $_{Bc}$	100.00	50.00 $_{Cb}$	14.28
	None	8.33 $_{Ab}$	33.33 $_{Ba}$	-	58.33 $_{Ca}$	-
F. proliferatum GSh 6	*T. harzianum*	8.33 $_{Ba}$	8.33 $_{Cb}$	66.68	33.33 $_{Dc}$	0.00
	P. fluorescens	0.00 $_{Bc}$	8.33 $_{Cc}$	66.68	33.33 $_{Db}$	0.00
	None	8.33 $_{Bb}$	25.00 $_{Ca}$	-	33.33 $_{Da}$	-
F. semitectum GQa 1	*T. harzianum*	8.33 $_{Ba}$	8.33 $_{Ab}$	83.34	41.66 $_{Ac}$	50.00
	P. fluorescens	0.00 $_{Bc}$	0.00 $_{Ac}$	100.00	33.33 $_{Ab}$	60.00
	None	8.33 $_{Bb}$	50.00 $_{Aa}$	-	83.33 $_{Aa}$	-
P. ultimum GSh 9	*T. harzianum*	8.33 $_{Ca}$	16.66 $_{Ab}$	50.00	50.00 $_{Bc}$	0.00
	P. fluorescens	0.00 $_{Cc}$	8.33 $_{Ac}$	75.00	50.00 $_{Bb}$	0.00
	None	0.00 $_{Cb}$	33.33 $_{Aa}$	-	50.00 $_{Ba}$	-
Un-treated		0.00	0.00		0.00	

*Efficacy was calculated, after 45 days from planting of geranium transplants.
**Root rot severity was assessed on a modified scale of Sánchez-Hernández *et al.* (2001).
***A, B, C: Data were analyzed by Duncan Multiple Range Test at probability of 0.05 to identify significant effect of an isolates, while a, b, c: Data were analyzed by Duncan Multiple Range Test at probability of 0.05 to identify significant effect of a treatments.

B-Plant growth Parameters

Results presented in Figure 1A indicated that treatment with *T. harzianum* (TCNu1) increased main shoot length of geranium plants grown in soil infested with *F. anthophilum* (75.55%), *F. proliferatum* (64.05%), *F. semitectum* (28.46%) and *P. ultimum* (36.23%), while treatment of *P. fluorescens* (strain3339) increased main shoot length with fungal isolates of *F. anthophilum* (68.57%), *F. proliferatum* (66.68%), *F. semitectum* (21.43%) and *P. ultimum* (37.79%). Results presented in Figure (1B) indicate that treatment of *T. harzianum* (TCNu1) increased main root length of geranium plants grown in soil infested with *F. anthophilum* (74.61%), *F. proliferatum* (85.43%), *F. semitectum* (72.72%) and *P. ultimum* (20.02%), while treatment of *P. fluorescens* (strain3339) increased main root length with fungal isolates of *F. anthophilum* (76.13%), *F. proliferatum* (84.99%), *F. semitectum* (61.11%) and *P. ultimum* (41.36%). Results presented in Figure 1C indicated that treatment of *T. harzianum* (TCNu1) increased number of leaves of geranium plants grown in soil infested with fungal isolates of *F. proliferatum* (34.33%) and *F. semitectum* (42.16%), while it had no significant effect with fungal isolates of *F. anthophilum* and *P. ultimum*. Treatment of *P. fluorescens* (strain3339) increased number of leaves with fungal isolates of *F. proliferatum* (19.16%), *F. semitectum* (54.54%) and *P. ultimum* (21.72%), while it had no significant effect with fungal isolates of *F. anthophilum*. Results presented in Figure 1D indicated that treatment of *T. harzianum* (TCNu1) increased number of flowers of geranium plants grown in soil infested with fungai solates of *F. semitectum* (100.00%) and *P. ultimum* (100.00%), while it had no significant effect with fungal isolates of *F. anthophilum* and *F. proliferatum*. Treatment of *P. fluorescens* (strain3339) increased number of flowers by (100.00%) for both *F. semitectum* and *P. ultimum*, while it had no significant effect with fungal isolates of *F. anthophilum* and *F. proliferatum*. Results presented in Figure 1E indicated that treatment of *T. harzianum* (TCNu1) increased fresh weight of geranium plants grown in soil infested with *F. anthophilum*(42.90%), *F. proliferatum* (43.21%), *F. semitectum* (38.14%) and *P. ultimum* (54.87%). Meanwhile treatment of *P. fluorescens* (strain3339) increased fresh weight with fungal isolates of *F. anthophilum* (17.65%), *F. proliferatum* (34.15%), *F. semitectum* (32.58%) and *P. ultimum* (31.54%).

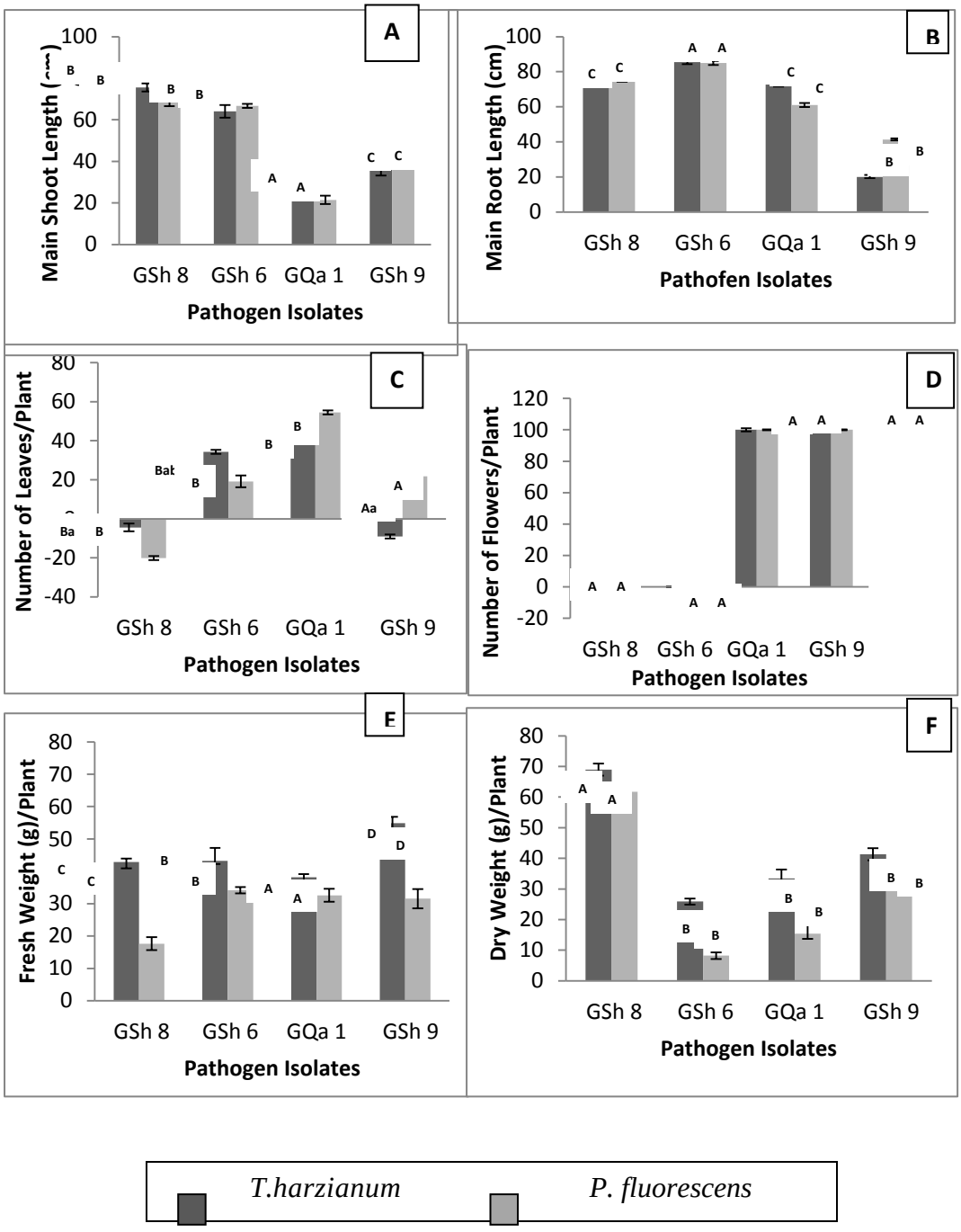

Figure 1. Efficacy of antagonists: *Trichoderma harzianum* (TCNu1) ( ) and *Pseudomonas fluorescens* (strain3339) ( ) on some growth characters of geranium plants, grown in artificially infested soil, after 60 days from transplanting, under greenhouse conditions.

Results presented in Figure (1F) indicated that treatment of *T. harzianum* (TCNu1) increased dry weight of geranium plants grown in soil infested with *F. anthophilum* (68.98%), *F. proliferatum* (25.89%), *F. semitectum* (33.39%) and *P. ultimum* (41.35%), while treatment of *P. fluorescens* (strain3339) increased dry weight with fungal isolates of *F. anthophilum* (61.71%), *F. proliferatum* (8.28%), *F. semitectum* (15.40%) and *P. ultimum* (34.28%).

Treatment of both *Trichoderma harzianum* (TCNu1) and *Pseudomonas fluorescens* (strain3339) had best significant effect on main shoot length and fresh weight of plants. Meanwhile treatment of both *T. harzianum* and *P. fluorescens* had the same effect on the main root length and dry weight of plants. It had no significant effect on number of flowers, while *P. fluorescens* was a more significant than *T. harzianum* on number of leaves.

The positive effect on growth of geranium plants due to treatment with bio-agent have also been reported in other studies (Haggag and Abdel-latif, 2001; Weller, 2007; Alwathnani and Perveen, 2012; Sallam *et al.*, 2013), where *Trichoderma harzianum* and *Pseudomonas fluorescens* strains had ability to increase plant growth and vigor, and this increase was attributed to reduce negative effects on diseased root or may be due to the production of plant growth promoters (auxins, gibberellins and cytokines) or through indirect stimulation of nutrient uptake and by producing siderophores or antibiotics to protect plants from deleterious rhizosphere organisms (Harman, 2006; Weller, 2007; Sivasakthi *et al.*, 2014).

Additional work is necessary in order to demonstrate the bio-control efficacy of these isolates under commercial greenhouses conditions as a component of integrated control strategy of root rot diseases in ornamental nurseries.

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