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FACTORS AFFECTING BASAL ROT DISEASE OF ROSE CUTTINGS

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ABSTRACT

Fusarium solani (three isolates), *Fusarium* sp. (one isolate), *Rhizoctonia solani* (two isolates), *Aspergillus flavus* (one isolate), *Mucor* sp. (one isolate) and *Rhizopus stolonifer* (one isolate), were isolated from naturally infected cuttings of rose, collected from El-Minia and El- Giza Governorates. *F. solani*, *Fusarium*.sp. and *Rhizoctonia solani* could induce basal rot of rose cuttings, while A.*flavus, Mucor* sp. and R. *stolonifer* were nonpathogenic fungi for rose cuttings. The linear growth of the tested isolates of *F. solani* and *R. solani* grew in a wide range of temperature (15 - 35^{o}C) and atmospheric humidity (50 -100%R.H.). The optimum temperature for *F. solani* was 25^{o}C, whereas for *R. solani* it was 20^{o}C. In the same time, the best growth was at 95-100% R. H. Both tested fungi failed to grow at 5^{o}C and 14.5% R.H. *Trichoderma viride* and *Bacillus. subtlis* were able to inhibit the growth of all tested isolates of *F. solani* and *R. solani in vitro*, but the two bioagents varied in their ability to antagonistic effect. In general, *T. virida* exhibited the higher antagonistic effect toward the tested pathogens than *B. subtlis*. R. *solani* was more sensitive to the two antagonistic agents than *F. solani*. Addition of the two bio–control-agents to the soil, one week before sowing, significantly increased the percentages of growing cuttings under artificial infection with either *F. solani* or *R. solani* in greenhouse conditions. The available literature revealed that this study is the first about cutting rot disease in rose in Egypt.

Keywords: Rose, biological control, *F. solani* and *R. solani*

INTRODUCTION

The rose *(Rosa gallica* L.) is one of the most important ornamental plants grown in gardens, offices and homes. Roses are known worldwide for their fragrant showy blooms. Rose plants could be used in landscape gardening and design purposes (Essa, 1992).Rose plants are subject to attack by many different pathogenic fungi during different stages of growth. The fungi that cause root, basal stem and cuttings rots can be found in most soils (Manici *et al.* 2012). *Rhizoctonia* spp. was reported as a major plant pathogenic fungus causing severe economic damage to many species of ornamental plants. Occurrence of *Rhizoctonia* spp. on Miniature rose was reported by Priyatmojo *et al*. (2001) in Japan. They isolated 153 isolates of *Rhizoctonia* spp. from infected roots and stems. Of 153 isolates, 9 had binucleate and 144 (identified as *R. solani*) had multinucleate vegetative hyphal cells. Five isolates from each group caused severe rot and mortality on cuttings during rooting.

At EL-Minia Governorate, rot of rose cuttings constitutes a serious problem in most growing nurseries of rose. The recent field observations have shown that the disease is widely spread causing highly destructive looses. However, no studies have been carried out on any of the diseases, particularly, cutting rots, affecting rose at El-Minia Governorate. The dwarf roses are propagated from cuttings and are typically grown in greenhouses (Leahy, 1994). This method of propagation is relatively simple; and, the risk of disease development can be quite high depending on cultural and management practices (Horst, 1983).

Physiological characters of *Fusarium solani* and *R. solani* concerning effects of culture media, temperature, relative humidity, medium pH on growth of pathogens were studied by several researchers. Qazi and Quebral (1970) stated that temperature in the range 24-28^{o}C was the best for mycelial growth of *R. solani* in culture, and 16-20^{o}C for sclerotial formation. Chi and Hanson (1964) reported that optimum temperature for spore germination and growth of 2 isolates of *F. solani* was 28^{o}C. Dorrance *et al.* (2003) and Infantino *et al.*(2006) reported that damping-off disease caused by *R. solani* is most severe in cooler soils. Mostafa (1972) reported that the optimum temperature for mycelia growth of *F. solani* and *R. solani* was 25 and 30^{o}C, respectively. Abdel-Latif (1976) also found that some soil borne fungi, i.e. *Fusarium fusarioides, F. equesiti, Neocosmospora vasinfectum* and *Aspergillus flavus,*that isolated from rotted pods of peanut were able to grow at a wide range of temperature, ranged between 10 and 45^{o}C,. although, the best relative humidity for all tested fungi ranged between 95 and 100% levels.

The integrated control treatments are still not easy and costly in application. However, they can serve as the best control measures under greenhouse conditions. In addition, their

applications are safe, unhazardous for human, animals and avoid environmental pollution. Lacicowa and Pieta (1996) reported that the pea seeds that dressed with microbiological materials, prepared from *Trichoderma koningi* and *T. viride,* were most efficient in protecting pea from *R. solani* and *Fusarium* spp. in infested soil. Sunick *et al.* (1997) recorded that *Bacillus* sp. gave a highly antagonistic effect against some phytopathogenic fungi including *Fusarium solani* and *Rhizoctonia solani.* In 1997, Nazim *et al.* reported that biological control approach is still problematic in application on a large scale under field conditions. Ragab *et al.* (1999) reported that *T. harzianum and Bacillus subtlis* showed antagonistic ability against the causal organisms of pea root–rot disease, i.e. *F. solani, R. solani* and *Phytophthora* sp. Recently, Hassan *et a*l. (2013) tested the effect of *T. harzianum* (as commercial product named Plant Gard) and *Bacillus subtlis* (as Rizo-N) on the linear growth of *Fusarium semitectum, F. oxysporum, F. moniliforme, F. solani* and *Rhizoctonia solani*. They found that all pathogenic fungi tested were sensitive to both tested biocontrol agents. They reported also that treating faba bean seeds with the bioagents before sowing in artificially infested soil significantly decreased the percentages of the infection with seedling damping off, root rot and dead plants. Mosa *et al.* (2013) found that *Bacillus subtlis*, *T. harzianum*and *Pseudomonas fluorescence* reduced the percentages of dead strawberry plants planted in soil infested with *R. solani, F. solani* or*Macrophomina phaseolina.*

The present investigation was conducted to isolate and identify the causal organism(s) related with rose cutting rot, to study the effect of temperature and humidity on the growth of the main pathogens, and the effect of some bioagents on controlling rose cutting rot incidence.

MATERIALS AND METHODS

1- Sampling, isolation and identification.

Natural rotted cuttings of rose grown in experimental greenhouse in Faculty of Agriculture, El-Minia University, and in some commercial nurseries in Abo-Qurqas, El-Minia Governorate and Kerdasa, Giza Governorate, were collected during December 2011 to March 2012. Rotted cuttings were used for isolating the pathogen(s). Collected rotted cuttings were surface sterilized by dipping in 0.1% mercuric chloride solution for 2 minutes and washed several times in sterilized distilled water, then were cut into small pieces, 2 to 5 mm long cuttings. Two cutting pieces were transferred onto potato dextrose agar (PDA) medium containing penicillin (40 units/plate). The inoculated plates were incubated at 25^{o}C for 3 days. The developed fungal growth was subcultured on sterilized PDA medium. The isolated fungi were purified using hyphal tip (Brown, 1924) or single-spore (Booth, 1971) techniques. Test tubes (10 ml) containing inoculated PDA slants were

kept in refrigerator at 5°C as stock cultures for further studies. The established fungal isolates were identified on the basis of morphological and microscopically characteristics according to Gilman (1957), Booth (1971) and Barnett and Hunter (1972) and was further verified by Division of Fungal Taxonomy, Plant pathology Research Institute, Agriculture Research Center, Giza. Egypt.

2-Pathogenicity test.

Pathogenicity trials with the isolated fungi were carried out in the greenhouse of Plant Pathology Department, Faculty of Agriculture, Minia University, using cuttings of rose, *Rosa gallica* var. *aegyptiaca*, obtained kindly from Prof. Dr. M. Abdel Hady, Department of Ornamental Plants, Faculty of Agriculture, Minia University. Cuttings (about 15-25 cm long) were planted in clay pots (30 cm. in diameter) containing sterilized Nile loamy-clay soil (4 kg/pot) infested with the different fungi. Pots and soil sterilization was carried out (15 days before soil infestation) by autoclaving the soil for two hours at 2 kg/cm^3 pressure and dipping the pots in 5% formalin solution for 5 minutes, then soil was serrated for 15 days before being infested. The basal portions of rose cuttings were surfacely sterilized (to exclude the probability of accidental infection with saprophytic rotted bacteria or fungi) by dipping in mercuric chloride (0.1%) solution for two minutes andthen washed with several changes of sterilized water before being planted in tested pots. Two methods of inoculation were studied, the first: by dipping the basal part of cuttings in suspension of the tested fungi (10^5 CFU/ml) for half of hour, then were planted in autoclaved soil. The second was carried out by soil infestation. Inocula of the isolated fungi were prepared, separately, either on sterilized barley grains (150 gm grains + 200 ml water/ 500-ml Erlenmeyer flask) or in Czapek's liquid media for soil infestation or dipping cuttings of rose, respectively. Inoculated flasks were kept at 25°C for 15 days then used for cutting inoculation or soil infestation. Soil infestation was applied 7 days before planting, by thoroughly mixing 2% grams of inoculum, representing a barley culture of one fungus, with the soil in each pot. The infested soil was irrigated daily till planting. Three replicates, each consisting of 4 cuttings grown in one pot, were used in each treatment. Sterilized and uninoculated barley or Czapek's media was used in the check treatment. The pots were watered when necessary. Plants were regularly examined for disease symptoms, but final results were recorded 30 and 90 days after planting by recording the number of diseased plants, then the percentages of infected plants were calculated. Re-isolation was carried out from diseased cuttings to satisfy Koch's postulates.

3-Laboratory studies.

These experiments were carried out to study the effect of temperature, relative humidity and some bioagent antagonist on growth of fungi

pathogenic to rose cutting under laboratory conditions. The fungi used in these experiments were mostly those that proved to be highly pathogenic to cuttings of rose, viz. *Fusarium solani* (isolates 1 and 2) and *Rhizoctonia solani* (isolates 4 and 5).

In the following experiments, mycelial linear growth was measured as criterion for evaluating the effect of treatment. Except where otherwise mentioned, all experiments were performed on PDA medium. The pH value of the media was adjusted before sterilization to about 7 with 0.1N NaOH or 0.1N HCl. Media were autoclaved (for 15 minutes at ½ kg/cm^3 and then inoculated with 5 mm discs cut out with a sterile cork borer from the advancing margins of the fungal cultures to be tested. Three replicate plates were used in each treatment.

3-1. Effect of temperature on linear growth of the tested fungi:

The effect of temperature on the linear growth of the tested fungi was studied by keeping inoculated plates containing PDA medium at 5, 10, 15, 20, 25, 30, and 35^{o}C. The linear growth of each fungus was measured, 7 days after inoculation.

3-2. Effect of relative humidity on linear growth of the tested fungi:

To study the effect of relative humidity (RH) on fungal linear growth, the method described by Solmon (1951) was used. Petri dishes containing PDA medium were inoculated with fungal discs and turned upside down. Ten milliliters of appropriate concentrations of KOH or NaCl were poured into the lid of each dish to give relative atmospheric humidity levels, i.e. 14.5, 50, 80, 95 and 100%. The linear growth of each fungus was measured, 5 days after incubation at 25^{o}C.

3-3. Effect of antagonistic reaction between rose-cutting rot pathogens and *Trichoderma viride* and *Bacillus subtlis in vitro*, (dual culture interaction):

An isolate of each *Trichoderma viride* and *Bacillus subtlis* were isolated from rhizosphere of potato plants, purified and identified by Hassan (2013). Bioagents were grown on PDA and nutrient glucose agar (NCA) media, respectively, and then were incubated at 20^{o}C for 6- or 2-days, respectively, then were used as inocula. The antagonistic effects of the used bioagents were performed according to the methods adopted by Bell *et al*. (1982) and Ferreira *et al*. (1991).

A disk of *T. viride* (5mm in diameter) culture, or a loop from *B. subtlis* growth were inoculated on PDA or nutrient glucose agar media, in one side in Petri plates and the opposite side was inoculated by the pathogen isolated from rose cutting rot (viz. *F. solani* or *R. solani*). Inoculated plates were incubated at 25^{o}C. Three replicates were used for each bioagent and also for each pathogen. Inoculated plates with *F. solani* or *R. solani*, free of the antagonistic bioagent were used as control. Two to five days after inoculation, the linear growth of either

F.solani or *R.solani* was measured. The inhibition percent of growth was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

4- Pot experiments:

4-1- Effect of bioagents on rose-cutting-rot incidence:

An experiment was applied in greenhouse to study the effect of soil infestation either with *T. viride* or *B. subtlis* on basal rot of rose incidence. Experiment was carried out during 2013 and 2014 growing seasons.

4-1-1- Preparation of *Trichoderma viride* and *Bacillus subtlis* inocula.

The propagules (colony forming unit, CFU) suspensions of either *T. viride* or *B. subtlis* were prepared in sterile distilled water from 7 days-old-cultures on PDA (Rojo *et al.,* 2007) or NGA (Sallam *et al.,* 1978). The fungal or bacterial inoculum was harvested by flooding the culture with sterile distilled water and then rubbing the culture surface with sterile glass rod. The fungal or bacterial propagules concentration (in each suspension) were determined by counting, using a haemocytometer slide, then were adjusted to 10^5 spores/ml of the fungus and10^8 CFU/ml of bacteria. A mixture of milted soybean and talc powder (1:1 w/w) was used as a carrier mixture for antagonistic organism propagules. A carrier mixture was added at rate of 1:1 w/v to fungal and bacterial suspensions and mixed to even distribution of antagonistic agent propagules (Abd EL-Khair and El-mougy, 2003)

4-1-2- Evaluation of antagonistic activity of bioagents in pot experiment:

Antagonistic activity of both *T. viride* and *B. subtlis* against *Fusarium solani* and *Rhizoctonia solani* (one isolate of each); the rose cutting rot inducing pathogens, was evaluated in pots under artificially infestation condition. Frist, soil was infested with each pathogenic fungus separately in different pots and the pots were irrigated for 7 days before bio-control agents inoculation. Next, soil was inoculated with either *T.viride* or *B.subtlis* at 5g/kg soil, and then pots were watered for 7 days before sowing. Four rose cuttings (*Rosa gallica* var. *aegyptiaca)* were sown, in 15 February, in each pot and three pots were used as a replicate for each treatment as well as the control (untreated pots). Pots were kept under greenhouse conditions till the end of the experiment, 90 days after sowing. The percentages of disease incidence and survival of rose plants were recorded at the end of the experiment.

Statistical analysis:

The experimental designs of all experiments were completely randomized with 3 replicates, analysis of variance (ANOVA) of the data was performed using the Statistical

Analysis System (SAS Institute, Inc., 2004) statistical software. Means were compared according to the least significant differences (LSD 0.05) following Doncan's test

EXPERIMENTAL RESULTS

1- Isolation and identification of the pathogens.

Nine isolates of fungi, belonging to five genera (Table 1), were isolated and identified as *Fusarium solani* (three isolates), *Fusarium* sp. (one isolate), *Rhizoctonia solani* (two isolates), *Aspergillus* flavus (one isolate), *Mucor* sp. (one isolate) and *Rhizopus stolonifer* (one isolate) according to their morphological and microscopical characteristics using the keys given by Gilman (1957) and Booth (1971) and as verified by the Division of Fungal Taxonomy, Plant Pathology Research Institute, ARC, Giza, Egypt..

2- Pathogenicity tests.

The effect of soil infestation and soaking the basal part of cuttings in suspension of the fungi isolated from field rotted rose cuttings on the incidence of basal rot in rose (*Rosa gallica* var. *aegyptiaca*)are shown in Table (2). The results clear that *Fusarium solani*, *Fusarium*.sp. and *Rhizoctonia solani* could induce basal rot of rose cuttings, while *Aspergillus flavus*, *Mucor* sp. and *Rhizopus stolonifer* were nonpathogenic fungi for rose cuttings. The highest percentages of cutting infection were obtained, 90 days after sowing, with both *F. solani* and *R. solani* (58.3 - 100% infection) when cuttings were soaked in fungal suspension, while the percentages of infection ranged between 83.3-100% when cuttings were planted in infested soil. *Fusarium* sp. was induced the lowest percentages of rose cutting infection (16.6 and 25 %, in the two methods of infection, respectively).*Fusarium solani* isolates differed in their ability to induce the disease, i.e. in case of inoculation of rose cuttings with fungal isolates tested, the percentages of infection were 83.3 and 91.6% after 30 days of sowing for isolates No 1 and 2, respectively, and were 83.3 and 100% after 90 days of sowing, whereas, the percentages of disease incidence were 58.3% caused by isolate No. 3. Also, isolates of *R. solani* were differed in this respect, the percentages of infection were 75.0 and 66.6% for isolates number 4 and 5, respectively, 30 DAS, whereas, the incidence of disease percentages were 83.3 and 66.6% after 90 days of sowing. In case of soil infestation, isolates No 1 and 2 of *F. solani* and isolates No. 4 and 5 of *R. solani* caused 100% infection, 90 DAS. All cuttings of rose dipped in *F. solani* and *R. solani* suspensions were completely failed to continue grow after 90 days of planting.

The symptoms on infected cutting expression below the soil line consisted of an extensive dark brown to black soft rot, basal stem rot. This stem rot extended to the soil line (Figure 1). The infected cuttings were failed growth.

3- Laboratory studies:

In these studies, the effect of different temperature, relative humidity on the growth of *Fusarium solani* and *Rhizoctonia solani*, the causal pathogens of rose basal rot cuttings, were tested in the laboratory.

3-1. Effect of temperature:

Fungal linear growth (in mm) of *F. solani* and *R. solani*, the most pathogenic fungi to cuttings of rose, was estimated, 7 days after culturing in PDA medium. The obtained results (Table 3) show that all 4 isolates tested grew in a wide range of temperature (15 - 35^{o}C). The optimum temperature for growth of *F. solani* was 25^{o}C, whereas for *R. solani* it was 20^{o}C. Both tested fungi failed to grow at 5^{o}C.

Table (1): Locality and Frequency of fungi isolated from rotted rose cuttings.

Fungus	Number of Isolates	Locality of sample	Frequency
Fusarium solani	3	Minia	33.3
Rhizoctonia solani	2	Giza	22.2
Fusarium sp.	1	Giza	11.1
Aspergillus flavus	1	Minia	11.1
Mucor sp.	1	Minia	11.1
Rhizopus stolonifer	1	Minia	11.1

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Figure (1): Symptoms of basal rot on rose cuttings grown in artificially infested soil with *Fusarium solani* (right). Healthy plants (left).

Table (2): Percentages infested cuttings of rose (*Rosa gallica* var. *aegyptiaca*) using two methods of inoculation with fungi isolated from field- rotted cuttings, 30 and 90 days after sowing (DAS).

Fungus	Isolate	% of infested cuttings, by			
		dipping in fungus suspension		Soil infestation	
		30[1)]	90DAS	30	90DAS
Fusarium solani	1	91.6[2)]	100.0	100.0	100.0
	2	83.3	83.3	91.6	100.0
	3	58.3	58.3	75.0	83.3
*Rhizoctonia solan*i	4	75.0	83.3	91.6	100.0
	5	66.6	83.3	100.0	100.0
Fusarium sp.	6	16.6	16.6	25.0	25.0
Aspergillus flavus	7	0.0	0.0	0.0	0.0
Mucor sp.	8	0.0	0.0	0.0	0.0
Rhizopus stolonifer	9	0.0	0.0	0.0	0.0
Control		0.0	0.0	0.0	0.0
L.S.D 5% for dipping	A (fungi)=15.761		B(days)=N.S	AB=N.S	
L.S.D 5% for soil	A(fungi)= 17.392		B(days)=N.S	AB=N.S	

[1)] Data was recorded at 30 and 90 days after sowing.

[2)] Each reading is average of 3 replicates, each containing 4 cuttings.

Table (3): Effect of different degrees of temperature on linear growth (in mm) of rose cutting rot pathogens, grown on PDAmedium.

Fungi	Isolate No.	Fungal linear growth (mm) at						
		5	10	15	20	25	30	35
Fusarium solani	1	0	33	39	76	90	84	59
	2	0	18	31	67	87	75	56
Rhizoctonia solani	4	0	21	58	90	83	72	52
	5	0	33	61	90	86	67	42
L.S.D5% for A(fungi) =1.406, B (temperature) =3.090 AB=6.192								

3-2. Effect of relative humidity:

To study the effect of atmospheric humidity on the linear growth of *F. solani* and *R. solani*, 14.5, 50, 80, 95 and 100% of relative humidity (RH) were tested. Data in Table (4) show that all the tested fungi could grow in range of 50-100% RH. Increasing the RH of atmosphere from 50-100% gradually increased the linear growth of any of the tested two fungi. The best growth of two tested fungi was obtained at 95 and 100% RH. However, differences between the obtained values of all tested fungal growth at 80 and 95% levels of RH were statistically insignificant. It is

also clearly that all tested fungi failed to grow at 14.5% RH.

3-3. Antagonist between rose basal-rot-inducing pathogens and biotic agents *in vitro*:

The ability of either *T. virida*e or *B. subtlis* to inhibit the mycelial growth of *F. solani* and *R. solani*, the rose cutting-rot-pathogens, in dual culture was determined on PDA and NGA media, respectively. Data presented in Table (5) indicate that both bioagents *T. viride* and *B. subtlis* were able to inhibit the mycelial growth of all isolates tested of both rose cutting-rot-pathogens, i.e. *F. solani* and *R. solani in vitro*, but the two bioagents varied in their ability to antagonistic effect. In general, *T. virida* exhibited the higher antagonistic effect toward the tested pathogens than *B. subtlis*. Data show, also, that *R. solani* was more sensitive to antagonistic agents when compared with *F. solani*. Reduction in growth of *F. solani* isolates against *T. virida* ranged between 60 - 66.3%, while the growth of *R. solani* isolates reduced by 86.7 and 76.7% with no significant differences between them. In case of bacteria, the percentage of reduction in growth of *F. solani* and *R. solani* ranged between 50 - 66.7% and 50%, respectively.

Table (4): Effect of relative humidity on the linear growth (in mm) of rose basal cutting-rot inducing fungi.

Fungi, isolate number		Fungal linear growth (mm) at Relative humidity (%)				
		14.5	50	80	95	100
Fusarium solani	1	0	65	79	85	90
	2	0	51	81	81	90
Rhizoctonia solani	4	0	58	81	90	90
	5	0	42	80	82	90
L.S.D5% for A(fungi)=3.078, B(RH.)= 2.740 and AB=5.480						

4- Effect of bioagent on controlling rose- cutting- rot disease in pot experiment:

The effect of soil treatment with biocontrol agents *(Trichoderma viride* and *Bacillus subtlis*) on cutting rot incidence was studied during two successive seasons 2013 and 2014 under greenhouse conditions. Results of this study presented in Tables (6 and 7) indicate that addition of the two bio–control- agents to the soil, one week before sowing,has influenced significantly and increased the percentages of growing cuttings under artificial infection with either *F. solani* or *R. solani* in greenhouse conditions. The percentages of disease incidence, during 30 and 90 DAS caused by *F. solani* under application of *T. viride* and *B. subtlis* were in the range of 8.33-25% compared to 25-75% (control), respectively. At 30 DAS, *T.*

viride gave the highest reduction to disease incidence (66.7%) followed by *B. subtlis* (33.3%). At 90 DAS, both *T.viride* and *B.subtlus* gave about 66.7% reduction of disease incidence. Whereas, the disease incidence caused by *R. solani* under application of *T. viride* and *B. subtlis* ranged between 16.7% (after 30 DAS) and 25-33.3%, at 90 DAS. The highest reduction (62.7%) showed 90 DAS when *T. viride* was applied. The percentage of survival rose plants was in the range of 66.7 – 75.0% when compared with the control treatment (25 - 33.3% of healthy plants). The percentages of the increase of healthy plants were ranged between 100-200%. Data in the two successive seasons were similar in the most cases.

Table (5) Effect of *Trichoderma viride* and *Bacillus subtlis* on the growth of *F. solani and R. solani, in vitro.*

Fungi	Isolate	Linear growth (mm) of the pathogen when soil infested with		% inhibition of pathogen growth, antagonistic tester	
		T. virida	*B. subtlis*	*T. virida*	*B. subtlis*
F. solani	1	36.0	45.0	60.0	50%
	2	33.0	51.0	63.3	66.7%
Control		90.0	90,0	0.0	0.0
R. solani	4	21.0	45.0	86.7	50.0
	5	20.0	45.0	76.7	50.0
Control		90.0	90.0	0.0	0.0

L.S.D5% for A (fungi) =16.142, B (Antagonistic) =4.587 and AB=N.C

Table (6): The effect of *Trichoderma viride* and *Bacillus subtilis* treatments on rose - cutting rot incidence under greenhouse condition. during 2013 growing seasons.

Pathogen	Bioagent	Disease assessment, after					
		30 DAS		90 DAS		Survival plants,%	
		D.I., %	Reduction, %	D.I., %	Reduction, %	Healthy Plants	Increase, %
F. solani	*T. viride*	8.33	66.7	25	66.7	75.0	200.0
	B. subtlis	16.7	33.3	25	66.7	75.0	200.0
	Control	25	0.0	75	0.0	25.0	0.0
R. solani	*T. viride*	16.7	50	25	66.7	75.0	125.0
	B. subtlis	16.7	50	33.3	50.0	66.7	100.0
Control	Control	33.3	0.0	66.7	0.0	33.3	0.0
	T. viride	0.0	-	0.0	-	100.0	-
	B. subtlis	0.0	-	0.0	-	100.0	-

L.S.D5% for A= (bioagent+fungi) 10.7, B (days) = 6.2 and AB=17.4

Table (7): The effect of *Trichoderma viride* and *Bacillus subtilis* treatments on rose- cutting rot incidence under greenhouse condition, during 2014 growing seasons.

Pathogen	Bioagent	Disease assessment, after					
		30 DAS		90 DAS		Survival plants,%	
		D.I., %	Reduction,%	D.I.,%	Reduction, %	Healthyplants	Increase, %
F. solani	*T. viride*	16.7	60.0	25.0	70.0	75.0	349
	B. subtlis	16.7	60.0	25.0	70.0	75.0	349
	Control	41.7	0.0	83.3	0.0	16.7	0.0
R. solani	*T. viride*	16.7	66.6	16.7	77.7	75.0	125
	B. subtlis	8.3	83.4	33.3	55.6	66.7	100
	Control	50.0	0.0	75	0.0	33.3	0.0
Control	*T. viride*	0.0		0.0		100.0	
	B. subtlis	0.0		0.0		100.0	

L.S.D5% for A (bioagent+fungi) =16.3, B (days)=5.3 and AB=15.0

DISCUSSION

Roses (*Rosa gallica* L.) are best known ornamental plants grown for their flowers in the garden and sometimes indoors. They have been also used for commercial perfumery and commercial cut flower crops. Roses are subjected to attack by various disease pathogens which frequently induce severe losses in its plantations (Salamone *et al.,* 2011). Basal cutting rot disease is considered as one of the among fungal diseases of rose. The original intention of this work was to isolate and identify the causal organism(s) responsible for rose cutting rot disease prevalent at Minia and Giza nurseries, and to find an effective control measures against this disease.

Field-rotted cuttings of rose (R. *gallica* L.) collected from Minia and Giza Governorates during winter season of 2012 were used for isolating the pathogenic fungi associated with the disease. It was possible to identify nine fungal isolates representing 6 species, these were *Fusarium solani* (three isolates), *Rhizoctonia solani* (two isolates), *Fusarium* sp (one isolate), *Aspergillus flavus* (one isolate), *Mucor* sp. (one isolate) and *Rhizopus stolonifer* (one isolate).

R. *solani* (Priyatmojo *et al*., 2001), *Pythium helicoides* (Kageyama *et al.,* 2002 and Li *et al*., 2007), *Phytophthora citrophthora* (Salamone, 2011) and *Cylindrocladium scoparium*; teleomorph of *Calonecteria morganii* (Leathy, 1994 and Ryan, 1994) have been found to cause basal-cutting, root and stem rots of rose. The present study revealed that F. *solani*, *Fusarium* sp. and *R. solani* are able to induce the disease (basal rot of rose cuttings), whereas *A. flavus*, *Mucor* sp. and R. *stolonifer* are not.

The pathogenic fungi, isolated from rotted cuttings of rose, in the present investigation, varied considerably as regards degree of severity to rose cuttings. *F. solani* caused 58.3 - 100% infection at 30

DAS and 58.3 - 100% at 90 DAS, whereas R. *solani* caused 66.6- 100%, 30DAS and 83.3-100%, 90 DAS. Also, the percentages of infection were differed depending on the method of infection. All cuttings of rose soaked in suspension of either *F. solani* or *R. solani* were completely failed to grow. Then, isolates No.1 of *F. solani* and isolate No 4 of *R. solani* were the most pathogenic ones caused 100 and 83.3% infection, respectively when cuttings were sown in infested soil. The percentages of infection were 16.6 and 25% when the basal parts of cuttings were soaked in suspension of *Fusarium* sp. or when cultivated in infested soil, respectively. Such results are in close agreement with those of (Priyatmojo *et al*., 2001)

All the tested isolates were able to grow at a wide range of temperature (15 - 35^{o}C). The optimum temperature for growth of *F. solani* was 25^{o}C, whereas for *R. solani* it was 20^{o}C. Both tested fungi failed to grow at 5^{o}C.This was similar to the results of studies on isolates AG-2-2 obtained from soybean (Liu and Sinclair, 1991) and bent grass (Hyakumachi *et al*., 1998). The results for the pathogenicity tests indicate that the disease incidence caused by *R. solani*or *F. solani* was highest at the optimum tested temperature of growth (20 and 25^{o}C, respectively) at which the disease naturally occurred in winter. Priyatmojo *et al.* (2001) observed that the optimum temperature for growth rate of isolates of AG-2-2 of *R. solani* isolate, isolated from miniature rose cutting rot, was 28^{o}C, and it was able to grow at 35^{o}C.

The forenamed two cutting rot causing fungi could grow in range of 50-100% RH. Increasing the RH of atmosphere from 50 to 100% gradually increased the linear growth of any of the tested two fungi. The best linear growth showed at 100% relative humidity (RH). However, differences between the obtained values of all tested fungal growth at 80 and 95% levels of RH were statistically insignificant. It is also clear that all tested fungi failed to grow at 14.5% RH. Harfoush (1970) and Abdel-gawad (1978) found that the best rate of growth of *R. solani*o ccurred at 73-100% RH and of *F. oxysporum* was 100%.

Biological control of soilborne pathogens by introduced micro-organisms has been studied for over 100 years (Weller, 1988), but during most of that time it has not been considered commercially feasible. The main target of using biological and chemical treatments is to protect the cultivated plants throughout their growing period against plant disease pathogens. The present study showed that both *Trichoderma virida* and *Bacillus subtlis* were able to inhibit the mycelial growth of all isolates tested of both *F. solani* and *R. solani* (the rose cutting-rot-pathogens) *in vitro*, but the bio agents varied in their ability to antagonistic effect. In general, *T. virida* exhibited the higher antagonistic effect toward the tested pathogens (caused 60 - 66.3% reduction in growth of *F. solani* and

86.7 and 76.7% of *R. solani*), then *B. subtlis* which reduced the growth of *F. solani* and *R. solani* by 50 - 66.7% and 50%, respectively.

The introducing bioagent and fungicide as well into the soil is facing undesirable conditions. Therefore, they must withstand these conditions in order to achieve the proposed approach. Many investigators suggested such phenomena. Papavizas (1982) reported that the high population density of *T. harzianum*, introduced through soil treatment technique, enables the bioagent to adapt itself against environmental conditions and resulting in dominance of high population of the fungus. In 1981, Abd El-Moity stated that activity of *T. harzianum* acts through different mechanisms, i.e., production of gliotoxin, mycoparasitism, growing very fast and acts as a barrier between susceptible plant tissues and virulent pathogens. Many species of the genera *Bacillus and Trichoderma* are known to be potent producers of many antibiotics against soilborne-pathogens (Ahmed *et al.*, 2003 and Han *et al*., 2005). These suggestions may clarify the low cutting rot incidence in the present study when *T. viride* was introduced to the soil infested with either *F. solani* or *R. solani*. It could be suggested that the biological equilibrium between the introduced bioagent, *T. viride*, and other soil microflora seems to be in favor of bioagent against the disease pathogens; *F. solani* or *R. solani*, which resulted in reducing the disease incidence by 66.7% at 90 DAS. Similar results were obtained when a *Trichoderma* preparation mixed with soil artificially inoculated with *F. oxysporum* f.sp. *chrysanthemi*, the causal of chrysanthemum wilt (Locke *et al*., 1985).

The results of this investigation are closely agreement, also, with that obtained by Lacicowa and Pieta (1996) who found that dressing pea seeds with microbiological materials prepared from *T. koningi* and *T. viride* were most efficient in protecting pea from *R. solani* and *F.* spp. in soil Sunick *et al.* (1997) recorded that, *Bacillus* sp. gave a highly antagonistic effect against F.*solani* and R. *solani* and other pathoginc fungi tested. Elad *et al.* (1983) and Seema and Devaki (2012) explained the phenomenon of mycoparasitism as a complex process, involving recognition of the host, attachment to the mycelium, coiling round the hyphae, partial degradation of the cell wall and penetration of the host mycelium. Scanning electron microscope clearly has showed that the hyphae of *Trichoderma* coil around the hyphae of *Rhizoctonia solani* (the host), attached to host mycelium by forming hooks and produces approssoria at the tips of short branches (Elad *et al.*, 1983).

REFERENCES

Abdel-gawad T. I. 1978.Studies on basal stem rot disease of geranium cuttings. 1-108 pp, M. Sc. Thesis, Pl. Pathol. Dept., Fac Agric., El-Minia University.

Abd EL-Khair H. and El-mougy (Nehal) S. 2003. Field biological

approach under organic cultivation conditions for controlling garlic black mould disease infection during storage. Egypt. J. Appli. Sci., 18(6): 50 - 69.

Abd El-Moity, T.H. 1981. Further studies on the biological control of white rot disease of onion. 1-135pp, Ph. D. Thesis, Faculty of Agriculture, Minufiya University.

Abdel latif, M.R. 1976. Studies on some fungi causing pod rot of peanut in El-Minya governorate. 1-150 pp., M.Sc. Thesis. Faculty of Agriculture. El-Minia University.

Ahmed A.S., Ezziyyani M., Sachez C.P. and Candela M.R. 2003. Effects of chitin on biological control activity of *Bacillus* spp., and *Trichoderma harzianum* against root rot diseases of pepper (*Capsicum annum*) plants. Eur. J. Plant Pathol.,109 :633-637.

Barnett H. L. and Hunter B.B. 1972. Illustrated Genera of Imperfect Fungi,1-241 pp. Burgess Publishing Company, Minneapolis, Minnesota.

Bell D. K., Wells H.D. and Markham B.B. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72:379-382.

Booth C. 1971. The Genus *Fusarium*.1-237 pp, Commonwealth mycological Institute. Kew, Surrey, England.

Brown W. 1924. Two mycological methods: II- A method of isolating single strains of fungi by cutting a hyphal tip. Ann. Bot., 38:404.

Chi C.C. and Hanson W.E. 1964. Relation of temperature, pH and nutrition to growth and sporulation of *Fusarium* spp. from red clover. Phytopathology, 54: 1053-1058.

Dorrance, A. E.; Kleinhenz, M. D.; McClure, S. A. and Tuttle, N. T. (2003). Temperature, moisture, and seed treatment effects on *Rhizoctonia solani* root rot of soybean. Plant Dis., 87 (5): 533-538.

Elad Y., Chet I., Boyle P., and Henis Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*: scanning electron microscopy and fluorescence microscopy. Phytopathology, 73: 85 – 88.

Essa Z. M. 1992. Physiological studies on some rose varities. 1- 233 pp., Ph. D. Thesis, Faculty of Agriculture, Ain Shams University.

Ferreira J. H. S. Matthe F.N. and Thomas A.C. 1991. Biological control of *Eutypa lata* on grapevine by an antagonistic strain of *Bacillus subtlis.* Phytopathology, 81: 283-287.

Gilman J.C. 1957. A manual of Soil Fungi, 2nd ed. The Iowa State College Press.

Han J.S., Cheng J.H., Yoon T.M., Song J., Rajkarnikar A., Kim W.G., Yoo I.D., Yang Y.Y., Suh J. W. 2005. Biological control

agent of common scab disease by antagonistic strain *Bacillus* sp. sunhua. J. Applied Microbiology, 99:213-221.

Harfoush (Doria) I. 1970. Pathological and histological studies on damping-off disease of some solanaceous plants. M.Sc. Thesis. Fac. Agric., Ain Shams University.

Hassan M.M.E. 2013. Studies on *Rhizoctonia Canker* and Black Scurf Disease of Potato. 1-161 pp. Ph.D.Thesis, Plant Pathology Dept., Fac. Agric., Assiut University.

Hassan E.A., Abdel-Ghany R.A. and Gendy E.K. 2013. Effect of some fungicides and bioagents on controlling seed borne diseases on *Faba bean*. Egypt. J. Phytopathol., 41 (1): 67-87.

Horst K.R. 1983. Compendium of Rose Diseases. 1-50 pp. The American Phytopathological Society. St. Paul, Minnesota. USA.

Hyakumachi M., Mushika T., Ogiso Y.,Toda T..Kageyama K and Tsugo T. 1998.Characterization of anew cultural type (LP) of *Rhizoctonia solani* AG 2-2 isolated from warm-season turfgrasses, and its genetic differentiation from other cultural types. Plant Pathol. 47: 1 – 9.

Infantino A. Kharrat M., Riccioni L., Coyne C. J. Mc Phee K.E. and Grunwald N.J. 2006.Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica, 147: 201-221.

Kageyama, K. Aoyagi T., Sunouchi R. and Fukui. 2002. Root rot of Miniature roses caused by *Pythium helicoides*. Jurnal of General Plant Pathology, 68(1): 15-20.

Lacicowa B. and Pieta D. 1996. The efficiency of microbiological dressing of pea seeds (*Pisum sativum* L.) against pathogenic soil borne fungi. *Roczniki nauk rolniczych*. Seria E., Ochrona Roslin, 25 (112): 15-21. (cited from Ragab *et al.*,1999. Egypt. J. of Phytopathol., 27: 65-81.

Leahy, R.M. 1994. *Cylindrocladium* root and crown rot of roses. Plant Pathology Circular No.364, March/April 1994. Fla. Dept. Agric. & Consumer Services, Division of Plant Industry.

Li L., Kageyama K., Kinoshita N. and Yu. W. 2007. Development of bioassay for screening of resistant roses against root rot disease caused by *Pythium helicoides* Drechsler. Engei Gakkai zasshi, 76(1): 79-84.

Liu Z. and Sinclair J.B. 1991. Isolates of *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. Plant Dis.,75: 682-687.

Locke J.C., Marois J.J. and Papavizas G.C.1985. Biological control of *Fusarium* wilt of greenhouse grown chrysanthemums. Plant Diseases, 69: 167-169.

Manici L. Donatelli M., Fumagalli D. Lazzari A. and Bregaglio S.

2012. Potential response of soil-borne fungal pathogens affecting crops to a scenario of climate change in Europe. iEMSs Proceedings, pp 6-8.

Mosa (Olfat) M., Soliman N.E.K., Tolba A.F. and El-Sayed (Ayat) M. 2013. Evaluation of different mixtures of bioagents and antioxidants with bioagents on root rot in strawberries. Egypt. J. Phytopathol., 41(1): 109-119.

Mostafa H. M. 1972. Physiological and pathological studies on some fungi causing root rot to tomato. M. Sc.Thesis. Fac. of Agric., Ain Shams University.

Nazim M., Khalifa E. Z., El-Desouky S.M. and Amer G.A. 1997. Effect of time and method of application of *Trichoderma harzianum* in controlling soybean damping-off caused by *Macrophomina phaseolina* and *Sclerotium* rolfsii. Proc. 1st International Conference of Plant Pathology, Giza, Egypt, 8-13 pp.

Papavizas G.C. 1982. Survival of *Trichoderma harazianum* in soil and in pea and bean rhizospheres. Phytopathology, 72: 121-125.

Priyatmojo A., Yotani Y., Hattori K., Kageyama K. and Hyakumachi M. 2001. Characterization of *Rhizoctonia* spp. Causing root and stem rot of Miniature rose. Pland Dis., 85:1200-1205.

Qazi M.Z.and Quebral F.C. 1970. Effect of temperature on the growth of *Rhizoctonia solani* Khun. Agriculture Pakestan, 21(4): 447-451. (Rev of Pl. Path., 53: 884).

Ragab M.M., Aly M.D.H., Ragab (Mona) M.M. and El-Mougy (Nehal) S. 1999. Effect of fungicides, biocides and bioagents on controlling of pea root-rot disease. Egypt. J. Phytopathol., 27: 65-81.

Rojo F. G., Reynoso M. M., Sofia M. F. and Chulzel. 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Rop protection, 26: 549-555.

Ryan A. 1994. *Cylindrocladium* root and crown rot of roses. Plant Pathology Circular (unknown binding-1994). Fla. Dept. Agric. & Consumer Services, Division of Plant Industry. Amazon.com

Salamone, A. Scarito, G., Pane, A. and Cacciola S.O. 2011. Root and basal stem rot of rose caused by *Phytophthora citrophthora* in Italy. Plant Disease, 95 (3): 358 - 360.

Sallam A.A, Abd-Elrazek A.A and Rushdi M.H. 1978. Antagonistic effect of *Bacillus subtlis* against *Cephalosporium maydis*. Egypt. J. Phytopathol., 10:97-105.

Seema M. and Devaki N.S. 2012. *In vitro*, evaluation of biological control agents against *Rhizoctonia solani*. Journal of Agricultural Technology, 8 (1): 233- 240.

Solmon M.F. 1951. Control of humidity with potassium hydroxide, sulfuric acid, or other

solutions. Bull. Ent. Res., 42: 543-553.

Sunick K., Sungioon Y. and Honggi K. 1997. Selection of antagonistic bacteria for biological control of ginseng diseases. Korean J. of Plant Pathol., 13: 342-348.

Weller D. M.1988. Biological control of soilborne plant pathogens in the rhizospher with bacteria. Annual Review of Phytopathology, 26:379-407.

الملخص العربى

العنوان

الباحثين

تم عزل وتنقية ثلاث عزلات للفطر *Fusarium solani*، وعزلتان للفطر *Rhizoctonia solani* وعزله واحدة لكل من الفطريات *Fusarium* sp.، *Aspergillus flavus*، *Mucor* sp. و *Rhizopus stolinifer* من عقل ورد مصابة بالعفن تم جمعها من محافظتى المنيا والجيزة. أظهرت عزلات الفطران *F. solani* و *R. solani* قدرة على إصابة عقل الورد سواء الملوثة بالفطر المختبر أو المزروعة فى تربة ملوثة. ولم تحدث أى إصابة بالفطريات *A. flavus*، *Mucor* sp. و *Rhizopus stolinifer*. نمت العزلات المختبرة للفطرين *Fusarium solani* و *Rhizoctonia solani* فى مدى واسع من الحرارة (15 إلى 35 ^{o}م) ومدى واسع من الرطوبة الجوية (50 – 100% رطوبة نسبية). وكانت درجات الحرارة المثلى 25^{o}م للفطر *F. solani* و 20 درجة للفطر *R. solani* ، والرطوبة المثلى كانت 95–100%. أظهرت كل من عزلتى الفطر *Trichoderma viride* والبكتريا *Bacillus subtliis* القدرة على تثبيط نمو كل من الفطرين المختبرين *F. solani* و *R. solani* فى البيئة مع اختلاف قدرتهما على إحداث التثبيط. وعموما، سبب الفطر *T. viride* تأثيرا أقوى من البكتريا. وكان الفطر *R. solani* أكثر حساسية لكلا الكائنين. كما أدى خلط كل من الفطر *T. viride* والبكتريا *B. subtlis* فى التربة التى سبق عدواها بأى من الفطرين *F. solani* أو *R. solani* إلى زيادة معنوية لنسبة العقل النامية تحت ظروف العدوى الصناعية فى الصوبة الزجاجية.