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EFFECT OF ROOT ROT AND PLANT GROWTH PROMOTINGS INFECTION ON SOME BIOCHEMICAL CHANGES OF CUCUMBER AND SQUASH PLANTS

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ABSTRACT

Infection with the pathogenic root rot fungi (*Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc.) to cucumber and squash increased peroxidase activity. The highest activity of peroxidase enzyme was detected in cucumber roots, while the lowest one was observed in squash roots. Infection also increased the polyphenol oxidase activity in the roots of both hosts. Inoculation with the pathogenic fungi and coating of seeds with plant growth promoting rhizomicroorganisms resulted in an increase in the percent of total phenol contents. Furthermore, the coating of seeds with plant growth promotings increased the plant roots content of Indol Acetic Acid (IAA) and Gibberellic Acid (GA3).

Keywords : Cucumber, squash, causal organisms (*Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc.), root rot, biochemical changes, plant growth promoting rhizomicroorganisms (PGPR).

INTRODUCTION

Cucumber (*Cucumis sativus* L.) and squash (*Cucurbita pepo* L.) are considered the most important vegetable crops in many countries. Yield-losses in cucumber and squash caused by soil-borne pathogens cause endemic problems. Soil borne pathogens have been reported to attack both crops from seedling to maturity, causing root rot diseases and severe damage as well as high reduction in quantity and quality of yield.

Enzymes played an important role in decreasing or increasing the

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pathogens infection. The enzymes activity were higher in infected tissues than in healthy ones, Arun and Arya (1991) and Aly *et al.* (2001).

Biotic agents can induced systemic resistance (ISR) against fungi, by lipopolysaccharide (LPS), sidrophores, jasmonic acid (JA) and ethylene perception (Chen *et al.,* 1999). Plant growth promoting rhizobacteria (PGPR) strain of *B. subtilis* and *Pseudomonas fluorescens* treatment increased phenylalanine ammonia-lyase (PAL) and peroxidase activity, Chen *et al.,* (2000).

Bacillus sp. could be a potential agent to protect maize, horsebean, and rice (*Oryza sativa* L.) plants from infection with pathogenic fungi *in vivo* and had properties as a plant growth-promoting endophytic bacterium (PGPE) (Huili *et al.*, 2009).

Schroeder *et al.* (2009) found that soil borne pathogens can be particularly difficult to quantify. Unlike foliar disease symptoms caused by soil borne pathogens such as *Pythium* spp. and *Rhizoctonia* spp. are not observable, making it difficult to estimate pathogen populations. Soils were collected over a large geographic region of Eastern Washington in 2005, 2006 and 2007. Total DNA was extracted from the pathogens and species-specific primers for three species of *Rhizoctonia solani* and three to nine species of *Pythium* were used with a Roche Light Cycler to quantify pathogen DNA in these soils. The prevalence of *Pythium* species is favoured by higher precipitation zones. The diversity can also very greatly with as many as nine or as few as one species being detected in a single soil sample. Conversely, *R. solani* kühn AG-8 is quantified in low amounts in the higher precipitation zones and favors areas with less than 300 mm of annual precipitation. *Rhizoctonia oryzae* is less affected by precipitation, being prevalent in most regions. This work has also revealed correlations between the presence of certain species of these necrotrophic root pathogens with specific host plants. For example, *R. solani* AG-2-1 is favored by rotations with brassica crops. Using these real-time PCR assays, disease risk models are

being created to develop this procedure into a preplant tool for improved disease management.

Galvez *et al.* (2009) investigated "DNA barcoding" as a tool that aims to become a taxonomic method using a short standard genomic sequence, present in all the taxons of interest and showing sequence variation, enough to discriminate among species. Barcoding could provide an easy and reliable method to overcome these problems.

This work was designed to : 1- Isolate and identificate the cucumber and squash root rot causal organisms. 2- The differentiation between the isolates of cucumber and squash root rot fungi using RAPD PCR was also undertacken. 3- The effect of infection with fungal root rot pathogens (*R. solani* and *F. solani*) and seed coated with plant growth promoting rhizomicroorganisms (PGPR) on some biochemical changes of cucumber and squash plants (oxidative enzymes, growth hormones and phenolic compounds) was also studied.

MATERIALS AND METHODS

Isolation, Purification and Identification of the Causal Organisms from Cucumber and Squash Rotted Roots

The collected naturally infected roots from different districts (El-Zagazig, El-Salhia and Abou-Hammad) in El-Sharkia governorate were washed thoroughly several times with tap water then cut into small pieces and divided into two groups. The first one was surface disinfested by immersing in sodium hypochlorite 2% for two minutes then washed thoroughly using several changes of sterilized water to get rid of excess sodium hypochlorite residues, while the second one was used without disinfestation to isolate the internal and extrenal microorganisms.

The naturally infected pieces were dried between two sterilized filter papers, planted on plain agar in Petri-dishes then incubated at 28 ˚C for 3 – 5 days. All the dishes were daily examined binocularally for the fungal growth then picked and subcultured on potato dextrose agar (PDA) medium. The isolated fungi were purified using hyphal

tip and / or single spore techniques according to Kiet (1915) and Brown (1924) then transferred to PDA slopes medium and stored in refrigerator at 5 ˚C for further studies.

The isolated fungi were identified according to Barnett (1998) in the laboratory of Plant Pathology, Agric. Bot. and Plant Pathology Dept., Fac. of Agric., Zagazig Univ. All isolates were renewed every two weeks and restored in refrigerator. The frequency of the obtained fungi from roots was calculated and tabulated.

Differentiation Between the Isolates of Cucumber and Squash Root Rot Causal Organisms

In this study the differentiation between the tested isolates of cucumber and squash root rot causal organisms were done using random amplified polymorphic (RAPD) DNA.

Seven different random primers namely A01, A15, A18, A19, B18,C10 and C11 as shown in Table 1 were used to discriminate the pathogenic isolates different genera.

Table 1. List of primers names and their nucleotide sequences.

Primer names	Sequence
A01	5'CAGGCCCTTC3'
A15	5'TTCCGAACCC3'
A18	5'AGGTGACCGT3'
A19	5'CAAACGTCGG3'
B18	5'CCACAGCAGT3'
C10	5'TGTCTGGGTG3'
C11	5'AAAGCTGCGG3'

Samples preparation

Isolates tested were grown on tap water agar amended with 250 chloramphenicol in order to get rid of bacterial contamination, then transferred into a Defined Nutrient Medium (DNM) according to Keijer *et al.,* (1996).

Samples extraction (DNA isolation)

For each isolate, mycelium was freeze-dried over night, pulmerized with a pestle in a reaction tube, the DNA extraction was carried out as described in Keijer *et al.,* (1996) and Schneider *et al.,* (1997).

Amplification reaction mixture

RAPD reactions were preformed in a total volume of 25 µl containing a IX Polymerase Chain Reaction (PCR) reaction mixture (Promega); 2.5 mM $MgCl_2$; 50 uM of each dNTP (Promega); 0.3 uM of primer; 50 ng of genomic DNA; and 5 units of Taq polymerase. A negative control (distilled deionized water) was used instead of the target DNA included to test for contamination. Amplification conditions of the thermocycler MJ research model PTC-200 were as shown in Table (1) for each used primer.

The amplified products and the DNA marker were loaded on 1.5% agarose gels, separated by electrophoresis in, 0.5x TBE buffer (45mM Tris-borat and ImM EDTA), stained with Ethidium bromide (Sambrook *et al.,* 1989), and photographed under UV light using Herolab gel documentation system model Mididoc (Herolab, Germany) attached with vedio copy processor Mitsubishi. Dendrograms were calculated using computer soft ware statistical.

Pathogenicity Tests

Pathogenicity tests for the isolated fungi were carried out using Madina cucumber cultivar and Eskandrany squash cultivar seeds under greenhouse conditions of the Agricultural Botany and Plant Pathology Dept., Fac. Agric., Zag. Univ. Pots (25 cm in diameter) were sterilized by using formalin solution. Pots were filled at a rate of 3 kg sandy loam soil/pot and infected with the pathogenic fungi *(Rhizoctonia solani* and *Fusarium solani)* at the rate of 5% of soil weight..

The above mentioned infested pots were watered and mixed thoroughly with the inocula and left for one week to ensure even distribution of the inocula (Whithehead, 1957). Ten surface disinfected seeds of cucumber and squash/pot were sown. The disease assessments and plant growth reduction percent were calculated after 45 days from sowing.

Root-Rot Diseases Incidence as Affected by Coated Seeds with Plant Growth Promoting Rhizomicroorganisms (PGPR) on Cucumber and Squash Plants

A greenhouse experiment was conducted to assess the biocontrol activity induced by PGPR against both cucumber and squash plants root-rot causal organisms using cucumber (Madina cv.) and squash (Eskandrany cv.).

Treatments of each experiment were divided into four groups of pots arranged in complete randomized block design with three replications as follows:

- The first group, seeds were sown without neither coating with PGPR nor soil inoculation with the pathogens to serve as control.
- The second one, the seeds were sown in pots pre infested separately with *Rhizoctonia solani* Kühn and/or *Fusarium solani* (Martins) Sacc.
- The third group having seeds pre coated individually with the growth promoting rhizomicroorganisms i.e. *Trichoderma viride, Actinomyces* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* then seeded in soil free of any of the causal pathogens.
- The fourth group, pots were separately inoculated with the two pathogenic fungal genera then each group of pots was sown with seeds previously coated individually with the different PGPR. Seeds of cucumber and squash were planted at depth of approximately 1 cm in 25 cm^3 plastic pots (2 seeds/pot) containing 3 kg of sandy loam soil.

Plant growth reduction percent was determined after 60 days and plant growth parameters of the healthy survivals (plant height (cm), stem length (cm), number of leaves, root length (cm), root weight (gm), number of flowers, number of branches, fresh weight (gm) and dry weight (gm), were investigated.

Effect of Infection with Fungal Root Rot Pathogens and Seed Coated with Plant Growth Promoting Rhizoorganisms on Some Biochemical Changes of Cucumber and Squash Plants

Root system was separated from these plants and oxidative enzyme activity (Peroxidase and polyphenol oxidase) and some plant growth hormones (Indol acetic acid "I.A.A." and Gibberellic Acid "GA_3" as well as

total phenols were determined in the plant roots of both of cucumber and squash plants.

Determination of oxidative enzyme activities

One-gram of root tissues from treated plants was used to determine peroxidase and polyphenoloxidase activities. Each sample was cut into small pieces and grinded in a porcelain mortar by the pestle in the presence of purified sand and 2 ml of buffer phosphate (pH 7.0) as described by Goldschmidt *et al.* (1968). The obtained extract was quantitatively completed to 10 ml then centrifuged at 5000 rpm for 15 minutes according to the methods described by Malik and Singh (1990). The resulted supernatant was used to determine peroxidase and polyphenoloxidase activities.

Peroxidase activity

Peroxidase activity was determined according to the method described by Allam and Hollis (1972).

The reaction mixture contained 0.5 ml phosphate buffer (pH 7.0); 0.2 ml peroxidase enzyme (sample extract), 0.3 ml of 0.05 m pyrogallol, 0.1 ml of 1.0% (v/v) H_2O_2 and distilled water to obtain final volume 3.0 ml. The reaction mixture was incubated at 30 °C for five minutes, then the reaction was inactivated by adding 0.5 ml of 5.0% (v/v) H_2SO_4 (Kar and Mishra, 1976) and the absorbance was recorded at wave length of 425 nm using spectrophotometer. One unit of peroxidase activity was expressed as the change in absorbance at 425 nm/minute/ 1.0 g fresh weight.

Polyphenoloxidase activity

The polyphenol oxidase activity was quantitatively determined in sample according to the method described by Matta and Dimond (1963).

Determination of phenolic compounds

Free and total phenols were determined using the colorimetric methods as described by Snell and Snell (1953).

Identification of plant growth hormones from root of cucumber and squash

Plant growth hormones were determined according to the method described by (Wasfy et al., 1975).

RESULTS AND DISCUSSION

Isolation, Purification and Identification of the Causal Organisms from the Cucumber and Squash Rotted Roots

Data gives in Table 2 indicate that *Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc. were the most frequently isolated fungal genera from randomly 100 root rotted samples collected from different districts of El-Sharkia governorate.

Rhizoctonia solani Kühn and *Fusarium solani* (Martins) Sacc. were previously reported by several investigators as the incidents of root rot diseases (Mehl and Epstein 2006). The virulence of the isolates differed according to the host genotype and fertile perithecium formed in some dual cultures between field isolates (Nagao, *et al.* 1994).

Differentiation Between the Isolates of Cucumber and Squash Root Rot Causal Organisms

Phylogeny relationship among fungal isolates based on amplified RAPD fragments (bands)

Eighty six bands were detected as PCR products for the seven primers over all the six isolates fungi. The number of bands varied from 6 to 23 bands per primer and from 1 to 11 bands per isolate. The size of the amplified fragments ranged from 0.075 Mw to 3.107 Mw.

The amplified bands could be divided into three types :

1. monomorphic bands which were present in all samples.
2. polymorphic bands which presented in some samples and absent in others.
3. Unique bands (positive specific bands) present only in one sample and absent in the others.

Agarose gel electrophoresis for the seven RAPD PCR products shown in Fig. 1 reveal that, both primers A01 and A18 had the highest number of different PCR banding patterns (23 and 19). Meanwhile, the primers C10 and C11 showed the lowest number of different RAPD patterns (6 bands). This indicated that, the primers A01 and A18 were the highest among the tested seven primers in their ability to plank the DNA sequences of the six isolates tested in this work.

Table 2. Frequency and occurrence of the isolated fungi from root-rotted cucumber and squash collected from different districts of El-Sharkia governorate during 2003 growing season

Isolated fungi	Cucumber						Squash					
	El-Zagazig		El-Salhia		Abou-Hammad		El-Zagazig		El-Salhia		Abou-Hammad	
	Isolates	Frequency %	Isolates	Frequency %	Isolates	Frequency %	Isolates	Frequency %	Isolates	Frequency %	Isolates	Frequency %
Rhizoctonia solani	17	60.71	15	48.39	14	50.00	13	52.00	10	40.00	9	45.00
Fusarium solani	11	39.28	16	51.61	14	50.00	12	48.00	15	60.00	11	55.00
Total	28		31		28		25		25		20	

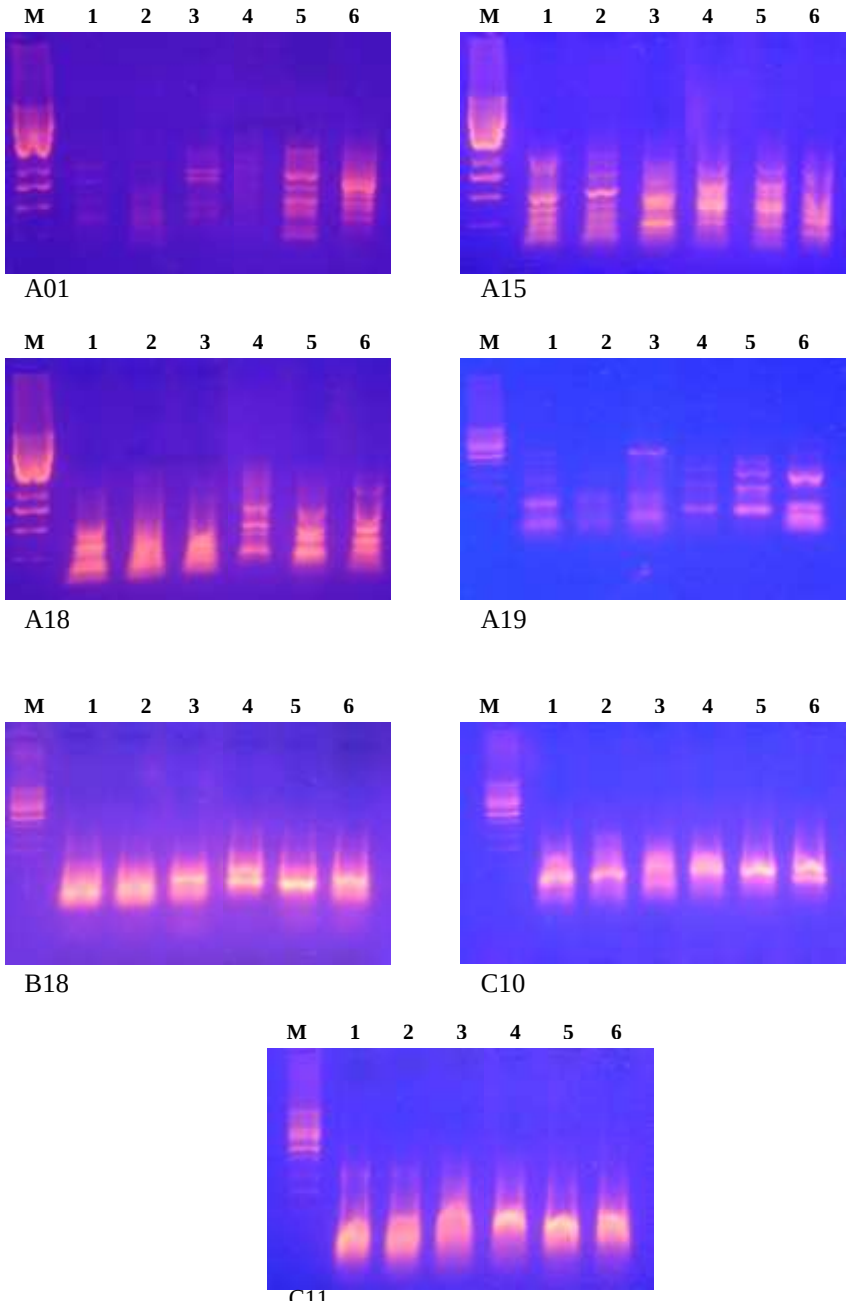

Fig. 1. RAPD-PCR fragments produced by the seven primers in the six fungal isolates

The results revealed the possibility of DNA analysis to differentiate between the isolates. The Majority of random primers examined gave distinctly reproducible patterns among all isolates studied. Whatever, the primers vaired in the extent of information that generated. Some produced highly polymorphic patterns, whereas others produced less polymorphic products, some DNA fragments were apparently similar in size among the six isolates (monomorphic). However, others were unique to a particular isolates that could be used as markers for such isolates.

These results demonstrated the usefulness of the RAPD-PCR technique for detecting DNA polymorphism in isolates.

The similarity coefficient values among isolates based on band polymorphisms generated by RAPD-PCR after using all the presented primers in Table 3. The highest similarity coefficient value (0.928) was found between *Fusarium* (2) and *Fusarium* (1). The lowest similarity coefficient value (0.454) was found between *Rhizoctonia* (2, 3) and *Fusarium* (2, 3).

The potential application of RAPD analysis to relationships among fungal isolates was assessed through cluster analysis of those six different isolates with large number of scorable polymorphic markers from 7 different random primers.

Genetic distances, calculated as the total number of band differences between fungal isolates, are presented in Table 4. The minimum distance (2.646) was found between *Fusarium* (2) and *Fusarium* (1) isolates, while the maximum distance (7.280) was found between *Rhizoctonia* (2, 3) and *Fusarium* (2, 3).

The dendrogram of genetic distances among six fungal isolates based on band polymorphisms generated by RAPD PCR using all primers is shown in Fig. (2). It is clear that six fungal isolates are segregated in two clusters. The first group involved three isolates, i.e. *(Fusarium* 1, 2, 3). The second main group was found to have three isolates which were *(Rhizoctonia* 1, 2, 3). The lowest degree of fungal isolates differentiation of *Fusarium* (1) and *Fusarium* (2) is shown by the low genetic distance between them.

Table 3. Similarity coefficients (Dice measure) between fungal isolates on their RAPD-PCR markers

Fungal isolates	*Fusarium* (1)	*Fusarium* (2)	*Fusarium* (3)	*Rhizoctonia* (1)	*Rhizoctonia* (2)	*Rhizoctonia* (3)
Fusarium (1)		.928	.845	.588	.485	.485
Fusarium (2)	.928		.856	.557	.454	.454
Fusarium (3)	.845	.856		.536	.454	.454
Rhizoctonia (1)	.588	.557	.536		.711	.691
Rhizoctonia (2)	.485	.454	.454	.711		.835
Rhizoctonia (3)	.485	.454	.454	.691	.835	

Table 4. Genetic distances, calculated as the total number of band differences out of all RAPD-PCR markers, between fungal isolates

Fungal isolates	*Fusarium* (1)	*Fusarium* (2)	*Fusarium* (3)	*Rhizoctonia* (1)	*Rhizoctonia* (2)	*Rhizoctonia* (3)
Fusarium (1)		2.646	3.873	6.325	7.071	7.071
Fusarium (2)	2.646		3.742	6.557	7.280	7.280
Fusarium (3)	3.873	3.742		6.708	7.280	7.280
Rhizoctonia (1)	6.325	6.557	6.708		5.292	5.477
Rhizoctonia (2)	7.071	7.280	7.280	5.292		4.000
Rhizoctonia (3)	7.071	7.280	7.280	5.477	4.000	

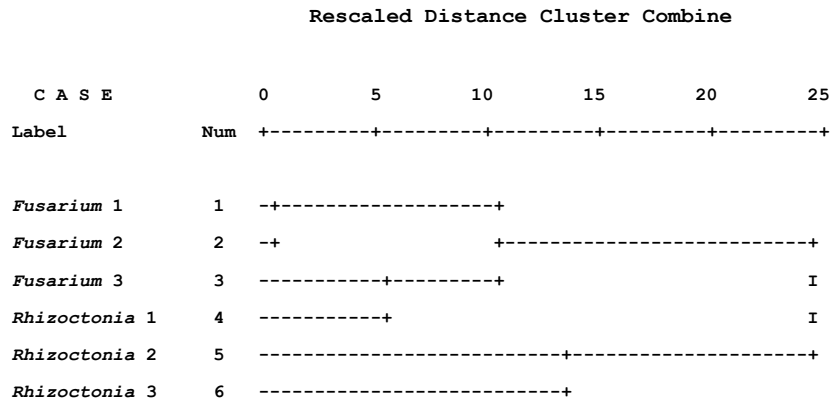

Fig. 2. Dendrogram of the six fungal isolates genetic distances generated by RAPD-PCR

In conclusion, this study demonstrated that RAPD or PCR analysis could be considered as a valuable tool in determining the genetic relationships among isolates of *Rhizoctonia* spp. Thus, the genomic variability is associated or could explain the pathological variation among the isolates (Fouly *et al.,* 2002).

Similar results were also obtained with *F. solani* infected squash. The discoloration and collapse noticed on the diseased seedlings might be due to different oxidates metabolites produced by the pathogenic fungal genera that cause chlorosis, necrosis, and epinasty for diseased plants (Kinoshita and Ichitani, 1998).

Pathogenicity Tests

Pathogenicity tests of the most frequently isolated fungi namely *Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc. were carried out under greenhouse conditions in Agric. Bot. and Plant Path. Dept., Fac. of Agric., Zagazig University.

Data presented in Table 5 indicate that *Fusarium solani* (Martins) Sacc. show similar percentages of infection being (50.00%) to cucumber and squash plants. While, the percentage of infection with *R. solani* differed in cucumber and squash plants.

Table 5. Pathogenicity tests of *Rhizoctonia solani* and *Fusarium solani* causing root rot of cucumber Madina cv. and squash Escandarany cv. plant

Treatment	Cucumber (Madena cv.)		Squash (Escandarany cv.)	
	Root rot %	Healthy survival %	Root rot %	Healthy survivual %
Control	**16.66 c**	**83.33 a**	**00.00 c**	**00.00 a**
Rhizoctonia solani	**50.00 a**	**50.00 b**	**33.33 ab**	**66.66 c**
Fusarium solani	**50.00 a**	**50.00 b**	**50.00 a**	**50.00 d**
L.S.D.$_{0.05}$	**3.21**	**4.32**	**3.88**	**2.21**

Pathgoenicity tests of the isolated fungi of cucumber and squash showed that *Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc. were pathogenic and differed in their virulence. In case of root-rot these fungi destroyed the tissues of older roots preventing the absorption of nutrient and water. These results were supported with those obtained by Vakalouakis and Fragkiadakis (1999). Differences in pathogenicity tests might be due to physiological reduction and anatomical changes essential for their pathogenicity and also to host-parasite - environment interactions (Tello *et al.,* 1990 and Moulin *et al*., 1994).

Root-Rot Disease Incidence as Affected by Plant Growth Promoting Rhizo-microorganisms (Seed Coating) on Cucumber and Squash Plants Regarding Plant Growth Parameters

Cucumber plants

The effect of four plant growth promoting rhizo-microorgansism (PGPR) *(Trichoderma viride, Actinomyces* sp. *Bacillus subtilis* and *Pseudomonas fluorescens* on root rot disease incidence and growth parameters of cucumber, was tested under greenhouse conditions.

Data in Table 6 indicate that application of the PGPR as seed coating before planting was

Table 6. Cucumber root rot incidence as affected by plant growth promoting rhizomicroorganisms (seed coating) regarding plant growth parameters

Treatment		Disease reduction percent (%)	Plant height (cm)	Stem length (cm)	Number of leaves	Root length (cm)	Root weight (gm)	Number of flowers	Number of branches	Fresh weight (gm)	Dry weight (gm)
Control		**50.00**	**36.40 j**	**34.50 h**	**19.00 ef**	**5.42 hi**	**4.22 h**	**7.00 h**	**1.00 b**	**117.1 h**	**17.14 g**
Rhizoctonia solani		**33.33**	**35.40 j**	**33.20 h**	**17.00 fg**	**5.55 h**	**1.03 j**	**6.00 hi**	**1.00 b**	**116.4 h**	**15.22 g**
Fusarium solani		**33.33**	**35.42 j**	**34.00 h**	**17.00 fg**	**5.50 h**	**1.40 j**	**6.00 hi**	**1.00 b**	**116.7 h**	**15.23 g**
Trichoderma viride		**100.00**	**69.40 a**	**65.50 a**	**31.00 a**	**14.00 a**	**8.50 a**	**18.00 a**	**2.00 a**	**145.4 a**	**24.05 a**
***Actinomyces* spp.**		**100.00**	**56.00 b**	**54.60 b**	**30.00 a**	**9.80 def**	**7.62 bcd**	**14.00 c**	**1.00 b**	**137.3 c**	**23.20 abc**
Bacillus subtilis		**100.00**	**54.00 c**	**50.00 c**	**29.00 ab**	**11.50 bc**	**7.43 b-e**	**16.00 b**	**1.00 b**	**136.3 c**	**23.00 a-d**
Pseudomonas fluroscens		**100.00**	**57.00 b**	**55.60 b**	**30.00 a**	**12.00 b**	**8.01 ab**	**19.00 a**	**2.00 a**	**142.4 b**	**23.55 ab**
Rhizoctonia solani	***Trichoderma viride***	**100.00**	**45.50 f**	**44.00 e**	**25.00 cd**	**9.50 ef**	**7.60 bcd**	**12.00 de**	**2.00 a**	**126.3 de**	**21.96 b-e**
	***Actinomyces* spp.**	**83.33**	**45.20 f**	**44.30 de**	**23.00 d**	**9.00 f**	**7.02 def**	**10.00 fg**	**1.00 b**	**125.4 ef**	**21.20 c-f**
	Bacillus subtilis	**100.00**	**42.50 gh**	**40.00 f**	**25.00 cd**	**9.20 f**	**6.85 ef**	**11.00 ef**	**1.00 b**	**123.5 fg**	**20.54 ef**
	Pseudomonas fluroscens	**100.00**	**41.00 h**	**40.20 f**	**25.00 cd**	**10.00 def**	**7.95 abc**	**11.00 ef**	**1.00 b**	**126.3 de**	**21.54 b-f**
Fusarium solani	***Trichoderma viride***	**100.00**	**53.00 cd**	**45.60 d**	**29.00 ab**	**10.60 cde**	**7.35 b-e**	**13.00 cd**	**2.00 a**	**140.9 b**	**21.69 b-e**
	***Actinomyces* spp.**	**100.00**	**52.00 d**	**50.00 c**	**27.00 bc**	**10.50 cde**	**7.30 cde**	**13.00 cd**	**1.00 b**	**127.9 d**	**21.50 b-f**
	Bacillus subtilis	**100.00**	**47.90 e**	**45.00 de**	**27.00 bc**	**10.50 cde**	**6.85 ef**	**14.00 c**	**1.00 b**	**127.5 de**	**21.65 b-e**
	Pseudomonas fluroscens	**100.00**	**52.40 d**	**50.00 c**	**27.00 bc**	**11.00 bcd**	**7.65 bcd**	**13.00 cd**	**2.00 a**	**127.9 d**	**21.50 b-f**
L.S.D$_{0.05}$			**1.691**	**1.389**	**2.140**	**1.235**	**0.6755**	**1.903**	**0.5095**	**2.375**	**2.062**

accurate as biological control way against root-rot of cucumber.

Data in the same Table indicate that the plant seeds treated with PGPR reveal high significant reduction percentage of root-rot followed with high plant growth parameters.

Treated plants with PGPR in the presence of the pathogenic fungi led to a reduction in percent of root-rot and high values of plant growth parameters even when plants were infected with *Rhizoctonia solani* and/or *Fusarium solani* in the three different treatments. *Actinomyces* spp. exhibit lower effect with *R. solani* being (83.33%) if compared with the other bioagents.

Squash plants

The effect of four plant growth promoting rhizomicroorganisms (PGPR) *(Trichoderma viride*, *Actinomyces* sp. *Bacillus subtilis* and *pseudomonas fluorescens* on root-rot disease incidence and growth parameters of squash under greenhouse conditions, was tested.

Data reveal significant higher percentage of root-rot than the control treatment in case of *Fusarium solani* in the three different treatments.

Data in Table 7 indicate that application of the PGPR as seed coating before planting was the most effective biological way to control root-rot of squash if compared with the application of the PGPR even after inoculation with the different pathogenic fungi (soil drench).

Data in the same table indicate that the plants treated with PGPR reveal a high reduction percentage of root-rot followed with high plant growth parameters when coating seeds, were investigated.

Plants treated with PGPR in the presence of the pathogenic fungi led also to a reduction percent of root rot with high values of all investigated plant growth parameters.

The effects of *Trichoderma* spp. on plants, including induced systemic or localized resistance, are also very important. These fungi colonize the root epidermis and the outer cortical layers and release bioactive molecules that cause walling off of the *Trichoderma* spp. thallus. At the same time, the transcriptome and the proteome of plants are substantially altered. As a

Table 7. Squash root rot incidence as affected by plant growth promoting rhizomicroorganisms (seed coating) regarding plant growth parameters

Treatment		Disease reduction percent (%)	Plant height (cm)	Stem length (cm)	Number of leaves	Root length (cm)	Root weight (gm)	Number of flowers	Number of branches	Fresh weight (gm)	Dry weight (gm)
Control		**83.33**	**50.00 o**	**4.50 f**	**15.00 d**	**8.50 ef**	**2.713 fg**	**3.00 cd**	**1.00 c**	**240.6 j**	**34.82 g**
Rhizoctonia solani		**16.66**	**45.00 j**	**4.50 f**	**10.00 h**	**10.20 d**	**1.40 jk**	**2.00 d**	**1.00 c**	**231.4 j**	**30.54 hi**
Fusarium solani		**50.00**	**40.00 k**	**5.00 f**	**10.00 h**	**8.40 efg**	**1.01 k**	**2.00 d**	**1.00 c**	**221.1 j**	**28.42 i**
Trichoderma viride		**100.00**	**97.50a**	**13.0 d**	**24.00 a**	**13.5 c**	**6.66 a**	**4.00 bc**	**2.00 b**	**995.9 a**	**66.51 a**
***Actinomyces* spp.**		**100.00**	**91.32 b**	**20.0 a**	**17.00 c**	**15.0 b**	**5.23 c**	**4.00 bc**	**2.00 b**	**798.8 b**	**65.73 a**
Bacillus subtilis		**100.00**	**93.00 b**	**15.0 cd**	**20.00 b**	**7.0 g**	**3.95 d**	**6.00 a**	**3.00 a**	**976.4 a**	**66.36 a**
Pseudomonas fluroscens		**100.00**	**91.50 b**	**19.0 ab**	**20.00 b**	**16.5a**	**5.23 c**	**4.00 bc**	**2.00 b**	**770.7 c**	**53.32 b**
Rhizoctonia solani	***Trichoderma viride***	**100.00**	**78.60 e**	**13.5 d**	**14.00 de**	**7.5 fg**	**2.68 fgh**	**4.00 bc**	**1.00 c**	**546.0 e**	**46.59 c**
	***Actinomyces* spp.**	**100.00**	**69.50 f**	**13.0 d**	**15.00 d**	**13.2 c**	**3.57 de**	**3.00 cd**	**1.00 c**	**541.4 e**	**41.40 e**
	Bacillus subtilis	**83.33**	**69.00 f**	**17.0 bc**	**14.00 de**	**16.5 a**	**6.53 a**	**4.00 bc**	**2.00 b**	**466.8 g**	**38.57 f**
	Pseudomonas fluroscens	**100.00**	**69.20 f**	**17.0 bc**	**13.00 ef**	**7.5 fg**	**2.48 gh**	**3.00 cd**	**1.00 c**	**452.2 g**	**29.35 hi**
Fusarium solani	***Trichoderma viride***	**100.00**	**66.50 g**	**13.0 d**	**15.00 d**	**8.5 ef**	**2.12 hi**	**3.00 cd**	**2.00 b**	**445.7 g**	**24.49 h**
	***Actinomyces* spp.**	**100.00**	**63.00 h**	**8.0 e**	**11.00 gh**	**9.5 de**	**3.25 ef**	**2.00 d**	**1.00 c**	**227.0 j**	**19.75 k**
	Bacillus subtilis	**83.33**	**63.50 h**	**10.0e**	**12.00 fg**	**8.2 efg**	**3.05 feg**	**3.00 cd**	**1.00 c**	**291.2 i**	**19.77 k**
	Pseudomonas fluroscens	**100.00**	**66.00 g**	**20.0 a**	**11.00 gh**	**10.0d**	**5.25 c**	**3.00 cd**	**2.00 b**	**346.0 h**	**20.04 k**
L.S.D$_{0.05}$			**2.254**	**2.226**	**1.662**	**1.447**	**0.5773**	**1.086**	**0.9247**	**22.88**	**2.739**

consequence, in addition to induction of pathways for resistance in plants, increased plant growth and nutrient uptake occur (Harman (2006). *Bacillus brevis* produced gramicidin S and acted as an antifungal compound. It would appear that from the *in vitro* studies. The target site of this antifungal was the plasma membrane (although other internal membranes e.g. the mitochondrial membranes are not excluded) with the loss of membrane integrity and selective membrane permeability) (Droby and Chalutz, 1994). Improving the effectiveness of biological control by fluorescent *Pseudomonas* spp. might be established by using combinations of strains that have different mechanisms of disease suppression, such as competition for iron and ISR. Combining SA-dependent and SA-independent ISR is another possibility to increase effectiveness. Several *Pseudomonas* strains produce SA under conditions of low iron availability and potentially are able to induce the SA-dependent signal transduction pathway. However, ISR by these SA-producing strains does not appear to depend on SA, and it is speculated that in most cases the SA is channeled into SA-containing siderophores. Manipulating SA production in these bacteria by either uncoupling SA production from the biosynthesis of SA-containing siderophores or by transfer of SA biosynthesis genes into non-SA producers seem effective. Production of SA by strains that already possess determinants that effectively trigger SA-independent ISR may create strains that induce both signal transduction pathways simultaneously (Bakker *et al.*, 2007).

Effect of Infection with Fungal Root Rot Pathogens and Seed Coated with Plant Growth Promoting Rhizoorganisms (PGPR) on Some Biochemical Changes of Cucumber and Squash Plants

Peroxidase activity

Data presented in Table 8 show the effect of *Rhizoctonia solani* kühn and *Fusarium solani* (Martins) Sacc. on peroxidase activity in cucumber and squash roots. Pathogenic fungi led to increased the peroxidase activity compared with the control 0.40 and 0.26 unit/g fresh weight for

Table 8. Effect of pathogenic fungi tested combined with PGPR on peroxidase activity (unit/g fresh weight)

Treatments		Peroxidase activity (unit/g fresh weight)	
		Cucumber	Squash
Control		**0.40**	**0.26**
Rhizoctonia solani		**3.33**	**2.42**
Fusarium solani		**3.43**	**2.56**
Trichoderma viride		**0.47**	**0.33**
***Actinomyces* sp.**		**0.53**	**0.60**
Bacillus subtilis		**0.47**	**0.53**
Pseudomonas fluroscens		**0.64**	**0.26**
Average		**1.32**	**0.99**
Rhizoctonia solani	***Trichoderma viride***	**2.50**	**2.10**
	***Actinomyces* sp.**	**1.40**	**1.73**
	Bacillus subtilis	**2.21**	**2.35**
	Pseudomonas fluroscens	**1.96**	**1.50**
Fusarium solani	***Trichoderma viride***	**2.12**	**2.02**
	***Actinomyces* sp.**	**1.34**	**1.65**
	Bacillus subtilis	**2.11**	**2.30**
	Pseudomonas fluroscens	**1.56**	**1.32**

cucumber and squash, respectively.

Furthermore, the highest activity of peroxidase enzyme was detected in cucumber roots, while squash roots exhibited lower activity.

The highest peroxidase activity was detected when roots of cucumber and squash plants were infected with *Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc. and treated with *T. viride* followed by *B. subtilis*. The lowest peroxidase activity was detected when roots of cucumber and squash plants were infected with the two tested pathogenic fungi and treated with either *Actinomyces* sp. and/or *Pseudomonas fluroscens*.

Polyphenoloxidase activity

The inoculation with *Rhizoctonia solani* Kühn and *Fusarium solani* (Martins) Sacc. increased the polyphenol oxidase

Table 9. Effect of tested pathogenic fungi on polyphenoloxidase activity (unit/g fresh weight).

Treatment		Polyphenoloxidase activity (unit/g fresh weight)	
		Cucumber	Squash
Control		0.42	0.21
Rhizoctonia solani		3.11	3.02
Fusarium solani		3.01	2.95
Trichoderma viride		1.07	0.49
***Actinomyces* sp.**		0.74	0.79
Bacillus subtilis		1.04	0.91
Pseudomonas fluroscens		0.1	0.35
Rhizoctonia solani	***Trichoderma viride***	2.55	1.65
	***Actinomyces* sp.**	2.60	2.21
	Bacillus subtilis	3.00	1.83
	Pseudomonas fluroscens	2.43	1.65
Fusarium solani	***Trichoderma viride***	2.42	1.58
	***Actinomyces* sp.**	2.56	2.11
	Bacillus subtilis	2.81	1.72
	Pseudomonas fluroscens	2.33	1.51

activity in the roots of both cucumber and squash plants, being 3.11, 3.02, 3.01, 2.95, respectively compared with the control treatment.

On the other hand, infection of cucumber and squash plants with the pathogenic fungi and seed coated with plant growth promoting rhizomicroorganisms caused a slight increase of polyphenoloxidase activity compared with the control treatment.

The higher polyphenoloxidase activity was obtained in cucumber roots compared with the squash roots.

Enzymes activity played an important role in plant disease resistance through increasing of plant defense mechanisms which are considered the main tool of varietal resistance (Takuo *et al*, 1993). Data obtained revealed that, the inoculation with the tested pathogenic fungi (especially *F. solani*) gave higher increase in both peroxidase and

polyphenoloxidase activity comparing with the control treatment especially in the susceptible cultivar. It is also concluded that, peroxidase activity is frequently increased in plants infected by pathogens and the level of its activity correlated with disease resistance (Guirgis and Clark, 1989). This might be due to such enzymes seem to be important in oxidize phenols to quinines which more fungal toxic (Singh and Singh, 1994). These were in line with the results obtained by Tohamy *et al.* (1987); Cherif *et al.* (1994) and Chen *et al.* (2000).

Determination of total phenols

It is noticed from Table 10 that the inoculation with *R. solani* and *F. solani* and seed coated with plant growth promoting rhizomicroorganisms increased the total phenol contents in the roots of both cucumber and squash cvs. compared with the control treatment which reached 30.9, 28.1 unit/g fresh weight, respectively.

Table 10. Effect of pathogenic fungi tested combined with PGPR on total phenol contents in the roots of cucumber and squash plants (unit/g fresh weight)

Treatment		Total phenol contents (unit/g fresh weight)	
		Cucumber	Squash
Control		**30.9**	**28.1**
Rhizoctonia solani		**70.10**	**66.11**
Fusarium solani		**68.0**	**65.0**
Trichoderma viride		**63.7**	**53.5**
***Actinomyces* sp.**		**47.6**	**51.0**
Bacillus subtilis		**60.1**	**33.5**
Pseudomonas fluroscens		**44.1**	**40.29**
Rhizoctonia solani	***Trichoderma viride***	**88.5**	**81.60**
	***Actinomyces* sp.**	**85.60**	**74.13**
	Bacillus subtilis	**71.12**	**66.11**
	Pseudomonas fluroscens	**74.23**	**86.90**
Fusarium solani	***Trichoderma viride***	**87.3**	**81.1**
	***Actinomyces* sp.**	**85.6**	**73.1**
	Bacillus subtilis	**70.1**	**65.7**
	Pseudomonas fluroscens	**72.2**	**86.7**

In addition the treatment of treated cucumber seeds infected with *Rhizoctonia solani* Kühn and/or *Fusarium solani* (Martins) Sacc. with *T. viride* caused the highest phenolic content. In case of treated squash seeds with *P. fluroscens* lead also to highest phenolic content. The highest total phenol contents were obtained in cucumber roots as compared with the squash treatment.

The increase of the total phenolic compounds in the infected roots was highest in case of infection by *Rhizoctonia solani* followed by *Fusarium solani*. These results were supported by Abou-Taleb *et al.* (1998). The difference in total phenol content between plants might be due to the different resistant degrees of these plants Xue *et al.* (1998).

Identification of plant growth hormones from root of cucumber and squash

Data concerning the effect of infection with *F. solani,* the most pathogenic fungus to cucumber and squash cvs., on plant growth hormones activity are presented in Table 11 showed that seed of cucumber and squash coated with plant growth promoting rhizomicroorganisms increased the content of Indol Acetic Acid (IAA) and Gibberellic Acid (GA3) in the roots of the cucumber and squash infested with *T. viridie* compared with the control treatment.

Table 11. Identification of plant hormones from root of cucumber and squash inoculated with *Fusarium solani*

Treatment		IAA (mg/100 g)		GA3 (mg/100 g)	
		Cucumber	Squash	Cucumber	Squash
Control		0.139	21.26	0.444	6.58
Fusarium solani		0.056	15.64	0.243	2.15
Trichoderma viride		1.699	511.54	7.323	20.90
Actinomyces sp.		0.303	286.68	2.937	15.67
Bacillus subtilis		0.241	301.66	1.719	17.44
Pseudomonas fluroscens		0.293	908.12	2.675	20.59
Fusarium solani	*Trichoderma viride*	0.217	208.96	1.458	14.4
	Actinomyces sp.	0.197	102.88	0.382	6.63
	Bacillus subtilis	0.093	136.42	0.299	3.26
	Pseudomonas fluroscens	0.113	154.53	0.482	10.9

Infected squash plants either of coated seeds with plant growth promoting microorganisms or non, exhibited greatest amount of Indol acetic acid if compared with the similar treatments of cucumber. On the other hand squash roots showed higher amount of GA_3 rather than cucumber of all treatments.

The presence of fungus *(F. oxysporum)* in submerged culture of *T. harzianum* (T8) in the absence of growth regulators increases the ability of *T. harzianum* to control *F. oxysporum* by increasing the chitinase production. On the other hand, the presence of plant growth regulators in *T. harzianum* culture medium decrease both the chitinase production and mycelia growth of *T. harzianum* which could decrease the mycoparasitim of this fungus on phytopathogens Badri *et al.* (2007).

REFERENCES

Abou-Taleb, E.M., M.A. Abd-El-Rehim and A. Tohamy. 1998. The role of protein content and certain phenol oxidase in wilt disease of cotton. Alex. J. Agric. Res., 33 : 201 – 214.

Allam, A.I. and S.P. Hollis. 1972. Sulfide inhibition of oxidases in rice root. Phytopathology, 62 : 634-639.

Aly, A.Z., M.S. Shalaby and Y.O. Fotouh. 2001. *Fusicoccum* sp. as the causal organism of citrus twigs gummosis. Zagazig J. Environmental Res., 4 : 53 – 66.

Arun, A. and A. Arya. 1991. Production of pectolytic and celuloytic enzymes by four pathogenic fungi of *Phomopsis* spp.. Advances in plant Sciences, 4 (2) : 304 – 309.

Badri, M., M.R. Zamani and M. Motallebi. 2007. Effect of plant growth regulators on *in vitro* biological control of *Fusarium oxysporum* by *Trichoderma harzianum*, Pakistan J. Biological Sciences, 10(17) : 2850 – 2855.

Bakker, P.A.H.M., C.M.J. Piaters and L.C. Vanloon. 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology, 97 : 239 – 243.

Barnett, H.L. 1998. Illustrated genera of the imperfect fungi. Burgess publishing company, Minneapolis, Minn. USA, 218 pp., Fourth Ed.

Brown, N. 1924. Two mycological methods. II. A method of isolated single strain fungi by cutting a hyphal tip. Ann. Bot., 38 : 402 – 406.

Chen, C., Belanger, R., Benhamou, N. and T. Paulituz. 1999. Role of SA in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber. Eur. J. Pl. Patho., 105 : 477 – 486.

Chen, C.Q., R.R. Belanger, N. Benhamou; T.C. Paulitz and C.Q. Chen. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiological and Molecular Plant Pathology, 56(1) : 13-23. (c.f. CAB, 1998 – 2000).

Cherif, M., A. Asselin and R.R. Belanger. 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. Phytopathology, 84 (3) : 236 – 242.

Droby, S. and E. Chalutz. 1994. Successful biocontrol of postharvest pathogens of fruits and vegetables. Brighton Crop Protection Conference – Pests and Diseases, 3 : 1265 – 1272.

Fouly, H.M., M.A. Kararah and M.A. Ahmed. 2002. Use of Random Amplified Polymorphic DNA (RAPD) procedure to study the genetic variability among isolates of *Rhizoctonia* sp. exhibiting varied degrees of pathogenicity in wheat roots. Second congress of recent technologies in Agriculture. Giza, 28 – 30 Oct., 111 : 788 – 798.

Galvez, E.J., L. Franco-Lara, S. Restrepo and P. Jimenez. 2009. Search of a DNA barcode for identification of the genus *Fusarium*. Phytopathology, 99 (6) : S 39.

Goldschmidt, E.G.; R. Goren and S.P. Monselise (1968). The IAA oxidase system of citrus roots. Planta, 72 : 313-22.

Guirgis, A.A. and R.L. Clark. 1989. Peroxidase isozyme variability and its association with cucumber fruit rot resistance. Proceeding of the first Arab Conference of Genetic. Fayoum, Egypt, 1 : 341 – 352.

Harman, G.E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96 (2) : 190 – 194.

Huili, W., W. Kai, Z. Xiuyun, W. Xuedong, L. Aiying and H. Huazhu. 2009. The inhibitory activity of endophytic *Bacillus* sp. Strain CHM1 against plant pathogenic fungi and its plant growth-promoting effect. Phytopathology, 99 (6) : S 56.

Kar, M. and D. Mishra. 1976. Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Pl. Physiol., 57 : 315-319.

Keijer, J., P.M. Houterman, A.M. Dullemans and M.G. Korsman. 1996. Heterogeneity in electrophoretic karyotype within and between anastomosis groups of *Rhizoctonia solani*. Mycological Research 100 : 789 – 797.

Kiet, M.M. 1915. Single technique for isolating single spore strains of certain types of fungi. Phytopathology, 5 : 266 – 269.

Kinoshita, T. and T. Ichitani. 1998. Hyphal interactions between *Pythium ultimum* and a mycoparasite, *P. oligandrum*-Light microscopic observations of their interface in and around cucumber roots in soil. Nippon Kingakukai Kaiho, 39(4) : 159-168. (c.f. Rev. Pl. Path. 1999., Vol. 78, No. 8).

Malik, C.P. and M.B. Singh. 1990. Extraction and estimation of amino acids and Kito acids. In : Plant Enzymology and Histo-enzymology. New Delhi-Lud Hana, India.

Matta, A. and A.E. Dimond. 1963. Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology, 53 : 574-587.

Mehl, H.L. and L. Epstein. 2006. Diagnosis and control of *Fusarium solani* f.sp. *cucurbitae* race 1 (teleomorph, *Nectria haematococca* mating population 1). Phytopathology, 96 (6) : S 82.

Moulin, F., P. Lemanceau and C. Alabouvette. 1994. Pathogenicity of *Pythium* species on cucumber in peat-sand, rockwool and hydrponics. European Journal of Plant Pathology, 100(1) : 3 – 17. (c.f. Rev. Pl. Path. 1995., Vol. 74, No. 5).

Nagao, H., K. Sato and S. Ogiwara. 1994. Susceptibility of *Cucurbita* spp. to the cucurbit root-rot fungus, *Fusarium solani* f.sp. *cucurbitae* race I. Agronomy, 14(2) : 95-102.

Sambrook, J., E.F. Fritsch and T. Maniats. 1989. Molecular cloning. A laboratory manual. Second Edition, Cold Spring Harbor Laboratory Press, USA.

Schneider, J.H.M., O. Salazar; V. Rubio and J. Keijer. 1997. Identification of *Rhizoctonia solani* associated with field grown tulips using ITS rDNA polymorphism and pectic zymograms. Eur. J. Pl. Pathol., 103 : 607 – 622.

Schroeder, K.L., T.C. Paulitz and P.A. Okubara. 2009. Incidence and spatial distribution of *Rhizoctonia* and *Pythium* species determined with real-time PCR. Phytopathology, 99 (6) : S 115.

Singh, S. and S. Singh. 1994. Influence of soil amendements on the efficacy of fungicides against Rhizoctonia seedling rot of cowpea (Crop Research Histar) 8(1) : 159 – 161. (c.f. Rev. Pl. Path., 75 (4) 1556).

Snell, F.D. and G.F. Snell. 1953. Colurimetric methods of analysis. Van Mastrond, Rein Hold and Co., New York.

Takuo, S., S. Tatsuji, H. Johan and V. Erick. 1993. Pectin, pectinase and protopectinase : Protection, properties and applications. Adv. Appl. Microbiol., 39 : 213 – 294.

Tello, J.C., J. Gomez, P. Camporota and A. Lacasa. 1990. Parasitic capacities of *Pythium aphanidermatum* and *Rhizoctonia solani* (Kühn) on cucumber and melon. Boletin de Sanidad Vegetal, Plagas, 16(4) : 733-741. (c.f. Rev. Pl. Path. 1993, Vol. 72, No. 11).

Tohamy, M.R.A., A.Z. Aly, Dawlat A. Abd El-Kader, and Ahlam M. Gowily. 1987. Studies on damping-off disease of some legume plants. Egypt. J. Appl. Science, 2nd Issue, 2 : 783 – 790.

Vakalounakis, D.J. and G.A. Fragkiadakis. 1999. Genetic diversity of *Fusarium oxysporum* isolates from cucumber : differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting, Phytopathology, 89(2) : 161-168.

Wasfy, W., Shindy and Orrin E. Smith. 1975. Identification of plant hormones from cotton ovules. Plant physiol., 55 : 550 – 554.

Whithehead, M.D. 1957. Sorghum, a medium suitable for the increase of inoculums for studies on soil-borne and certain other fungi. Phytopathology, 47 : 450.

Xue, L., P.M. Charest and S.H., Jabaji-Hare. 1998. Systemic induction of peroxidase, 1,3-beta-glucanases, chitinases, and resistance in bean plants by binucleate *Rhizoctonia* species. Phytopathology, 88 : 359 – 365.

تأثير الإصابة بكل من مسببات عفن الجذور وكائنات الجذور الدقيقة المحفزة لنمو النبات على بعض التغييرات الكيموحيوية فى نباتات الخيار والكوسة

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تعتبر نباتات الخيار والكوسة من أهم نباتات العائلة القرعية والتى تتعرض إلى فقد فى المحصول نتيجة لتعرضها للمسببات المرضية القاطنة فى التربة والتى تسبب أمراض عفن الجذور. أوضحت نتائج العزل والتعريف أن فطرى الريزوكتونيا سولانى والفيوزاريوم سولانى كانا أكثر الفطريات تكراراً فى عملية العزل. كما تم دراسة معرفة الاختلافات أو درجة التشابه ما بين العزلات المختلفة لهذين الفطرين بإحدى الطرق الحديثة من طرق البيولوجيا الجزيئية. وقد أشارت النتائج إلى أن البوادئ العشوائية أ١. و أ١٥ وب١٨ أعطت أكبر عدد من الحزم فى اختبار تفاعل البلمرة المتسلسل عن البوادئ العشوائية الأخرى والتى تم تطبيقها مع عزلات الريزوكتونيا والفيوزاريوم المختبرة وأوضحت النتائج أيضاً أن أعلى درجة تشابه كانت بين عزلتى الفيوزاريوم (١) ، (٢). هذا وقد بينت النتائج أيضاً أن معاملة النباتات بكائنات الجذور الدقيقة المحفزة لنمو النبات بطريقة تغطية البذور أعطت أعلى نسبة من النباتات السليمة وكذا قياسات النمو النباتى. كما أدى معاملة بذور نباتات الخيار والكوسة بكائنات الجذور الدقيقة المحفزة لنمو النباتات إلى زيادة فى نشاط إنزيمى البيروكسيديز والبولى فينول أكسيديز والفينولات الكلية والأندول أستيك أسيد والجبريلك أسيد فى جذور نباتات الخيار والكوسة المصابة مقارنة بالكنترول.