Comparative Studies on Some Isolates of Fungi Responsible for Soybean Root, Foliage Diseases and Response of Some Cultivars to Infection

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Received: 1/3/2006

Abstract: *Soybean (Glycine max (L.) Merr.) is able to infection with several diseases, such as damping-off, root-rot and anthracnose. Survey of these diseases in 2001 and 2002 growing seasons, indicated their presence in six governorates. Rhizoctonia solani, Fusarium solani, Marophomina phaseolina and Colletotrichum dematium were the main pathogens. Anastomosis groups test was used to identify R. solani isolates. This method revealed that all R. solani isolates are multinucleated and were classified into two anastomosis groups. The main pathogenic isolates were determined by using RAPD. Under greenhouse conditions,the eight tested soybean cultivars were different in their reactions to these diseases. Crawford and Giza 82 cultivars were the least susceptible to root-rot and anthracnose diseases, respectively, while Giza 21 cultivar was the most susceptible to both diseases. All cultivars tested varied in their protein patterns. There were remarked changes in protein patterns of all cultivars infected with R. solani, M. phaseolina and C. dematium. Crawford, Giza 21 cultivars (healthy and infected samples) and R. solani have unique band at molecular weght 19. While, Crawford, Giza 21 cultivars (healthy and infected samples) and M. phaseolina have unique band at molecular weight 33. Also, Giza 82, Giza 21 cultivars (healthy and infected samples) and C. dematium have unique band at molecular weight 34.*

Key word: *Root and Foliage Diseases, Soybean, Glycine max, and Cultivars reaction*

INTRODUCTION

Soybean plants are severly attacked by many pathogenic fungi, which infect different plant parts causing severe losses in soybean yield quality and quantity (Sinclair, 1982). Damping off, root rot, charcoal rot, (Hassanein, 1978) and anthracnose (Abd-El-Rahman, 2001) are considered the most important diseases of this crop.

Fusarium solani, Marophomina phaseolina and Rhizoctonia solani with nine pathogens were tested on soybean plants under greenhouse conditions and were found to be highly pathogenic (Arafa, 1994). A method for testing soybean plants for susceptability to anthracnose disease was described by Manandhar *et al.* (1988) who found that defoliation and necrosis in stem tips were occurred.

Characters of isolates of *Rhizoctonia solani* associated with soybean in Brazil were studied according to hyphal anastomosis reaction, mycelial growth rate, thiamine requirements, sclerotia production and RAPD molecular markers (Fenille *et al.*, 2002).

Random amplified polymorphic DNA (RAPD) method was used to differentiate *R. Solani* isolates (Yang *et al.*, 1996), and *Colletotrichum dematium* isolates (Abd-El-Rahman, 2001).

Soybean cultivar reactions were tested to infection with *M. phaseolina* (Gangopadhyay and Wyllie, 1973), *C. dematium* infection (Rahman and Fakir, 1985 and Abd-El-Rahman, 1995) and *R. solani* (Camara *et al.*, 1988) on soybean plants. All the tested cultivars of soybean varied in their protein patterns, as the susceptible and moderatly susceptible cultivars were compared with the resistant cultivars, also the proteins were highly induced in the susceptible and moderately susceptible cultivars compared with resistant ones after infection by *C. dematium* (Abd-El-Rahman, 2001).

The aim of this study was to look for and survey root-rot, charcoal rot and anthracnose of soybean in six governotates of Egypt, to identify the causal organisms by the classic and modern methods and to test soybean cultivars against these diseases. Furthermore, determination of the changes in proteins extracted from healthy and infected cultivars by *R. solani, M. phaseolina and C. Dematium* were also taken into consideretion.

MATERIALS AND METHODS

A-Survey of Root-Rot, Charcoal Rot and Anthracnose Diseases:

Soybean fields in Beheira, Kafr El-Sheikh, Sharkia, Giza, Beni-Seuf and Menia governorates were surveyed in 2001-2002 growing seasons for the presence of the diseases. Infection was recorded and samples of the diseased plants were collected.

B-Isolation and Identification of The Causal Organisms:

1-Classic methods:

The infected plant samples were washed carefully with tap water, dried between sterilized filter papers and cut into small pieces. The pieces were surface sterilized by immersing in sodium hypochloride solution (2%) for two minutes, then rinsed for several times in sterilized water and dried between two sterilized filter papers. Plant pieces were placed on the surface of water agar medium in Petri-dishes and incubated at 25-28°C for several days. They were investigated daily.

The isolated fungi were purified by using the singal spore technique according to Hildebrand (1948) and /or by the hyphal tip technique according to Brown (1924). The detected fungi were transferrd to slants of Potato Dextrose Agar (PDA) medium and were kept at 5C for further studies.

Identification of the isolated fungi was carried out according to Glements and Shear (1975), Kulshrestha *et al.*, (1976). and Sinclair (1982).

2- Modern methods.

a-Hyphal anastomois grouping of *Rhizoctonia solani*:

The methods based on the observation of the hyphal anastomosis and determination of the anastomosis was used as groups described by Ogoshi (1976). Anastomosis was tested opposing isolates on 2% water agar in Petri dishes. Mycelial transfers from the growing margins of young colony on PDA were plated 2.3 cm apart in 9 cm Petri dishes and incubated at 25 C until advancing hyphae made contact and slightly overlapped, this portion was observed under microscope. Hyphal Fusion Frequency (FF %)

1- FF= probability of fusion between a hyphal and anther hypha under a certain condition. The ability of hyphal fusion of one isolate is, therefore, expressed as that frequency.

2- FF(%)= The sum of contact points in the same fields.(Parmeter *et al.*, 1967)

b- Differentiation of different fungi isolates using (RAPD) technique:

1- DNA Extraction and Isolation:

Five *R. solani* isolates, five *M. phaseolina* and four *C. dematium* isolates were used to study the genetic variations. Random amplified polymorphic DNA technique (RAPD) was used in this study. Czapek's solution medium was distributed in 100 ml Erlenmeyer flasks, 50 ml each and autoclaved for 15 min, flasks were inoculated with 5 mm fungal discs from 7 days old cultures of *R. solani* and *M. phaseolina* and from 10 days old culture of *C. dematium*. Flasks inoculated were incubated at 28°C to *R. solani* and *M. phaseolina* and 25°C for *C. dematium* for 10 days, then the mycelia growth was filtered off on filter paper, washed three times with deionized distilled water and dried in a freezing dried for 30 min. Two grams of the dried mycelia were homogenized with a pestle in liquid nitrogen using apre-chilled mortar. Two ml of Tries-HCL buffer solution (pH 6.8 and 0.0625M, containing 10% glycerol and 1ml M Dithiotheritol (D.D.T) were added. Then the mixture was centrifuged for 20 min at 18.000 rpm. The supernatant was obtained to extract soulble proteins (Kuramae-Izioka, 1997).

DNA was isolated from 50 mg of organism using Qiagen kit for DNA extraction. The extracted DNA was dissolved in 100 ul of elution buffer. The concentration and purity of the obtained DNA was detemined by using "Gen qunta" system-pharmacia Bio-tech. The purity of the DNA for all samples was between 90-97% and the ratio between 1.7-1.8. Concentration was adjusted at 6 ng/ml for all samples using TE buffer pH 8.0 (Kuramae-Izioka, 1997).

2-Random Amplified Polymorphism DNA technique (RAPD):

Thirty ng from the extracted DNA were used for amplification reaction according to protocol of Williams *et al.* (1990).

C-Pathogenicity Tests:

Pathogenicity tests were carried out for all tested fungi in pots under greenhouse conditions using soybean Clark cultivar.

1-Soil infestation:

Discs (5 mm in diam) of 7 day old culture of the desired fungus (*Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii*) were cut and each disk was placed in 500 ml conical flasks cotaining autoclaved sorghum-sand medium, flasks were then incubated at 28C for 15 days according to Arafa (1994). Soil infestation was carried out by adding the inoculm of the fungus to the sterilized soil at the rate of 5% soil weight (w/w). The fungal inoculum was mixed thoroughly with the infested soil, which was watered for 7 days to enhance fungal growth. The control was treated with the same amount of autoclaved sorghum-sand medium without fungal inoclum.

Seeds of soybean Clark cultivar were surface sterilized by immersing them in sodium hypochloride solution 2% for 2 minutes and then washed several times with sterilized water. The dried seeds were sown at the rate of five seeds per pot(20 cm diam) and three pots were used for each treatment. Disease incidence was recorded as pre-and post-emergence damping-off after 15 and 30 days from sowing date and root rot and healthy survivaed plants were recorded after 60 days from planting. Reisolation was done from the parts infected by root rot.

2- Foliar inoculation:

The four isolates of *Colletotrichum dematium* were used to inoculate soybean seed extract agar medium (the most suitable medium for sporulation) and incubated at 25C for ten days. Spore suspension (5×10^6 spore/1ml) which gave suitable inoculum potential, was prepared by adding sterilized water to the culture and was then gently rubbed using smooth brush.

Forty five days old soybean plants were sprayed with spore suspension (5×10^6 spore/ 1ml) of 10 days old culture of *C. dematium*. The plants were covered with polyethytlene bags for 48 h to maintain high relative humidity. Inoculated plants were kept under daily obsevations for 7 days. Control treatments were handled in the same way but without fungus inoculation. Pots (20 cm diam) were sown with five soybean seeds (cv. Clark) and treatments were replicated three times. Disease severity was recorded 7 days after inoculation using a modified disease scale of Manandhar *et al.*, (1988). Disease severity (%) was calculated using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (n \times v)}{5N} \times 100$$

Where:

(n) = number of plants in each category.

(v) = numerical values of symptoms category.
(N) = total number of plants.
(5) = maximum of numerical values of symptoms categories.

D- Control studies:

Cultivar reactions:

(a) Soil infestation:

These trials were carried out in pots in greenhouse to study the reactions of different soybean cultivas, viz. Giza-111, Giza-83, Giza-82, Giza-21, Giza-22, Clark and Crawford, to select the resistant and susceptible cultivars.

Pots (20 cm diam) were sterilized by immersing them in 5% formaline solution for 15 minutes and then left for seven days before using and soil was sterilized with 5% formaline solution and then left for two weeks for formaline evaporation. Every pot was filled with 2kg of sterilized soil. The soil was infested with the inoculum of the desired fungus,i.e. *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* as mentioned before. Surface sterilized seeds of the eight tested soybean cultivars were sown in the infested soil at the rate of five seeds in pot. Three replicates were used for each treatment. Percentages of pre-and post-emergence damping-off, root rot and healthy survived plants were recorded.

(b) Foliar inoculation:

Reaction of different soybean cultivars, viz. Giza-111, Giza-83, Giza-82, Giza-21, Giza-22, Clark and Crawford, against *C. dematium* was evaluated.

Plants, 45 days old, were sprayed with spore suspension (5× 10^6 spore/ 1ml) of *C. dematium* until the run-off point. All plants were covered with polyethylene bags for 24 h to maintain high relative humidity, then kept in greenhouse. Five seeds were sown in each pot, and four replicates were used for each treatment, plants were examined and disease severity was recorded 7 days after inoculation as mentioned before.

Cultivar reactions were calculated according to Ganzhelo and Sichkar (1985) as following:

Degrees of Resistance	Symbol	Diseased Plants
Highly Resistance	H R	0 – 5 %
Resistance	R	6 – 15 %
Moderately Resistance	MR	16 – 30 %
Moderately Susceptible	MS	31 – 50 %
Susceptible	S	51 – 75 %
Highly Susceptible	HS	76 – 100 %

E- Protein patterns:

Changes in protein patterns extracted from soybean cultivars infected by *R. solani, M. phaseolina* and *C. dematium* by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) were measured.

Polyacrylamide gel electrophoresis (PAGE) was used to determine the quantitative changes that occur in resistant and susceptible cultivars such as; Crawford and Giza-21 inoculated plants with *R. solani* and uninoculated plants, Crawford and Giza-21 inoculated plants with *M. phaseolina* and uninoculated. also Giza-83 and Giza-21 inoculated plants with *C. dematium* and uninoculated plants.

(a) Protein extraction:

Leaves of similar age were taken from inoculated and uninoculated plants. Two grams from each sample were ground in 0.05 M sodium acetate buffer + sea sand with a mortar in liquid nitrogen at 4C. After that, 50 mg of the extract plus 0.7 ml of ES (4% SDS, 5% sucrose and 50 % mercaptoethanol) were shacked for 10 min at room tempreature with gentle stirring. The extract was centirfuged at 18.000 rpm for 30 min and the clear supernatant was heated at 100C for 2-5 min and then cooled to room temperature. Proteins were precipitated by adding cold (-20C) acetone (8x volume of the supernatant). Protein content of the supernatant was determined using the method described by Ekramoddoullah and Davidson (1995). In the same time, bovine serum albumin (BSA) was used as a standard.

(b) Staining of protein bands:

Sliver staining method for protein described by Hochstrasser *et al.*,(1988) was used. Where the membrane was stained with 0.1% coomassie blue R-250 in 50% methanol, was then rinsed with water for 10 min. the stained membrane was then scanned by a laser scaner. Protein separated by SDS-PAGE was electrophoretically transferred to immobilon - Pmembrane, as described by Matsudaira (1987).

The protein patterns of isolates of *R. solani* no. 2 and *M. phaseolina* no. 2 were compared with the protein patterns of two different soybean cultivars (Crawford, resistant and Giza-21, susceptible) and the protein patterns of isolate *C. dematium* no.1 was compared with the protein patterns of two different soybean cultivars (Giza-83, resistant and Giza-21, suceptible). which were prepared as mentioned before to study the relationship between them.

RESULTS

A-Isolation and identification of the causal organisms:

1-Classic methods:

Data in Table (1) show that five different fungi (15 isolates) were associated with soybean roots and one fungus was associated with soybean petioles and leaves (4 isolates). The fungi isolated from roots were identified using the classic methods as *Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Macrophomina phasolina* and *Sclerotium rolfsii* and the fungus isolated from foliar parts was *Colletotrichum dematium*. *Fusarium solani* and *F. oxysporum* isolates (both 2 isolates) were presented in infected samples collected from Beheira and Sharkia governorates. *R.*

solani isolates (5) were isolated from all governorates except Kafr El-Sheikh, while *M. phaseolina* isolates (5) were isolated from all governorates except Menia. *Colletotrichum dematium* isolates (4) were isolated from infected samples collected from all governorates except Beni-Sweif and Menia, while *Sclerotium rolfsii* (one isolate) was isolated from Giza governorate.

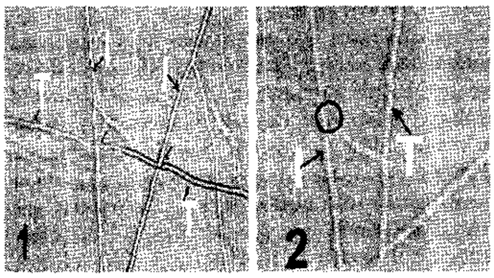

Figure (1): Identification of *Rhizoctonia solani* isolates into anastomosis groups; (1) no anastomosis between a tester (T) and an isolate (I), (2) hyphal anastomosis between an isolate and AG-4 tester composed similar to H character.

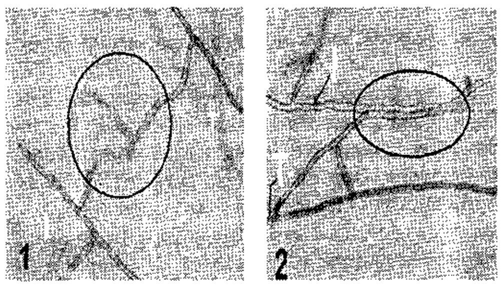

Figure (2): Identification of *Rhizoctonia solani* isolates into anastomosis groups; (1) imperfect fusion between hyphae of a tester (T) and an isolate (I), note plasmolysis of cells, and (2) hyphal anastomosis side by side between hyphae of a tester (T) and an isolate (I).

2- Modern identification methods:

a- Anastomosis tests and identification of the tested isolates into AGS:

Tested isolates of *R. solani* proved to be *Rhizoctonia solani*, where all of them had multinucleated hyphae.

Anastomosis tests were done in 2 stages according to the availability of AGs tester. In the first stage, anastomosis tests were conducted between the tested isolates and each others without testers. In the second stage, The tested isolates were assigned with 6 testers (AG – 4 Hg I, AG – 4 Hg II, AG2-1, AG 2-2, AG-3 and AG-F). Ferquency of fusion (FF) was used as a parameter only in the first stage, where the occurrence of anastomosis was often frequently observed.

The five tested isolates were expected to fall in different four groups according to data presented in Tables (2 & 3). Anastomosis was freqauently observed between pair discs of the same isolate with FF values of 90, 85, 93, 97 and 88%, for isolates 1-5, respectively.

Anastomosis was occurred also between pairs of isolates 1 & 4 with FF value of 68% respectively. Data in Table(3b) and Figs (1 & 2) show that *R. solani* isolate 1 and 4 were AG 2-2. *R. Solani* isolate 2 was AG-4 Hg II, *R. Solani* isolate 3 was AG-4 Hg I, while, *R. solani* 5 isolate was AG 2-1.

b- Differentiation of different fungi isolates using (RAPD) technique:

One primer was used in the study. This primer was successed in amplification with isolates of the fungi *R. solani, M. phaseolina* and *C. dematium*.

Data in Figs (3and4) show that this primer differentiate the five isolates of *R. solani* into three subclusters, the first one consists of two isolates,i.e. *R. solani* 1 and *R. solani* 3 with similarity level 99.21%. The second group consists of three isolates, i.e. *R. solani* 1, *R. solani* 3 and *R. solani* 4 with similarity level 98.99% and the third group consists of four isolates,i.e. *R. solani* 1, *R. solani* 3, *R. solani* 4 and *R. solani* 2 with similarity level 97.95%, while the overall similarity level was 94.25%.

Also, data in Figs (5 & 6) show that the same primer was located the five isolates of *M. phaseolina* 1, *M. phaseolina* 2 and *M. phaseolina* 4, *M. phaseolina* 5 with similarity level 93.97 and 93.77%, respectively. The second group consists of three isolates *M. phaseolina* 1, *M. phaseolina* 2 and *M. phaseolina* 3 with similarity level 89.27%, while the overall similarity level was. 82.22%.

Also, the same primer in Figs (7 & 8) distingate the four isolates of *C. dematium* into two subclusters, the first group consists of two isolates.i.e. *C. dematium* 1 and *C. dematium* 2 with similarity level 98.03 and the second group consists of three isolates *C. dematium* 1, *C. dematium* 2 and *C. dematium* 3 with similarity level 97.05, while the over all similarity level was 93.70.

Generally, the primer used was not appropriate to detect the genetic variations among the genotypes of the isolates of each fungus from the three fungi.

B- Pathogenicity Test:

1- Soil infestation:

Data in Table (4) show that all the tested fungi were pathogenic to soybean Clark cultivar and caused pre- and post-emergence damping-off. At the end of the pathogenicity test, plants showing root rot symptoms and healthy survival plants showed significant differences between the most tested isolates.

Generally, the most pathogenic isolates were *R. solani* (1&2), *M. phaseolina* (1&2) and *F. solani(1)*.

2- Foliar inoculation:

Data in Table (5) indicate that all isolates of *C. dematium* were pathogenic to soybean plants with

Table (1): Fungi isolated from infected soybean plants collected from six governorates of Egypt

Governorates	Isolated fungi	
	Number of Fungi	Isolates Number
Beheira	5	1, 4, 7, 12 & 18
Kafr El- Sheikh	2	10 & 19
Sharkia	5	2, 3, 8, 13, & 16
Giza	4	5, 11, 15, & 17
Beni-Sweif	2	9 & 14
El-Menia	1	6
1 = *Fusarium solani* 1	2 = *Fusarium solani* 2	3 = *Fusarium oxysporum 1*
4 = *Fusarium oxysporum 2*	5 = *Rhizoctonia solani 1*	6 = *Rhizoctonia solani 2*
7 = *Rhizoctonia solani 3*	8 = *Rhizoctonia solani 4*	9 = *Rhizoctonia solani 5*
10 = *Macrophomina phaseolina 1*	11 = *Macrophomina phaseolina 2*	12 = *Macrophomina Phaseolina 3*
13 = *Macrophomina phaseolina 4*	14 = *Macrophomina phaseolina 5*	15 = *Sclerotium rolfsii*
16 = *Colletotrichum dematium 1*	17 = *Colletotrichum dematium 2*	18 = *Colletotrichum dematium 3*
19 = *Colletotrichum dematium 4*		

Table (2): The expected anastomosis grouping (EAGs) within the selected tested isolates of Rhzoctonia solani as investigated during the preliminary anastomosis test without testers

***Rhizoctoni* isoates**	**R. h 1**	**R. h 2**	**R. h 3**	**R. h 4**	**R. h 5**	**EAGS**
R. h1	+	-	-	+	-	I
R. h 2	-	+	-	-	-	II
R. h 3	-	-	+	-	-	III
R. h 4	+	-	-	+	-	I
R. h 5	-	-	-	-	+	V

Table (3a): Frequency of fusion (FF) among selected tested isolates of Rhizoctonia solani as investigated during the preliminary anastomosis test without testers

Rhizoctonia isolates	**R. h1**	**R. h2**	**R. h3**	**R. h4**	**R. h5**	**EAGS**
R. h1	90%	0.0%	0.0%	68%	0.0%	I
R. h2	0.0%	85%	0.0%	0.0%	0.0%	II
R. h3	0.0%	0.0%	93%	0.0%	0.0%	III
R. h4	68%	0.0%	0.0%	97%	0.0%	I
R. h5	0.0%	0.0%	0.0%	0.0%	88%	V

Table (3b): Frequency of fusion (FF) among selected tested isolates of Rhizoctonia solani as investigated during the preliminary anastomosis test without testers

Tester / ***Isolates***	**AG-4HgI**	**AG-4Hg II**	**AG2-1**	**AG2-2**	**AG-3**	**AG-F**
R. h1	-	-	-	+	-	-
R. h2	-	+	-	-	-	-
R. h3	+	-	-	-	-	-
R. h4	-	-	-	+	-	-
R. h5	-	-	+	-	-	-

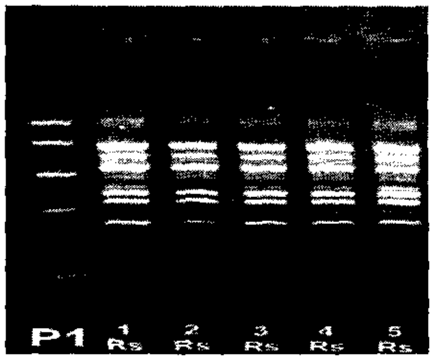

Figure (3): Dendrogram showing the polymorphism of DNA of *R. solani* isolates by PCR-based RAPD primer no. 1 using polyacrylamide gel as a support medium: P1 = Primer no.1, 1Rs = *R. solani* isolate no.1, 2Rs = *R. solani* isolate no.2, 3Rs = *R. solani* isolate no.3, 4Rs = *R. solani* isolate no.4, 5Rs = *R. solani* isolate no.5

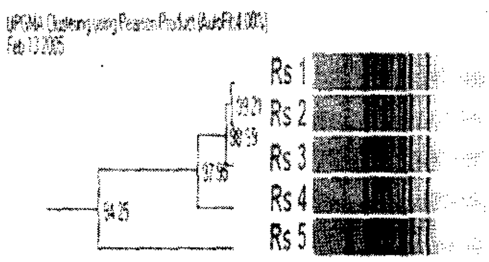

Figure (4): Cluster analysis showing the similarity polymorphism of DNA of *R. solani* isolates by PCR-based RAPD primer no.1 using polyacrylamide gel as a support medium.

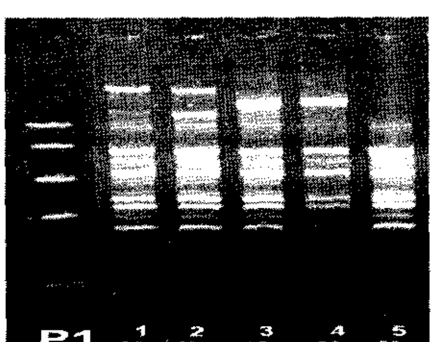

Figure (5): Dendrogram showing the polymorphism of DNA of *M. phaseolina* isolates by PCR-based RAPD primer no. 1 using polyacrylamide gel as a support medium:
P1 = Primer no.1, 1Mp = *M. phaseolina* isolate no.1, 2Mp = *M. phaseolina* isolate no.2, 3Mp = *M. phaseolina* isolate no.3, 4Mp = *M. phaseolina* isolate no.4, 5Mp = *M. phaseolina* isolate no.5

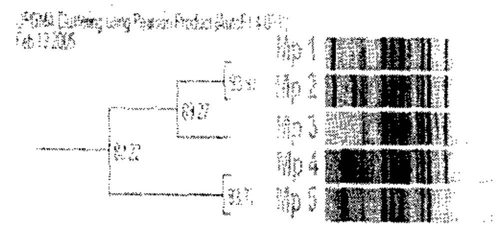

Figure (6): Cluster analysis showing the similarity polymorphism of DNA of *M. phaseolina* isolates by PCR-based RAPD primer no.1 using polyacrylamide gel as a support medium

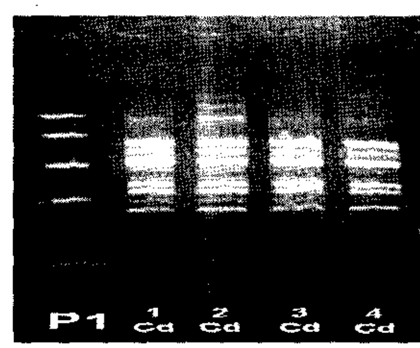

Figure (7): Dendrogram showing the polymorphism of DNA of *C. dematium* isolates by PCR-based RAPD primer. no. 1using polyacrylamide gel as a support medium:
P1 = Primer no.1, 1Cd = *C. dematium* isolate no.1, 2Cd = *C. dematium* isolate no.2, 3Cd = *C. dematium* isolate no.3, 4Cd = *C. dematium* isolate no.4, 5Cd = *C. dematium* isolate no.5

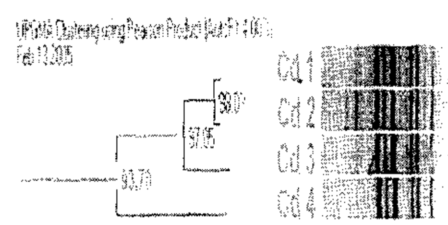

Figure (8): Cluster analysis showing the similarity polymorphism of DNA of *C. dematium* isolates by PCR-based RAPD primer no.1using polyacrylamide gel as a support medium.

different degrees. *C. dematium* 1 isolate was the most virulent followed by *C. dematium* 2. *C. dematium* 3 and *C. dematium* 4.

According to the obtained data isolates of *F. solani* (1), *M. phaseolina* (2) and *C. dematium* (1) were chosen to complete the further studies.

C- Control studies:

1- Cultivars reaction

(a) Soil infestation:

Data in Table (6) indicate that there is a significant difference between the tested soybean cultivars. Cvs Crawford and Giza-111 showed the lowest percentage of pre-emergence damping – off and root rot and the highest percentage of healthy survived plants followed by cvs Giza–22 and Clark, while cultivars Giza-21 and Giza –35 showed the highest percentage of pre-emergence damping – off and root rot the lowest percentage of healthy survived plants followed by Giza-83 and Giza–82cultivars.

Data in Table (6) show that no significant difference between the tested soybean cultivars on the percentage of post-emergence damping–off were detected.

(b) Foliar inoculation

Data in Table (7) and Fig.(9) exhibited significant differences between the tested soybean cultivars. Cultivars Crawford, Giza–21, Giza–22 showed the highest percentage of disease severity, followed by cvs. Clark and Giza–35, while Giza–83 Giza–82, and Giza–111cvs recorded the lowest percentage of disease severity.

D- Changes in protein patterns extracted from soybean cultivars infected by *R. solani, M. phaseolina* and *C. dematium*:

1-Soybean cultivars infected by *Rhizoctania solani*:

Data presented in Table (8) and Figs. (10 & 11) show protein banding patterns of soybean roots (cvs. Crawford and Giza-21) healthy and infected with *R. solani*. The uninfestated (healthy) and infected root samples were fractianated into 8 and 13 bands, respectively, for cv. Crawford and into 14 and 12 bands, respectively, for cv. Giza-21. While *R. solani* was fractionated into 24 bands.

Data in Figs.(10&11) show that number of induced proteins in soybean cultivars infected by *R.solani* were 8 for cv. Crawford and 6 for cv. Giza – 21, respectively. While, disappeared proteins in soybean cultivars due to infection by *R. solani* were 3 for cv. Crawford and 8 for cv. Giza – 21, respectively.

Crawford and Giza-21 cultivars (healthy and infected samples) and *R. solani* have unique band at molecular weight 19.

2- Soybean cultivars infected by *Macrophomina phasealina:*

Table (8) and Figures (10&11) show protein banding patterns of soybean roots (cvs. Crawford and Giza-21) healthy and infected with *M. phaseolina*. The healthy and infected root samples were fractionated into 9 and 9 for cv. Crawford and into 14 and13 bands, respectively,for cv. Giza- 21. While *M. phasealina* was separated into 18 bands.

Data also show that number of induced proteins in soybean cultivars infected by *M. phasealina* were 6 for cv. Crawford and 10 for cv. Giza – 21, respectively. While, disappeared proteins in soybean cultivars due to infection by *M. phasealina* were 6 for Crawford and 11for cv. Giza – 21, respectively. cv.Crawford and Giza-21 cultivars (healthy and infected samples) and *M. phasealina* have unique band at molecular weight 33

3- Soybean cultivars infected by *Colletotrichum dematium.*

Data in Table (9) and Figures (10 & 11) show protein panding pattrens of soybean leaves healthy and infected with *C. dematium* (cv. Giza-82 and Giza-21).

The healthy and infected leaves samples were fractianated into 18 and 18, respectively for cv. Giza-82 and into 16 and 13, respectively, for cv. Giza-21. While *C. dematium* was fractianated into 23 bands.

Data also show that number of induced proteins in soybean cultivars infected by *C. dematiuam* recorded 21 for cv. Giza - 82 and 10 for cv. Giza–21, respectively. While, disappeared proteins in soybean cultivars due to infection by *C. dematiuam* were 12 for cv. Giza-82 and 13 for cv. Giza – 21, respectively. Giza-82 and Giza-21 cultivars (healthy and infected samples) and *C. dematium* have unique band at molecular weigth 34.

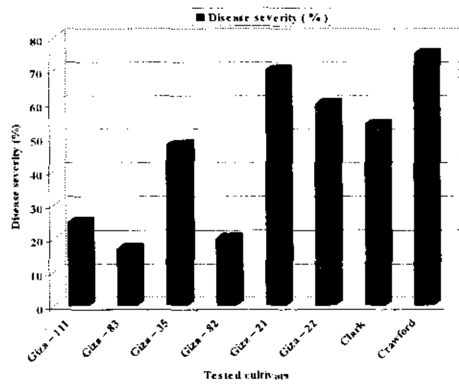

Figure (9): Evaluation of eight soybean cultivars to infection with *C.dematium* in pots.

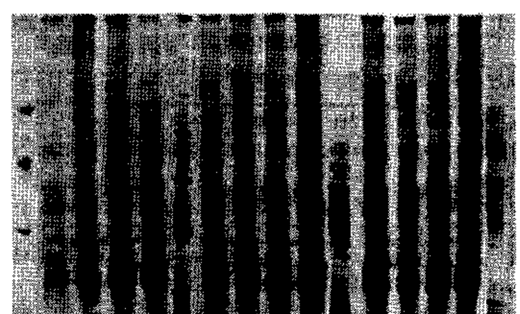

Figure (10): Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein extracted from healthy and infected roots and leaves of different soybean cultivars.

Table (4): Ability of the isolated fungi on inducing pre–and post-emergence damping–off and root rot and their effect on healthy survived plants cv. Clark under greenhouse conditions.

Isolates	Damping – off		Root rot (%)	Healthy survival (%)
	Pre-emergence (%)	.Post-emergence (%)		
Fusarium solani 1	40.00	6.7	13.33	40.00
Fusarium solani 2	40.00	6.7	6.7	46.7
Fusarium oxysporum 1	13.33	13.33	20.00	53.32
Fusarium oxysporum 2	6.7	6.7	20.00	66.7
Rhizoctonia solani 1	53.33	13.33	13.33	20.00
Rhizoctonia solani 2	46.7	13.33	13.33	26.7
Rhizoctonia solani 3	33.33	13.33	6.7	46.7
Rhizoctonia solani 4	26.7	6.7	13.33	53.33
Rhizoctonia solani 5	20.00	6.7	13.33	60.00
Macrophomina phaseolina 1	40.00	13.33	20.00	26.7
Macrophomina phaseolina 2	33.33	13.33	20.00	33.33
Macrophomina phaseolina 3	20.00	6.7	13.33	60.7
Macrophomina phaseolina 4	13.33	6.7	6.7	73.33
Macrophomina phaseolina 5	13.33	6.7	6.7	73.33
Sclerotium rolfsii	33.33	6.7	20.00	40.00
Control	0.00	0.00	6.7	100.00
L.S. D at 0.05	21.51	n.s.	3.61	25.22

Table (5) : Pathogenicity of *C.dematium* isolates to soybean plants cv. Clark, in pots under greenhouse conditions.

Isolates	**Disease severity (%)**
*Colletotrichum dematium*1	54.33
Colletotrichum dematium2	51.75
Colletotrichum dematium3	39.74
Colletotrichum dematium4	38.66
Control	0.00
L.S.D at 0.05	4.90

Table (6): Evaluation of pre-,post-emergence damping–off, percentage of root rot and healthy survival plants of eight cultivars infested with three pathogens, in pot experiment,under greenhouse conditions.

Soybean cultivars	*Damping off*						Root rot (%)			*Healthy survival plants (%)*		
	Pre-emergence (%)			Post-emergence (%)								
	F.s	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*
Giza -111	33.33	40.00	26.33	6.66	13.33	0.00	6.66	6.66	20.00	53.33	40.00	53.33
Giza - 83	60.00	66.33	53.33	6.66	13.33	6.66	6.66	6.66	13.33	26.66	13.66	26.66
Giza - 35	66.66	73.33	46.66	13.33	13.33	6.66	6.66	6.66	13.33	13.66	6.66	33.33
Giza -82	53.33	60.00	40.00	6.66	13.33	13.33	13.33	6.66	20.00	26.66	20.00	26.66
Giza -21	86.66	86.66	73.33	6.66	6.66	6.66	0.00	0.00	13.33	6.66	6.66	6.66
Giza -22	40.00	46.66	26.66	6.66	6.66	6.66	6.66	6.66	13.33	46.66	33.33	53.33
Clark	40.00	46.66	33.33	6.66	13.33	0.00	13.33	6.66	20.00	40.00	33.33	33.33
Crawford	26.66	40.00	20.00	6.66	6.66	0.00	6.66	0.00	6.66	60.00	60.00	73.33
L.S.D at 0.05	27.71	24.91	24.30	n.s.	n.s.	n.s.	n.s.	n.s.	3.10	24.10	30.61	21.40

F.s = Fusarium solani.
R.s = Rhizoctonia solani.
M.p = Macrophomina phaseolina

Table (7): Evaluation of eight soybean cultivars to infection with *C.dematium*, in pots under greenhouse conditison.

Cultivars	Disease severity (%)	Symbol	Diseased Plants
Giza – 111	25.00	MR	16-30%
Giza – 83	17.00	MR	16-30%
Giza – 35	48.00	MS	31-50%
Giza – 82	20.00	MR	16-30%
Giza – 21	70.00	S	51-75%
Giza – 22	60.00	S	51-75%
Clark	54.00	S	51-75%
Crawford	75.00	S	51-75%
L.S.D.at 0.05	5.53		

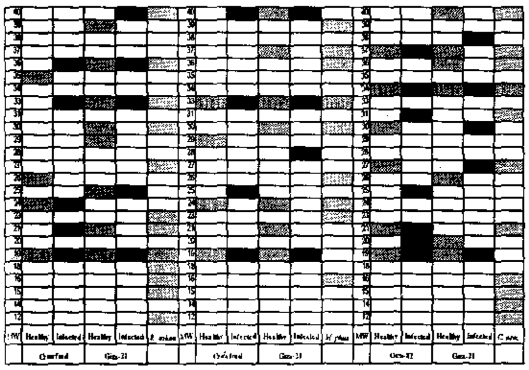

Figure (11): Protein banding patterns obtained by SDS-PAGE from roots and leaves of three soybean cultivars, healthy and infected plants with *R. solani, M. phaseolina* and *C. dematium* at three periods

DISCUSSION

Soybean (*Glycine max* (L.) Merr) is one of the most important field crops all over the world. It's importance comes from the amounts of oil and protein in its seeds, and their high value for human and animal consumption.

Several serious diseases can attack soybean plant during its various growth stages from seedling till maturity causing serious economic losess in yield quality and quantity (Sinclair, 1982).

Damping–off, root rot, charcoal rot and anthracnose are the most important diseases of this crop. The present work showed that damping – off, root rot and charcoal rot diseases are incited by a group of soil borne fungi including *Rhizoctonia solani, Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina and Sclerotium rolfsii.* These results are in agreement with these obtained by Acimovic (1966); Saharam and Cupta (1972); Orellana *et al.* (1976); Hassanein (1978) Sinclair (1982); Abou – Zeid *et al.*, (1987); Dang and Gilly (1992); Vyas (1994); Lichangsong *et al.* (1996) and Naito and Itoh (1999). Also, the anthracose disease is incited by *Colletotrichum dematium.* This is in agreement with Schneider *et al.* (1974); Kulshretha *et al.* (1976); Sinclair (1982); El -Wakil (1985) and Abd - El - Rhman (1995).

The pathogens were isolated in 2001 and 2002 growing seasons from infected soybean plants collected from different governorates,viz. Beheira and Kafr El–Sheikh governorates (represent the north governorates) and Giza, Beni–Sweif and Menia governorates (represent the souuthern governorates). Five isolates of *R. solani*, two isolates of *F. solani*, two isolates of *F. oxysporum*, one isolate of *S. rolfsii*, five isolates of *M. phaseolina* and four isolates of *C. dematium* were isolated from infected soybean plants. These finding suggest that root rot diseases are endemic in Egyptain governorates. On the other hand, the variations in the incidence of both diseases (rot-root and anthracnose) is not surprising because the environmental conditions vary among the governorates and may contribute greatly in development of both diseases. Supporting evidence come from Hassanein (1978) and Morsy *et al.* (1986) for root rot incidence and El-Wakil (1985) and Abd-El Rahman (1995) for anthracnose. Kraft *et al.* (1986) reported that root rot and wilt of food legumes increase by soil compaction, poor drainge, excess or deficit water and extremes of temperatures. Anthracnose diseases was not found in both Beni-Sweif and Menia governorates. Sinclair (1982) demonstrated that soybean anthracnose conidia are short lived and sensitive to drying. Five hours of air drying can reduce germination by 98%.

In Egypt, certain studies were carried out on the identification of pathogens causing pre-and post–emergence damping–off, root rot, charcoal rot and anthracnose of soybean using classical methods. In the present work the identification of fungi was carried out using the classical and modern methods. The modern identification involved anastomsis grouping test between *Rhizoctonia solani* isolates and the finger print technique between the pathogens isolates.

Results of hyphal anastomsis grouping test reported that all *Rhizoctonia solani* isolates were multinucleated hyphae. Also, results reported that isolates of *R. solani* are classified into two anastomosis groups (AGs). *R. solani* 1 and 4 isolates were AG 2-2, *R. Solani* isolate 2 was AG-4 Hg II, *R. solani* isolate 3 was AG-4 Hg I. While, *R. solani* isolate 5 was AG 2-1. This is in agreement with the results obtained by Wasfy *et al.*(1984); Ogoshi (1985); Liu and Sinclair (1991); Moustafa (1991); Muyolo *et al.*(1993); El-Akkad (1997); Priyatmojo *et al.* (2001); Carling *et al.* (2002) and Harikrishnan and Yang (2004).

Random Amplified Polymorphic DNA (RAPD) analysis was used to evaluate the genetic diversity of *R. solani, M. phaseolina* and *C. dematium* isolates, which were obtained from different governorates. Results of RAPD analysis using one primer revealed that *R. solani* isolates No 1 and 2 in one subcluster (SI 99.21%, respectively). While islates Nos 1, 3 and 4 in one subcluster (SI 98.99%, respectively). Isolates Nos

Table (8): protein banding patterns obtained by SDS-PAGE from healthy and infected roots of two soybean cultivars were with *R solani* and *M. phaseolina.*

	Crawford		Giza-21				Crawford		Giza-21		
Mw	Healthy	Infected	Healthy	Infected	*R.solani*	Mw	Healthy	Infected	Healthy	Infected	*M.phaselina*
93	0.00	0.00	0.00	0.00	0.00	93	0.00	0.00	0.00	0.00	2.97
92	0.00	0.00	0.00	0.00	0.00	92	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	.080	90	0.00	0.00	0.00	0.00	0.00
88	0.00	0.00	0.00	0.00	0.00	88	0.00	0.00	0.00	3.07	0.00
87	4.08	0.00	0.00	0.00	0.00	87	0.00	3.44	3.83	0.00	0.00
86	0.00	3.64	3.64	4.35	1.66	86	4.54	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00	0.00	85	0.00	0.00	0.00	0.00	1.27
84	0.00	0.00	0.00	0.00	0.00	84	0.00	0.00	0.00	0.00	0.00
83	0.00	0.00	0.00	0.00	0.00	83	0.00	0.00	0.00	1.93	0.00
82	0.00	0.00	0.00	2.51	0.00	82	0.00	0.00	2.14	0.00	0.00
81	0.00	0.00	0.00	0.00	0.00	81	0.00	0.00	0.00	0.00	0.00
80	0.00	0.00	0.00	2.67	1.50	80	0.00	3.95	2.11	0.00	1.60
79	5.06	5.42	7.56	0.00	0.00	79	4.01	0.00	0.00	2.16	0.00
78	0.00	0.00	0.00	0.00	0.00	78	0.00	0.00	0.00	0.00	0.00
77	0.00	0.00	0.00	0.00	1.00	77	0.00	0.00	0.00	0.00	0.00
75	0.00	0.00	0.00	0.00	0.00	75	0.00	0.00	0.00	0.00	1.35
74	0.00	2.09	0.00	0.00	0.00	74	0.00	0.00	0.00	2.86	0.00
73	0.00	0.00	0.00	0.00	1.92	73	0.00	0.00	0.00	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	72	0.00	0.00	0.00	1.57	0.00
71	0.00	0.00	0.00	0.00	0.00	71	0.00	0.00	0.00	0.00	0.00
70	0.00	0.00	0.00	0.00	0.00	70	0.00	0.00	0.00	4.01	0.00
69	9.93	5.92	0.00	0.00	1.79	69	0.00	13.15	10.27	0.00	0.00
68	0.00	0.00	6.96	9.12	0.00	68	10.28	0.00	0.00	0.00	0.00
67	0.00	0.00	0.00	0.00	0.00	67	0.00	0.00	0.00	0.00	2.53
65	0.00	0.00	0.00	0.00	0.00	65	0.00	0.00	0.00	0.00	0.00
64	3.00	1.79	0.00	2.79	0.00	64	0.00	0.00	4.26	0.00	0.00
63	0.00	0.00	2.77	0.00	3.62	63	0.00	0.00	0.00	3.28	0.00
61	0.00	0.00	0.00	0.00	0.00	61	0.00	0.00	8.55	0.00	3.89
59	0.00	0.00	0.00	0.00	0.00	59	0.00	0.00	0.00	0.00	0.00
58	0.00	0.00	14.88	0.00	0.00	58	0.00	0.00	0.00	6.49	0.00
57	0.00	0.00	0.00	0.00	0.00	57	0.00	7.44	0.00	0.00	0.00
56	0.00	9.12	0.00	14.89	1.77	56	0.00	0.00	0.00	0.00	0.00
55	0.00	0.00	0.00	0.00	0.00	55	0.00	0.00	0.00	0.00	0.00
54	0.00	0.00	0.00	0.00	0.00	54	17.90	4.22	3.65	0.00	0.00
53	0.00	5.12	0.00	0.00	0.00	53	0.00	0.00	0.00	2.08	0.00
51	0.00	0.00	0.00	0.00	0.00	51	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00	4.51	50	0.00	0.00	0.00	0.00	6.82
48	0.00	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	0.00
46	0.00	0.00	0.00	0.00	8.27	46	0.00	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00	45	0.00	0.00	0.00	0.00	6.28
44	0.00	0.00	0.00	0.00	1.47	44	0.00	0.00	0.00	0.00	0.00
43	0.00	20.22	0.00	11.93	0.00	43	0.00	0.00	0.00	0.00	0.00
42	0.00	0.00	12.71	0.00	0.00	42	21.76	0.00	0.00	0.00	2.40
41	0.00	0.00	0.00	0.00	0.00	41	0.00	0.00	0.00	24.12	0.00
40	0.00	0.00	0.00	8.11	3.25	40	0.00	22.99	21.75	0.00	0.00
39	0.00	0.00	8.02	0.00	0.00	39	0.00	0.00	0.00	0.00	4.62
38	0.00	0.00	0.00	0.00	0.00	38	0.00	0.00	0.00	0.00	0.00
37	0.00	0.00	0.00	0.00	0.00	37	0.00	0.00	2.59	0.00	1.72
36	0.00	2.76	3.13	2.91	5.50	36	0.00	0.00	0.00	0.00	2.59
35	55.34	0.00	0.00	0.00	0.00	35	0.00	0.00	0.00	0.00	0.00
34	0.00	0.00	0.00	0.00	0.00	34	0.00	0.00	0.00	0.00	0.00
33	0.00	6.03	6.24	6.24	2.26	33	6.43	6.39	6.30	6.37	7.14
31	0.00	0.00	0.00	0.00	0.00	31	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	6.30	0.00	8.75	30	0.00	0.00	6.94	0.00	9.03
29	0.00	0.00	2.02	0.00	0.00	29	13.30	0.00	0.00	0.00	0.00
28	0.00	0.00	0.00	0.00	0.00	28	0.00	0.00	0.00	36.22	0.00
27	0.00	0.00	0.00	0.00	3.64	27	0.00	0.00	0.00	0.00	0.00
26	4.08	0.00	0.00	0.00	0.00	26	0.00	0.00	0.00	0.00	4.81
25	0.00	0.00	20.16	31.41	0.00	25	0.00	33.96	0.00	0.00	0.00
24	13.04	29.97	0.00	0.00	0.00	24	16.77	0.00	25.40	0.00	2.96
23	0.00	0.00	0.00	0.00	9.00	23	0.00	0.00	0.00	0.00	3.10
21	0.00	3.48	2.74	0.00	1.58	21	0.00	0.00	3.29	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	20	0.00	0.00	0.00	0.00	0.00
19	4.87	3.73	2.88	3.08	1.51	19	5.02	4.46	0.49	4.08	0.00
18	0.00	0.00	0.00	0.00	7.54	18	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	9.25	16	0.00	0.00	0.00	0.00	34.89
15	0.00	0.00	0.00	0.00	5.83	15	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	14	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	2.77	12	0.00	0.00	0.00	0.00	0.00

Table (9): protein banding patterns obtained by SDS-PAGE from healty and infected leaves of two soybean cultivars with *C.dematium*.

MW	Giza-82		Giza-21		
	Healthy	Infected	Healthy	Infected	*C.dematium*
93	0.00	0.00	0.00	0.00	0.00
92	0.31	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	1.15	0.00
88	2.06	2.79	3.78	0.00	0.00
87	0.00	0.00	0.00	0.00	0.00
86	0.00	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00	0.00
84	0.93	0.00	0.00	0.00	0.00
83	0.00	3.38	1.91	0.00	0.00
82	0.75	0.00	0.00	0.00	1.19
81	0.00	0.00	1.81	0.00	0.00
80	0.00	0.00	0.00	0.00	0.00
79	1.78	0.00	0.00	0.00	0.00
78	0.00	0.00	0.00	11.27	2.09
77	0.00	2.41	1.78	0.00	0.00
75	1.89	0.00	0.00	0.00	0.97
74	0.00	0.00	1.82	0.00	0.00
73	1.07	0.00	0.00	0.00	1.71
72	0.00	2.86	0.00	0.00	0.00
71	3.22	0.00	0.00	0.00	0.00
70	0.00	3.58	5.57	0.00	0.00
69	0.00	0.00	0.00	1.01	0.00
68	0.00	0.00	0.00	0.00	0.00
67	0.00	0.00	0.00	0.00	0.00
65	0.00	1.92	2.67	2.64	5.09
64	1.85	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	0.00	2.09
61	0.00	0.00	0.00	0.00	0.00
59	0.00	4.27	8.76	0.00	0.00
58	6.39	0.00	0.00	10.03	0.00
57	0.00	3.80	0.00	0.00	3.98
56	0.00	0.00	0.00	0.00	0.00
55	0.00	2.33	0.00	0.00	0.00
54	5.06	0.00	4.76	0.00	2.52
53	0.00	0.00	0.00	0.00	0.00
51	0.00	1.38	0.00	0.00	2.84
50	0.00	0.00	0.00	0.00	5.13
48	0.00	0.00	0.00	5.64	0.00
46	0.00	0.00	0.00	0.00	2.41
45	0.00	0.00	0.00	6.62	0.00
44	0.00	0.00	0.00	0.00	0.00
43	0.00	0.00	0.00	0.00	0.00
42	0.00	0.00	0.00	0.00	4.68
41	20.32	20.38	0.00	11.42	0.00
40	0.00	0.00	20.97	0.00	4.74
39	0.00	0.00	0.00	0.00	0.00
38	0.00	0.00	0.00	3.68	0.00
37	2.79	2.34	3.09	0.00	1.84
36	0.00	0.00	1.94	0.00	2.13
35	0.00	0.00	0.00	0.00	0.00
34	7.46	7.58	8.77	7.82	4.00
33	0.00	0.00	0.00	0.00	0.00
31	0.00	6.31	0.00	0.00	8.25
30	7.35	0.00	0.00	1015	0.00
29	0.00	0.00	0.00	0.00	0.00
28	0.00	0.00	0.00	0.00	0.00
27	28.37	0.00	0.00	26.94	5.51
26	0.00	0.00	28.11	0.00	0.00
25	0.00	26.89	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00
21	2.77	1.70	0.00	0.00	2.35
20	0.00	3.94	2.16	0.00	0.00
19	5.64	2.15	2.20	1.64	0.00
18	0.00	0.00	0.00	0.00	0.00

1,3,4 and 2 in one Subcluster (Sl 97.95%, respectively).

The same primer revealed that *M. phaseolina* isolates Nos 1,2 and 3,4 in one subcluster (Sl 93.97% and 93.77%, respectively), while isolates No 1, 2 and 3 in one subcluster (Sl 89.27%, respectively).

Also, the same primer revealed that *C. dematium* isolates Nos 1 and 2. in one subcluster (Sl 98.03%, respectively), while isolates Nos, 3 and 4 in one subcluster (Sl 97.05%, respectively). These results in are line with those reported by Freeman *et al.*, (1993), Achenbach *et al.*(1996); Yang *et al.* (1996); Morsy (2000); Abd-El-Rhman (2001); Fenille *et al.* (2002) Martinez–Culebras *et al.*(2002); Jana *et al.*(2003).

Generally, the primer used was not appropriate to detect the genetic variations among the genotypes of the isolates of each fungus from the three fungi

Results of the pathogenicity tests using soil infestation by soilborne fungi showed that all isolates were pathogenic but varied in virulence for both pre- and post-emergence damping–off and in their behaviour of infection. The most pathogenic isolates were *R.solani* (land2) and *M. phaseolina* (1 & 2). This is in agreement with the results obtained by Gangopdhyay and Wyllie (1973); Kunwar *et al.*(1986); Barkar (1992); Arafa (1994), Lichangsong *et al.*(1997) and Uma and Thapliyal (1999). Results of the pathogenicity test using the foliar inoculation by *C. dematium* indicated that all isolates were pathogenic to soybean plants with different degrees. This is in agreement with the results obtained by Morgan and Johnson (1964); Manandhar *et al.*(1988) and Abd-El-Rhman (1995).

At the same time, results indicated significant variations in the host respone to anthracnose disease. Giza–83 and Giza–82cvs were resistant,they recorded the lowest percentage of disease severity. While,cvs. Crawford and Giza-21 were susceptible. The other examined cultivars ranged as a moderate resistant and / or susceptible ones. The variation in response of soybean cultivars to infection by root rot and anthracnose diseases may be due mainly to two factors :-

1- The viulence of the pathogen that may resisted by a specific gene or polygenes.

2- Mechanical or photo chemical of host resistance which also maybe controlled by gene or polygenes in cultivated cultivars. Interaction between these factors may explain the mechanism of host resistance against *R. solani*, *F. solani*, *M. phaselina* and *C. dematium*. This is in agreement with the results obtained by Machado and Carvalho (1975); Rahman and Fakir (1985); El-Wakil and Abou-Zeid (1988) and Abd-El-Rhman(1995).

The healthy soybean cultivars showed considerable variation in their protein patterns. Infection by *R.solani* resulated in changes and induction of new proteins and disappearance of some proteins. Molecular weights from 12 to 40 were important to appear PR-proteins. Resultes indicated that Crawford, Giza-21 cultivars (healhty and infected root samples) and *R. solani* have unique band at molecular weight 19. Crawford, Giza-21cvs. (healhty and infected root samples) and *M. phaseolina* have unique band at molecular weight 33. Giza–82, Giza -21cvs. (healthy and infected leaves) and *C. dematium* unique band at molecular weight 34. This is in agreement with that reported by Sang and Joo (1992); Liu *et al.* (1994); and Abd - El - Rhman (2001).

REFERENCES

Abd-El-Rhman, Saieda. S. A. 1995. Phytopathological studies on soybean anthracnose disease in A. R. E. M. Sc. Thesis, Fac. Agric., Cairo Univ., 130.

Abd-El-Rhman, Saieda. S. A. 2001. Further studies on soybean Anthracnose disease in Egypt. Ph. D. Thesis, Fac. Agric., Cairo Univ., 159

Abou-Zeid, N. M., ,K. A. Abada and M. M. Ragab. 1987. Effect of different fungicides *in vitro* and *in vivo* on the control of soybean damping-off disease. Agric. Res. Rev., 65 (2):263-269.

Achenbach, L. A., J. Patrick and L. Gray. 1996. Use of RAPD markers as a diagnostic tool for the identification of *Fusarium solani* isolates that cause soybean sudden death syndrome. Plant Dis. 80:1228- 1232.

Acimovic, M. 1966. *Macrophomina phaseolina* as a parasite of soybean in Yugoslavia. Savr. Poltjopr., 11:271-280. (Rev. Appl. Mycol., 43:2775).

Arafa, M. K. M. 1994. Studies on some diseases of soybean. Ph. D. Thesis. Fac., Agric., Al- Minia Univ., 174.

Barkar, S. G. 1992. Influence of weather on Sclerotium root rot and wilt in soybean. Indian J. Mycology and Plant Pathology, 22 (2): 192-194.

Brown, W. 1924. Two mycelial methods. II. A. method of isolation single strains of fungi by cutting out a hyphal tip. Ann. Bot., 38: 402 404.

Camara- M,L. Herrera, Isla, and I. V. Guevara . 1988. Varietal resistance to *Rhizoctonia solani Kuhn* in French bean and soybean. Minist. Agric., Conakry, Guinea. Centro-Agricola. 1988. 15: 2,24-29.

Carling, D. E., R. E. Baird, R. D. Gitaitis, K. A. Brainard and S. Kuninaga. 2002. Characterization of AG- 13, a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology 92: 893-899.

Dang- Vu Thi Thanh and A. Gilly . 1992. Simultaneous occurrence and symptoms of *Fusarium solani* (Martius) Sacc., *Fusarium oxysporum* Schlecht and *F. tracheiphiphilum* Armst. Et Armst. on soybean seedlings. No Venvedelem 28 (11): 466- 473.

Ekramoddaullah, A. K. M. and J. J. Davidson. 1995. A method for the determination of conifer foliage protein extracted using sodium dodecyel sulphate and mecaptoethanol. Phytochem. Anal., 6:20-24.

El-Akkad, Salwa, A. F. 1997. Studies on anastomosis groups of *Rhizoctonia solani*. Ph. D. Thesis., Fac., Agric. Cairo. Univ.,182.

El-Wakil, A. A. 1985. Soybean anthracnose, a new disease in Egypt. Proceeding of 1st Nat. Conf. of Pests and Dis.of Veg.and Field Crops in Egypt, Ismailia, 918-921.

El-Wakil and N. M. Abou-Zeid. 1988. Survey,pathological studies,and control of soybean anthracnose (*Colletotrichum dematium*). Agric. Res. Rev., 66: 185-196.

Fenille, R. C., N. L. D. Souza and E. E. Kuramae. 2002. Characterization of *Rhizoctoni solani* associated with soybean in Brazil. European. J. of Plant Pathology 109 (8): 783-792.

Freeman, S., M. Pham and R. J. Rodriguez. 1993. Molecular genotyping of *colletotrichum* species based on arbitrarily primed PCR, A+Trich DNA, and nuclear DNA analyses. Exp. Mycol. 17: 309-322.

Gangopadhyay, D. and T. D. Wyllie. 1973. Biochemical comparison of nine soybean varieties in relation to their susceptibility to *Macrophominaphaseolina*,Indian. J. Mycol. and Pl. Path., 3:131-140.

Ganzhelo, O. I., and V. I. Sichkar. 1985. Resistance of colleated and bred forms of soybean to *Fusarium* under artificial infection Selektsionno-Geneticheskogo Instituta No. (3): 51-55.

Glements, F. E., and G. L. Shear . 1975. The Genera of fungi Houfer Pubis, Co. New York, 496.

Harikrishnan, R., and X. B. Yang. 2004. Recovery of anastomosis groups of *Rhizoctonia solani* from different latitudinal positions and influence of temperatures on their growth and survival. Plant Dis. 88: 817- 823

Hassanein, A. M. 1978. Studies on root rot disease of soybean in A. R. E. M. Sc. Thesis, Fac. Agric., Zagazig Univ., 145.

Hildebrand A. A. 1948. An occurrence of brown stem rot of soybean in Antario. Sci. Agric. 28:261-263.

Hochstrasser, D. F., M. G. Harington,A. C. Hochstrasser, M. J. Miller and C. R. Merril . 1988. Methods for increasing the resolution of two dimensional protein electrophoresis. *Annl. Biochem.*, **173**: 424 435.

Jana, T. Sharma, T. R. Prasad, R. D. and Arora, D. K. 2003. Molecular characterization of *Macrophomina phaseolina* and *Fusarium* species by a single primer RAPD technique. Microbiol Res. 158: 249-257.

Kraft, J. M.; M. P. Haware and M. M. Hussein. 1986. Root rot and wilt disease of food legumes. In World Crops: Cool season food legumes. Ed. by R. S. Summer field. Pp 565-575. Kumes Aca. Pub.

Kulshrestha, D. D., S. B. Mathur and P. Neergaaed . 1976. Identification of seed borne species of *Colletotrichum*. Friesia, 11:116-125

Kunwar, I. K., T. Singh, C. C. Machado and J. B. Sinclair. 1986. Histopathology of soybean seed and seedling infection by *Macrophomina phaseolina*. Phytopathology, 76: 532-535.

Kuramae-Izioka, E. E. 1997. A rapid easy and high yield protocol for total genomic DNA isolation from *Colletotrichum gloeosporioides* and *Fusarium oxysporum* for RAPD, Revista Unimar 19: 683-689.

Lichangsong,Luo Ruiwu, Yang Ghongliang, Zhao Jiuhua, Shang Youfen, Xin Xiangei, Xing Han, and Zhao jing Rong. 1996. Isolation and identification of the pathogenic fungi causing soybean root rot in Yellow River and Huaihe River basins [***Fusarium solani***]. Acta phytophylacica Sinica 23 (2): 187-188.

Lichangsong,Zhao jiuhua, Yang Chongliang, Shang Youfen, lou Rei Wu, Xin Xiangoi,Xing Han and Zhao JingRong. 1997. Preliminary study on differentiation of pathogenicity of *Fusarium solani* causing soybean root rot. Acta Phytopathologica Sinica 27 (2): 129-132.

Liu, Z. and J. B. Sinclair,1991. Isolates of *Rhizoctonia solani* anastomosis group2-2 Pathogenic to soybean. Plant Dis. 75: 682-687.

Liu, L. j.; Y. Chen and M. j. Gao. 1994. Detection of soybean SMV pathogenesis – related protein and acquisition of tolerance. Soybean Science. 13 (4): 345-348.

Manandhar, J. B., G. L. Hartman and J. B. Sinclair . 1988. Soybean germplasm evaluation for resistance to *Colletotrichum truncatum*. Plant Dis., 72: 56-59.

Machado, J. DA., and M. G. DE. Carvalho. 1975. Response of commercial soybean varieties to isolates of *Colletotrichum truncatum* and transmission of the pathogen by seed according to the time of plant infection. Experientiae 19: 119-148. (C. F. Rev. Pl. Pathol. 55: 454. 1976).

Margon, F. L. and H. W. Johnson 1964. Leaf symptoms of soybean anthracnose. Phytopathology 54: 625.

Martinez- Culebras, P.V., E. Barrio, M.B. Suarez – Fernandez , M. D. Garcia- Lopez and A. Querol. 2002. RAPD analysis of *Colletotrichum* species isolated from strawberry and the design of specific Primers For the indentification of *C. fragariae*. J. Phytopath. 150:680-686.

Matsudaria, P. 1987. Sequence from picomole quantities of proteins electroblotted into polyvinylidene difluoride membranes. J. Bio. Chem., 262: 10035-10038.

Morsy, A. A., A. F., Sahab, M. M., Diab, and M. A. Nofal . 1986. Determining seed health of soybean *Glycine max* by the effect of seed borne fungi on gemination, invasion and occurrence in culture. Egypt. J. phytopath., (1982, publ. 1985) 14(1/2): 75-82.

Morsy, K. M. M. 2000. Studies on improving resistance of faba bean to chocolate spot disease. Ph. D Thesis, Fac. Agric., Cairo-Univ., 180.

Moustafa, A. M. 1991. Studies on behavoir of *Rhizoctonia solani* isolates. M. Sc. Thesis, Fac. Agric., Ain Shams-Univ.,175.

Muyolo, N. G., P. E. Lipps and A. F., Schmitthenner. 1993. Anastomosis grouping and variation in virulence among isolates of *Rhizoctonia solani* associated with drybean and soybean in Ohio and Zaire. Phytopath. 83: 438-444.

Naito, S. and N. Itoh. 1999. Occurrence of soybean root rot disease caused by *Rhizoctonia solani* A G 2-3 in Japan. Annual Report of the Society of Plant Protection of North Japan No. 50, 54-57.

Ogoshi, A. 1976. Studies on the grouping of *Rhizoctonia solani* kuhn with hyphal anastomosis and on the perfect stages of groups. Bul. Nat. Inst Agr. Sci. Ser. C. No. 30: 1-63.

Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia.* Fitopatholoia Brasiliera 10:372-390.

Orellana, R. G., C. Sloger and V. L. Miller. 1976. Rhizoctonia Rhizobium interactions in relation to yield parameters of soybean. Phytopathology, 66: 464-467.

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

Rahman, M. H., and G. A. Fakir. 1985. Reaction of some cultivars of soybean to anthracnose. Bangladesh Journal of Plant Pathology, 1: 45 50. (C.F. Rev. Pl. Pathol. 66:34,1987).

Saharam, G. S. and V. K. Cupta. 1972. Pod rot and collar rot of soybean caused by *Fusarium semitectum.* Pl. Dis. Reptr., 56: 693-694

Sang, G. K. and Y. Y. Joo . 1992. Changes in protein patterns resulting from infection of rice leaves by *Xanthomonas oryzae* pv. *oryzae.* Molec. Plant Microbe Interact., Vol. 5, No. 4, pp. 356- 360.

Schneider, R. W., O. D. Dhingra, J. F. Nicholson, and J. B. Sinclair. 1974. *Colletotrichum truncatum* borne wthin the seed coat of soybean. Phytopathology, 64: 154-155.

Sinclair, J. B. 1982. Compendium of Soybean Diseases. 2nd ed. The American Phytopathological Society, 104 pp.

Uma Singh and P. N. Thapliyal. 1999. Fungi rerponsible for seedling emergence problem in different soybean cultivars in Tarai region. Indian Phytopath. 52 (1): 79-81.

Vyas, S. C. 1994. Integrated biological and chemical control of dry root- rot on soya bean. Indian, Journal of Mycology and Plant Pathology, 24(2): 132-134.

Wasfy, E. H., H. M. Sheir and M. M. Weesh. 1984. Comparative studies on some isolates of *Rhizoctonia solani* in Egypt. Phytopathologische Zeitschrift, 4: 346- 353.

Williams J. G. K.; Kuberlik A. R. and Kenneth J. L. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6231-6235.

Yang, J., P. D. Kharbanda, H. Wang and D. W. Mcandrew1996. Characterization, virulence and genetic variation of *Rhizoctonia solani* AG- 9 in Alberta. Plant Dis. 80: 513-518.

دراسات مقارنة لبعض العزلات الفطرية المسببة لأمراض الجذور والسيقان فى فول الصويا واستجابة بعض الأصناف للإصابة

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يتعرض محصول فول الصويا للاصابة بالعديد من المسببات المرضية التى تسبب أمراض سقوط البادرات وعفن الجذور والأنثراكنوز، ولقد تم عمل حصر فى ست محافظات فى جمهورية مصر العربية خلال موسمى ٢٠٠١ و ٢٠٠٢ حيث وجد أن الفطريات *Rhizoctonia solani,Fusarium solani, Marophomina phaseolina and Colletotrichum dematium* كانت المسببات الرئيسية لهذه الأمراض.

استخدم اختبار المجاميع الارتباطية Anastomosis لتعريف عزلات فطر *R. Solani*. أتضح ان عزلات هذا الفطر عديد الأنوية ووضعت تحت مجموعتين من مجموعات الـ AG ، كما تم التفريق بين عزلات الفطريات المعزولة والمسببة لهذه الامراض باستخدام طريقة عمل بصمة وراثية للـ DNA بواسطة طريقة الـ RAPD.

تم أختبار ردود الأفعال المختلفة لثمانية أصناف من فول الصويا لمدى الاصابة بالامراض السابق ذكرها تحت ظروف الصوبة ، حيث وجد أن الاصناف كراوفورد وجيزة ٨٢ كانا أقل الأصناف أصابة بعفن الجذور والأنثراكنوز بينما كان الصنف جيزة ٢١ أكثر الاصناف قابلية للإصابة.

أظهرت كل الأصناف المختبرة (كراوفورد وجيزه ٢١ وجيزه ٨٢) اختلافات واضحة فى تفريد البروتين حيث كانت هناك أختلافات واضحة فى كل من الأصناف المختبرة السليمة والمصابة بالفطريات الثلاثة *R.solani* و *M. phaseolina* و *C.dematium*. كان لصنفى كراوفور وجيزة ٢١ السليمة والمصابة بالفطر *R. solani* نطاق *band* متفرد (مميز) عند الوزن الجزيئي ١٨ ، بينما كان لصنفى كراوفور وجيزة ٨٢ وجيزة ٢١ السليمة والمصابة بالفطر *M. phaseolina* نطاق متفرد عند الوزن الجزيئي ٣٣ . ولصنفى جيزة ٨٢ وجيزة ٢١ السليمة والمصابة بالفطر *C. dematium* هذا النطاق المتفرد عند الوزن الجزيئي ٣٤ جزيء.