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EFFECT OF PLANTING DATE AND HOST CULTIVAR AND THREE COMMERCIAL MICROBIAL PRODUCTS ON DEVELOPMENT OF DAMPING-OFF, ROOT ROT AND WILT OF SOYBEAN PLANTS

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ABSTRACT

Soybean (*Glycine max* (L.) Merr) is subject to stand injury and yield loss due to several diseases. *Fusarium oxysporum, Macrophomina phaseolina, Rhizoctonia solani and Sclerotium rolfsii* are found to be pathogenic to soybean causing damping off, root rot and wilt diseases. All isolates of the tested fungi were pathogenic but varied in virulence for pre and post- emergence damping off and their behavior. The highest level of pathogenicity was exhibited by *R.solan.* Chemicals are effective in controlling these diseases but, these chemicals are expensive and not environmental friendly. There are great efforts to reduce environmental pollution by reducing the dependence on agrochemicals to control pests. Biological control and resistant soybean cultivars are used means to control many diseases. Some microbial products such as Mycostop®, active microbial ingredient; *Streptomyces griseoviridis*; Bio-ARC® *Bacillus megaterium* and Biozeaid ®, *Trichoderma a/bum* were evaluated as compared with the fungicide Rhizolex for their efficacy against *Fusarium oxysporum, Macrophomina phaseoli, Rhizoctonia solani and Sclerotium rolfsii*. , all tested microbial products or fungicide were significantly reduced the percentage of damping-off, infested plants and increesed the `ercentage of healthy survival plants. Susceptibility of some soybean cultivars was tested in potó and under field condition. In pots experiment, none of the cultivars was completely resistant, however Giza 111 cultivar could be c• nsidered tolerantl while Gmza 22 cultivar considered thå most susceptible one. Under natõrally infested soil in the fiåld conditions,(the reactions of seven cultivars (Giza 111, Giza 22, Giza 35, Giza 82, Giza21, Kilarce and Crawford) to root-rot and wilt diseases complex were evaluated in two seasons. In 2006/2007 season Giza 111 had the least incidence % of diseases plants but Giza 35 cultivar had the highest one. In 2007/2008 season, the same trend was noticed with light grade. Plant weight and plant height did not correspond with the level resistance.

Delaying of soybean planting from May to July caused increasing in damping-off. Where, the percentage of damping-off increased from about 13.3 to 53.3%; 20 to 56.6% and from 33.3 to 60% with delayed planting from May to July in presence of *F.oxysporum, M.phaseoli and S.rolfsi* respectively. But it decreased from 46.7% t0 33.3% in presence of *R.solani*.

Keywords: Soybean, Biological control, Mycostop®, BioARC®,, BioZaied®, *Streptomyces griseovirides*, *Bacillus megaterium, Trichoderma album Fusarium, Rhizoctonia solani*, *Macrophomina phaseolina, Sclerotium rolfsii*

INTRODUCTION

Soybean (*Glycine max* (L.) Merr) has many benefits for human and animal nutrition It can be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming systems (Nassiuma and Wasike 2002., Akande *et al* 2007).

Soybean is susceptible to damping off, root rot and wilt diseases , caused by *Fusarium oxysporum, Macrophomina phaseoli, Rhizoctonia solani and Sclerotium rolfsii* which are the most serious pathogen of soybean in many countries, causing considerable damage and loss in seed yield (Amer,2005; Hashem, 2004; Luiz et. al., 2006; Wrather et. al., 2007; Haikal , 2008 and Sweets, 2008).

These pathogens are difficult to control because of their persistence in the soil and wide host range. Some Chemicals are effective in controlling these diseases but, these chemicals are expensive and not environmental friendly. Therefore, alternative control methods are needed for managing these pathogens. Several alternative measures are being tested. Natural resources such as biological control, resistance and good agricultural practices found to be good and safe means of diseases control. Smith and Carvil, 1997; Bradley *et al.*, 2005 and Pabon *et al.*, 2006 evaluated certain soybean cultivars for resistance and susceptibility to *M. phaseoli* and *R. solani* in the field. Amer, 2005 and Bahaa_Eldin, 2005 evaluated eight soybean cultivars against *F. oxysporum*, *R. solani, M. phseolina*, *Sclerotum rolfsii* and *Colletotricum sp* under greenhouse and filed conditions. They showed that cultivars varied significantly in their susceptibility to damping off- and root rot diseases. Many researchers have used the biological control as an alternative control method to fungicides against soil-borne plant diseases (Tahvonen *et al*.1994; El-Sharkawy *et al*. 1998; Koch, 1999, Hassanein *et al*, 2000; El-Barougy and El-Sayad, 2003, Bahaa_Eldin, 2005 and Bayaa, 2006).Several diseases of soybean are related to Planting date and susceptible cultivar in presence of the pathogen (Almeida and Corso, 1991; Grau*, et.al*., 1994).Wrather, 2003 determine the effects of planting dates with one susceptible soybean cultivar, on seed infection by *Phomopsis* spp . Landa *et al*., 2004 reported that sowing date was the factor with the greatest effect on Fusarium wilt and yield of chickpea. Severity of Soybean seedling blight caused by some soil borne fungi varies with the environmental conditions (*Sweets*, 2008).

The aim of the present work was to study the efficacy of three commercial microbial products, (Mycostop® developed in Finland, BioARC® and BioZeaid® developed Locally in Egypt) compared with the fungicide Rhizolex for controlling damping off, root rot and wilt diseases of soybean under pot conditions.Also to evaluate resistance of some soybean cultivars to root rots and wilt diseases. And to determine the effect of planting dates and their interactions with four soybean cultivars on soybean damping off, root rot and wilt diseases in pots-grown plants under artificial infection and in field- grown plants under natural condition.

MATERIALS AND METHODS

1-Sample collection, isolation and identification:

Soybean plants showing root rot and wilt symptoms were collected from different localities of Ismailia Governorate. Ten plants (Two from the center and two near each corner) were removed from each field. Root rotted samples were first washed in running tap water to remove the adhering soil particles and then surface sterilized in 5 % sodium hypochlorite solution for two minutes. The sterilized plant parts were rinsed several times in sterilized distilled water and dried between sterilized filter paper then cut into small pieces and directly placed on potato-dextrose agar medium (PDA) in Petri-dishes. The Petri dishes were incubated at 25 °C for 5-7 days. The hyphal tips of the growing hyphae were taken from the growing colonies and transferred to PDA plates and purified using single-spore technique as described by Toussoun and Nelson (1968). Isolated fungi were identified according to their morphological characters according to Booth (1971), Nelson *et al.* (1983) and Barnett and Hunter (1986).

2-Pathogenicity tests:

The pathogenicity of some isolated fungi, *Fusarium oxysporum*, *Rhizoctonia solani* , *Macrophomina phaseolina* and *Sclerotium rolfsii* was tested at Ismailia Agric . Res. Station, by sowing seeds in artificially infected soil.

Preparation of fungal inoculum soil infestation was carried out as following: Sterilized sorghum medium (250 g sorghum / bottle (1Liter) and enough water to cover the sorghum) was used for preparation of fungal inoculum. The medium was autoclaved then inoculated with each of the isolated fungi and incubated at 27 ° C for 20 days.

Pots (30 cm in diameter) were filled with unsterilized soil .The soil was infested with the fungal inoculum at rate of 3 % (W/W) of soil weight. Inoculated soils were watered and mixed thoroughly for one week to insure even distribution of the inoculum. Soybean seeds (Giza 35 cultivar) were sown at the rate of 10 seeds / pot (30 cm in diameter). A set of four replicates were used for each fungus .Four pots containing non-inoculated soil were used as control. Percentages of pre and post emergence damping off were recorded 15 and 30 days after planting, respectively. Plant growth parameters (Shoot weight, plant height /plant (aver. of 5 plants) were recorded three months after planting. Infested survival plants were evaluated 3 months after sowing by cutting longitudinally through each plant (stem and root) and any discoloration of internal tissue was recorded while, healthy plants which had no visual evidence of disease. Disease severity of wilt and root rot and any discoloration of internal tissue were recorded Severity of inside browning of internal tissue was recorded and conducted with scale proposed by Haware and Nene (1980) based on 0-4 scale according percentage of foliage yellowing or necrosis (0=0%, 1=1-33%, 2=34-66%,3=67-loo%, 4= dead plant).

3- Cultivar reactions to root rot and wilt fungi:

3.1- Pots experiment:

Four soybean cultivars "Giza111, Giza 22, Giza35 and Kilarce" were tested for their reaction to *F. oxysporum*, *R. solani, M.paseolina* and *Sclerotium rolfsii* isolated from rotted root and wilted soybean plants. Ten seeds of each cultivar were planted in each pot (30cm in diameter), filled with 5 kg of soil and infested with each pathogen at the rate of 3% as mentioned before.

Three pots were used for each treatment. Percentages of pre- post-emergence damping-off and survival plants were calculated at 10 and 30 days after planting, respectively.Whereas root rot/wilt was recorded as severity infection after 90 days. According to the scale proposed by Haware and Nene (1980).Parameter growth (plant height and Shoot weight) were recorded three months after planting.

3.2- **Field experiment:**

Soybean cultivars (Giza 111, Giza 22, Giza 21, Giza 35, Giza 82, Kilarce and Carfowerd) were tested for their reaction against naturally infection under field conditions. Treatments were arranged in a complete randomized block designed with four replicates. The field plots were 2 × 3 m ^{2} with 5 rows, 200 seeds were sown in each plot. Incidence (%) of diseased plants (Total number of dead plants/ Total number of plants at plots (%) was calculated 2 and 3 months after planting. However, plant growth parameters (Shoot weight and plant height) were recorded four months after planting, Disease severity was also recorded on a random sample of plants of the plots (20 plants) four months after planting. Disease severity indexing (DSI) of root rot and any discoloration of tissue were recorded according to based on 0-4 scale according percentage of foliage yellowing or necrosis Haware and Nene (1980) based on *(0=0%,* 1=1-33%, 2=34-66%,3=67-loo%, 4= dead plant) . Scores <l and >3 were considered as resistant (R) and susceptible (S) reactions, respectively. Scores in between were considered as moderately susceptible.

4-Biological control:

Effect of microbial products and fungicide for controlling damping off, root rot and wilt diseases in soybean plants :

Three microbial products, i.e. Mycostop® (*Streptomyces griseovirides*) produced by Kemira (Kemira Agro Oy), Finland, Bio-ARC® (*Bacillus megaterium*) and (Biozeid)® (*Trichoderma album*) are Egyptian products, were used to evaluate their efficiency in controlling damping-off, root rot and wilt diseases in soybean plants in pot experiment .

Soybean seeds (Giza 35) were soaked in the solution of each microbial product for 2h. at the rate of 4g/Lfor Bio-ARC and BioZeaid and 2g/L Mycostop . The soaked seeds were left to dry in the air for 1/2 hour before sowing in the potted infested soil with the pathogenic fungi. This experiment was conducted in 30 cm diameter clay pots. Seeds were sown at the rate of 10 seeds / pot .A set of four replicates were used for each treatment.

There were six treatments as follows:

1-Soil infested with pathogen + Mycostop (soaked seeds at rate of 2g/L).

3- Soil infested with pathogen + Bio-ARC (soaked seeds at rate of 4 g/L).

4- Soil infested with pathogen + BioZeaid (soaked seeds at rate of 4gm/ L).
5- Soil infested with pathogen + Rhizolex (soaked seeds at rate of 1.5g/ L).
6- Soil infested with pathogen (Control 1).
7- Uninfested soil (Control 2).

Percentage of damping off and survival plant were recorded 30 and 90 days after seeding respectively. Infected survival plants were evaluated 3 months after sowing by cutting longitudinally through each plant (stem and root) and any discoloration of internal tissue was recorded .Healthy survival plants = no visual evidence of disease.

4-- Planting date:

4.1-Pots experiment:

Effect of planting date on damping off and survived soybean seedling grown in soil infested with some pathogenic fungi in pots:

Data used in this study were obtained from pot experiments conducted at Ismailia Agric. Res. Station. Soil in the pots (30 cm in the diameter) was artificially infested with one of the tested fungi (*F. oxysporum*, *R. solan,i M.paseolina* and *Sclerotium rolfsii*) before sowing. The treatments comprised all combinations of three levels of sowing dates (First May, June 7and July12). **Preparation of fungal inoculum and soil infestation**:

Sterilized sorghum medium inoculated with the fungi and incubated at 27 ° C for 15 days. Pots (30 cm in diam.) filled with unsterilized soil were simply infested with each of the tested fungi at the rate of 3 % (W/W) of soil weight. The infested soil was watered and mixed thoroughly for one week to insure even distribution of the inoculum. A set of 4 pots for each treatment were cultivated by 10 soybean seeds Giza 35 cv/pot. Percentages of damping-off and survived plants were calculated 30 and 90 days after planting, respectively.

4.2 – Field trial:

Effect of planting date on root rot and wilt diseases complex of soybean plant under field condition:

Field trial was carried out at Ismailia Agric. Res. Station to determine the effects of planting date on root rot and wilt diseases complex of soybean plant under natural condition. Treatments were arranged in a complete randomized block designed with four replicates. The field plot was 3x4 m with 5 rows, 200 seeds were sown in each plot. Planting dates (First May, June 7 and July12) were the main plots and 4 soybean cultivars "Giza111, Giza 22, Giza35 and Kilarce" were the subplots The parameters measured were, percentage of survival plants calculated 3 months after planting. Also, soil temperature was daily monitored throughout the experiment by soil thermometer plots. Soil temperature were recorded three times daily, at 8:00 am , 12:00 pm, and at 2:00pm, at 20 cm depth (Table, 1)

Table (1): Soil temperature during the experimental period (1stMay to 1st July)

Month	The mean temperature(ºC) at 20 cm depth During ,May, June and July	
	Minimum	Maximum
May	20	26
June	27.5	39
July	29	41.5

Statistical analysis:

All the data were statistically processed by the analysis of variance and by determining the significance threshold using Duncan's test (Duncan, 1955).

RESULTS

Isolation and identification of the causal pathogens:

Rotting, wilting and damping off of soybean seedlings were observed in soybean fields.

Isolation trails from rotted and wilted soybean plants collected from different localities of Ismailia Governorate (Fig.1) yielded the following fungi which were identified as *Fusarium oxysporum*, *Rhizoctonia solani* , *Macrophomina phaseolna* and *Sclerotium rolfsii*

A B

A B

Fig (1): Typical symptoms of root rot and wilt diseases complex (natural infection)

A : Healthy soybean plant B- Naturally infected soybean plants

2-Pathogenicity test:

Disease symptoms attributed to F.*oxysporum, R. solani, M. phaseolina* and *S. rolfsii* were observed on soybean plants (Giza 35 cv.) grown in soil artificially infested with the tested fungi in pots experiment. Symptoms were almost similar to those noticed under the field conditions; the fungi produce wilt symptoms at any stage of the plant development.

Data were recorded as percentage of pre, post emergence damping-off and survival plants (healthy and infested plants) at 15, 30, 90 days after planting, respectively. Data presented in Table (2) indicate that all the tested fungi proved to be pathogenic and caused different degrees of pre and post-emergence damping off. Results revealed that the highest percentage of pre-emergence damping off was caused by *R.solani* (46.7 %) followed by *S. rolfsii* , *M.phaseolina* and *F. oxysporum* (33.3 %, 20% and 16.7 %, respectively as compared with the control which showed 3.3 %. The highest percentage of post emergence damping off (26.7 %) was detected by *M.phaseolina* infection followed by both of *R. solani*, *F. oxysporum* and *S. rolfsii* (16.7%. 13.3 % and 10 %)respectively. Data also revealed that percentage of infected survival plants ranged from 10 to 23.3% and the lowest percentage of healthy survival plants (26.6%) was caused by *R. solani*. For diseases severity, the highest degree was obtained from plants grown in soil infested with *R. solani* (3.9) followed by *M.phaseolina,* S. *rolfsii* and F*. oxysporum* which recorded 3.8, 3.2 and 2.4 respectively.

Table (2):Pathogenicity test with certain fungi isolated from diseased soybean Giza 35 cultivar.

Soil infested with	Percentage and severity of damping off on Giza 35 cultivar				
	%Damping off		%Survival plants		Disease severity Score
	Pre-emergende	Post emergende	Infested survival	Healthy survival	
F. oxysporum	16.7 bc	13.3b	16.7 a	53.3 b	2.4^{b}
R. solani	46.7 a	16.7 ab	10 ab	26.6 c	3.9^{a}
M.phaseoli	20 b	26.7 a	13.3 a	36.6 bc	3.8^{a}
S. rolfsii	33.3 ab	10 bc	23.3a	33.4 b	3.2^{ab}
Control	3.3c	3.3c	3.4b	90a	1.3^{c}
LSD	19.6	9.5	13.3	23.3	1.01

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

Effect of inoculation with the tested fungi on parameter growth (plant height and Shoot weight) of 4 soybean cultivars growing in pot experiments:

Data presented in Table (3) reveal that the infection with F*.oxysporum, R. solani, M. phaseolina* and *S. rolfsi* reflected on plant height and weight of Giza 35 cultivar. It was noticed that reduction in height and weight of the inoculated plants compared with uninoculated ones Fig. (4)The highest reduction in plant height and weigh (66.8% and 68.2 % respectively) was recoded from plants grown in soil infested with *R. solani* and the lowest reduction (54.3 % and 57.6 %) for plants grown in soil infested with F.*oxysporum.* (Table 3 and F.ig. 2)

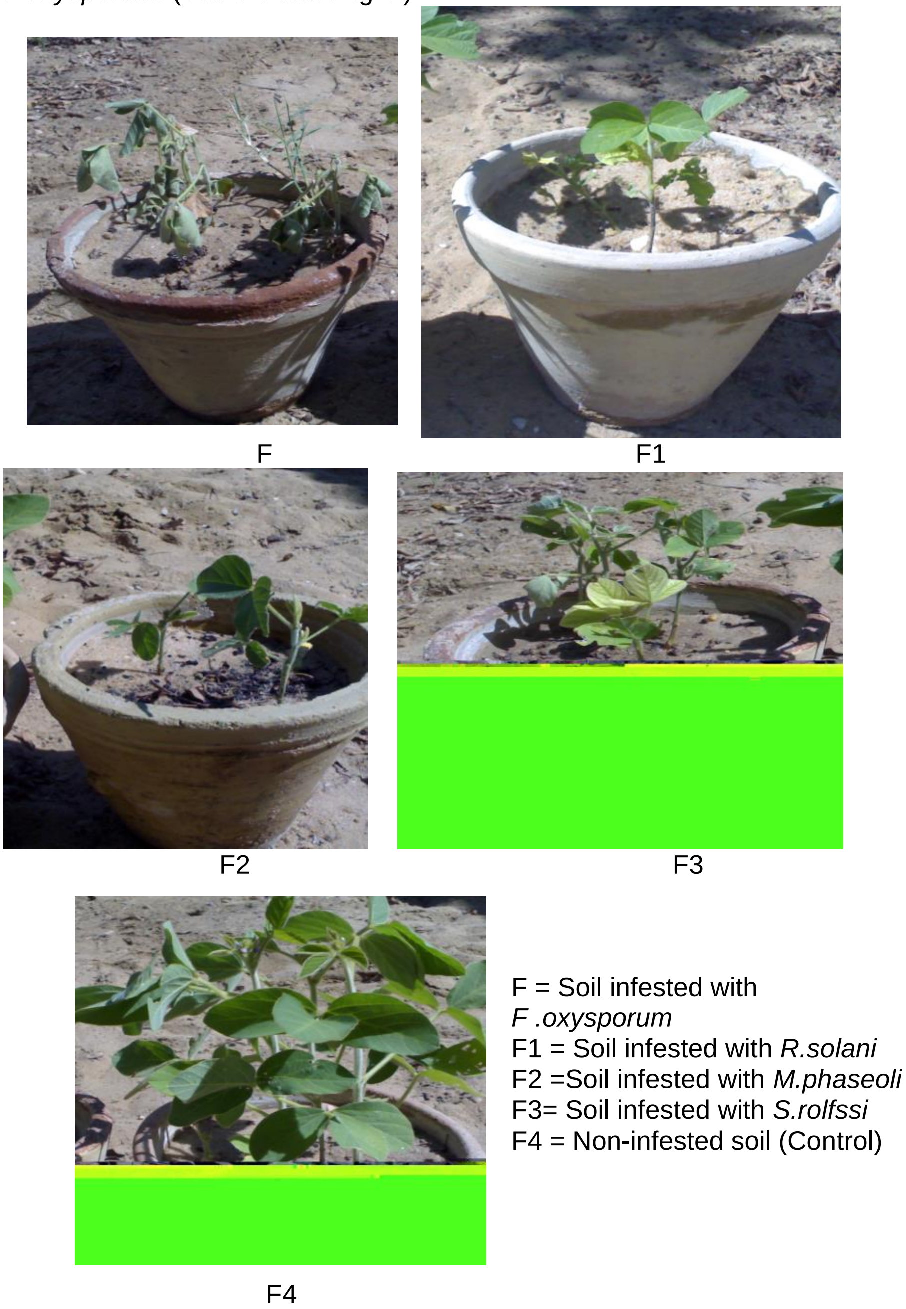

Fig. (2) Artificial inoculation in soil infested with some soil borne fungi one weak, before sowing soybean seeds of Giza 35 cultivar.

Table (3):Effect of inoculation with F.*oxysporum, R. solani* , *M. phaseolina* and *S. rolfsii* on plant height and Shoot weight of Giza 35 soybean cultivar pot experiments.

Soil infested with	Plant height (in cm)		Shoot weight (in gram)	
	Plant height (in cm)	% reduction over control	Shoot weight (in gram)	% reduction over control
F. oxysporum	16.8 b	54.3	13.9b	57.6
R. solani	12.2c	66.8	10.4 b	68.2
M.phaseolina	14.3bc	61.1	11.2 b	65.8
S. rolfsii	13.4 bc	63.5	10.6 b	67.6
Control	36.8a	-	32.8a	-
L.S.D	4.9		4.85	

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

3-Cultivar reaction:

3.1-In pot experiment :

Susceptibility of four soybean cultivars to *F.oxysporum, R,solani, M.phseolina and S.rolfsii* added to the soil one day before seeding :

Data in Table (4) cleare that all the tested soybean cultivars were susceptible to infection with *F.oxysporum*, R*. solani, M.phaselina* and *S. rolfsii* at different degrees. In soil infested with *F.oxysporum,* Giza 111 showed the highest percentage of healthy survival plants (80%) followed by Kilarce (56.7%) and Giza 35 (53.3 7%) while Giza 22 was more susceptible cultivar showing only 40 % of healthy plants.

Table (4): Susceptibility of certain soybean cultivars to some pathogenic fungi

Cultivars	*F. oxysporum*				*R,solani*				*M.phseoli*				*S.rolfsii*			
	Damping off %		Survival plants %		Damping off %		Survival plants %		Damping off %		Survival plants %		Damping off %		Survival plants %	
	pr	Po	I	H	pr	po	I	H	pr	po	I	H	pr	po	I	H
Giza 35	16.7 b	13.3 a	16.7 ab	53.3 ab	40 a	233 a	10 a	26.7 b	233 b	20 a	10 a	46.7 ab	33.3 a	10 b	23.3 a	33.3 ab
Giza 111	10 C	0 b	10 b	80 a	23.3 a	13.3 a	6.7 a	56.7 a	20 b	13.3 a	6.7 a	60 a	26.7 a	10 b	20 ab	43.3 a
Giza 22	23.3 a	16.7 a	20 a	40 ab	43.3 a	10 a	10 a	36.7 b	53.3 a	3.3 a	16.7 a	26.7 b	36.7 a	13.3 a b	23.3 a	26.7 b
Kilarce	13.3 bc	0 b	16.6 ab	56.7 ab	23.3 a	13.3 a	20 a	33.3 b	0.7 b	13.3 a	16.7 a	43.3 ab	30 a	23.3 a	10 b	36.7 ab
LSD	4.41	7.7	7.6	24.7	24.9	16.3	23.1	17.1	28.7	26.6	16.3	17.2	16.36	12.15	12.1	17.1

In soil infested with *R.solani*, Giza 35 gave the lowest percentage of healthy survival plants (26.7%). While, Giza 111 had the highest percentage (56.7 %), followed by Giza 22 (36.7 %) and Kilarce (33.3 %)).

In presence of *M.phaseoli*, Giza 22 was highly susceptible to *M.phaseolina*, where the lowest percentage of healthy survival plants (26.7 %) is found. Whoever, Giza 111 showed the highest percentage (60%). In the presence of *S.rolfsii*, Giza 22 recorded the lowest percentage of healthy survival plants being 26.7 %. Meanwhile, Giza 111 recorded the highest one (43.3 %).

Concerning pre emergence damping off, Giza-22 was the most susceptible cultivar to all fungi where it recorded the highest percentage of pre emergenc damping off (53.3%, 43.3%, 36.3% and 23.3%) in presence of *M.phaseolina* , *R.solani*, *S.rolfsii* and *F.oxysporum* respectively. while Giza 111 recorded the lowest value (10%, 20%, 23.3% and 26.7%) in presence of *F.oxysporum, M.phaseolina , R.solani,* and *S.rolfsii* respectively.For infested survival, it is obvious that the highest percentage reached 23.3% in presence of *S.rolfsii* for both of the two cultivars (Giza35 and Giza 22) but, Giza 111 recorded the lowest percentage (6.6%) for both of *R.solani* and *M.phaseolina*. Generally, Giza 111 was less susceptible to all tested fungi.

Disease severity was recorded in Table (5), data revealed that the highest degree of disease severity (4.1) was obtained from kilarce cultivar in presence of *R.solani* but the lowest one (2.2) was observed on Giza 111 in presence of *M.phaseolina.*

Table (5): Severity of disease reaction of soybean cultivars inoculated with some soil borne fungi

Soil infested with	Cultivars			
	Giza 111	Giza 35	Giza 22	Kilarce
F. oxysporum	2.6 [a]	3.5a	3.4a	2.3b
R. solani	3.3[a]	3.7a	3.2ab	4.1a
M.phaseolina	22 [a]	2.5a	3.7a	2.3b
S. rolfsii	2.3 [a]	3.7a	2.3b	2.3b
LSD	1.27	1.07	1.23	1.25

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

3.2 – Field trial

Reaction of soybean cultivars to root-rot and wilt diseases complex under natural infection in the field:

In this experiment seven soybean cultivars, Giza 111, Giza 21. Giza 22, Giza 35, Giza 82, Kilarce and Crawford were evaluated to root rot and wilt disease complex under natural infection during two seasons (2006/2007 and 2007/2008).Incidence % of root rot and wilt of soybean cultivars under natural infection was recorded at two stage of growth (2 and 3 months). Results in Table (6) indicate that , In 2006/3007 season, Giza 111 had the lowest percentage of disease incidence (4.5 %) of 2 months after planting followed by Giza 21 (7.5%) and Crawford (9%) Also, Giza 111 showed the lowest percentage of disease incidence (8.25%) of 3 months after planting followed by Giza 22 and Crafowerd reached 16.7% and 20.5 %, respectively. In 2007/2008 season, almost the same trend was noticed with light more grade where, Giza 111 recorded the lowest percentage of disease incidence 2 months after sowing followed by Giza 22 and, Crafowerd with averages of 7.12%, 9.8% and 12.8%, respectively and Giza 35 was the most susceptible cultivar, where it recorded the highest incidence % (39) 3 months after planting followed by Kilarce and Giza 22 with averages of 36% and 30%, respectively.

Table (6): Reaction of soybean cultivars to root-rot and wilt diseases complex under naturally infection in the field at (2006/2007 and 2007/2008) growing seasons.

Cultivars	Incidence (%) of diseases plants *					
	During 2006/2007season		During 2007/2008eason		Mean	
	2months after planting	3month after planting s	2months after planting	3months after planting	2months after planting	3months after planting
Giza111	4.5c	8.25 d	7.12c	12.6b	6.8	10.4
Giza35	15.5 ab	34.5 a	25a	39a	20.3	36.7
Giza 22	13.6 abc	16.7cd	9.8bc	30a	11.2	23.4
Giza 82	9 .5 abc	27.25 abc	21a	24.5ab	15.2	25.8
Giza 21	7.5 bc	35 a	18.3ab	27.5a	12.9	31.3
Kilarc	17.25 a	30 ab	12.8bc	36a	15.03	33
Crafowerd	9 abc	20.5 ab	10.15	13.3	9.06	16.9

***Total number of dead plants/ Total number of plants at plots (%)**
(a–c) those values within a given column followed by the same letter are not significantly different at 5% level. based on Duncan's multiple range test

In this experiment, disease severity was recorded on a random sample of 20 plants of each plot, 3 months after sowing (Table 7). Results show that , Giza 35cultivar gave the highest value of degree (3.5 and 3.75) in 2006 /2007 and 2007 /2008 season respectively while, Giza 111 cultivar revealed the lowest value of degree (1.25 and 1.5) from the sample were noticed in 2006 /2007 and 2007 /2008 season. Generally, data in Table (7) showed that Giza 111 was the most resistant cultivars during two seasons but Giza 35 cultivar was highly susceptible, followed by Giza 21, Kilarc, Giza 82 and Giza 22 .

Table (7): Severity of root rot and wilt diseases complex on certain soybean cultivars under field condition:

Cultivars	diseases severity Index**		
	During 2006 /2007 season	During 2007 /2008 season	Mean
Giza111	1.25 d	1.5 d	1.38 c
Giza35	3.5 a	3.75 a	3.63 a
Giza 22	1.75 cd	2.25 cd	2 bc
Giza 82	2 c	2.75 b	2.37 b
Giza 21	3 ab	2.5 bc	2.7 b
Kilarc	2.75 b	2.5 bc	2.5 b
Crafowerd	1.5 cd	1.75 cd	1.63 c
LSD	0.66	0.78	0.62

****Disease severity of root rot and any discoloration of tissue were recorded according to. Haware and Nene (1980) based on 0-4 scale according percentage of foliage yellowing or necrosis *(0=0%,* 1=1-33%, 2=34-66%,3=67-loo%, 4= dead plant) . Scores <l and >3 were considered as resistant (R) and susceptible (S) reactions, respectively. Scores in between were considered as moderately susceptible**

4-Biological control:

Effect of microbial products compared with the fungicide Rhizolex® for controlling damping off, root rot and wilt disease in soybean plants in pots:

Three commercial microbial products namely Mycostop®, BioARC® and Bioziead® and beside the fungicide Rhizolex were used singly to study their effect on damping off, root rot and wilt diseases development in soybean in pots. Data in Table (8) show that all commercial microbial products and the fungicide decreased damping off and percentage of infested survival plants and increased healthy survival plants compared with the control treatment.

Data indicated that soil infested with *F. oxysporum* used as control, showed the highest percentage of damping off (43.3%) but the lowest percentage which reached 16.7% was obtained from both of Rhizolex and Mycostop while, BioARC and BioZeid recorded 26.7% and 30% respectively.Significant differences were realized between control No.1 (infested soil), and the other treatments with respect to damping off percentage observed in such treatments. Concerning the percentage of healthy survival plants, the most effective treatment was Mycostop at (63.3%) followed by Rhizolex, BioARC and BioZeid showing 56.7%, 46.7% and 43.3%,. while the control showed 23.3 % survival.

Soil infested with *R.solani* used as a control, without fungicide or microbial products revealed the highest percentage of damping off (83.3%) but, the lowest percentage (10%) was obtained from non-infested soil (control No.2).For healthy survival plants, the lowest percentage (6.7%) recorded from infested soil (control No.1) compared with the highest percentage (86.7%) recorded from non-infested soil (control No.2). The most effective treatment was obtained from Rhizolex, 80% of healthy plants followed by 73.3%, 63.3% and 53.3 recorded from Mycostop, BioZeid and BioARC respectively. . (Table 8)

Fungicide Rhizolex when used to control *M.phaseoli* resulted in the lowest percentage of damping off (20%) as compared with Mycostop, BioZeid and BioARC which recorded 40%, 43.3% and 53.3% respectively, whereas the control was characterized by 66.7%.The highest percentage of healthy survival plants at 63.3% was recorded when Rhizolex was used in controlling the pathogen compared with the lowest of 23.3% 0btianed in the control treatment. (Table 8)

Data presented in Table (8) also, reveal that Soil infested with *S.rolfsii* used as a control revealed the lowest percentage of healthy survival (16.7%), but the highest percentage was obtained when Mycostop was used followed by BioZeid ,Rhizolex and BioARC (56.6%, 43.3% and 36.7% respectively).The lowest percentage of Daming off was obtained when Rhizolex was used (3.4%) followed by BioZeid (16.7%) and both of BioARC and Mycostop gave the same value (26.7%).

5- Planting date:

5.1 Pots experiment:

Effect of planting date on damping off and survived soybean seedling grown in soil infested with some pathogenic fungi:

Data used in this study were obtained from a pot experiment conducted in Ismailia Agric. Res. station to evaluate the effects of planting date on damping off and survived soybean seedling grown in soil infested with *F.oxysporum, R.solani, M.phaseolina and S.rolfsi*. Data in table (9) show that in presence of the tested fungi*, F.oxysporum, R.solani, M.phaseoli and S.rolfs,i* first May planting date had higher percentage of Healthy survival plants (43.3, 33.3, 43.3 and 46.7)than those in July plantings (36.7, 30, 16.7 and 23.3 respectively) On the other hand, delaying of soybean planting from First May to July12 caused increasing in damping off. where, the percentage of damping off increased from about 13.3% to 53.3% ; 20% to 56.6% and from 33.3% to 60% with delayed planting from May to July in presence of *F.oxysporum, M.phaseoli and S.rolfsi* respectively. But it decreased from 46.7% t0 33.3% in presence of *R.solani* . Similar effect was observed on Infested survival plants with all fungi in the three planting date.

5.2 – Field trial

Effect of planting date on root rot and wilt diseases complex of soybean plant under field condition:

A field experiment was conducted during 2006-2007 to determine the effects of soybean planting date on percentage of root rot and wilt diseases complex of 4 cultivars in three planting dates (First May, June7 and July12).Data in Table (10) reveal that the percentage of survival plants in all cultivars were higher in First May planting date than in July 12 plantins. The highest percentages (82.5, 78.25, 76.5and 74.5) were recorded for Giza 111, Kilarc, Giza 22 and Giza 35 respectively, in First May planting date. On the other hand, delaying of soybean planting from May to12 0f July caused decreasing of survival plants%, the lowest percentage (25%) was recorded in July planting date for Giza 22. Significant differences were recorded between first May planting date and both of June7 and July12 planting date in all cultivars.

T8

Table(9): Effect of planting date on damping off and survived soybean seedling grown in soil infested with some pathogenic fungi :

Planting date	*F. oxysporum*			*R,solani*			*M.phseolina*			*S.rolfsii*		
	Damping off	Survival plants		Damping off	Survival plants		Dampin g off	Survival plants		Damping off	Survival plants	
		I	H		I	H		I	H		I	H
First May	13.3b	43.4	43.3	46.7a	20	33.3a	20b	36.7	43.3a	33.3b	20	46.7a
June7	40a	26.7	23.3b	23.4a	60	16.6ab	40ab	40	20a	56.7a	10	33.3ab
July12	53.3a	10	36.7ab	33.3a	36.7	30a	56.6a	26.7	16.7b	60a	16.7	23.3b
l.s.d.	14.8		14.8	19.97		14.8	26.6		22.8	14.8		14.7

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

Table (10): Effect of planting date on root rot and wilt diseases complex of soybean plant under natural field infection.

Planting date	Survival plants % Cultivars			
	Giza111	**Giza22**	**Giza 35**	**Kilarc**
First May	82.5a	76.5a	74.5a	78.25a
June7	46.7b	41.75b	37.5b	36b
July12	48.7b	25b	34.7b	42.2b
L.S.D	6.6	12.4	11.3	8.1

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

DISCUSSION

Isolation trails from wilted and rotted soybean plants yielded *Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseoli, and Sclerotium rolfsii* conforming to other reports (Amer,2005; Bahaa_Eldin,2005, Luiz et. al., 2006, Pabon *et. al.,*2006, Wrather et. al., 2007 and Sweets, 2008).They reported that these diseases are very common in soybean plants growing areas. In pots experiment, pathogenicity tests carried out on Giza 35 soybean cultivar. It was conducted and led to symptoms which were almost similar to those noticed under field conditions conforming to Almeida *et al.,* 2004. The present investigation demonstrated that the isolated fungi from naturally infested field could reduce seedling emergence and healthy plants and could directly affect yield and the isolates were pathogenic but varied in virulence for both pre and post- emergence damping off. The highest level of pathogenicity was exhibited by *R.solani*.. This is in agreement with results obtained by Roseli *et al*., 2003; Estevez *et al*., 2004; Bahaa_Eldin,2005 and Amer,2005.

Nowadays the world is suffering from environmental pollution due to the use of agrochemical. Recently, an increasing desire to reducing the use of pesticides and there are the attempts to use Natural resources such as resistance, good agricultural practices and other nonchemical treatments such as biological control is high on the list of potential alternative methods . However, the use of fungicides will continue but at lower rates whenever necessary. Therefore, it was thought to be of value to evaluate the cultivars for resistance and susceptibility to the pathogens and to evaluate a series of microbial products in comparison to fungicides to be included in the protection of the crops.

The effect of important microbial products Mycostop (developed in Finland) and the locally producet Bio-ARC® and Biozeid)® and the fungicide Rhizolex were evaluated in the greenhouse against *Fusarium oxysporum, Macrophomina phaseoli, Rhizoctonia solani and Sclerotium* infecting soybean plants. The obtained results revealed that percentage of healthy plants remaining in the pot, depending on the different treatments. All pots treated with microbial products or fungicide showed better effect on plant health than untreated when the pathogen was involved. These results are in agreement with Tahvonen *et al*., 1994, El-Barougy.1997 ; El-Sharkawy,*et al*., 1998; Hassanein, *et al*.,2000 they tested powdery biological preparation containing *St.griseoviridis* (Mycostop) in the greenhouse and in field experiments against some soil borne fungi. Koch (1999) tested five microbial products (Supresivit®, active® microbial ingredient *Trichoderma harzianum;* TRI 002®, *T. harzianum*; Ecofit®, *T.viride;* Soilgard® *Giocladium virens;* and Protus®, *Talaromyces flavus*) in the greenhouse against soil-borne pathogens on peat. They found that Soilgard® was the only biocontrol products that gave significant control of *Pythium ultimum* on cucumber. Also, Soilgard was as efficient as the chemical standard pencycuron aginst *Rhizoctonia solani* on peas. El-Barougy and El-Sayed (2003) evaluated the effect of microbial products; Mycostop & GlioMix & Plant guard and Rhizo-N and the fungicide (Topsin-M) in a greenhouse and field against *F.oxysporum* infecting lupin plants. Bahaa El-Din, 2005 found that soybean Seed treated with biocides (BioARC and BioZeid) decreased percentage of pre and post emergence damping off of soybean plants in pots and in filed experiments. He found that BioARC was the most effective biocides followed by BioZeid. Andrea 2006 reported that the biofungicide Mycostop was very effective against *Fusarium oxysporum* f.sp. *lycopersici* and *Verticillium dahliae*

Evaluation of 4 soybean cultivars (Giza 111, Giza 22, Giza 35 and Kilarce) revealed that all of them were susceptible with different degree of infection with *F.oxysporum, R. solani, M. phaseolina, and S. rolfsii*. Results showed that none of the cultivars was completely resistant, however Giza 111 had the highest percentage of healthy survival plants and could be considered resistant while, Giza 22 cultivar should be the most susceptibility and recorded the lowest percentage of healthy survival plants. This is in agreement with results obtained by Bahaa_Eldin,(2005). He found that Giza 111 was moderately resistant in pots and it was resistant under field condition. While, Giza 21 was highly susceptible where as Giza 35 was susceptible. Under naturally infested soil in the field conditions the reactions of 7 cultivars (Giza 111, Giza 22, Giza 35, Giza 82, Giza21, Kilarce and Crawford to root-rot and wilt diseases complex were evaluated in two seasons. In 2006/2007 season Giza 111 had the least incidence % of diseases plants followed by Crawford , Giza 22, Giza 82 ,Giza21, Kilarce and Giza 35 with averages of 6.37, 7.3, 15.5, 18.7, 21.7, 23.7 and 25 %, respectively., In 2007/2008 season, the same trend was noticed with light grade. Plant weight and plant height did not correspond with the level resistance This might be due to nature of the cultivar. Using resistance of the host plant found to be one of the most efficient and safe means of diseases control. The results were in line with those reported by Smith, and Carvil, 1997; Bradley *et al.*, 2005; Pabon *et al.*, 2006. They evaluated certain soybean cultivar for resistance and susceptibility to *M. phaseolina* and *R. solani* in the field. Amer, 2005 and Bahaa_Eldin, 2005 evaluated eight soybean cultivars against *F. oxysporum*. *R. solani , M. phseolina. Sclerotum rolfsii* and *Coletotricum sp* under greenhouse and filed condition. They showed that cultivars varied significantly in their susceptibility to damping off- and root rot diseases.

Advancing sowing dates may contribute to control wilt and root rot diseases.

In the present study the percentage of diseased soybean plants in all cultivars were less in First May planting date than in July12 planting dates. On the other hand, delaying of soybean planting from May to July caused decreasing of survival plants% and increasing in damping off% caused by *F.oxysporu*m, *M.phaseolina and S.rolfs*. Such results might be due to the various climatic conditions The soil was warm and moist in First May but hot and dry in June and July. These conditions were responsible for stronger plants for first May than for June and July planting dates and soybeans susceptible seedling stage may grow out and escape damping-off in First May planting date. Such effect could be attributed, as was previously observed Landa *et al*., 2004; to the relatively higher monthly average temperature in July than In May. The results were in line with those reported by Navas Cortés *et al., 2000 and* Landa *et al*., 2004 they confirmed that sowing date was the factor with the greatest effect on Fusarium wilt and yield of chickpea also, and they reported that temperature was the primary determinant of the time to Fusarium wilt disease of chickpea onset. Sweets, 2008 reported that severity of soybean diseases such as seed decay, seedling blights and root rots caused by *Rhizoctonia, Macrophomina* and *Fusarium* vary with the environmental conditions. Bastidas *et al.,* 2008 .revealed that several diseases of soybean are related to stressful growing conditions.

These results indicate the importance of the early planting during May specially in Ismailia Governorate because high temperature.

In view of the apparent planting date, susceptibility of the cultivars tested, biocontrol could provide a means for reducing the incidence of wilt and root rot of soybean in addition to avoiding the use of fungicides.

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تـأثير مواعيـد الزراعـة و المقاومـة البيولوجيـة و حساسـية الأصـناف علـى مـرض موت البادرات و أعفان الجذور و الذبول في فول الصويا

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يصاب فول الصويا بالعديد من الأمراض و يعتبر مرض الذبول و أعفان الجذور من أهم الأمراض التي تصيب فول الصويا.وقد أجرى هذا البحث في محطة البحوث الزراعية بالإسماعيلية لاختبـار حساسـية أربعـة أصـناف مـن فـول الصـويا للإصـابة بفطـر فيوزاريـوم أوكسيسـبورم والريزوكتونيا سولانى والماكروفومينا فاسيولينا و الأسكليروشيم رولفسيي المسببة لأمراض مـوت البادرات وعفن الجذور الذبولى فى نباتات فول الصويا و ذلك تحت ظروف العدوى الصناعية حيث لـوحظ تبـاين فـى درجـة حساسية ألأربعـة أصـناف (جيـزة ١١١ , جيـزة ٣٥ , جيزة٢٢ و الصـنف كلارك) و كان الصنف جيزة ١١١ أكثز الأصناف مقاومة لكل الفطريات المختبرة .

كـذلك تـم اختبـار حساسية سبعة أصـناف مـن فـول الصـويا (جيـزة ١١١ , جيـزة ٣٥ , جيزة٢٢, جيزة٨٢, جيزة٢١ ,الصنف كارفود و الصنف كلارك) لمقاومـة أمراض موت البادرات وعفن الجذور الذبولى تحت ظروف العدوى الطبيعيـة فـى الحقل وكـان أيضـا الصـنف جيـزة ١١١ أكثز الأصناف مقاومة مع ملاحظة عدم ارتباط مقومـة الصنف بارتفـاع أو وزن النبـات وقد يرجـع هذا لطبيعة الصنف.

كمـا أجـرى هـذا البحـث لأسـتخدام بعـض المنتجـات الميكروبيـة مثـل الميكوسـتوب (استربتوميسس جريسيوفيريدس) , بيوزيد (تريكوديرمـا ألبيم), بيـوأرك (باسيلس ميجـاتيريم) مـع استخدام الريزولكس كمبيد فطرى فى مقاومـة أمراض مـوت البـادرات و اعفلان الجـذور و الـذبول تحت ظروف الصوبة. و قد أثبتت النتائج أن كل المعـاملات أدت الـى زيـادة نسبة النباتـات السليمة مقارنة بالكنترول الغير معامل الا أنه لوحظ اختلاف فى الكفاءة بين المعاملات المختلفة.

كذتك أجرى هذا البحث لدراسة تأثير ثلاثة مواعيد للزراعة على مرض موت البارد ات و أعفان الجذور والذبول تحت ظروف العدوبالصناعية ببعض الفطريات الكمنـة فـى التربـة وكـذلك تحت ظـروف العـدوى الطبيعيـة فـى الحقـل . وقـد كـان لميعـاد الزراعـة تـأثير معنـوى علـى نسـبة الأصابة وكان أنسب ميعاد للزراعة هو بدايـة شـهر مـايو بالنسبة لكل الأصـناف حيـث كانـت درجـة الحرارة مناسبة لنمو سريع و قوى للنباتات مما يـؤدى لسـرعة هـروب النبـات مـن الأصـابة ببعض الفطريلت الكامنـة فـى التربـة مقارنـة بارتفـاع درجـة الحـرارة فـى شـهر يوتيـه ويولبـو فـى محاقظـة الأسماعيلية و قد كانت استجابة الأصناف مختلفة لميعاد الزراعة حيث سجل الصنف جيزة ١١١أقل نسبة اصابة عند زراعته في بداية شهر مايو مقارنة بالأصناف الأخرى.

Table (8): Effect of microbial products and one fungicide on damping off, wilted plants, healthy plants of soybean seedling infected with some pathogenic fungi

Treatment	*Fusarium oxysporum*			*Rhizoctonia solani*			*Macrorhomina phaseol*			*Sclerotium rolfsii*		
	Damping off%	Survival plants %		Damping off%	Survival plants %		Damping off%	Survival plants %		Damping off%	Survival plants %	
		I	H		I	H		I	H		I	H
Mycostop	16.7cd	20b	63.3b	13.4c	13.3a	73.3ab	40b	10a	50bc	26.7b	13.3bc	60b
BioARC	26.7bc	26.8ab	46.7c	30b	16.7a	53.3c	53.3ab	10a	36.6C	26.7b	36.6abc	36.7c
BioZeid	30b	26.7ab	43.3c	23.4b	13.3a	63.3b	43.3b	10a	46.7C	16.7b	26.7abc	56.6b
Rhizolex	16.7cd	26.6ab	56.7c	13.3c	6.7a	80a	20c	16.7a	63.3B	3.4c	53.3a	43.3c
Infested soil (Control 1)	43.3a	33.4a	23.3d	83.3a	10a	6.7d	66.7a	10a	23.3D	60a	23.3bc	16.7d
Non-infested soil (Control2)	10d	3.3c	86.7a	10c	3.3a	86.7a	10c	3.3a	86.7A	10c	3.3e	86.7a
LSD	11.8	9.37	16.23	15.1	14.52	15.68	16.2	18.7	15.6	13.2	28.1	8.36

= Infested Survival plants H = Healthy Survival plants

Figres in the same column followed by the same letters are not significantly different (p> 0.05) based on