Egypt. J. Phytopathol., Vol. **44**, No. 2, pp. 35-50 (2016)

Efficacy of Two Natural Extracts and Antioxidants in Comparison with Fungicides in Reducing Soybean Damping-off and Root-rot Diseases

Hala M. El-Gendy*, Sahar A. El-Sayed* and Abeer M. Mousa**

**Plant Pathol. Res. Instit., ARC, Giza, Egypt.*

*** Soil Water Res., Dept., Nuclear Res. Centre, Atomic Ener. Authority, Cairo, Egypt.*

Isolation trials from the rotten roots of soybean roots collected from three Egyptian governorates, *i.e.* Giza, Menoufia and Gharbia yielded many fungal isolates. The isolated fungi were purified and identified as *Alternaria* spp., *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Rhizoctoniasolani* and unknown fungi. Laboratory, greenhouse and field studies were conducted to study the effect of two natural extracts and antioxidants in comparison with two fungicides on the reduction of damping-off and root-rot diseases. *In vitro* studies showed that these treatments significantly reduced the linear growth of the tested fungi compared with control treatment.Greenhouse experiment revealed that all the tested treatments significantly reduced damping-off and root-rot compared to the untreated plants. The most effective treatment was obtained by the extracts of neem and Eucalyptus followed by the antioxidants salicylic and oxalic acid, which reduced both damping-off and root-rot diseases. Crop parameters, *i.e.* plant height, number of branches, number of pods, seed yield / plant, weight of 100 seed as well as the average weight of seed yield (kg)/plot, were recorded at harvest. Also, total N and crude protein in the seeds were greatly increased due to the tested treatments as well as the treatment with two fungicides: Vitavax-T and Monceren.

Keywords: Antioxidants, crop parameters, damping-off, fungicides, natural extracts, pathogenic fungi, root-rot and soybean.

Soybean (*Glycine max* L.) is one of the most important legume crops in Egypt and many other countries in the world. It is extensively, cultivated since it has high value and level of oil and protein seed content, which is used for animal feed, human food and medicine to treat a number of disorder (Sinclair and Backman, 1989). In Egypt, the total cultivated area with soybean in 2015 reached to 33893 feddan, which produced about 46671 ton (Annual Reports of Agricultural and Statist Department, Ministry of Agriculture, A.R.E, 2015). Soybean is liable to attack with several fungal, bacterial and viral diseases in addition nematode and physiological disorder (Abo-Zeid*et al*., 1987; Hartman *et al*., 1999 and Perez-Brandán*et al*., 2014). However, fungal diseases, especially damping-off and root-rot disease are the most constrain ones affecting the quality and quantity of seed production (Tachibana *et al*., 1971; Rizvis and Yang, 1996 and Carling *et al*., 2001). *Fusarium* spp., *Pythium* spp. and *R. solani* isolated from diseased soybean seedling in Iowa (USA) were the major

causal fungi associated with seedling disease complex of soybean and control of these pathogens have depended mainly on fungicides (Waraitch *et al*., 1986; Vyas, 1994 and Zein Al-Abdean*et al*., 2013). Application of garlic extract resulted in significant decrease in damping-off and root-rot incidence of cantaloupe caused by *Fusarium semitectum* (Michail and El-Khateeb, 1985) and root-rot bean (Gaffar*et al*., 1989). Soil amendments with neem (*Azadirachta indica*) seed cake are effective against *F. oxysporum, M. phaseolina* and *R. solani* (Etheridge and Bateman, 1998). Hussein (2005); El-Sayed and El-Shennawy (2009) and Abdel-Aziz (2011) found that plant extracts of many plants such as neem, lemon grass leaves and Eucalyptus resulted in great reduction to damping-off and root-tot diseases of many crops. In addition, several reports have been published on the use of salicylic acid and its derivative as potential activators for induction resistance in plants (Kanss *et al*., 1992; Karabulut *et al*., 2003; Smilanick *et al*., 2006 and El-Shennawy *et al*., 2009).

The objective of the present work is investigating the effect of two plant extracts and antioxidants in comparison with two fungicides on the growth of fungi responsible for causing damping-off and root-rot of soybean. The work was expanded to control both damping-off and root-rot diseases of soybean under greenhouse and field conditions, in addition to assessing the effect of the infection with both diseases on protein content in the produced seeds from the treated plants with the tested treatments.

Materials and Methods

Disease survey of soybean root-rot in different governorates:

Survey was carried out in three governorates in Egypt, namely Giza, Menoufia and Gharbia. Infected plants of soybean showing root-rot symptoms were collected from the surveyed fields. The average percentage of disease incidence was calculated as the number of rotted plants (infected plants) in relative to the total number of examined plants.

Disease incidence (%)=Number of rotted plant/Total number of plant assessed x 100

Isolation, purification and identification of fungi associated with soybean diseased plants:

Infected soybean roots were cut into small fragments, washed thoroughly with tap water, then sterilized with sodium hypochlorite solution (1 %), then dried between two sterilized filter papers. Fragments were then placed on potato dextrose agar (PDA) medium in Petri-dishes and incubated at 25°C for 7 days and observations were recorded (Chrictensen, 1975). The isolated fungi were purified using hyphal tip and single spore techniques then transferred individually to new PDA plates. Identification was carried out depending on the morphological features of the isolated fungi depending on the description of Gilman (1957); Booth (1971) and Domsch *et al*. (1980).

Pathogenicity tests:

Pathogenicity tests were carried out under greenhouse conditions at Sers El-Layian, Agric. Res. Stat. throughout the year 2013. At first, all fungal isolates which were isolated from rotten roots of soybean were tested for their pathogenic

potentialities on the susceptible cv. Giza 35 under greenhouse conditions in order to select the highly pathogenic isolates.

Pots (25 cm in diameter) were sterilized by dipping in 5% formalin solution for 15 min. and then left in open air until dryness. Soil was sterilized using 5% formalin solution by mixing thoroughly, then the treated soil was covered with plastic sheet for one week then plastic sheet was removed in order to allow complete formalin evaporation (Whitenhead, 1957). Fungi were individually grown on sand-barley (SB) medium (25 g clean sand, 75 g barley and enough water to cover the mixture). Flasks containing sterilized medium were inoculated with each fungus and incubated at 25°C for two weeks. Soil infestation with each individual fungal was carried out at the rate of 3% of soil weight (Metwelly, 2004). Potted soil was watered daily for a week to enhance fungal growth. Soil of control pots was mixed with the same amount of sterilized fungus-free sand barley (SB) medium. Ten soybean seeds were surface sterilized using 2 % sodium hypochlorite for 2 min, washed several times with sterilized water and then sown. Three replicate pots with a total of 30 seeds were used for each treatment.

Disease assessment:

Percentages of pre- and post-emergence damping-off as well as the healthy survived in each treatment were determined 15, 30 and 45 days after sowing, respectively using the formula according to El-Helaly *et al*. (1970):

Pre-emergence (%) = Number of non-germinated seeds/Total number of sown seeds x 100

Post-emergence (%) =Number of dead seedlings/Total number of sown seeds x 100

Survived plant (%) =Number of survived plants/Total number of sown seeds x 100

Laboratory experiment:

This experiment was conducted to investigate the effect of the natural extracts of neem and Eucalyptus leaves on the growth of the tested fungi. The extract was prepared by lending 10 g frozen plant material with 100 ml water for 60 minutes. The resultant was strained through a double cheese cloth fabric and centrifuged for 10 minutes at 3000 rpm. Sterilization was made by 0.2 um millipore filters. The extract was added at the rate of 1.5 ml/plate to gliotoxin fermentation medium (GFM) as described by Brain and Hemming (1945). Inoculation was done with *Fusarium solani, Rhizoctonia solani* and *Fusarium moniliforme*, control (check) plates were incubated under similar conditions at 25°C. Salicylic and oxalic acid at the rate of 10mM were prepared and added separately to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri-plates (9 cm in diameter). Plates were individually inoculated at the center with equal disks (5 mm) of 5 days old culture of the pathogenic fungi and incubated at 25°C for 5 days. The average of the fungal linear growth was then recorded. The effect of two fungicides Vitavax-T (carboxin thiram) and Monceren (pencycuron) on the fungal growth was tested at the concentration of 200 ppm based on the active ingredient. The prepared concentration of any of both fungicides was added to PDA medium just before solidification and poured in Petri-

dishes. Three Petri-dishes as replicates were used for each treatment. The percentages of reduction in fungal growth were calculated as the growth in different treatments relative to those in check treatment.

Greenhouse experiment:

Greenhouse experiment was carried out to evaluate the effect of the treatments of plant extracts, neem and Eucalyptus, antioxidants salicylic acid and oxalic acid as well as the fungicides Vitavax-T and Moncerenon the incidence of soybean damping-off and root-rot severity in pot experiment. Sterilized pots (25 cm in diameter) containing autoclaved sand-clay soil were inoculated with any of the tested pathogenic fungi inocula (Mazen, 2004). The inoculum was added to the autoclaved soil at the rate of 3% inoculum level and mixed thoroughly at the upper layer of the soil. The treatments were watered and left for one week to ensure the distribution of the inoculum to sand. Natural extract of neem and Eucalyptus, antioxidants (salicylic and oxalic acid) and the fungicides Vitavax-T and Monceren were tested for their efficacy in reducing the incidence of soybean damping-off and root-rot. Plant extract of neem and Eucalyptus leaves was prepared as 90 ml sterilized water + 10 ml plant extract (Hussein, 2005). Meanwhile, salicylic and oxalic acid were prepared at 10 mM (Ismail *et al*., 2004), Soybean seeds (cv. Giza 35) were soaked for two hr. in the tested plant extracts and antioxidants as well as water only as control treatment, just before sowing in the soil. Also, soybean seeds dressed with the fungicides Vitavax-T and Moncerenat the rate of 3 g/kg seeds (super film was used as adherent material at the rate of 10 ml/kg seeds) were used as a check. The same aforementioned methods were used without fungicides as control. Five seeds were sown in each pot and three replicates were presented for each treatment. Disease incidence was recorded as a percentage of pre- and post-emergence damping-off 15 and 30, respectively. Also, the survived plants were counted 45 days after sowing and root-rot severity was then assessed using the devised scale (0-5 %) according to Salt (1982) as follows:

$$DS\ \% = \text{Sum of } (n \times v)/5N \times 100$$

WhereDS = disease severity,n = number of roots in each category,v = numerical value of each category, N = total number of roots in samples.

Effect of the tested treatments on the incidence of damping-off and root-rot diseases as well as some crop parameters under field conditions:

These experiments were conducted in naturally infested soil at Sers El-Layian Agric. Res. Stat. The land has a back history of high infestation with the causal of damping-off and root-rot of many legume crops. The land was prepared for sowing soybean as usual. Seeds were sown in the first week of June during 2014 and 2015 growing seasons. The land was divided into plots (3x3.5 m) in complete randomized block design with three replications for each treatment. Each replicate has 4 rows of 3.5 m long. Two seeds were sown in each hill with 25 cm in between.

Seed treatment:

Soybean seeds cv. Giza 35 were treated with the plant extracts for two hours according to Hussein (2005). The dry method depends on mixing the seed with talcum powder laden with the extract and Arabic gum. The dried preparation was obtained by mixing 250 g of talc powder with 250 ml of the extract and spread till

drying. Antioxidants in controlling soybean damping-off and root-rot were assessed under field conditions. Soybean seeds were soaked in aqueous solution of 10 mM oxalic and salicylic acid for 12 h., while Vitavax-T and Monceren were used at the rate of 3 g/kg seeds. Three replicates were used for each treatment. Disease assessment was estimated and recorded as mentioned before. Also, yield components were assessed.

Disease assessment:

Pre-emergence damping-off was assessed 15 days after sowing and post-emergence damping-off and survived plants were assessed 30 and 45 days after sowing, respectively. Ten randomized plants in each plot were pull-off 60 after sowing to assess root-rot severity according to Salt (1982). Some crop parameters, i.e. plant height, number of branches, number of pods per plant, weight ofone hundred seeds per plant as well as the average weight of seed (kg)/ plot were recorded at harvest.

Determination of protein:

Seed samples were taken, dried in an electric oven at 70° till constant weight and ground for determination of total N calorimetrically by using orange G dye method according to Hafez and Mikkelsen (1981). Crude protein of seeds was calculated by multiplying total N % × 6.25.

Results

Isolation, purification and identification of the causal organisms:

Isolation trials from the rotten roots of soybean roots collected from three Egyptian governorates, *i.e.* Giza, Menoufia and Gharbia yielded many fungal isolates. The isolated fungi (Table 1) were purified and identified as *Alternaria* spp., *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Rhizoctonia solani* and unknown fungi (18 isolates). Concerning the occurrence of the isolated fungi, samples of Gharbia governorate recorded the highest occurrence through 97 isolates, followed by samples of Giza governorate (94 isolates) then samples of Menofia governorate were 88 isolates. The fungus *R. solani* followed by *F. moniliforme* recorded the highest occurrence with 50 and 49 isolates, respectively. Meanwhile, *F. oxysporum* and *Alternaria* spp. recorded the lowest occurrence with 28 and 31 isolates, respectively, whereas the occurrence of the unknown fungi recorded 42 isolates.

Table 1.Occurrence and frequency of the isolated fungi from rotten roots of soybean collected from three governorates

Isolated fungi	Giza		Menofia		Gharbia		Total	
	Occ[1]	% Freq[2]	Occ[1]	% Freq[2]	Occ[1]	% Freq[2]	Occ[1]	% Freq[2]
F. semitectum	10	10.6	13	14.8	15	15.5	38	14.0
F. solani	14	14.9	12	13.6	15	15.5	41	15.1
F. moniliforme	16	17.0	15	17.1	18	18.6	49	18.1
F. oxysporum	9	9.6	10	11.4	9	9.3	28	10.3
R. solani	15	16.0	17	19.3	18	18.3	50	18.5
Alternaria sp.	12	12.8	10	11.4	9	9.3	31	11.4
Unknown Fungi	18	19.2	11	12.5	13	13.4	42	15.5
Total	94	------	88	------	97	-------	271	------

1. Occ = Occurrence and 2. % Freq = % Frequency.

Pathogenicity test:

In this experiment, three *Fusarium* spp., i.e. *F. moniliforme*, *F. solani* and *R. solani* were selected from the isolated fungi from the inspected governorate to test their pathogenic capabilities. Results presented in Table (2) revealed that all the tested isolates infected soybean cv. Giza 35 caused pre- and post-emergence damping-off. The highest infection was recorded by *Fusarium solani* (Menoufia isolate) followed by *F. moniliforme* (Giza isolate). Nevertheless, all tested isolates caused post emergence damping-off infection.

Table 2. Pathogenicity tests of the fungi isolated from rotten soybean roots

Isolated fungi	Damping-off (%)		Survived plant (%)
	Pre-emergence	Post-emergence	
F. moniliforme	6.77	10.00	83.23
F. moniliforme	13.33	6.67	80.00
F. moniliforme	3.33	6.67	90.00
F. solani	20.00	13.33	66.67
F. solani	13.33	10.00	76.67
F. solani	6.67	3.33	90.00
R. solani	13.33	16.67	70.00
R. solani	16.67	6.67	76.66
R. solani	3.33	10.00	86.67
L.S.D. at 0.05	6.25	5.13	9.23

Effect of the tested plant extracts, antioxidants and fungicides on the linear growth of the pathogenic fungi in vitro:

The effect of the tested treatments on the mycelial growth of the tested fungi (Table 3) indicated that the tested plant extracts, antioxidants and fungicides significantly reduced the linear growth of the pathogenic fungi compared to the

control. Neem was the most effective plant extract followed by Eucalyptus in concern compared to control. The antioxidants salicylic acid and oxalic acid revealed a significant reduction of fungal linear growth. Salicylic acid was the most effective antioxidant in reducing fungal linear growth compared to control. Also, the fungicides Vitavax-T and Monceren significantly reduced the linear growth of the pathogenic fungi.

Effect of two plant extracts and antioxidants in comparison with two fungicides on the incidence of damping-off, survived plants and root-rot severity under greenhouse conditions:

Results in Table (4) indicated clearly that the plant extract reduced significantly pre- and post-emergence damping-off infection of soybean plants infected with the causal pathogens comparing with control. While the antioxidants salicylic acid and oxalic acid reduced significantly pre- and post-emergence damping-off. Also, the fungicides more effective from plant extracts and antioxidants compared to control.Vitavax-T was the best followed by neem and salicylic acid, respectively. On the other hand, the lowest effective were Eucalyptus and oxalic acid. In this respect, (Table 5) the highest percentage of healthy survived plants obtained when Vitavax-T, neem and salicylic acid were used, respectively. The best treatment was fungicides Vitavax-T and Monceren compared to the control.

Table 3.Effect of two plant extracts and antioxidants in comparison with two fungicides on the linear growth of three tested pathogens *in vitro,* after incubation at 25°C for 5 days

Treatments	*F. moniliforme*	*F. solani*	*R. solani*	Mean
Neem	20	20	30	23.3
Eucalyptus	30	30	40	33.3
Salicylic acid	35	20	30	28.3
Oxalic acid	40	25	35	33.3
Vitavax-T	0.0	0.0	0.0	0.0
MoncerenCont	0.0	10	20	10.0
rol	90	90	90	90
Mean	20.8	17.5	25.8	-

L.S.D. at 0.05: Treatments (T) = 1.9, Fungi (F) = 2.1, T × F = 4.0

Table 4.Influence of two plant extracts and antioxidants in comparison with two fungicides on the incidence of soybean damping-off, greenhouse experiment

Treatments	% Pre-emergence			Mean	% Post-emergence			Mean
	F. solani	*R. solani*	*F. moniliforme*		*F. solani*	*R. solani*	*F. moniliforme*	
Neem	25.0	23.3	13.3	20.5	16.7	16.7	10.0	14.5
Eucalyptus	25.0	25.0	16.7	22.2	20.0	20.0	13.3	17.8
Salicylic acid	30.0	25.0	13.3	22.8	20.0	16.7	13.3	16.7
Oxalic acid	10.0	28.0	20.0	19.3	22.2	20.0	16.7	19.6
Vitavax-T	13.3	0.0	0.0	4.4	10.0	10.0	0.0	6.7
MoncerenContr ol	33.3	10.0	3.3	15.5	10.0	6.7	3.3	6.7
	22.3	30.0	25.0	25.8	30.0	25.3	22.2	25.9
L.S.D. at 0.05	Pathogen (P) = 6.3 Treatments(T) = 6.9 P × T = 12.2				= 3.1 = 4.9 = 9.2			

Table 5. Influence of two plant extracts and antioxidants in comparison with two fungicides on the percentages of survived plants and root-rot severity, greenhouse experiment

Treatments	% survived plants			Mean Mean	Disease severity (%)			Mean
	F. solani	*R. solani*	*F. moniliforme*		*F. solani*	*R. solani*	*F. moniliforme*	
Neem	63.3	60.0	76.7	66.7	25.6	30.0	25.6	27.1
Eucalyptus	55.0	55.0	70.0	60.0	28.8	33.3	30.0	30.7
Salicylic acid	55.0	58.3	73.3	62.2	25.6	30.0	25.0	26.9
Oxalic acid	47.8	52.0	63.3	54.4	33.3	33.3	35.3	34.0
Vitavax-T	80.0	90.0	100.0	90.0	11.1	6.7	6.7	8.2
MoncerenContro l	76.7	80.0	93.3	83.3	13.3	10.0	10.0	11.1
	36.7	44.7	52.8	44.7	65.6	60.0	55.6	60.4
Mean	59.2	62.9	75.6	-----	29.0	29.0	26.9	-----
L.S.D. at 0.05	Pathogen (P) = 7.5 Treatments(T) = 8.9 P × T = 13.9				= 5.9 = 6.0 = 11.1			

Table 6.Effect of treating soybean seeds with the tested plant extracts, antioxidants and fungicides on the incidence of damping-off and root-rot severity during 2014 and 2015 growing seasons under field conditions

Treatments	Season 2014				Season 2015			
	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survived plants (%)	Root rot severity (%)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survived plants (%)	Root rot severity (%)
Neem	3.33	6.67	90.00	7.77	3.33	6.67	90.00	8.22
Eucalyptus	10.00	10.00	80.00	8.88	6.67	10.00	83.33	9.99
Salicylic acid	3.33	10.00	86.67	8.88	6.67	6.67	86.66	8.88
Oxalic acid	13.33	10.00	76.67	11.11	13.33	13.33	73.34	12.22
Vitavax-T	0.00	3.33	96.67	6.66	3.33	3.33	93.34	5.55
MoncerenControl	3.33	6.67	90.00	7.77	6.67	3.33	90.00	8.11
	20.00	25.00	55.00	18.88	25.00	25.00	50.00	23.33
L.S.D. at 0.05	5.23	6.22	10.00	1.63	5.98	8.73	12.09	1.37

Table 7.Effect of soaking soybean seeds in the tested treatments on some crop parameters of soybean under field conditions during 2014 and 2015 growing seasons

Treatments	Season 2014					Season 2015				
	Plant height (cm)	No. of branches / plant	No. of pods / plant	100 seeds (g)	Yield (kg)/ plot	Plant height (cm)	No. of branches / plant	No. of pods / plant	100 seeds (g)	Yield (kg)/ plot
Neem	90.17	4.00	89.66	16.00	9.87	87.33	4.00	87.00	16.00	9.12
Eucalyptus	85.13	3.91	85.24	15.60	8.77	83.16	3.90	83.19	15.55	8.60
Salicylic acid	87.33	4.00	87.23	15.95	9.14	85.00	4.00	84.97	15.95	8.87
Oxalic acid	80.17	3.85	85.00	15.70	8.75	83.00	3.70	82.43	15.50	8.60
Vitavax-T	92.17	4.57	95.66	17.16	10.51	90.66	4.20	90.00	17.00	10.23
MoncerenControl	90.00	4.37	91.46	16.30	9.88	88.00	4.20	87.00	16.00	9.75
	75.23	3.25	81.00	15.57	8.00	72.12	3.00	75.00	15.00	7.92
L.S.D. at 0.05	2.11	0.91	2.08	1.98	2.19	1.95	0.89	1.75	1.18	1.79

Table 8.Total nitrogen and protein constitutes of soybean dry seeds as affected by plant extracts, antioxidants and fungicides

Treatments	Season 2014		Season 2015	
	Total nitrogen (%)	Protein (%)	Total nitrogen (%)	Protein (%)
Neem	5.80	36.25	5.78	36.13
Eucalyptus	5.70	35.63	5.60	35.00
Salicylic acid	5.75	35.94	5.63	35.19
Oxalic acid	5.50	34.38	5.40	33.75
Vitavax-T	6.10	38.13	6.00	37.50
Monceren	5.90	36.88	5.95	37.19
Control	5.20	32.50	5.15	32.19
L.S.D. at 0.05	0.68	2.86	0.62	2.57

Effect of treating soybean seeds with plant extracts, antioxidants and fungicides on root-rot under field conditions:

Data shown in Table (6) show the influence of plant extracts, antioxidant and fungicides on root-rot of soybean under field conditions during two seasons. The results show significant reduction to both pre- and post-emergence damping-off compared to control. Vitavax-T and Monceren were the best in reducing pre- and post-emergence damping-off and consequently increased the survived plant compared to the control followed by Neem, Salicylic acid, Eucalyptus and Oxalic acid. In general, the protection degree offered by the fungicides and plant extracts application was much higher than antioxidants.

Effect of plant extracts, antioxidants and fungicides on some crop parameters of soybean under field conditions:

Data in Table (7) indicate that the tested plant extracts, antioxidants and fungicides significantly increased plant height, compared to untreated ones. The obtained results show that all treatments increased the number of pods, seeds weight (kg)/ plant, 100 seeds weight and seed yield (kg)/plot. Vitavax-T was the best treatment followed by Neem extract then Salicylic acid, respectively. All the tested treatments showed also a significant protection against the disease over the control. Data in Table (8) show that during the two studied seasons total nitrogen (N) and crude protein concentration in cv. Giza 35 soybean seeds were increased significantly each of total N and protein comparing to the control.

Discussion

Damping-off and root-rot diseases of soybean are considered critical factor in soybean production worldwide. The results of this study revealed that such diseases are caused by several soil borne pathogens including *Rhizoctonia solani*, *Fusarium solani* and *Fusarium moniliforme*. These pathogens have been widely reported as the causal organism of soybean damping-off and root-rot (Rizvis and Yang, 1996; Hartman *et al*., 1999; Carling *et al*., 2001; Kiprovsk *et al*., 2012 and Abdel-Monaim

et al., 2012). As for the factors affecting soybean root-rot disease *in vitro*, *in vivo* and field conditions, the results indicated that all the tested plant extracts significantly reduced the linear growth of all the tested fungi, where both salicylic and oxalic acid were effective antioxidants. Moreover, application of the plant extracts and antioxidants under greenhouse conditions reduced significantly pre- and post-emergence damping-off and increased the survived plants of soybean plants compared with the control. These results are in agreement with those obtained by Michail and El-Khateeb (1985); Gaffer *et al*. (1989); Nafie and Mazen (2008) and Kiprovski *et al*. (2012).

Acetyl salicylic acid (ASA) is a commercially available form of salicylic acid. It is known that in aqueous solutions, ASA is hydrolyzed almost entirely to SA, which is active ingredient (Mitchell and Broadhead, 1967). However, salicylic acid is an endogenous growth regulator with phenolic nature, which participates in regulation of several physiological processes in plants, such as stomatal closure, ion uptake, inhibition of ethylene biosynthesis and transpiration (Khan *et al*., 2003; Shakirova, *et al*., 2003 and Foyer and Noctor, 2005) .

Doubrava *et al*. (1988) mentioned that inducing acquired resistance is persistent and generally is pathogen nonspecific. Also, Larcke (1981) reported that phytoalexines accumulation, which is elicited at the site of application, may be responsible for localized protection and induces systemic acquired resistance that sensitizes the plant response rapidly after infection. These responses induce phytoalexines accumulation and lignifications and enhance activities of chitinase and β-glucanase (Dean and Kuc, 1985 and Metranx and Boller, 1986 and Singh *et al*., 2012). Kessmann *et al*. (1994) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad-spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection. In addition, Vernooij *et al*. (1994) mentioned also that salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance to plant pathogens, but is required in signal transduction. So, resistance might be correlated with the production of oxidative enzymes in the treated healthy and diseased plant tissues. In this respect, Melo *et al*. (2006) mentioned that polyphenoloxidase and peroxidase are enzymes of broad spectrum among plants catalyze the hydroxylation of monophenols to O-diphenols and their oxidation to o-diquinones. He added that quinines are highly reactive molecules that can spontaneously complex various types of molecules into large types.

The obtained results indicated that Neem extract was more effective than that of Eucalyptus in reducing the growth of the tested fungi. In addition, under greenhouse conditions, using of Neem and Eucalyptus extracts reduced significantly pre- and post-emergence damping-off infection as well as increased significantly the survived plants compared to the control. Similar results were mentioned by Etheridge and Bateman (1998); Hussein (2005); El-Sayed and El-Shennawy (2009) and Abdel-Aziz (2011). Concerning the effect of the fungicides, *in vitro* results indicated that both Vitavax-T and Monceren decreased significantly the fungal linear growth of the tested pathogenic fungi compared with control treatment. Under greenhouse

conditions, both fungicides reduced significantly pre- and post-emergence damping-off compared with control treatment. It could be concluded that, application any of plant extracts, antioxidants and fungicides under field conditions reduced significantly pre- and post-emergence damping-off compared to untreated plant (control). The obtained results of 2014 and 2015 seasons, revealed a significant effect of plant extracts, antioxidants and fungicides on plant height, number of pods/plant, 100 seed weight and yield (kg)/plot. The obtained data are in accordance with those obtained by Karabulut *et al*. (2003), Smilanic *et al*. (2006) and El-Shennawy *et al*. (2009).

References

Abdel-Aziz, N.M. 2011. Pathological studies on damping-off, root-rot and wilt diseases of pea in Egypt and Algeria. Ph.D. Thesis, Instit. Res. and Afr. Studies, Cairo Univ.

Abdel-Monaim, M.F.; Ismail, M.E. and Morsy, K.M. 2012. Induction of systemic resistance in soybean plants against Fusarium wilts disease by seed treatment with benzothiadiazole and humic acid. *Afr. J. Biotechnol.,***11**(10): 2454-2465.

Abou-Zeid, N.M.; Abada, K.A. and Ragab, Mona M. 1987. Effect of different fungicides *in vitro* and *in vivo* on the control of soybean damping-off disease. *Agric. Res. Rev.*,**65**: 262-269.

Annual Reports of Agricultural and Statist Department, Ministry of Agriculture, A.R.E., 2015.

Booth, C. 1971. The Genus *Fusarium* spp. 1-11 Commonwealth Mycological Institute, Kew, Surrey, England.

Brain, P.W. and Hemming, H.G. 1945. Gliotoxin a fungistatic metabolic product of*Trichoderma viride*. *Ann. Appl. Biol.,***32**: 214-220.

Carling, D.E.; Kuninage, S. and Brainard, K.A. 2001. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctoniasolani*. Anartomosis (Group-2 "AG-2" and AG-B1). *Phytopathology*, **91**: 43-50.

Christensen, C.M. 1975. Survey of faba bean (*Viciafaba*L.) diseases in the main faba growing regions of Syria. *Arab J. Plant Protec.,***17**: 113-116.

Dean, R.A. and Kuc, J. 1985. Induced systemic protection in plants. *Trends Biotechnol.,***3**: 125-128.

Domsch, K.H.; Games, W. and Anderson, T.H.A. 1980. Compendium of soil fungi, Vol. 1 and 2 Academic press, London.

Doubrava, N.; Dean, R. and Kuc, J. 1988. Induction of systemic resistance to anthracnose caused by *Colltotrichum lagenarum* from spinach and hubar leaves. *Physiol. Mol. Plant Pathol*., **33**: 60-70.

El-Helaly, A.F.; Elarosi, H.M.; Assawah, M.W. and Abd-Wafa, M.T. 1970. Studies on damping-off and root-rots of bean in UAR (Egypt). *Egypt J. Phytopathol.*,**2**: 41 - 57.

El-Sayed, Sahar A. and El-Shennawy, Rania Z. 2009. Control of root-rot and wilt diseases of lupine. *J.Advan. Agric. Res.*, Alexandria Univ. Fac. Agric., Saba Basha, **14**(3): 655 – 676.

El-Shennawy, Rania Z.; El-Sayed, Sahar A. and Ismail, I.A. 2009. Evaluation of the efficiency of some environmentally safe means for controlling graymold of lentil. *J. Advan. Agric. Res.*, Alexandria Univ. Fac. Agric., Saba Basha, **14**(4): 839 – 856.

Etheridge, J.V. and Bateman, G.L. 1998. Fungal diseases of white lupine (*Lupine albus*) and their control. Brighton crop protection conference. Pests of diseases volumes 3 Proceedings of an Inter. Conf., Brighton, UK, 16-19 November **1**: 1005 – 1010.

Foyer, R.C.H. and Noctor, G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ.*,**8**(8):1056–1071.

Gaffar, A.A.; El-Khateeb, Sanaa R.; Khalif, A.E.Z. and Mousa, M.M. 1989. Control of bean damping-off and root-rot by plant extracts growth regulators and fungicides in relation to root nodules bacteria. *Menoufia J. Agric. Res.*,**14**: 64-72.

Gilman, J.C. 1957. A Manual of Soil Fungi. The Iowa State College Press, Iowa, USA., 450 p.

Hafez, A. and Mikkelsen, D.S. 1981. Colorimetric determination of nitrogen for evaluating the nutritional status of rice. *Commnu.*,**12**(1): 61 – 69.

Hartman, G.L.; Sinclair, J.B. and Rupe, J.C. 1999. Compendium of Soybean Diseases. Fourth Edition, The American Phytopathological Society, 128 p.

Hussein, Manal Y. 2005. Evaluation of some plant extracts in controlling damping-off and root-rot of sugar beet. *Minufiya J. Agric. Res.*,**3**: 867 – 876.

Ismail, Y.M.; Soliman, G.L.; Mosa, A.A. and Gamil, W.A. 2004. Inducer resistance against chocolate spot disease of faba bean. *J. Agric. Res. Sci., Mansoura Univ.*,**29**(3): 1205 – 1211.

Larcke, P. 1981. Alternative chemical agents for controlling plant diseases. *Phil. Trans. Res. Soc.*, **2**: 83 – 101.

Kanss, H.; Theisinger-Hinkel E.; Mindermann, R. and Conroth, U. 1992. Dichloroisonicotinic acid and salicylic acid inducer of systemic acquire resistance enhance fungi elicitor response in parsley cells. *The Plant J.*, **2**(5): 655 – 660.

Karabulut, O.A.; Pursa, G. and Mansour, M. 2003. Near-harvest applications of *Metschniikowiafructicola*, ethanol and sodium bicarbonate to control post-harvest diseases of grape in control California. *Plant Dis.*,**87**: 1384 – 1389.

Kessmann, H.; Sataub, T.; Hofmann, C.; Meatzke, T. and Herzog, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Ann. Rev. Phytopathol*., **32**: 439 – 459.

Khan, W.; Prithiviraj, B. and Smith, D.I. 2003. Photosynthetic response of corn and soybean to foliar application of salicylates. *J. Plant Physiol.*,**160**: 485.

Kiprovski, B.; Malencic, D.J.; Popovic, M.; Budakov, D.; Stojsin, V. and Balesevic-Tubic, S. 2012. Antioxidant systems in soybean and maize seedlings infected with *Rhizoctoniasolani*. *J. Plant Pathol*.,**94**(2): 313-324.

Mazen, M.M. 2004. Resistance induction against disease of faba bean crop. Ph.D. Thesis, Fac. Agric. Suez Canal Univ., Egypt.

Melo, G.A.; Shimizu, M.M. and Mazzafera, P. 2006. Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochemistry*., **67**: 277-285.

Metranx, J.D. and Boller, T. 1986. Local and systemic induction of chitinase in cucumber plants in response to fungal, bacterial and viral infections. *Physiol. Mol. Pathol.*, **28**: 161 – 169.

Metwelly, M.M.M. 2004. Resistance induction against diseases of faba bean crop. Ph.D. Thesis, Plant Pathol. Fac. Agric. Suez Canal Univ. Egypt.

Michail, S.H. and El-Khateeb, Sanaa, R. 1985. *Fusarium semitectum* a causal organisms of fruit rot, damping-off and root-rot of cantaloupe with reference to biological control. *Menoufia J. Agric. Res.*,**10**: 631 – 636.

Mitchell, A.G. and Broadhead, J.F. 1967. Hydrolysis of solubilized aspirin. *J. Pharm. Sci.*, **56**(10): 1261- 1266.

Nafie, E. and Mazen, M. 2008. Chemical-induced resistance against brown stem rot in soybean: The effect of benzothiadiazole. *J. Appl. Sci. Res.*,**4**: 2046-2064.

Perez-Brandan, C.; Huidobro,J.; Grümberg, B.; Scandiani, M.M.; Luque, A.G.; Meriles. J.M. and Vargas-Gil, S. 2014. Soybean fungal soil-borne diseases: a parameter for measuring the effect of agricultural intensification on soil health. *Can. J. Microbiol.*,**60**(2):73-84.

Rizvis, S.A. and Yang, X.B. 1996. Fungi associated with soybean seedling disease in Iowa. *Plant Dis.*,**80**: 57 – 60.

Salt, G.A. 1982. Factors affecting resistance to root-rot and diseases (eds. Hawtin, G. and Webb, C.); faba bean improvement. ICARDA, Aleppo, Syria, 260-270.

Shakirova, F.M.; Sakabutdinova, A.R.; Berzukova, M.V.; Fathutdinova, R.A. and Fatkhutdinova, D.R. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.*,**164**: 317.

Sinclair, J.B. and Backman, P.A. 1989. Compendium of soybean diseases (3rded). American phytopathology Society press, St Paul M.N.

Singh, S.; Verma, A. and Dubey, V.K. 2012. Effectivity of anti-oxidative enzymatic system on diminishing the oxidative stress induced by aluminium in chickpea (*Cicer arietinum* L.) seedlings. *Braz. J. Plant Physiol.,* **24**(1): 47-54.

Smilanick, J.L.; Mansour, M.P. and Sorenson, D. 2006. Pre- and post-harvest treatments to control green mould of citrus fruit during ethylene degree a sing. *Plant Dis.*,**90**: 89 – 96.

Tachibana, H.; Jowett, D. and Fehr, W.R. 1971. Determination of losses in soybeans caused by *Rhizoctoniasolani*. *Phytopathology*, **61**: 1444 – 1446.

Vernooij, B.; Friedrich, L.; Morse, A.;Reist, R.; Kolditz-Jawhar, R.; Ward, E.; Uknes, S.;Kessmann, H. and Ryals, J. 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant Cell*,**6**(7): 959–965.

Vyas, S.C. 1994. Integrated biological and chemical control of dry root-rot on soybean.*Indian J. Mycol. Plant Pathol.*,**24**: 132 – 134.

Waraitch, K.S.; Kanwar, R.S. and Pen-Kumar, B.B. 1986. Fungicidal control of Sclerotium root-rot of sugar beet (*Beta vulgaris*) caused by *Sclerotium rolfsii*. *Indian Pathol.*,**39**: 100 – 120.

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

Zein Al-Abdean, W.; Abo-Elyousr, K.A.M.; Hassan, M.H.A. and El-Sheakh, M.M. 2013. Molecular characterization of *Rhizoctoniasolani* isolates the incident of soybean root-rot. *Archives Phytopathol. Plant Protec.*,**46**(9): 1108 – 1117.

(Received 10/06/2016;
in revised form 20/07/2016)

فاعلية بعض المستخلصات الطبيعية ومضادات الأكسدة والمبيدات فى الحد من الإصابة بمرضي سقوط البادرات وعفن الجذور فى فول الصويا

هالة محمد رشاد الجندى*، سحر عباس السيد*، عبير محمد موسى أبوصير**

* معهد بحوث أمراض النبات – مركز البحوث الزراعية – الجيزة – مصر

** قسم بحوث الأراضى والمياه – مركز البحوث النووية – هيئة الطاقة الذرية – القاهرة- مصر

أُجريت هذه الدراسة فى كلٍ من المعمل والصوبة والحقل بغرض استحثاث المُقاومة فى نباتات فول الصويا ضد مرض عفن الجذور فقد أدت إضافة هذه المواد الطبيعية إلى تثبيط النمو الميسليومى المُمرض مُقارنةً بالكنترول.تحت ظروف الصوبة وجد أن هذه المُعاملات قد أدت إلى انخفاض نسبة موت البادرات وزيادة عدد النباتات التى تم مُعاملتها بالنيم والكافور ومُضادات الأكسدة والمبيد الفيتافاكس تى والمونسرين كمُقارنة وذلك بمُقارنة هذه المُعاملات بالكنترول غير المُعامل.تحت ظروف الحقل وجد أن مُعاملة البذور بمُستخلص النيم والكافور ومضادات الأكسدة أو بالمبيد الفطرى الفيتافاكس تى والمونسرين أدت إلى انخفاض نسبة موت البادرات وكذلك زيادة عدد القرون ووزن الـ100 بذرة وزيادة فى النسبة المئوية لكلٍ من النيتروجين والبروتين فى البذور الجافة بالرغم من أن المُعاملة بالمبيد أعطيت أعلى المُعاملات تأثيراً ولكننا نُوصى باستخدام المُستخلصات الطبيعية كمُستخلص النيم والكافور ومُضادات الأكسدة كحمض السلسيليك والأوكسيليك بدلاً من استخدام المُبيدات المُقاومة للأمراض لِمَا تُسببه من خطرٍ على صحة الإنسان والميكروفلورا فى التربة، لذا نُوصى باستخدام المُستخلصات الطبيعية ومُضادات الأكسدة بدلاً من استخدام المُبيدات لمُقاومة أمراض عفن الجذور فى فول الصويا.