Safety Alternatives to Control Soybean Root and Foliar Diseases

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Abstract: *Damping-off, root rot, charcoal rot and antheracnose are important diseases which attack soybean causing great losses in quality and the total yield. All plant extracts used inhibited linear growth of F. solani, R. solani, M. phaseolina and C. dematium. Allium cepa was the most effective followed by Atrpilex sp., Mentha viridis, Allium sativum and Eucalptus globules. On the other hand, seed treatment with plant extracts decreased percentage of disease incidence in pots conditions. Atrpilex sp., and Allium sativum were the most effective followed by Allium cepa, Mentha viridis and Eucalptus globules under greenhouse conditions. At the same time, spraying soybean plants with the extracts 24 h before inoculation with anthracnose pathogen reduced disease severity, Atrpilex sp., and Allium cepa were the most effective. Application of chemical inducers in pots as seed treatment resulted in a great reduction in percentage of soil borne incidence. Also spraying soybean plants with chemical inducers before inoculation with C. dematium diminished anthracnose disease. EDTA and SA were the most effective inducers and increased percentage of soil borne diseases incidence in pots. Bio-ARC was the most effective followed by BioZied and Rhizo-N. Also, Bio-ARC was the most effective biocide as foliar treatment in controlling anthracnose followed by BioZied and Rhizo-N.*

INTRODUCTION

Soybean plants are severely attacked by many pathogenic fungi 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. It is well established that extensive application of chemical treatments in controlling plant diseases resulted in harmful effects on the environments. Therefore, search for an alternative safe effective and economic ways for controlling plant pathogens is greatly needed.

It is well established that extensive application of chemical treatments in controlling plant disease resulted in harmful effects on the environments. Yousef (1991), Attia (1995) and Osman *et al.*, (1996) found that garlic extract caused reduction in the mycelial growth and inhabited spore germination of *Macrophomina phaseolina, Fusarium solani, Rhizoctonia solani and Sclerotinia sclerotium.*

Induced or acquired resistance is the one of environmentally safe method to control plant diseases. EDTA, SA, K_2 HPO_4, Cu Cl are all this chemical inducers which play role in induced resistance by stimulating biosynthesis of different families of P–R - proteins (Raskin,1992) and increasing the activities of chitinase, peroxidase and B–13– glucanase (Abd– El – Kareem *et al.*, 2001, Prachi and Singh, 2002 and Tilak *et al.*, 2002). Seed treatment with EDTA was in line with those reported by Walters and Murray (1992) and Marry *et al.* (1995). On the other hand, Doubrava *et al.*, (1988), Gottstein and Kuce (1989) indicated that treatment with K_2HPO_4 or phosphate is associated with systemic resistant to different diseases. Where as Hassanein *et al.* (2000) and Abou-Zeid *et al.* (2003) evaluated the *Bacillus subtilis* which was biologically controlled damping –off and root rot diseases in different crops.

Therefore the present work was designed to study the effect of using natural plant products, induced resistance and bio-agents for controlling soybean root and stem diseases as safety alternatives in environment.

MATERIALS AND METHODS

1- Plant Products:

Plant extracts were prepared by mixing 100 gm fresh plant material (leaves of *Eucalyptus globules* (Camphor), *Mentha viridis* (Mint), and *Atrpilex sp* (Atrpilex), *Allium sativum* (Onoin bulbs) and *Allium cepa* (Garlic cloves)) with 100 ml of water using electric blender for 5 minutes. The plant extracts were filtrated through double layers of chees cloth. Centrifuged at 3000 rpm for 15 minutes and sterilized using Watman filter paper.

a- In vitro

(1) Effect of different concentrations of plant extracts on linear growth and sporulation:

The plant extracts were added to autoclaved PDA medium (45°C) to obtain final concentrations of 10, 15, 20, and 25% and then poured into Petri dishes. Plates used as control. Plates were then inoculated at the center by equal discs (5mm in diam) taken from 7 days old cultures of the pathogenic fungi, *i.e. F. solani, R. solani, M. phaseolina* and from 10 days old culture of *C. dematium*. Four replicates were used for each concentration. Plates were incubated at 28 C for fungi *F. solani, R. solani and M. phaseolina* and 25 C for *C. dematium*. Linear growth of each tested fungus was measured when any of the pathogenic fungi completely coverd the surface of the medium in the control treatment by taking two perpendicular diameters (in mm) and averaged.

The inhibition percent was calculated according to Abd– El– Rhman (2001). Also, number of spores/ml of *F. solani* and *C. dematium* was calculated using a

haemocytometer slide, and the percentage of inhition sporulation was calculated.

(2) Effect of different concentrations of plant extracts on spore germination of *F. solani* and *C. dematium*:

Different concentrations from each plant extract, *i.e.* 10, 15, 20, and 25 were prepared using distilled water to study their effect on spore germination of *F. solani* and *C. dematium*.

The previous mentioned concentrations were used to prepare spore suspension of *F. solani* and *C. dematium*. Drop from spore suspension of each concentration was pipetted by sterilized pipette on germination slides, other germination slides with drops from spore suspension prepared using distilled water were used as control. *F. solani* slides were incubated at 28 ℃ and *C. dematium* slides was incubated at 25 ℃ for 18 h, then percentage of spore germination was recorded through microscopic examinations. Also percentage of inhibition of germination was calculated as mentioned before.

b- In vivo:

(1)Effect of different plant extracts on disease incidence:

This experiment was carried out under greenhouse conditions using sterilized pots (20 cm diam.). Pots were immerse them in 5% formaline solution for 15 minutes and then left for seven days before using, then were filled with sterilized clay soil. Soil was infested with *F. solani, R. solani,* and *M. phaseolina* by adding the inoclum of each the tested fungi to the sterilized soil at the rate of 5% soil weight (w/w). The fungal inoculum was mixed thoroughly with the 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 growth. Surface sterilized soybean seeds (cv. Giza-35) were treated with 4% solution of carboxymethyl cellulase (CMC) as sticker, then air dried. Seeds were soaked for 1h in the plant extracts at concentrations 25 and 50%. The same mentioned methods were used without plant extracts for control treatment.

Five seeds were sown per pot, and three replicates were used for each treatment. Percentage of pre-and post- emergence damping- off, root rot and healthy survived plants were recorded.

(2) Effect of different plant extracts on each severity of *anthracnose disease:*

The crude extracts of plant extracts were diluted to 25 and 50% concentration using distilled water and sprayed as foliar spray on 45 days old soybean plants (cv. Giza-35), 24 h either before or after spraying with the spore suspension of *C. dematium* (5×10^6 spore/ ml). The same mentioned methods were used without plant extracts for control treatment. All plants were covered with polyethylene bags for 48 h to maintain high relative humidity. Plants were kept in greenhouse under daily observation for 7 days. Four replicates were used for each treatment and five seeds were sown for each replicate. Disease severity was determined using the scale of Manandhar *et al.* (1988).

2- Resistance Induction:

a- *In vivo:*

(1) Effect of chemical inducers on disease incidence:

This experiment was carried out in sterilized pots (20 cm diam.) containing sterilized clay soil under the greenhouse conditions. Soil was infested with each of ***F. solani, R. solani,*** and ***M. phaseolina*** as mentioned before.

Soybean seeds (cv.Giza-35) were soaked, before sowing, in solutions of six chemical inducers, Salicylic acid (SA) at concentrations (2.5,5.0, and 7.5 Mm), Oxalic acid (OA) at concentrations (100, 150, and 200 ppm), K_2HPO_4 at (5, 10, and15Mm), Ethephon at (200, 400, and 600 ppm), CuCl at (5, 10, and 15 ppm) and EDTA at (5, 10, and15 Mm) for 1h. Seeds were soaked in tap water, some to serve as control.

Five seeds were sown per pot, and three replicates were used for each treatment. Percentage of pre-and post- emergence damping-off, root rot, and healthy survivals were recorded.

(2) Effect of the chemical inducers on severity of *anthracnose* disease:

Chemical inducers *i.e.* salicylic acid , oxalic acid, K_2HPO_4, Ethephon, CuCl, EDTA were sprayed as foliar spray at concentrations of 25 and 50%, on forty five days old soybean plants (cv. Giza-35), 24 h either before or after spraying with spore suspension *of C. dematium* (5×10^6 spore/ ml). The same aforementioned methods were used without inducers as control. All plants were coverd with polyethylene bags for 48 h to maintain high relative humidity. Plants were kept in greenhouse under daily observation for 7 days. Three replicates were used for each treatment and five seeds were sown for each replicate. Disease severity was determined as mentioned before.

3-: Biological control.

Efficiency of three biocides, Bio–ARC, Biozeid and Rhizo-N (Table 1) on the disease incidence was evaluated.

a- *In vitro*

Effect of antagonistic bioagents on linear growth of the tested fungi:

Petri dishes containing PDA medium were inoculated each with a disc (5 mm in diam.) taken from 7 day old cultures of the pathognic fungi, *i.e. F. solani, R. solani, M. phaseolina* and from 10 days old culture of *C. dematium*. The pathogenic fungi were inoculated at one side, whereas the opposite side was inoculated with either disc of antagonistic fungus *Trichoderma album* (Biozeid) or with streak for antagonistic bacteria *Bacillus megaterium* (Bio- ARC) and *Bacillus subtilis* (Rhizo-N) 6 cm apart (the antagonistic bacteria were inoculated before 24h from inoculation of the pathogenic fungi). Plates only inoculated with pathogenic fungi at one side were kept as control. Four replicates were used for each treatment. Plates were incubated at 28℃ for *F.solani, R.solani* and *M.phaseolina* and 25℃ for *C. dematium*. Linear growth of each tested fungi was measured when pathogenic fungi completely coverd the surface of the medium in the control treatment.

Table (1): Biocides used as seed treatment and foliar treatment

Trade name	Bio-agent	Rate of application	
		Per100/ L water	Per kg seed
Bio- ARC	***Bacillus megaterium***	500g	5g
Biozeid	***Trichoderma album***	500g	5g
Rhizo-N	***Bacillus subtilis***	400g	4g

The inhibition percent was calculated using the formula as follow: $I = C\text{-}T/C \times 100$
Where: **I** = percent of fungal growth inhibition. **C** = fungal growth of control. **T** = fungal growth of treatment.

b- *In vivo:*

(1) Effect of the tested biocides on disease incidence under greenhouse conditions:

This experiment was carried out in sterilized pots (20 cm diam.). Soil was infested with each of ***F. solani*** ,***R.solani*** and ***M. phaseolina*** separately as mentioned before. Soybean seeds (cv. Giza-35) were treated with the tested biocides, Bio-ARC, Biozeid and Rhizo - N (at the rate of application) after treated with 4% solution of carboxymethyl cellulose (CMC) as sticker. The same aforementioned methods were used without biocides as control. Five seeds were sown per pot, and three replicates were used for each treatment. Percentage of pre-and post- emergence damping- off, root rot and healthy survivals were recorded.

(2) Effect of the tested biocides on severity of anthracnose disease:

Biocides *i.e.* Bio-ARC, Biozeid and Rhizo-N were sprayed as foliar spray (rate of application) on 45 days old soybean plants (cv. Giza-35) 24 h either before or after spraying with spore suspension of *C. dematium* (5×10^6 spore/ml). /The same aforementioned methods were used without biocidesas to serve as control. All plants were covered with polyethylene bags for 48 h to maintain high relative humidity. Plants were kept in greenhouse under daily observation for 7 days. Three replicates were used and five seeds were sown in each replicate. Disease severity was determined as mentioned before.

RESULTS

1-Plant products:

a. In vitro

(1) Effect of different plant extracts on fungi growth inhibition:

Data presented in Table (1) show that *Allium cepa* extract completely inhabited growth of all fungi at 15% concentration. *Mentha viridis* completely inhibited growth of *F.solani* at 25% concentration, followed by *Allium sativum* and *Atrpilex sp*, which recorded 81.8 and 62.5 % growth inhibition at 25% concentration. Also, data show that *Mentha viridis* and *Atrpilex sp* completely inhibited growth of *R.solani* at 20% concentration, followed by *Allium sativum*, which recorded 68.1% growth inhibition at 25% concentration. While, *Allium sativum* and *Atrpilex sp* completely inhibited growth of *M. phaseolina* at 20% concentration, followed by *Mentha viridis* which recorded 84.1% growth inhibition at 25% *concentration.*

Data in Table (1) show that *Allium* cepa only completely inhibited growth of *C.dematium*, followed by *Atrpilex sp*, *Mentha viridis* and *Allium sativum*, which recorded 78.8, 71.1 and 51.4% growth inhibition at 25% concentration, respectively.

Generally, all the extracts were significantly inhibited mycelial growth. Increasing concentration of plant extracts increased the mycelial growth inhibition. This inhibition for *F. solani* and *C. dematium* was the heighest with *Allium cepa* extract followed by *Mentha viridis* and *Allium sativum*. Also, this inhibition for *R. solani* was the heighest with *Allium cepa* extract followed by *Mentha viridis* and *Atrpilex sp*. Mycelial growth inhibition of *M. phasolina* showed its highest values under the effect of *Allium cepa* and *Allium sativum* extracts.

(2) Effect of different plant extracts on sporulation inhibition:

Data presented in Table (2) indicate that the most effective concentration on sporulation inhibition of *F. solani* was 25%. *Allium cepa*, followed by *Mentha viridis*, *Atrpilex sp*, *Eucalyptus globules* and *Allium sativum* (100.00, 87.00, 84.60 and 76.10%, respectively) at conc. 25%. Also *Allium cepa* extract was the most effective on sporulation inhibition of *C. dematium* at conc. 10%, followed by *Allium sativum Atrpilex sp*, *Mentha viridis* and *Eucalyptus globules* (100.00, 95.10, 94.30, 90.50, and 80.80% respectively) at conc. 25%.

(3) Effect of different plant extracts on the inhibition of spore germination:

Data presented in Table (2) show that increasing concentration of plant extracts was associated with an increasing spore germination inhibition. In this respect, *Allium cepa* and *Mentha viridis* completely inhibited spore germination of *F.solani* at 15 and 25% concentration, followed by *Atrpilex sp*, *Eucalyptus globules* and *Allium sativum*, which recorded 90.1, 81.3 and 73.5% at 25% concentration, respectively.

Also, data show that *Allium cepa* completely inhibited spore germination of *C.dematium* at 10% concentration, followed by *Atrpilex sp*, *Allium sativum*, *Mentha viridis* and *Eucalyptus globules*, which recorded 69.6, 95.7, 93.3 and 84.2% at 25% concentration, respectively.

Table (1): Effect of different concentrations of plant extracts with different concentrations on the linear growth of the tested fungi

Plant extracts	Concentration%	Growth inhibition (%)			
		F.solani	*R.solani*	*M.phaseolina*	*C.dematium*
Eucalyptus globules	10	7.03	4.76	3.60	16.60
	15	17.03	6.90	7.30	16.90
	20	23.72	18.80	18.80	18.80
	25	28.51	21.40	24.10	21.10
Allium sativum	10	75.80	52.20	80.30	42.50
	15	79.20	57.70	93.30	46.60
	20	81.40	67.00	100.00	49.60
	25	81.80	68.10	100.00	51.40
Allium cepa	10	69.20	100.00	100.00	100.00
	15	100.00	100.00	100.00	100.00
	20	100.00	100.00	100.00	100.00
	25	100.00	100.00	100.00	100.00
Mentha viridis	10	69.60	44.40	49.90	31.80
	15	73.60	86.90	55.10	64.10
	20	86.90	100.00	56.30	67.70
	25	100.00	100.00	84.10	71.10
Atrpilex sp	10	47.00	58.10	3.30	63.60
	15	52.90	58.40	29.60	65.10
	20	57.70	100.00	100.00	70.30
	25	62.50	100.00	100.00	78.80
L.S.D. at 0.01	P	2.81	3.21	1.48	2.01
	C	1.46	2.43	2.20	1.15
	P×C	3.34	5.43	4.92	2.57

Plant extracts (P). Concentrations (C) Plant extract × Concentrations (Px C)

b – *In vivo*:

(1) Effect of the tested plant extracts on the disease incidence under greenhouse conditions:

Data in Table (3) show significant differences in disease incidence due to using the tested plant extracts. Pre-emergence damping –off percentage was decreased, while percentage of healthy survived plants was increased. Meanwhile, no significant differences were detected for post-emergence damping-off and root rot percentages caused by *F. solani* and *R.solani*. At the same time, no significant difference between plant extracts effect was found in all stages when soil was infested by *M. phaseolina*.

Atrpilex sp and *Allium sativum* were the most effective plant extracts at conc. 50% with all fungi were infestation, followed by *Allium cepa* at conc. 25 and 50%, while *Eucalyptus globules* and *Mentha viridis* were the least effective plant extracts at conc. 25 and 50 %.

Data show that the best applied concentration was 50%. Also, data show that the interaction between plant extracts and applied concentration was found to be insignificant.

(2) Effect of spraying soybean plants with different plant extracts on disease severity of anthracnose under greenhouse conditions:

Data presented in Table (4) indicate that five plant extracts significantly reduced disease severity of anthracnose compared with the control, when they were sprayed 24 h before inoculation with *C.dematium*. The most effective extracts were *Atrpilex sp* 24 h before inoculation at conc. 25 and 50 followed by *Allium cepa* and *Mentha viridis* at conc. 50% *and* 25%, followed by *Allium sativum* and *Eucalyptus globules* 50%. The extracts did not reduce disease severity when sprayed 24 h after inoculation with *C. dematium*. Also, data show t significant differences among the concentrations of plant extracts in reducing disease severity when sprayed 24 h before inoculation with *C. dematium*.

Table (2): Effect of different concentrations of plant extracts with different concentrations on sporulation and spore germination inhibition of *F.solani* and *C.dematium*

Plant extracts		Sporulation inhibition %		Germination inhibition %	
		F.solani	*C.dematium*	*F.Solani*	*C.dematium*
Eucalyptus globules	10	46.80	68.10	42.60	74.60
	15	64.20	68.60	62.00	78.20
	20	67.40	78.90	66.60	79.90
	25	84.60	80.80	81.30	84.26
Allium sativum	10	68.60	89.20	67.40	89.60
	15	73.10	90.70	73.60	89.80
	20	75.10	91.40	75.20	91.70
	25	76.10	95.10	78.30	95.70
Allium cepa	10	67.80	100.00	73.50	100.00
	15	100.00	100.00	100.00	100.00
	2	100.00	100.00	100.00	100.00
	25	100.00	100.00	100.00	100.00
Mentha viridis	10	63.90	78.20	59.60	79.80
	15	64.10	86.80	61.10	86.70
	20	70.10	89.30	64.90	88.50
	25	100.00	90.50	100.00	90.20
Atrpilex spp	10	81.30	92.30	81.30	93.30
	15	84.10	92.70	82.60	94.60
	20	86.80	93.30	86.60	95.60
	25	87.00	94.30	90.10	96.60
L.S.D. at 0.01	P	2.55	4.47	5.53	3.63
	C	2.09	1.78	2.29	0.96
	PXC	4.69	3.99	5.13	2.75

Plant extracts (P). Concentrations (C) Plant extract × Concentrations (Px C)

Table (3): Effect of different concentrations of some plant extracts on infection percentage with pre-and post-emergence damping – off, root rot and healthy survived soybean plants

Plant extract		Damping off						Root rot (%)			Healthy survivals (%)		
		Pre-emergence (%)			Post-emergence (%)								
	Conc (%)	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*
Eucalyptus globules	25	60.00	60.00	46.66	6.66	6.66	6.66	6.66	6.66	20.00	26.66	26.66	26.66
	50	53.33	53.33	46.66	6.66	6.66	6.66	6.66	6.66	13.33	33.33	33.33	33.33
Allium sativum	25	53.33	53.33	46.66	6.66	6.66	6.66	6.66	6.66	6.66	33.33	33.33	40.00
	50	40.00	40.00	33.33	0.00	6.66	6.66	6.66	6.66	6.66	53.33	46.66	53.33
Allium cepa	25	53.33	53.33	40.00	13.33	6.66	6.66	6.66	6.66	6.66	26.66	33.33	46.66
	50	46.66	46.66	33.33	6.66	6.66	6.66	6.66	6.66	13.33	40.00	40.00	46.66
Mentha viridis	25	66.66	66.66	46.66	13.33	13.33	6.66	13.33	6.66	33.33	6.66	13.33	13.33
	50	53.33	53.33	46.66	6.66	13.33	6.66	6.66	6.66	13.33	33.33	26.66	33.33
Atrpilex sp	25	46.66	46.66	40.00	6.66	6.66	6.66	6.66	0.00	6.66	40.00	46.66	46.66
	50	33.33	33.33	26.66	0.00	0.00	6.66	6.66	6.66	6.66	60.00	60.00	60.00
Control	0	66.66	73.33	46.66	13.33	13.33	6.66	6.66	6.66	33.33	13.33	6.66	13.33
L.S.D at 0.01	P	7.90	7.90	n.s	n.s	n.s	n.s	n.s	n.s	5.80	9.30	13.80	N.S
	C	11.10	7.70	n.s	n.s	n.s	n.s	n.s	n.s	6.01	12.70	11.30	13.80
	P×C	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Plant extracts (P). Concentrations (C) Plant extract × Concentrations (Px C)

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

Table (4): Effect of spraying soybean plants with different concentrations of some plant extracts before and after inoculation on anthracnose disease severity

Plant extracts	Conc.%	Disease severity (%)	
		Before inoculation	After inoculation
Eucalyptus globules	25	27.00	48.00
	50	24.60	47.60
Allium sativum	25	24.00	48.00
	50	22.60	47.60
Allium cepa	25	19.30	47.60
	50	15.30	47.60
Mentha viridis	25	19.60	48.00
	50	16.60	48.00
Atrpilex spp.	25	15.30	47.60
	50	12.00	46.60
Control	0	48.00	48.00
L.S.D. at 0.05	D	2.20	n.s
	C	1.10	n.s
	DXC	2.20	n.s

D = Disease severity (%) C = Extract concentrations DXC = Disease severity x Extract concentrations

2- Induction of acquired resistance:

a- ***In vivo:***

(1) Effect of the chemical inducers on the disease incidence under greenhouse conditions:

Data in Table (5) show a significant difference between the tested inducers in decreasing percentage of pre-emergence damping – off and increasing percentage of healthy survived soybean plants. On the other hand, no significant difference was found between the percentage of post – emergence damping – off and root rot. EDTA and SA were the most effective inducers against all fungi followed by K_2HPO_4 and OA. Also, data show that there was no significant effect between ethephon and CuCl compared with the control, so that ethephon and CuCl were not used in field experiments.

Data also, show significant differences between the concentrations of inducers in decreasing percentage of pre-emergence and increasing percentage of healthy survived soybean plants. While, no significant differences between the percentage of post-emergence damping –off and root rot were found. The best applied concentrations were EDTA at (15 Mm), SA at (7.5 M m), K_2HPO_4 at (10 Mm) and OA at (200 ppm). Also, data show that the interaction between inducers and applied concentration was found to be insignificant.

(2) Effect of spraying different chemical inducers on disease severity of soybean anthracnose under greenhouse conditions:

Data presented in Table (6) indicate that four chemical inducers significantly reduced disease severity of soybean anthracnose compared with the control when sprayed 24 h before inoculation with *C.dematium*. The chemical inducers were not able to reduce disease severity when they were sprayed 24h after inoculation with *C dematium*. The most effective inducers were EDTA at (15 Mm) and SA at (7.5 Mm) followed by, K_2HPO_4 at (10 Mm) and OA at (200 ppm). Data also show that ethephon and CuCl insignificantly reduced disease severity of anthracnose compared with the control.

3- Biological control

a. ***In vitro:***

Effect of different bioagents on linear growth of the tested fungi:

Data presented in Table (7) indicate that all bio-agents tested significantly decreased the linear growth of the pathogenic fungi on PDA medium compared with the control.

Data show that *T. album* (Biozeid) inhibited the growth of *F. solani, R. solani, M. phaseolina* and *C. dematium* by 63.3, 64.1, 60.7 and 73.2 % reduction, respectively, while *B. megaterium* (Bio-ARC) and *B. subtilis* (Rhizo – N). retarted colony growth of the pathogen at a distance by production of inhibitory zone against the pathogen and inhibited the growth of *F. solani* by 33.3 and 25.1%, growth of *R. solani* by 36.9 and 28.8%, growth of *M.phaseolina* by 35.1 and 25.8 and growth of *C.dematium* by 52.9 and 45.1% respectively. Generally, *T. album* (Biozeid) was the most effective bio-agent followed by *B. Megaterium* (Bio-ARC) and *B. subtilis* (Rhizo – N), respectively.

b. ***In vivo:***

(1): Effect of the tested biocides on the disease incidence under greenhouse conditions:

Date in Table (8) exhibited a significant difference between the tested biocides as they decreased percentage of pre-emergence damping–off and increased percentage of healthy survived plants. While, no significant differences were found among biocides for infection with each of post-emergence, damping–off

Table (5): Effect of some chemical inducers with different concentrations on infection of soybean with pre-and post–emergence damping–off, root rot and healthy survived plants

Inducer		Damping off						Root rot (%)			Healthy survivals (%)		
		Pre-emergence (%)			Post-emergence (%)								
	Conc (%)	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*	*F.s*	*R.s*	*M.p*
	2.5M.m	46.66	46.66	40.00	13.33	13.33	6.66	13.33	13.33	20.00	26.66	26.66	33.33
SA	5 M.m	40.00	46.66	33.33	6.66	6.66	6.66	6.66	6.66	13.33	46.66	40.00	46.66
	7.5 M.m	26.66	26.66	20.00	6.66	6.66	0.00	6.66	6.66	13.33	60.00	60.00	66.66
	5 M.m	46.66	53.33	40.00	13.33	6.66	6.66	20.00	13.33	13.33	20.00	20.00	33.33
K_2HPO_4	10 M.m	33.33	40.00	33.33	6.66	6.66	0.00	6.66	6.66	13.33	53.33	46.66	53.33
	15 M.m	46.66	46.66	40.00	13.33	13.33	6.66	13.33	13.33	13.33	26.66	26.66	40.00
	100 ppm	60.0	66.66	40.00	13.33	13.33	6.66	13.33	6.66	13.33	13.33	13.33	40.00
OA	150 ppm	46.66	60.0	33.33	13.33	6.66	6.66	13.33	6.66	6.66	26.66	20.00	53.33
	200 ppm	40.00	40.00	33.33	6.66	6.66	0.00	6.66	13.33	13.33	46.66	40.00	53.33
	200 ppm	60.00	66.66	46.66	13.33	13.33	6.66	13.33	6.66	26.66	13.33	13.33	26.66
ETH	400 ppm	66.66	66.66	46.66	13.33	13.33	6.66	6.66	6.66	20.00	13.33	13.33	20.00
	600 ppm	73.33	66.66	46.66	13.33	13.33	6.66	0.00	6.66	20.00	13.33	13.33	26.66
	5 ppm	66.66	73.33	46.66	13.33	13.33	6.66	6.66	6.66	20.00	13.33	6.66	26.66
CuCL	10 ppm	66.66	66.66	40.00	13.33	13.33	6.66	6.66	13.33	20.00	13.33	6.66	33.33
	15 ppm	73.33	66.66	46.66	13.33	13.33	6.66	0.00	13.33	20.00	13.33	6.66	26.66
	5 M.m	53.33	53.33	40.00	13.33	13.33	6.66	6.66	6.66	6.66	26.66	26.66	46.66
EDTA	10M.m	40.00	40.00	26.66	13.33	13.33	6.66	6.66	6.66	13.33	40.00	40.00	53.33
	15 M.m	26.66	26.66	20.00	6.66	6.66	0.00	6.66	6.66	6.66	60.00	60.00	73.33
Control	0	66.66	73.33	46.66	13.33	13.33	6.66	6.66	6.66	33.33	13.33	6.66	13.33
L.S.D. at 0.05	I	10.30	9.10	8.70	n.s	n.s	n.s	n.s	n.s	8.11	12.30	13.10	10.70
	C	6.10	7.30	6.40	n.s	n.s	n.s	n.s	n.s	n.s	9.80	9.80	9.30
	I×C	14.90	n.s	n.s	n.s	n.s	n.s	22.70	n.s	n.s	n.s	n.s	n.s

Inducers (I) concentrations (C) Inducers × concentrations (I×C)

Table (6): Effect of spraying chemical inducers with different concentrations on anthracnose disease severity of soybean plants before and after inoculation

Inducers	Conc.	Disease severity (%)	
		Before inoculation	After inoculation
SA	2.5M m	6.30	47.60
	5 M m	5.60	47.60
	7.5 M m	4.80	47.30
K_2HPO_4	5 M m	7.00	48.00
	10 M m	5.60	48.00
	15 M m	5.60	47.30
OA	100 ppm	12.60	47.60
	150 ppm	12.00	47.60
	200 ppm	10.30	47.00
Eth	200 ppm	48.00	48.00
	400 ppm	48.00	47.60
	600 ppm	48.00	47.00
Cucl	5 ppm	48.00	48.00
	10 ppm	48.00	48.00
	15 ppm	48.00	48.00
EDTA	5 M m	6.60	47.60
	10M m	6.30	47.30
	15 M m	4.60	47.30
Control	0	48.00	48.00
L.S.D. at 0.05	I	1.55	n.s
	D	0.72	n.s
	I×D	1.76	n.s

Inducers (I) Disease severity (D) Inducers × Disease severity (I×D)

and root rot. Bio-ARC was the most effective biocide when the soil was infested by each of *F. solani* and *R. solani* followed by Biozeid and Rhizo – N. Also, data show no significant differences between the tested biocides concerning percentage of pre – and post – emergence damping–off when the soil was infested by *M. phaseolina*. Meanwhile, infection with root rot was significantly decreased and consequently healthy percentage of survived plants was significantly increased. Generally, Bio-ARC was the most effective on pot plants, followed by Biozeid and Rhizo –N.

(2): Effect of spraying soybean plants with different biocides on anthracnose disease severity:

Data presented in Table (9) indicate that the tested biocides significantly reduced disease severity of anthracnose compared with the control when they were sprayed 24 h before inoculation with *C. dematium*. The tested biocides were not able to reduce disease severity when they were sprayed 24 h after inoculation with *C. dematium*. The most effective biocide was Bio-ARC followed by Biozeid and Rhizo – N.

DISCUSSION

It is well established that extensive application of chemical treatments in controlling plant diseases resulted in harmful effects on the environments. Therefore, search for an alternative safe, effective and economic ways for controlling plant pathogens is greatly needed.

All plant extracts tested inhibited the linear growth, sporulation and spore germination. Increasing concentration of the plant extracts was associated with great reduction in mycelial growth, sporulation and spore germination. Thus, *Allium cepa* was the most effective plant extract, it completely inhibited linear growth of all fungi, sporulation and spore germination of *F. solani* and *C. dematium*. *Atrpilex sp* completely inhibited mycelial growth of *R. solani* and *M. phaseolina* at 20 % concentration, followed by *Mentha viridis* which completely inhibited growth of *R. solani* and *F. solani* at 20% and 25 % concentration and completely inhibited sporulation and spore germination of *F. solani*. The results are in line with those reported by El– Shami *et al.* (1985) and Lakshmanan (1990). Favaron *et al.* (1993) reported that extract of *Allium sativum* contained factors that inhibited poly-galacturonases (PGS) produced *in vitro* by fungi.

On the other hand, all tested plant extracts decreased percentage of pre –and post – emergence damping – off and root rot and increased percentage of healthy survived plants. *Atrpilex sp* and *Allium sativum* were the most effective plant extracts at 50% concentrations, followed by *Allium cepa*. While, *Eucalyptus globules* and *Mentha viridis* were the least effective plant extracts. This is in agreement with the results obtained by Malhotra and Rai (1990) who found that *Datura metel* extract decreased the soybean mycoflora and increased seed germination.

Table (7): Effect of different bioagents on linear growth of the tested fungi

Bioagent	*F. Solani*		*R.. Solani*		*M. phaseolina*		*C. dematium*	
	Linear growth (cm)	Reduction (%)	Linear growth (cm)	Reduction (%)	Linear Growth (cm)	Reduction (%)	Linear growth (CM)	Reduction (%)
Bacillus megaterium	6.00	33.30	5.60	36.90	5.83	35.10	4.23	52.90
Trichoderma album	3.30	63.30	3.23	64.10	3.53	60.70	2.40	73.20
Bacillus subtilis	6.70	25.10	6.40	28.80	6.66	25.80	4.93	45.10
Control	9.00	-	9.00	-	9.00	-	9.00	-
L.S.D at 0.01	0.36	-	0.54	-	0.88	-	0.64	-

Table (8): Effect of different biocides on soybean infection with pre-and post-emergence damping–off, root rot and healthy survival soybean plants in pots

Biocides	Damping off						Root rot (%)			Healthy survivals (%)		
	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*
Bio -ARC	33.33	40.00	26.66	0.00	0.00	6.66	6.66	13.33	20.00	60.00	46.66	53.33
Biozeid	40.00	46.66	33.33	6.66	6.66	0.00	6.66	6.66	20.00	46.66	40.00	46.66
Rhizo-N	46.66	53.33	40.00	6.66	6.66	6.66	6.66	13.33	13.33	40.00	26.66	40.00
Control	66.66	73.33	46.66	13.33	13.33	6.66	6.66	6.66	33.33	13.33	6.66	13.33
L.S.D at 0.05	22.10	22.10	n.s	n.s	n.s	n.s	n.s	n.s	6.60	29.70	23.10	22.10

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

Table (9): Effect of spraying different biocides on severity of soybean anthracnose before and after inoculation

Biocides	Disease severity (%)	
	Before inoculation	After inoculation
Bio-ARC	6.00	46.30
Biozeid	20.30	47.60
Rhizo – N	20.60	46.60
Control	48.00..	48.00
L.S.D at 0.05	3.81	n.s

Khan and Fakir (1995), Osman *et al.* (1996) and Youssef (1991) reported that soaking of lentil seeds in juice of *Allium cepa* colves decreased the percentage of damped off seedlings.

Spraying soybean plants (cv. Giza – 35) with plant extracts 24 h before inoculation with *C. dematium* (protective treatment) reduced disease severity compared with the control. *Atrpilex sp* and *Allium cepa* were the most effective extracts at 50 % concentration. They caused the highest reduction in disease severity, followed by *Mentha viridis, Allium sativum* extracts. The obtained results are in line with those reported by Babu and Reddy (1986) who indicated that spraying plant extracts, as pre–inoculation treatment was more effective than post-inoculation in controlling *Colletotrichum gloesporioides* and *Botryodeplodia theobrome*. Abd-Rabboh (2000) and Abd -El - Rhman (2001) reported that spraying soybean plants with plant extracts before inoculation with *C. dematium* reduced disease severity compared with after inoculation.

One of the new environmentally safe means of disease control is the induced or acquired resistance. The importance of induced resistance has long been demonstrated. The foregoing study under greenhouse proved that soybean seed treatment with different concentrations of inducers reduced pre– and post-emergence damping–off and root rot and increased healthy survived plants. EDTA (15 mM) and SA (7.5 mM) were the most effective inducers for all fungi, followed by K_2 HPO_4 (10 mM) and OA (200 ppm). Seed treatment with ethephone and CuCl showed no significant effect compared with the control. The role of chemical inducers in induced resistance has been reported in different researches. Application of SA stimulated biosynthesis of different families of P–R - proteins (Raskin, 1992) and increasing the activities of chitinase, peroxidase andB–1.3–glucanase (Prachi and Singh 2002 and Tilak *et al.*, 2002). Results of seed treatment with EDTA are in line with those reported by Walters and Murray (1992) and Marry *et al.* (1995).

Spraying soybean plants grown in pots with chemical inducers 24 h before inoculation with *C. dematium* reduced disease severity compared with the control. The most effective inducers were EDTA (15 mM) an SA (7.5mM), followed by K_2HP0_4 (10 mM) and OA (200 ppm). Results indicated that treatment with any inducer significantly reduced disease severity of anthracnose compared with the control. The obtained results are in line with those reported by Marry *et al.* (1995). Abd – El – Kareem (1998) reported that foliar application of cucumber with K_2HP0_4 increased activity of chitinase and B – 1,3 – glucanase.

Different bioagents, *i.e. Bacillus megaterium, Bacillus subtilis* and *Trichoderma album* as commercial products, were used in the present investigation to study their effect in controlling soybean damping–off, root rot, charcoal rot and anthracnose. Results of *in vitro* tests, results obtained indicated that all the tested bioagents effectively inhibited the mycelial growth of *F. solani, R. solani, M. phaseolina* and *C. dematium* on PDA. *Trichoderma album* (Biozeid) was the most effective bioagent with all fungi, followed by *Bacillus megaterium* (Bio-ARC) and *Bacillus subtilis* (Rhizo–N). These results are in agreement with those obtained by Wu (1980), Deb (1990), Mohamed (1993) and Saber *et al.* (2003) reported that biocontrol induced by *T. viride* against the pathogenic fungi might be due to mycoparasitism or lysis of the pathogen due to competition for nutrition and / or antibiosis. Bhattacharyya and Purkayastha (1982) found that *B. megaterium* inhibited growth of *Colletotrichum corchorum* while culture filtrate of *B. megaterium* reduced spore germination of the same pathogen and caused 30% reduction in germ tube length. Abd - El - Rhman (2001) indicated that culture filtrates of the bioagents contained antagonistic material and / or lytic enzymes, which inhibited spore germination. Moreover, culture filtrate of *B. megaterium* causes malformed ungermenated spores and this might be attributed to some antibiotics produced by the bacterium.

In pots experiment, the obtained results showed that seed treatment with biocides decreased percentage of pre – and post – emergence damping – off and root rot and increased percentage of healthy survived plants. Bio-ARC was the most effective biocide followed by Biozeid and Rhizo – N. These results are similar to those obtained by Abd-El– Moity and Abou–Zeid (1988), and Hassanein *et al.* (2000), Abou – Zeid *et al.* (2003) suggested that some by – products of microganisms *(T. harzianum* and *B. subtilis*) stimulated plant growth and at the same time reduced population density of plant pathogens. Also, they found that using *B. subtilis* as seed coating and early–sowing date against *R. solani* and *Fusarium* spp decreased chickpea pre– and post–emergence damping–off. The results obtained during the progress in present investigation indicated that spraying soybean plants with biocides 24 h before inoculation with *C. dematium* (protective treatment) caused reduction in percentage of disease severity compared with the control, Bio-ARC was the most effective biocide followed by Biozeid and Rhizo–N. Results showed no significant reduction in disease severity when biocides were sprayed 24 h after incoulation by *C. dematium*. Results showed that Bio-ARC was the most effective bio-pesticide followed by Biozeid and Rhizo – N. The results are in line with those reported by Bhaswati and Purkayastha (1989) and Abd -El - Rhman (2001).

Generally, it could be concluded that the application of antagonistic microorganisms to seeds has been employed to control pre– and post–emergence damping–off and anthracnose disease of the leguminous crops, but also to reduce the density of soil – borne pathogens. Also, prospective application of biocntrol technique may help to decrease the use fungicides and to avoid environmental pollution.

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البدائل الآمنة لمقاومة أمراض الجذور والمجموع الخضرى لنباتات فول الصويا

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من الأمراض الهامة التى يتعرض محصول فول الصويا للاصابة بها أمراض سقوط البادرات وعفن الجذور والعفن الفحمى والأنثراكنوز، والتى تسبب بفقد كبير فى المحصول الكلى و جودته. كل المستخلصات النباتية المستخدمة أدت الى تثبيط النمو الخطى لفطريات *F. solani, R. solani, M. phaseolina* and *C. dematium.* وكان مستخلص *Allium cepa* الأكثر فاعلية يليه *Atrpilex sp.* و *Mentha viridis* و *Allium sativum* و *Eucalptus globules*. من ناحية أخرى ، تقلل معاملة البذرة بالمستخلص النباتى نسبة حدوث المرض تحت ظروف الحقل والأصص. كان الأكثر فاعلية تحت ظروف الصوبة مستخلصات *Atrpilex sp.* و *Allium sativum* يليها *Allium cepa* و *Mentha viridis* و *Eucalptus globule* . قلل رش نباتات فول الصويا بالمستخلصات قبل ٢٤ ساعة من الحقن بالمسبب لمرض الأنثراكنوز شدة الإصابة وكانت مستخلصات *Atrpilex sp.* و *Allium cepa* الأكثر فاعلية . نتج عن إضافة المحفزات الكيميائية كمعاملة للبذور فى الأصص الى تقليل نسبة حدوث الأمراض الكامنة بالتربة بدرجة كبيرة. رش نباتات فول الصويا بالمحفزات الكيميائية قبل الحقن بـ *C. dematium* قلل مرض الأنثراكنوز. كانت EDTA و حامض السالسيليك أكثر المحفزات فاعلية وأدت الى خفض نسبة حدوث الأمراض الكامنة بالتربة بالأصص. كان Bio-ARC الأكثر فاعلية يلية BioZied و Rhizo-N. أيضاً كانت المعاملة بالرش الورقى بـ Bio-ARC الأكثر فاعلية كمبيد حيوى فى مقاومة مرض الأنثراكنوز يلية BioZied و Rhizo-N.