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EVALUATION OF SOME ENVIRONMENTALLY SAFE CHEMICALS AND BIOAGENTS AGAINST *Fusarium solani* AND *Sclerotium rolfsii* INFECTED COWPEA PLANTS

Mohamed, Gehad M. ; Naglaa A. S. Muhanna ; Seham S. M. Ragab and S. M. H. Kamel
Plant Pathology Research Institute, ARC, Giza, Egypt.

ABSTRACT

Incidence of root rot and damping-off caused by *Fusarium solani* and *Sclerotium rolfsii* on cowpea plants was successfully controlled by some plant extracts, essential oils, bioagents, systemic resistance-chemical inducers and seed dressing fungicides which tested under laboratory, greenhouse and field conditions in two successive seasons (2008 and 2009). All tested plant extracts; *Artemisia absinthium*, *Ocimum basilicum* and *Mentha longifolia*; significantly reduced the linear growth of *F. solani* and *S. rolfsii*. The maximum inhibition in fungal radial growth was induced by *Ocimum basilicum* and *Artemisia absinthium* with averages of 81.29 and 61.99%, respectively. All the tested essential oils reduced the fungal growth of *S. rolfsii*, the most effective oils were *Syzygium aromaticum* and *Eucalyptus globulu*s with averages of 87.70 and 81.10 %, respectively. On the other hand, using culture filtrates of *Trichoderma harzianum*, *T. viride* and *Bacillus subtilis* significantly reduced the linear growth of *F. solani* and *S. rolfsii* with the average of 75.28, 69.62, and 54.36%, respectively. Results of chemical inducers revealed that salicylic acid have the highest inhibition effect on the mycelial growth of *F. solani* and *S. rolfsii* with the average of 20.00 and 29.63 %, respectively.

Under greenhouse conditions, all treatments caused significant decrease of disease incidence and increase of survival plants, as compared to the untreated plants. Soaking seeds in *Ocimum basilicum* extract and *S. aromaticum* oil decreased damping-off and had the highest percentage of healthy survival cowpea plants. Soaking cowpea seeds in Vitavax-captan or in the suspension of *T. harzianum*, (however, had the best effect) for decreasing damping-off and increasing survival plants. The disease could be controlled also by salicylic acid and ascorbic acid (4mM) which decreased damping-off and root-rot incidence and increased survival plants. On the other hand, there is a correlation between induced resistance and some biochemical changes in leaf tissues of cowpea healthy plants such as increasing the activity of peroxidase and Polyphenol oxidase enzymes. *T. harzianum*, salicylic acid and *S. aromaticum* produced the highest level of peroxidase and Polyphenol oxidase enzymes activity.

Under field conditions, Vitavax-captan, bioagents *T. harzianum*, plant extract; *Ocimum basilicum*, essential oil; *S. aromaticum* and resistance-inducing chemical salicylic acid significantly reduced disease incidence and increased seed yield production.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is belonging to the family leguminoceae. It is considered one of the most important crops in Egypt and as an inexpensive source of protein.

The most important problems of legumes production in Egypt are due to pests and diseases, which attack the plant causing great losses in the yield quality and quantity. *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* are the major pathogens causing cowpea diseases in Egypt (Tomader 2005).

In addition, cowpea proved unalterable diseases caused by soil borne fungi in many countries worldwide especially *Alternaria* spp., *Fusarium oxysporum, Fusarium solani* and *Sclerotium rolfsii* which attack roots (Adandonon *et al.,* 2004 and Infantiono *et al.*, 2006).

Because of hazards of pesticides in general and fungicides in specific on public health and environmental balance, there is a need to find alternatives to synthetic pesticides. Such products from higher plants and microbes are relatively broad- spectrum, bio-efficacious, economical and environmentally safe and ideal candidates for use as agrochemical (Macias *et al.*, 1997 and Cutler and Cutler, 1999).

Many natural compounds isolated from plants have been shown to have biological activities, and the essential oil from aromatic and medicinal plants is particularly interesting as described by many researchers (Deans and Ritchie 1987; Baratta *et al.*, 1998 and Laura *et al*., 2002).

Biological control considered one of the most important strategies to control soil borne diseases incidence of legumes are studied by several plant pathologists Xi *et al.* (1996); Kalphna-Bhatnagar and Bansal (2003) and Negi, *et al.* (2005). Also, several successful attempts have been made to control these pathogens using the species of *Trichoderma* and *Gliocladium*. Hwang and Chakravarty (1993), Kapoor *et al.* (2005) and Sendhilvel, *et al.* (2005) studied five isolates of *Pseudomonas fluorscens* isolated from cowpea rhizosphere region against *Macrophomina phaseolina*. They found that, the seeds and soil application with *P. fluorscens* svpf2 significantly reduced rot incidence and increased seeds germination percentage and vigor index of cowpea plants. In addition, Hwang and *et al.* (1996) and Wakelin *et al.* (2002) reported that pea's seeds inoculation with *Bacillus subtilis* reduced seed and root rot caused by *Pythium ultimum* and *Aphanomyces euteiches* and significantly increased seedling survival and shoot dry weight compared to non-treated seeds in infested soil.

Induced disease resistance can be defined as the process of active resistance dependent on the host plant physical or chemical barriers activated by biotic or a biotic agents Kloeper *et al.* (1992) and could be induced in plants by applying chemical elicitors Reglinski *et al.* (2001). Several investigators such as Dmitrier *et al*. (2003) and Achuo *et al*. (2004) used different inducers like salicylic, benzoic, citric, ascorbic and oxalic acids beside ribavirin. On the other hand, induced resistance may also affect other growth parameters; chlorophyll content, plant growth, accumulation of antifungal compounds and increasing activity of oxidative enzymes (Ziadi *et al.,* 2001; Malolepsza and Urbanek, 2002 and Fariduddin *et al.*, 2003).

The present investigation was conducted to control root rot and damping-off of cowpea using some plant extracts, essential oils, antagonistic bacteria and fungi and some chemical inducers *in vitro* and under both greenhouse and field conditions and to study their effect on some cowpea agronomic characters also, their impacts on activity of oxidative enzymes.

MATERIALS AND METHODS

1- Laboratory Experiment:

1.1- Isolation and identification of pathogenic fungi:

Roots of cowpea, showing typical symptoms of root-rot and wilt, were washed carefully with tap water, dried between two filter papers and then cut into small pieces. Pieces of infected roots were surface sterilized using sodium hypochlorite 3% for 2 min., washed with sterilized water, dried between sterilized filter papers, then directly transferred to Petri dishes containing potato dextrose agar (PDA) medium, and incubated at 25˚C for 5 days. The developed fungal hypha were isolated, and finally purified by the single spore or hyphal tip techniques Brown (1924) and Hawker (1960). The isolated fungal pathogens were identified by the aid of Department of Mycology, Plant Pathology Institute, Agricultural Research Center Giza, Egypt. Confirmation of pathogenicity of these isolates were done on the host from which it was isolated the pathogenicity tests revealed that these isolates were *Fusarium solani* and *Sclerotium rolfsii*.

1.2- Effect of plant extracts on the linear growth of the pathogens:

The effect of some plant extracts such as absinthe (*Artemisia absinthium* L.), mint (*Mentha longifolio* L. hupson) and basil (*Ocimum basilicum* L.) were tested against *F. solani* and *S. rolfsii*. Dry plants were grounded into fine powder and boiled in distilled water at the rate 1:1 (w/v) for four hours, and allowed to stand overnight Arjunan *et al*. (1994). The mixtures were filtered through two layers of cheesecloth, centrifuged for 10 minutes at 300 rpm and supernatant sterilized using zeits filter. 100 ml of the crude plant extract was incorporated into 900 ml of PDA media and poured into Petri dishes of 9 cm diameter at the rate of 15 ml/Petri dishe. Plates of agar medium without any plant extracts saved as control and four replicates were maintained for each treatment. The Petri dishes were inoculated with equal disks (4 mm in diameter) of pathogenic fungi (*F. solani* or *S. rolfsii*). The plates were incubated at 27˚C. Linear growth of each tested pathogenic fungi was measured after the diameter of the growth in control treatment reached the average of 9cm (Atia, 1995).

1.3- Effect of essential oils on the linear growth of the pathogens:

The effect of essential oils of Nigella (*Nigella sativa* L.), Clove (*Syzygium aromaticum*), Blue gume (*Eucalyptus globulus* Labill) and Bergamot (*Momordica fistulosa* L.) on the mycelial growth of the tested fungi was carried out under laboratory conditions. The crude oils were added to autoclaved PDA medium at (10000 ppm) prior to pouring the PDA medium Thakur *et al.* (1989). Amended medium was poured into sterilized Petri dishes of 9 cm diameter at the rate of 15 ml/Petri dish and others without any essential oil were prepared and served as control. Petri dishes were inoculated with equal discs (4 mm in diameter) of each tested fungi and incubated at 27˚C and the linear growths were measured as mentioned by (Atia, 1995).

1.4- Effect of different bioagents filtrates on the linear growth of the pathogens:

The interaction between culture filtrates of *Trichoderma harzianum*, *T. viride*, *Gliocladium vierus*, *Bacillus subtilis* and *Pseudomonas fluorescencs* and the pathogenic fungi was studied under laboratory conditions. Culture filtrates of *Trichoderma* spp. and *G. vierus* were obtained from liquid gliotoxin fermentation medium (GFM) developed by Brain and Hemining (1945) under complete darkness condition to stimulate toxin production Abd El-Moity and Shatla (1981).The bacterial culture filtrates were obtained in 500 ml flasks capacity each contain 150 ml liquid King s̀ B medium King *et al.* (1945). Ten ml from sterile culture filtrates were added to flasks contained 90 ml warm PDA medium. Then amended medium was poured into sterilized Petri dishes of 9 cm diameter at the rate of 15 ml/Petri dish and others without any culture filtrates were prepared and served as control. Petri dishes were inoculated with equal discs (4 mm in diameter) of each tested fungi and incubated at 27˚C. Growth reading was made after the pathogenic fungi in the control treatment, almost covered the medium surface. The mean diameter of the pathogenic fungi was measured in each treatment and the percentage of growth reduction due to the presence of different microorganisms filtrates were calculated from the following formula:

R= (B-C/B x100) where: R= the percentage of growth reduction, B= the mean diameter of the fungus in the control. C= the mean diameter of the pathogenic fungi in the treatment.

Effect of chemical inducers on the linear growth of the pathogen: 1.5-

Table (1): Chemical inducers and its some different properties.

Chemical Inducers	**Structure molecules**	**Mol. weight (M.W)**	**Concentration**
Ascorbic acid	$C_6H_8O_6$	176.00	4 mM
Salicylic acid	$HOC_6H_4CO_2H$	138.12	4 mM
Oxalic acid	HO_2CCO_2H	90.04	4 mM

The effect of chemical Systemic Acquired Resistance (SAR); ascorbic, salicylic and oxalic acids on the mycelial growth of the tested fungi was carried out under laboratory conditions. Chemical compounds were dissolved in ethanol (95%) and added to autoclaved PDA medium at one percent concentrate 4 mM Booth (1971). Amended medium was poured into sterilized Petri dishes (9cm diameter) at the rate of 15ml/petri dish and others with ethanol only at (95%) were prepared and served as control, and incubated at 27˚C and the linear growth were measured as mentioned previously.

2- Greenhouse Experiments:

Greenhouse experiments were conducted to evaluate the effect of the tested plant extracts, essential oils and bioagents on the plant growth, disease incidence and some physiological activities of cowpea plants infected with *F. solani* and *S. rolfsii.*

2.1- Effect of seed soaking in different plant extracts and essential oils on disease incidence:

Seeds of cowpea (Cream 7) were soaked for 12 hrs in the crude plant extracts and essential oils. Soil were infested with *F. solani* and *S. rolfsii* grown on sand wheat bran medium (1: 3 v/v) at the rate of 2% w/w and dispensed one weak before sowing in 25 cm diameter pots. Treated seeds were air-dried and then seven seeds were sown/ pot containing the tested fungi. Four pots were used for each treatment. Seeds soaked in the sterile distilled water were sown in the same manner as control. Pre and post emergence damping-off recorded after 15 and 30 days, respectively, healthy survival plants and fresh and dry weight of plants were determined after 40 days.

2.2- Effect of tested bioagents on disease incidence:

The effect of different biocontrol agents (*T. harzianum*, *T. viride*, *Gliocladium vierus, B. subtilis* and *P. fluorescence*) aganist disease incidence were tested. Seeds of cowpea (Cream7) were soaked in suspension of the antagonistic fungi and bacteria for 12 hrs. Spore suspension ($6x10^5$ spores/ml) of each fungus were added and mixed with germinated seeds, while germination seeds Khaleifa *et al.* (2007) were immersed in the bacterial cell suspension of concentrations ($3.1x10^7$ cuf/ml). The treated were incubated for 12 hrs at room temperature then they were sown in the infested soil.

2.3- Effect of seed treatments with chemical inducers.

Cowpea cv. Cream7 were soaked for 12 hrs in the solution of ascorbic, salicylic and oxalic acids at the rate of 4 mM to study their effects in inducing resistance in cowpea plants against root rot and wilt caused by *F. solani* and *S. rolfsii*.

2.4- Effect of seed treatments with Vitavax captan on disease incidence:

Seeds were dressed with the fungicide Vitavax captan at the rate of 3 gm/kg seeds and used as recommended treatment. Each treatment had four replicates.

3- Determination of chemical constitutes:

The activity of the oxidative enzymes; peroxidase (PO) and Polyphenol oxidase (PPO) in the 30-day-old treated and untreated cowpea plants were determined.

3.1- Determination of peroxidase activity:

Enzyme extraction from the leaves was prepared from 30 days-old plants as described by Maxwell and Bateman (1967). The leaf tissues were ground in a mortar with 0.1M sodium phosphate buffer at PH7.1 (2ml buffer/gm of fresh tissues). Triturated tissues were strained through four layers of cheesecloth and then filtrates were centrifuged at 3000 rpm for 20 min. at 6˚C. The supernatant fluid was used for enzyme assays.

Peroxdase activity was determined according to the method described by Allam and Hollis (1972) by the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425nm. The sample curette contained 0.5 ml of 0.1 potassium phosphate buffer at PH7 and 0.1m enzyme extract, 0.3m of 0.05M pyrogallol, 0.1ml of 1% H_2O_2 and distilled water to bring curette contents to 3ml. The rate of peroxdase activity was expressed as the change in absorbance at 425 nm/gram fresh weight/min.

3.2- Determination of polyphenol oxidase activity:

The activity of Polyphenol oxidase was measured as described by Matta and Dimond (1963). The reaction mixture consisted of 0.3ml. sample (1.0 ml. sodium phosphate buffer PH7, and 1.0 ml 10^3 M catechol) and completed with distilled water to 6.0 ml. The Polyphenol oxidase activity was assayed as mentioned above and expressed as the change in absorbency 1 minute/1g fresh weight.

4- Field Experiments:

The most efficient of plant extracts such as absinthe (*Artemisia absinthium* L.) and basil (*Ocimum basilicum* L.), essential oils as clove (*Syzygium aromaticum*) and blue gum (*Eucalyptus globulus* Labill), bioagents (*T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescence*) and chemical inducer (ascorbic and salicylic), which showed the highest reductions of disease incidence under greenhouse conditions were used in these experiments.

The field experiments were carried out at Etay El-Baroud Agricultural Experiments Station in two successive seasons during 2008 and 2009. The experiment unit 3x3 meter, contained four rows with distance 30 cm between hills and 2 seeds per hill. Cowpea cv. Cream7 was used in this investigation and sown in March for both seasons. Treatments were arranged in complete randomized block design with four replicates. Pre and post emergence damping-off were recorded after 30 and 45 days, respectively.

5- Statistical analyses:

Data collected were subjected to the statistical analyses according to the standard methods recommended by Gomez and Gomez (1984) using the computer program (costate). Means were compared using L.S.D. at the level 5% of probability.

RESULTS AND DISCUSSION

1- *In vitro* experiments:

Aqueous leaf extracts from *Artemisia absinthium*, *Ocimum basilicum* and *Mentha Longifolia* inhibited fungal growth. Table (2) showed that *O. basilicum* exhibited the maximum inhibition of fungal growth with an average of 81.29%, while the minimum inhibition of fungal growth was exhibited by *M. Longifolia* with an average of 39.07%. These results are in agreement with those reported by Srivastava and Bihari (1997), who reported that leaf extract of *O. basilicum* protected fruit rot of pea due to *Alternaria alternata* by 82.85%. Thymol and phenol present in *Lantana camara* and *O. basilicum* are toxic substances, which inhibited the growth of many fungi and bacteria Anonymous (1975) and (1976). In this respect, Cown (1999) reported that this effect may be attributed to plant contents of secondary metabolites (*e.g*., phenolic, alkaloids, flavonoids and terpenoids) that could adversely influences pathogen growth and development.

Table (2): Reduction percentage of mycelial growth of *F. solani* and *S.rolfsii* as affected by some plant extracts.

Plant extracts	Reduction of linear growth (%)		
	F. solani	***S. rolfsii***	**Mean**
Artemisia absinthium	64.37	59.63	61.99
Ocimum basilicum	83.33	79.23	81.29
Mentha longifolia	44.44	33.70	39.07
Mean	64.05	57.52	

	F. solani	***S. rolfsii***	**Interaction FxS**
L.S.D 5%	**4.23**	**3.45**	**N.S**

All of the oils tested against *F. solani* and *S. rolfsii* exhibited antifungal activity. Oils of *S. aromaticum* and *E. globulus* exhibited the highest reduction of the mycelial growth followed by *M. fistulosa* oil while *N. sativa* had slight reduction effect on mycelial growth Table (3). These results are in agreement with that mentioned by Antonov *et al.* (1997) who found that clove oil (*S. aromaticum*) reduced condium germination and germ tube length elongation and mycelial growth of *Botrytis cinera* with 67.00, 77.45 and 93.90%, respectively. Farag *et al.* (1989) found that essential oils of thyme, cumin, clove and rose mary caused complete inhibition of *Asperigillus parasitic* mycelial growth and aflatoxin production. In this respect, Tombe *et al.* (1995) reported that clove oil contains various volatile compounds *e.g* Eugenol, eugenol acetate and B- caryophllene. Eugenol inhibited radial growth and sporulation *F. oxysporum*, also the mycelia tips swelled, branched and distort. Zambonelli *et al.* (1996) observed that the essential oils of *Mentha piperita* and *Thymus vulagris* caused degeneration of the *F. solani* hyphae, which appeared emptied of their cytoplasmic content. Rahhal (1997) reported that several essential oils as clove, rose, bergamot, eucalyptus, nioul and lavender were able to prevent the growth of *B. cinerea in vitro*.

Table (3): Reduction percentage of mycelial growth of *F. solani* and *S. rolfsii* as affected by some essential oils

Essential oils	Reduction of linear growth (%)		
	F. solani	***S. rolfsii***	**Mean**
E. globulus	81.10	81.10	81.10
S. aromaticum	79.99	87.70	83.87
N. sativa	18.46	13.99	16.23
M. fistulosa	66.67	51.10	58.89
Mean	61.56	58.48	

	F. solani	***S. rolfsii***	**Interaction FxS**
L.S.D 5%	**1.43**	**1.01**	**0.45**

The biocontrol action of *T. viride, T. harzianum, G. virens, B. subtilis* and *P. florescens* filtrates on *F. solani* and *S. rolfsii* presented in Table (4) which showed that the culture filtrates of each *T. harzianum* and *T. viride* significantly reduced the radial growth of the tested pathogenic fungi significantly with averages of 75.28 and 69.62%, respectively. On the other hand, moderate reduction effect was obtained with *B. subtilis* with an average of 54.36%. *P. florescence* had the least effective reduction with an average of 49.66%. The same results are obtained by Shalaby and Atia (1996) and Nawal *et al.* (2006) where *T. harzianum*, *T. koningii* and *B. subtilis* had the highest percentages of reduction linear growth of *F. oxysporum*, *V. albo-atrum* and *F. solani*. The bioagents might effect the pathogen through excreting antimicrobial substance that effect the pathogen growth Bolar *et al.* (2000). It has been known that *Trichoderma* spp. inhibit the fungal growth by three mechanisms, competition (for space and nutrients), parasitism (deriving nutrients from the host) and antibiosis (production of an inhibition metabolite or antibiotic) Lorito *et al*. (1994) and Harman (2006), additionally, *B. subtilis* through phytoalexins production and increasing the activities of lyic enzymes (Sailaja *et al*., 1998).

Table (4): Reduction percentage of the mycelial growth of *F. solani* and *S. rolfsii* due to culture filtrates of the fungal and bacterial antagonists.

Culture filtrates	Reduction of in linear growth (%)		
	F. solani	***S. rolfsii***	**Mean**
T. viride	77.70	61.54	69.62
T. harzianum	82.67	67.88	75.28
B. subtilis	57.21	51.17	54.36
P. fluorescens	52.66	46.65	49.66
G. virens	54.07	47.07	50.57
Mean	64.93	54.86	

	F. solani	***S. rolfsii***	**Interaction FxS**
L.S.D 5%	**1.20**	**0.85**	**0.37**

Three antioxidants were used to evaluate their effects on the linear growth of *F. solani and S. rolfsii* Table (5). The tested antioxidants proved to be effective against these fungi. Salicylic acid was the most effective, which reduced the mycelial growth with an average of 46.85%. On the other hand, the minimum reduction was exhibited by oxalic acid with an average of 24.81%. These results are in agreement with the findings of Hussain (2009) who mentioned that salicylic, benzoic and oxalic acids inhibited the mycelial growth of *A. cucumerinum* with averages of 22.5, 23.7 and 28.7%, respectively.

Table (5): Effect of some chemical antioxidants on the mycelial growth of *F. solani* and *S. rolfsii.*

Antioxidants	Reduction of linear growth (%)		
	F. solani	***S. rolfsii***	**Mean**
Ascorbic acid	33.89	40.93	37.41
Salicylic acid	20.00	29.63	46.85
Oxalic acid	45.00	48.70	24.81
Mean	32.96	39.75	

	F. solani	***S. rolfsii***	**Interaction FxS**
L.S.D 5%	**4.841**	**3.952**	**N.S**

2- Greenhouse Experiments:

The effect of soaking cowpea seeds Cream 7 in the tested plant extracts, essential oils, bioagents and antioxidants on the pre and post emergence damping-off incidence has been studied under greenhouse conditions as the methods described by Atia *et al.* (2002). Seed soaking in *S. aromaticum* oil had the lowest percentage of pre emergence damping-off with the average of 20.83% for both *F. solani* and *S. rolfsii*, however, *O. basilicum* leaf extract showed the least percentage of damping-off for *F. solani* and *A. absinthium* for *S. rolfsii* and caused significant increases in survival plants with the average of 66.67 and 61.12 %, respectively Table (6). These results are in agreement with those obtained by Dwivedi and Singh (1998) and Atia *et al*. (2002) who studied the efficacy of (crude and aqueous) leaf extract of basil (*Ocimum basilicum*) and neem *(Azadirachta indica*) against major seed borne fungi of African yam bean. They reported that the incidence of seed borne fungi decreased and the seed germination and seedling emergence increased compared with the untreated control. The results indicated that the reduction effect of plant extracts on root rot of cowpea may be due to the absorption and translocation of the active substances into the seeds or seedling tissues and the remain during the seedling stage when most of disease occurs El-Shami, Mona *et al*. (1985) and Agha (1992). The same results were obtained by Atia *et al.* (*2002*), who found that under greenhouse conditions, seed soaking in garlic extract and dianthus oil gave the highest percentage of healthy survival of eggplants and pepper. These effects might be due to the presence of some antibiotic substances such as volatile sulpher compounds, phenolic compounds alkaloids.

Table (6): Effect of some plant extracts and essential oils as seed soaking on pre and post emergence damping-off of cowpea cv. Cream 7, under greenhouse conditions.

Treatments	Disease incidence %					
	F. solani			***S. rolfsii***		
	Pre emergence (%)	**Post emergence (%)**	**Survival**	**Pre emergence (%)**	**Post emergence (%)**	**Survival**
Plant extracts						
A. absinthium	33.33	6.67	60.00	33.33	5.55	61.12
M. longifolia	46.67	11.11	42.22	53.33	5.55	41.12
O. basilicum	33.33	0.00	66.67	40.00	0.00	60.00
Essential oils						
E. globulus	20.00	6.67	73.33	37.50	0.00	62.50
N. sativa	53.33	5.55	41.11	53.33	0.00	46.67
M. fistulosa	59.00	0.00	41.00	60.00	0.00	40.00
S. aromaticum	20.83	6.67	72.50	20.83	11.11	68.06
Vitavax captan	25.00	0.00	75.00	23.33	0.00	76.67
Infested-soil	63.33	0.00	36.67	66.67	0.00	33.33
Non infested soil	00.00	0.00	100.0	00.00	0.00	100.0
L.S.D 5 %	10.49	2.69	11.19	13.464	5.18	9.74

Results in Table (7) show that, seeds treated with the fungicide Vitavax captan and sown in infested soil with either *F. solani* or *S. rolfsii* caused an increase in cowpea fresh and dry weight with averages of 4.68, 1.82, 4.87 and1.97 (g), respectively, followed by *S. aromaticum* treatment which had averages of 4.56, 1.56, 4.31 and 1.88 (g), respectively.

Table (7): Effect of some plant extracts and essential oils as seed soaking on cowpea fresh and dry weight under greenhouse conditions.

Treatments	Growth parameters			
	F. solani		***S. rolfsii***	
	Plant fresh weight /g	**Plant dry weight/g**	**Plant fresh weight /g**	**Plant dry weight/g**
Plant extracts				
A. absinthium	3.58	1.29	4.21	1.56
M. longifolia	2.60	0.69	3.22	0.69
O. basilicum	4.08	1.84	3.58	1.14
Essential oils				
E. globulus	4.50	1.78	4.62	1.75
N. sativa	2.59	0.56	3.41	0.75
M. fistulosa	2.01	0.40	2.76	0.59
S. aromaticum	4.56	1.56	4.31	1.88
Vitavax captan	4.68	1.82	4.87	1.97
Infested-soil	1.75	0.31	1.70	0.25
Non infested soil	6.74	2.76	6.74	2.76
L.S.D 5 %	1.70	0.56	1.37	0.68

Seed soaking treatments with fungal and bacterial spore's suspension reduced the incidence of cowpea pre and post emergence damping-off caused by *F. solani* and *S. rolfsii.* The results in Table (8) indicate that Vitavax captan had the best effect followed by *T. harzianum*, which reduced the incidence of pre emergence damping-off caused by *F. solani* with averages of 20.83% and 23.34%, respectively. In contrast, *G. virens* and *P. fluorescens* exhibited the lowest effect. In case of *S. rolfsii*, *T. harzianum* was the most effective followed by *B. subtilis*, which reduced the disease incidence with averages of 29.17 and 33.33%, respectively, and caused significant increases in survival plants with averages of 70.83 and 66.67%, respectively. These results are in agreement with those obtained by El-Kafrawy *et al.* (2002) and Khaliefa *et al.* (2007), they reported that the culture filtrate of spore suspension of *T. harzianum* was generally the best treatment in decreasing damping-off and charcoal rot of sunflower as well as increasing survival plants while culture filtrates of *P. fluroesens* was the least effective in this respect. The same results were obtained by Atia *et al.* (2002) who found that filtrates of *Trichoderma* sp. and *B. subtilis* behaved the best results in controlling damping-off and root rot diseases of eggplants and pepper.

Table (8): Effect of cowpea seed soaking treatments with fungal and bacterial spores suspension on the pre and post-emergence damping-off % caused by *F.solani* and *S. rolfsii* under greenhouse conditions.

Treatments	Disease incidence %					
	F. solani			*S. rolfsii*		
	Pre emergence (%)	Post emergence (%)	Survival	Pre emergence (%)	Post emergence (%)	Survival
T. harzianum	23.34	00.00	76.67	29.17	00.00	70.83
T. viride	26.67	11.11	62.23	37.50	00.00	62.50
G. virens	37.50	11.11	51.39	40.00	16.66	43.34
B. subtilis	25.00	6.67	68.33	33.33	00.00	66.67
P. florescens	37.50	00.00	62.50	00.00	00.00	62.50
Vitavax captan	20.83	00.00	79.17	25.00	00.00	75.00
Infested-soil	65.00	00.00	35.00	70.00	00.00	30.00
Non infested soil	00.00	00.00	100.0	00.00	00.00	100.0
L.S.D 5 %	14.75	6.15	10.40	8.19	10.20	13.09

Results in Table (9) show that seed soaking in *T. harzianum*, *T. viride* and Vitavax-captan and sown in infested soil with *F. solani* caused an increased in cowpea fresh and dry weight (g). In case of treated seeds with bioagents; *T. harzianum* and *T. viride*, they had the best averages, these may be due to the availably of essential nutrient elements for plant and production of some growth regulators such as gibberellins (GA3 and GA4), IAA and some of vitamin B group which increased plant growth (Tong *et al.,* 1993).

Table (9): Effect of cowpea seed soaking with fungal and bacterial spores suspension on fresh and dry weight under greenhouse conditions.

Treatments	Growth parameters			
	F. solani		*S.rolfsii*	
	Plant fresh weight /g	Plant dry weight/g	Plant fresh weight/g	Plant dry weight/g
T. harzianum	5.00	2.78	4.70	1.80
T. viride	4.53	2.27	4.60	1.63
G. virens	3.45	1.50	3.04	1.45
B. subtilis	4.30	1.98	3.85	1.60
P. florescens	4.00	1.94	4.01	1.50
Vitavax captan	4.90	2.30	4.66	1.75
Infested-soil	1.70	0.29	1.65	0.26
Non infested soil	6.80	2.98	6.78	2.95
L.S.D 5 %	1.23	0.87	1.36	0.62

Chemical inducers significantly reduced damping-off % caused by *F. solani* and *S. rolfsii* compared with the control as it clear in Table (10). Salicylic acid at 4 mM followed by ascorbic acid recorded significant reduction effect and caused significantly increased in survival plants compared with control with the averages of 73.33, 62.50, 66.67 and 60.00. Maggie *et al.* (1996), Mahmoud and Gomah (2006), and Raju *et al*. (2008), who studied the effect of these chemical inducers on induction of plant resistance against soil born fungi showed that salicylic, citric and oxalic acids followed by hydrogen peroxide had the highest reduction on disease incidence and consequently increased fresh and dry weight. Kachroo *et al*. (2005), found that salicylic acid as foliar spray treatment caused a reduction in root rot disease in cowpea caused by *R*. *solani*. Salicylic acid has been identified as an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack Alvarez (2000). After a pathogen attack, SA levels often increases, induces the expression of pathogenesis related proteins, and initiates the development of systemic acquired resistance and hypersensitive response (Gruner *et al*., 2003).

Table (10): Effect of cowpea seed soaking with chemical inducers on pre and post emergence damping-off % caused by *F. solani* and *S. rolfsii* under greenhouse condition.

Treatments	Disease incidence %					
	F. solani			*S.rolfsii*		
	Pre emergence (%)	Post emergence (%)	Survival	Pre emergence (%)	Post emergence (%)	Survival
Ascorbic acid	37.56	00.00	62.50	40.00	00.00	60.00
Salicylic acid	33.33	00.00	73.33	33.33	00.00	66.67
Oxalic acid	46.67	00.00	53.33	46.47	6.67	46.67
Vitavax captan	20.00	00.00	80.00	23.33	00.00	76.67
Infested-soil	66.67	00.00	33.33	70.00	00.00	30.00
Non infested soil	00.00	00.00	100.0	00.00	00.00	100.0
L.S.D 5 %	14.53	ns	14.53	11.48	2.10	13.59

Results in the Table (11) show that from the tested chemical inducers, salicylic acid and ascorbic acid exhibited the highest values of plant fresh and dry weight (g) which were sown in infested soil with *F. solani* and *S. rolfsii*. In contrast, Citric acid was the least effective treatment.

Table (11): Effect of cowpea seed soaking treatments with chemical inducers on pre and post emergence damping-off on fresh and dry weight under greenhouse conditions.

Treatments	Growth parameters			
	F. solani		*S.rolfsii*	
	Plant fresh weight /g	Plant dry weight/g	Plant fresh weight /g	Plant dry weight/g
Ascorbic acid	3.59	1.57	3.58	1.45
Salicylic acid	4.43	1.69	3.85	1.65
Oxalic acid	3.06	1.46	3.28	1.40
Vitavax captan	4.51	1.78	4.20	1.56
Infested-soil	1.72	0.31	1.70	0.28
Non infested soil	6.45	2.70	6.46	2.70
L.S.D 5 %	1.26	0.73	1.03	0.74

2.1- Effect of different treatments on the changes of polyphenol oxidase (PPO) and peroxidase (PO) activities of infected plants:

Investigations of pathogen – host interactions problems are often encountered where a number of factors are involved; one is how the host defends itself. The high activities of peroxidase and catalase recorded in infected untreated plants could be considered as an antioxidant mechanism for protecting plants against the effects of pectinase on the plant cell walls. Activities of oxidative enzymes in any infected plants tissues are known to contribute to disease resistance mechanisms through the oxidation of phenols Tarrad *et al.* (1993) and Yehia *et al*. (2004). Data in Table (12) showed that all treatments increased the enzymes activities of peroxidase (PO) and polyphenoloxidase (PPO) compared with the infested soil without any treatment. In case of cowpea seeds treated with different agents and sown in infested soil with *F. solani* or *S. rolfsii,* leaves peroxidase activity increased sharply especially with treatments of *T. harzianum* and salicylic acid with averages of 1.611, 1.216, 0.526 and 0.650, respectively.

Table (12): Effect of different biotic and abiotic agents on peroxidase and polyphenol oxidase activities in leaves (30-day-old) of cowpea plants cv. Cream7 infected with *F. solani* and *S. rolfsii* under greenhouse conditions.

Treatments	Enzyme activity (z)			
	Peroxidase (PO)		Polyphenol oxidase (PPO)	
	F. solani	*S. rolfsii*	*F. solani*	*S. rolfsii*
Plant extracts				
A. absinthium	0.868	0.557	0.051	0.047
M. longifolia	0.784	0.390	0.046	0.044
O. basilicum	0.486	0.429	0.050	0.054
Essential oils				
E. globulus oil	0.830	0.509	0.066	0.062
N. sativa oil	0.320	0.274	0.028	0.022
M. fistulosa oil	0.393	0.400	0.040	0.034
S. aromaticum oil	0.633	0.442	0.068	0.053
Bioagents				
T. harzianum	1.216	0.526	0.133	0.102
T. viride	0.730	0.479	0.066	0.061
G. virens	0.520	0.450	0.046	0.041
B. subtilis	0.900	0.461	0.060	0.062
P. florescens	0.465	0.432	0.044	0.039
Chemical inducers				
Ascorbic acid	0.900	0.461	0.047	0.041
Salicylic acid	1.611	0.650	0.082	0.076
Oxalic acid	0.450	0.390	0.038	0.034
Vitavax-captan	0.685	0.595	0.043	0.038
Infested-soil	0.274	0.163	0.022	0.019
Non infested soil	0.349	0.349	0.032	0.032

Z: Activity of peroxidase, and polyphenol oxidase enzymes were expressed as change in absorbance / minute / 1.0 g fresh weight.

In contrast, polyphenoloxidase activity increased with the treatments of salicylic acid followed by *T. harzianum* with averages of 0.133, .082, 0.102

and 0.076 respectively. These results are in agreement with the results of Abd-El-Kareem (2007), Gailte *et al.* (2007) and Jayalakshmi *et al*. (2009), since they reported that the activities of both polyphenol oxidase and peroxidase increased after the treatment with *Trichoderma harzianum,* Mandal *et al*. (2009) mentioned that PO and PPO are important in the defense mechanism against pathogens, through their role in the oxidation of phenolic compounds to quinines, causing increasing in antimicrobial activity. Therefore, they may be directly involved in stopping pathogen development. In addition, protective effect of ascorbic acid and salicylic acid more related to reduce active oxygen species damage to essential protein and/or nucleic acid (Noctor and Foyer, 1998).

3- Field Experiments.

Data in Table (13) show that all seeds treatments decreased disease incidence percentage and increased cowpea seed yield per replicate (g) compared with the control treatment in the two successive seasons 2008 and 2009. From the above table it is clear that in the first season 2008 cowpea seeds treated with Vitavax-captan had the best effect in reducing the pre-emergence followed by salicylic acid, *T. harzianum, S. aromaticum* and *E. globulus* with averages of 18.10, 22.38, 24.29, 24.60 and 25.83%, respectively. In this respect, the present results are in harmony with those recorded by Khaleifa (1997) and Khaleifa *et al.* (2007) who reported that ascorbic acid and salicylic acid (2mM) significantly decreased the incidence of charcoal rot of sunflower in greenhouse and field experiments and increased survival plants. In case of the post emergence percentage, bioagents (*T. harzianum* and T. *viride*) had the least effect, in addation, the plant extracts (*A. absinthium* and *O. basilicum*), the antioxidant (salicylic acid), the plant oils (*E. globulus* and *S. aromaticum*) and the antibacteria (*B. subtilis*) prevented it completely and these results reflected on survival plants. Salicylic acid, *T. harzianum* and Vitavax-captan treatments had the best seed yield/replicate (g) with averages of 770.67, 768.33 and 754.00 (g), respectively. In the second season 2009 the same trend was noticed where Vitavax-captan and salicylic acid had the same effect in reducing the pre-emergence damping-off % followed by *T. harzianum* and *S. aromaticum*. Concerning to the post emergence damping-off %, the treatments which prevented it in the first season had the same effect in the second one in addition to *T. viride* and Vitavax-captan treatments. In case of cowpea seed yield/replicate, Vitavax-captan, salicylic acid and *T. harzianum* had the highest yield with the averages of, 771.67, 750.00 and 723.33, (g), respectively. These results are in agreement with those reported by El-Fiki *et al.* (2004) and Ibrahim (2006) who studied *Trichoderma* spp. as seed and soil treatments and reported that they gave sufficient control to charcoal rot of sunflower in greenhouse experiments and slightly effect in the disease incidence in field.

Table (13): Effect of different biotic and abiotic agents on disease incidence of cowpea damping-off and root rot diseases under field conditions, seasons (2008 and 2009).

Treatments	Disease incidence %							
	Pre emergence (%)		Post emergence (%)		Survival		Yield/Replicate	
	2008	2009	2008	2009	2008	2009	2008	2009
Plant extracts								
A. absinthium	38.75	41.67	00.0	2.15	61.25	57.62	507.67	475.00
O. basilicum	34.50	37.33	00.0	0.00	65.83	62.67	541.00 c	515.00
Essential oils								
E. globulus	25.83	29.78	00.0	0.00	74.17	70.22	533.67	523.33
S. aromaticum	24.60	26.67	00.00	0.00	75.40	72.22	656.00	658.00
Bioagents								
T. harzianum	24.29	25.00	2.50	2.15	74.88	74.28	768.33	723.33
T. viride	28.33	34.17	2.85	0.00	70.71	64.17	636.00	618.33
B. subtilis	30.19	34.67	00.0	0.00	69.81	65.33	610.67	603.33
P. florescens	32.86	37.33	1.79	2.14	66.55	68.86	607.67	596.67
Chemical inducers								
Salicylic acid	22.38	24.76	00.0	0.00	77.62	75.06	770.67	750.00
Ascorbic acid	32.5	33.33	1.72	1.79	66.92	65.74	512.67	527.00
Vitavax-captan	18.10	23.55	2.36	0.00	81.11	76.45	754.00	771.67
Control	48.57	55.75	2.86	1.43	50.48	45.77	157.67 c	150.00
L.S.D 5%	6.27	7.94	1.19	1.20	5.35	6.93	46.650	57.851

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تقييم بعض المركبات الكيماوية والحيوية الآمنة بيئياً ضد الفيوزاريم سولانى وسكلورشيم رولفزياى على نبات اللوبيا

جهاد محمد محمد ، نجلاء عبد الباقى سلام مهنا ، سهام سمير محمد رجب ، سعيد محمد حسن كامل
معهد بحوث أمراض النبات – مركز البحوث الزراعية

تم إستخدام العديد من المركبات الكيماوية والحيوية المختلفة مثل المستخلصات النباتية والزيوت الطيارة والكائنات المضادة والمواد المحفزة كمعاملة للبذرة بالإضافة إلى إستخدام المطهر الفطرى (فيتافاكس-كابتان) فى مقاومة مرض عفن الجذور وموت البادرات فى اللوبيا المتسبب عن فيوزاريم سولانى وسكلورشيم رولفزياى تحت ظروف المعمل والصوبة والحقل فى الموسمين 2008 – 2009 . تحت ظروف المعمل وجد ان المستخلصات النباتية (الدمسيسة و الريحان و النعناع البرى) تقلل من معدل النمو الميسليومى فى طبق بترى مقارنة بالكنترول. كانت أعلى نسبة تثبيط لمستخلص ا لريحان لكل من الفطرين فيوزاريم سولانى و سكلورشيم رولفزياى بنسبة 81,29 و61,99 % على الترتيب, كما أدت الزيوت الطيارة المستخدمة إلى تثبيط وتقليل معنوى للنمو الميسليومى لكلا الفطرين. وجد ان زيت القرنفل والكافور اعطى اعلى نسبة تثبيط للفطر سكلورشيم رولفزياى بنسبة 87,70 و 81,10 % على الترتيب, كما ان الراشح الفطرى تريكودرما هارزيانم و تريكودرما فيردى والراشح البكتيرى باسلس ستلس أعطى أعلى نسبة تثبيط مسليومى 75,28 ، 69,62 ، 54,36 % على الترتيب, وجد أن أفضل المواد المحفزة هو حمض السلسيلك اسد الذى كان اكثر تأثيرا فى تثبيط للنمو المسليومى لكلا الفطرين. فى تجربة الصوبة عند نقع بذور اللوبيا فى مستخلص الريحان أو زيت القرنفل فقد قلل من نسبة النباتات المصابة, كما أنة أعطى أعلى نسبة من النباتات الحية المتبقية. وجد ان معاملة البذور بالمطهر الفطرى فيتافاكس كابتان كان اكثر فاعلية فى تقليل نسبة الاصابة. الراشح الفطرى للفطر تريكودرما هارزيانم أفضل الرواشح الحيوية المختبرة فى تقليل نسبة النباتات المصابة, كما انة أعطى أعلى نسبة من النباتات الحية المتبقية. أدى نقع البذور فى محاليل كل من حمض الأسكوبيك وحمض ا لسلسليك إلى تقليل لشدة الإصابة و زيادة نسبة النباتات الحية المتبقية. أظهرت نتائج الدراسة أن إستخدام المواد المستحثة للمقاومة كمعاملة بذرة الى تحفيز بعض اليات الدفاع الكيمو حيوية مثل زيادة انزيم البيروكسيديز والبولى فينول اكسيديز وكانت معاملة البذرة بتريكودرما هارزيانم و حمض سلسليك و زيت القرنفل أفضل المعاملات فى هذا الصدد. وتحت ظروف الحقل أدى نقع بذور اللوبيا قبل الزراعة فى المبيد الفطرى (فيتافاكس-كابتان) الى نقص معنوى فى حدوث المرض وزيادة فى نسبة النباتات الحية المتبقية و وزيادة المحصول / مكررة فى كلا الموسمين يلية المعاملة با لكائنات المضادة (تريكودرما هارزيانم) و الزيوت الطيارة (زيت القرنفل) والمواد المحفزة (حمض سلسليك).

قام بتحكيم البحث

أ.د / على على عبد الهادى	**كلية الزراعة – جامعة المنصورة**
أ.د / محمد مجدى حمزه رحال	**مركز البحوث الزراعية**