SOME APPLICABLE METHODS FOR CONTROLLING SESAME CHARCOAL ROT DISEASE (*MACROPHOMINA PHASEOLINA*) UNDER GREENHOUSE CONDITIONS

BY

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ABSTRACT

Sesame charcoal rot disease (*Macrophomina phaseolina* Tassi (Goid)) was successfully controlled under greenhouse conditions by using several practices. Treating sesame seeds with *Trichoderma harzianum* produced the highest percentages of healthy mature plants (96.7%) followed by *Chaetomium bostrycoides* (90.0%), *Trichoderma* sp 5 (83.3%) and *T. hamatum* (80.0%). Moreover, soaking seeds in the filtered extracts of thyme, rhubarb or garlic or autoclaved extracts of cumin or azedrach maximized the healthy mature plants (83.3-86.7%). While, soaking seeds in IBA at 100 ppm or SA at 4 mM produced 100.0% and 96.7% healthy standing plants, respectively. The probable biochemical defense mechanisms that induced by these treatments in term of oxidative enzymes, phenol and sugars content were investigated and discussed.

Applying soil preparations of the VAM (vesicular arbuscular mycorrhiza) fungi showed no significant effect on the post-emergence and charcoal rot incidence. The soil preparation containing different *Glomus* spp. (G1+G2+G3+G4) produced the highest % healthy standing plants (86.7%) followed by G1 alone (76.7%), G3+G4 (70.0%), G2+G3 (63.3%), and G4 (63.3%) compared with control (36.7%). In another experiment, mixture of 4 VAM fungi grown in vitro on autoclaved barely-sand modified medium was used at different rates. Applying this mixture at rates of 2g/Kg soil produced the highest significant increase in healthy standing plants (70.0%) compared with control (30.0%). The incidence of charcoal rot was decreased significantly only at rates 1 and 2 g/Kg soil (2.5-7.5%) compared with control treatment (22.5%).

Key words: Sesame, charcoal rot, *Macrophomina phaseolina*, antagonistic microorganisms, plant extracts, resistant inducing agents, axenic culture, vesicular arbuscular mycorrhiza, VAM fungi.

INTRODUCTION

Macrophomina phaseolina Tassi (Goid), the causal of charcoal and/or root-rot is the main destructive pathogen on sesame plants especially in Upper Egypt **(Khalifa, 1997 and Abdou *et. al*., 2001)**. The charcoal rot and many other root pathogens could be controlled by the antagonistic fungi and bacteria, the vesicular arbuscular mycorrhizal fungi (VAMF) (**Perrtin, 1985; Schonbeck, 1987; Dinakaran and Marimuthu, 1997; Filion *et al.,* 1999; Mohan, 2000**) and plant extracts (**Osman (Nagwa) *et al*., 1996; Raja** and **Kurucheve, 1999).** Employing

the resistant-inducing agents was also used (**Shalaby and Saeed, 2000; Shalaby *et al.*, 2001 and Abdou *et. al*., 2001).** The present work was conducted to investigate effects of antagonistic fungi and bacteria, some autoclaved and filtered plant extracts, and chemicals inducing resistance to control of *Macrophomina phaseolina* under lab and greenhouse conditions. Effects of two different preparations of the vesicular arbuscular mycorrhizal fungi i.e. soil based and axenic cultures on incidence of charcoal rot disease under greenhouse were also evaluated.

MATERIALS AND METHODS

Effect of treating sesame seeds with some antagonistic microorganisms:

In this study, 7 isolates of *Trichoderma* sp. and one isolate of *Bacillus* sp. No. 3, (isolated from rhizosphere of healthy sesame plants) in addition to *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *Gliocladium penicilloides*, *Cheatomium bostrycoides*, *Bacillus subtilis,* and *B. megtella* (obtained from Biological Control and Onion and Oil Crops Res. Dept Agric., Res. Center Giza, Egypt) were used. Ten days PDA culture of each antagonistic fungus grown at 26 °C was flooded with sterile-distilled water, scraped with a camel brush then filtered thorough sterilized filter papers. Spore suspensions were adjusted to approx. $5x10^8$ condia/ml for *Trichoderma* spp and *G. penicilloides* and about 10^6 ascospores/ml for *C. bostrycoides*. Surface sterilized sesame seeds (0.5g) was thoroughly mixed with 2 ml spore suspension plus 1 ml of 1% Arabic gum solution as sticker (modified from **Harman *et al*., 1980**) and shacked slowly for 5 minutes. The tested antagonistic bacterial isolates was grown for 48 hrs at 26 °C on slants of King's medium B (KB), then bacterial growth was scraped and the re-suspended in mixture of 1.0% methyl cellulose and 0.1 M $MgSO_4$ (1:1 by volume). Surface sterilized sesame seeds (0.5g) were thoroughly mixed with 2 ml of bacterial suspension for 5 minutes then left to dry for 2 hrs in a laminar flow before planting **(Park *et al.* 1991)**. Bacterial population determined per seed was $1x10^8$ cells/seed according to dilution plate assay described by **Callan *et al*. (1990)**. The treated and non-treated seeds were sown in pots (ϕ 25 cm) containing soils infested with *M. phaseolina* (isolated by **Khalifa, 2003** from charcoal rotted sesame plants grown at Samalot "Menia governorate") at the rate of 10 sesame seeds (Giza32 cv.)/pot. Three replicates were used for each particular treatment. As for disease assessment, % pre-emergence damping off (15 days after sowing), post-emergence damping off and % healthy survived seedlings (45 d after sowing), charcoal rotted and healthy standing plants (90 d after sowing) were estimated as percentages of the number of seeds sown per each pot.

Effect of some filtered and autoclaved watery plant extracts:

Fresh plant parts of garlic (cloves), thyme and marjoram (herbs) and dried plant parts of clove (flower buds), roselle (sepals), ginger and rhubarb (roots), anise, fennel and cumin (seeds), eucalyptus and azedrach (leaves) were used in this study. The clean fresh plant material (50g) were blended in 250 ml sterilized distilled water. The filtered extracts were obtained by squeezing the homogenates through cheesecloth, filtered through filter paper Watman No.1, centrifuged at 5000 rpm for 30 min then the supernatant extracts were sterilized using Senter

glass G5. While, the autoclaved extracts were obtained by boiling the homogenates for one hour, filtered and centrifuged as above described then supernatants were autoclaved at 121°C for 15 min. Surface sterilized sesame seeds were soaked for 15 minutes in a tested plant extract plus 1% Arabic gum solution as sticker then left to air dry for 24 hrs before sowing in *M. phaseolina*-infested potted soils. Seeds treated with Arabic gum only were used in control pots. Three pots each planted with 10 sesame seeds were used for each treatment. The disease incidence as affected by the tested treatments was measured as described before.

Effect of treating seeds with different resistant inducing agents (RIA):

Surface sterilized sesame seeds were soaked for 2.5 hours in a known concentration of any tested resistant inducing agents (**Table 1)**. After 24 hours the air-dried seeds were sown at the rate of 10 seeds/pot in *Macrophomina phaseolina* infested potted soils. Three pots were used for each treatment. Seeds soaked in water served as control. The criteria of disease incidence were estimated as above mentioned. After 60 days from sowing, samples of treated and untreated healthy sesame plant-leaves were extracted according **Goldschmidt** *et al*. (**1968**). The leaf extracts were used for determining activities of peroxidase, catalase and polyphenol-oxidase enzymes using the procedures suggested by **Allam** and **Hollis** (**1972**). **Maxwell** and **Bateman** (**1967**) and **Matta** and **Dimond** (**1963**), respectively. The phenolic and sugar contents were also determined according to the methods described by **Snell** and **Snell** (**1953**) and **Thomas** and **Dutcher** (**1924**), respectively.

Table 1: List of the tested systemic resistant inducing agents and their concentrations.

Tested compound	Tested concentrations
Salicylic acid (SA)	2.0, 4.0 and 8.0 mM
Bion 500 WG *	2.0, 4.0 and 8.0 mM
Indole acetic acid (IAA)	100, 200 and 400 ppm
Indole butyric acid (IBA)	100, 200 and 400 ppm
Hydrogen peroxide (H_2O_2)	1.0, 2.0 and 4%
Potassium chloride (KCl)	1.0, 2.0 and 4%

* [50% Acibenzolar-5 methyl (lysoprosall), chemical name: benzol (1,2,3) thiadiazol-7-carbothioic acid 5-methyl ester (BTH)]

Effect of the soil and axenic culture preparations of the vesicular arbuscular-mycorrhizal (VAM) fungi:

Two experiments were conducted to evaluate effect of VAM fungi on disease incidence. In the first experiment, inocula of the VAM fungi *Glomus macrocarpum* (G1), *G. australe* (G2), *Glomus* spp. (G3), and Multi-VAM commercial product produced through the pot culture technique **(Sylvia and Jarstfer 1994)** were used. The inocula of G1 and G2 were maintained by **Khalifa**, **1997**, while, G3 and Multi-VAM were kindly obtained from Mycology and Plant Disease Survey Dept. Plant Pathology Res. Ins. ARC., Giza Egypt. The VAM-pot culture soil containing the VAM spores and colonized roots were added, singly or in combinations to

potted soil (infested with *M. phaseolina*) at the rate of 100g/pot (ϕ 25 cm) just before sowing seeds. Three pots were used for each particular treatment.

In the second experiment, cultures of 4 isolates of VAM fungi isolated from roots of onion, broad bean, maize and Swiss cheese and grown individually in axenic cultures for 3-4 weeks on a modified sand-barely medium **(El-Fiki, *et al.*, 2001)** were kindly provided by Prof. Dr. El-Fiki. The cultures of the 4 VAM isolates were mixed together at equal ratios (by weight) and added to sterilize soil (infested with *M. phaseolina*) at rates of 1, 2, 4 and 8g/Kg soil and 4 pots for each particular treatment were used. Four pots were used for each particular treatment. The treated and untreated (control) pots in each experiment were planted each with 10 sesame seeds. Effect of different treatments in each experiment on the disease incidence was estimated as described above. Root samples of healthy and diseased plants were taken and preserved for 21 days in FAA solution then examined for VAM colonization as described by **Phillips** and **Hayman** (**1970**).

RESULTS

Effect of some antagonistic microorganism:

Data in **Table (2)** reveal that, all tested antagonistic fungi were significantly effective in suppressing infection with *M. phaseolina* under greenhouse conditions compared with the control treatment. In this respect, *T. harzianum* and *C. bostrycoides* were the most effective since they completely suppressed incidence of pre- (0.0%), recorded the lowest incidence of post-emergence damping off (0.0-3.3%) and charcoal rot (3.3-6.7%) and produced the highest % healthy plants (90.0-96.7%). On the contrary, *Trichoderma* sp 10 was the least effective as it recorded no significant effect on the incidence of pre-emergence (23.3%), post-emergence (26.7%), charcoal rot (20.0%) and produced the lowest significant increase in the healthy plants (30.0%) compared with 30.0, 26.7, 43.3 & 16.7%, respectively in the control treatment.

Table (2): Effect of some antagonistic microorganisms on the disease incidence caused by *M. phaseolina* on sesame plants.

Antagonistic fungi and bacteria	% Disease incidence			
	At seedling stage		At maturity stage	
	Pre-emergence	Post-emergence	Charcoal rot	Healthy plants
B. megtella *	16.7	16.7	23.3	43.3
Bacillus sp 3 **	13.3	10.0	23.3	53.3
Bacillus subtillus *	10.0	13.3	20.0	56.7
Chaetomium bostrycoides *	0.0	3.3	6.7	90.0
Gliocladium penicilloides *	16.7	20.0	26.7	36.7
T. hamatum *	6.7	6.7	6.7	80.0
T. viride *	16.7	6.7	6.7	70.0
Trichoderma harzianum *	0.0	0.0	3.3	96.7
Trichoderma sp 10 **	23.3	26.7	20.0	30.0
Trichoderma sp 2 **	13.3	13.3	13.3	60.0
Trichoderma sp 3 **	16.7	23.3	16.7	43.3
Trichoderma sp 5 **	6.7	0.0	10.0	83.3
Trichoderma sp 6 **	13.3	6.7	10.0	70.0

Trichoderma sp 8 **	16.7	20.0	20.0	43.3
Trichoderma sp 9 **	20.0	6.7	16.7	56.7
Control	30.0	26.7	26.7	16.7
LSD. at 5%	8.17	9.05	7.96	9.53

* Obtained from Biological Control and Onion and Oil Crops Res. Dept Agric., Res. Center Giza, Egypt.
** Isolated from rhizosphere of healthy sesame plants.

Effect of some watery plant extracts and their sterilization method:

Data presented in **Table (3)** indicate that all disease criteria were significantly affected by the source of extract but not with sterilization method. It is indicate also that, the filtered (F) and autoclaved (A) extracts of a known source have different effects on the disease incidence. For example, the F-extracts of thyme, garlic and marjoram significantly better than the A-extracts for decreasing disease incidence and increasing the healthy plants at maturity stage. In fact, the A-extracts of garlic had no significant effect on disease incidence (% pre- & post-emergence and % charcoal rot) compared with the control treatment. On the contrary, the A-extracts in case of cumin, azederach, roselle and clove were significantly better than the F-extracts whereas, the efficiency of the F- and A-extracts of roselle and anise, however, were approximately equal. In general, the best disease control based on % healthy plants at maturity stage was produced by soaking sesame seeds in filtered extracts of thyme, rhubarb or garlic (83.3-86.7%) or autoclaved extracts of cumin or azedrach (86.7%). were more effective and

Table (3): Effect of filtered and autoclaved watery plant extracts on the disease incidence caused by *M. phaseolina* on sesame plants.

Source of plant extract	Sterilization method of extracts							
	Filtered extracts "F"				Autoclaved extracts "A"			
	% Pre-emergence	% Post-emergence	% Charcoal rot *	% Healthy plants **	% Pre-emergence	% Post-emergence	% Charcoal rot *	% Healthy plants **
Cumin	10.0	13.3	10.0	66.7	0.0	6.7	6.7	86.7
Ginger	13.3	23.3	16.7	46.7	13.3	13.3	16.7	56.7
Marjoram	10.0	6.7	6.7	76.7	13.3	23.3	20.0	43.3
Garlic	3.3	10.0	3.3	83.3	20.0	26.7	20.0	33.3
Rhubarb	6.7	3.3	6.7	83.3	3.3	13.3	3.3	80.0
Eucalyptus	10.0	16.7	23.3	50.0	16.7	10.0	13.3	60.0
Thyme	3.3	3.3	6.7	86.7	16.7	20.0	23.3	40.0
Anise	10.0	6.7	10.0	73.3	6.7	6.7	10.0	76.7
Rosselle	6.7	16.7	20.0	56.7	6.7	6.7	6.7	80.0
Fennel	16.7	20.0	20.0	43.3	13.3	20.0	16.7	50.0
Azedrach	13.3	16.7	13.3	56.7	0.0	6.7	6.7	86.7
Clove	10.0	16.7	16.7	56.7	6.7	6.7	6.7	80.0
Control	23.3	26.7	23.3	26.7	23.3	26.7	23.3	26.7
Mean	**10.5**	**13.9**	**13.6**	**62.1**	**10.8**	**14.4**	**13.3**	**61.6**

L.S.D. at 5% for:	Pre-	Post-	Rot	Healthy
Sterilization method	n.s.	n.s.	n.s.	n.s.
Source of extract	6.92	6.47	5.82	6.61
Interaction	9.79	9.16	8.23	9.35

Effect of different concentrations of some systemic resistance inducing agents:

1- Disease incidence:

Data presented in **Table (4-a)** show that all tested chemical agents were significantly effective for suppressing disease incidence particularly post-emergence and charcoal rot and increasing healthy plants at the mature stage. Among tested chemical agents, the hydrogen peroxide (H_2O_2) and Bion only had no significant effect on controlling the incidence of pre-emergence damping off (23.3%) compared with the control (26.7%). However, indole butyric acid (IBA) and salicylic acid (SA) were the most effective in this respect since they recorded the lowest incidence of pre-emergence (2.2-4.4%), post-emergence (2.2%), charcoal rot (1.1-4.5%) and highest increase in the mature healthy plants (91.1-92.2%) compared with the control treatment. The same results prove that, the higher tested concentration significantly increased % post-emergence and decreased % mature healthy plants compared with the lower and middle concentrations. In general, the IBA at the lower concentration (100 ppm) and SA at the middle one (4 mM) were the best treatments for controlling disease incidence and maximizing the mature healthy plants (96.7-100.0%). While, the higher concentration of H_2O_2 (4%) had no significant effect on % pre-emergence (26.7%), post-emergence (23.3%) and % healthy plants (36.7%) in comparison with the control treatment.

Table (4-a): Effect of different chemical agents and concentrations* as seed soaking on the disease incidence caused by *M. phaseolina* on sesame plants.

Agents	% Pre-emergence			% Post-emergence			% Charcoal rot			% Healthy plants		
	* I	II	III	* I	II	III	* I	II	III	* I	II	III
H_2O_2	23.3	20.0	26.7	16.7	13.3	23.3	13.3	16.7	13.3	46.7	50.0	36.7
KCl	16.7	16.7	10.0	20.0	13.3	3.3	20.0	13.3	16.7	43.3	56.7	70.0
IAA	6.7	13.3	16.7	3.3	3.3	10.0	3.3	10.0	13.3	86.7	73.3	60.0
IBA	0.0	3.3	3.3	0.0	0.0	6.7	0.0	6.7	6.7	100.0	90.0	83.3
SA	3.3	0.0	10.0	3.3	3.3	0.0	3.3	0.0	0.0	90.0	96.7	90.0
Bion	23.3	20.0	26.7	13.3	10.0	16.7	10.0	13.3	16.7	53.3	56.7	40.0
Control	26.7	26.7	26.7	23.3	23.3	23.3	23.3	23.3	23.3	26.7	26.7	26.7
Mean	14.3	14.3	17.2	11.4	9.5	11.9	10.5	11.9	12.9	63.8	64.3	58.1

* The tested concentrations of each compound were shown in Table (1).

LSD. at 0.05 for:	Pre	Post	Charcoal	Healthy plants
Chemical agents (A):	4.10	4.23	3.95	5.94
Concentrations (C):	2.90	N.S.	n.s.	4.20
A x C	8.19	8.45	7.90	11.87

2- Oxidative enzymes activity, phenolics and sugars contents in plant leaf extracts:

The data in **Table (4-b)** showed that the different tested chemical agents caused remarkable increases in the activities of the peroxidase, polyphenol oxidase and catalase enzymes in leaf extracts of sesame plants compared with the control treatment. IBA and SA recorded the highest increases in the activities of the peroxidase and polyphenol oxidase enzymes whereas, KCl recorded the highest increase in the activity of the catalase enzyme. Increasing conc. of the tested chemicals caused obvious decrease in the enzymes activities particularly

peroxidase. In general, the IBA treatment recorded the highest activities of the peroxidase and polyphenol oxidase and catalase enzymes at the lower conc. (100ppm), middle (200ppm) and higher one (400ppm), respectively. While, the lower conc. (2mM) of SA recorded the highest activities of the 3 enzymes. At meddle (2.0%) and higher (4.0%) conc., KCl increased activity of catalase enzyme more than the corresponding conc. of IBA and SA.

The data in **Table (4-c)** reveal that, IBA and SA treatments recorded the highest accumulation of free- (10.0 & 11.2mg), conjugated- (2.1 & 1.5mg) and total-phenols (12.0 & 12.8mg) compared with 6.2, 0.6 and 6.7mg, respectively in the control treatment. Both chemical agents induced highest accumulation of the free- and total-phenols at the lower conc. (100ppm). Also, IBA and SA recorded the highest amounts of reducing- (2.68 & 2.10mg) and total-sugars (3.02 & 3.0mg) whereas, KCl recorded the highest amounts of the non-reducing sugars (1.73mg) compared with the control **(Table, 4-d)**. The higher conc. (400ppm) of IBA and lower (2mM) and middle (4mM) conc. of SA induced the highest increase of the reducing and total sugars, respectively. On the contrary, the phenols **(Table, 4-c)** and sugars **(Table, 4-d)** contents seemed to be not affected by all conc. of the hydrogen peroxide (H_2O_2) compared with the control treatment.

Table (4-b): Effect of different concentrations of the tested chemical agents on the activities of peroxidase, polyphenoloxidase and catalase enzymes (as optical density/minute/g fresh weight) in leaf extracts of sesame plants.

Agents	Enzyme activity at different concentrations											
	Peroxidase				Polyphenoloxidase				Catalase			
	* I	II	III	Mean	* I	II	III	Mean	* I	II	III	Mean
H_2O_2	1.50	1.35	1.14	1.33	1.30	1.23	1.29	1.27	2.35	1.95	1.93	2.08
KCl	1.07	1.29	1.22	1.19	1.09	1.29	1.27	1.22	2.44	2.84	2.89	2.72
IAA	1.43	1.53	1.17	1.38	1.39	1.36	1.23	1.33	2.77	2.52	2.60	2.63
IBA	1.87	1.36	1.33	1.52	1.47	1.70	1.32	1.50	2.66	2.60	2.71	2.66
SA	1.97	1.44	1.18	1.53	1.51	1.36	1.26	1.38	2.70	2.60	2.46	2.59
Bion	1.57	1.29	1.02	1.29	1.17	1.24	1.05	1.15	2.66	2.51	2.45	2.54
Control	0.63	0.63	0.63	0.63	0.62	0.62	0.62	0.62	1.83	1.83	1.83	1.83
Mean	1.43	1.27	1.10		1.22	1.26	1.15		2.49	2.41	2.41	

* The tested concentrations of each compound were shown in Table (1).

Table (4-c): Effect of different concentrations of the tested chemical agents on the free-, conjugated- and total-phenols (mg/5g fresh weight) in leaf extracts.

Agents	Determined phenolic compounds and tested concentrations											
	Free phenols				Conjugated phenols				Total phenols			
	* I	II	III	Mean	* I	II	III	Mean	* I	II	III	Mean
H_2O_2	6.3	6.2	6.2	6.2	0.2	0.4	0.4	0.3	6.5	6.5	6.6	6.5
KCl	6.6	6.9	6.5	6.7	3.0	3.0	3.7	3.2	9.5	9.9	10.2	9.9
IAA	8.1	7.1	7.0	7.4	1.0	0.1	1.2	0.8	9.1	7.2	8.2	8.2
IBA	10.9	10.0	9.0	10.0	1.4	2.0	2.8	2.1	12.3	12.0	11.8	12.0
SA	13.3	10.7	9.6	11.2	3.7	0.4	0.6	1.5	17.0	11.1	10.2	12.8
Bion	7.0	6.3	6.4	6.6	3.7	2.4	1.3	2.4	10.7	8.6	7.7	9.0
Control	6.2	6.2	6.2	6.2	0.6	0.6	0.6	0.6	6.7	6.7	6.7	6.7

Mean	8.3	7.6	7.3		1.9	1.3	1.5		10.3	8.9	8.8	

* The tested concentrations of each compound were shown in Table (1).

Table (4-d): Effect of different concentrations of the tested chemical agents on sugars content (mg/5g fresh weight) in leaf extracts.

Agents	Determined sugars and tested concentrations											
	Reducing sugars				Non-reducing				**Total sugars**			
	* I	II	III	Mean	* I	II	III	Mean	* I	II	III	Mean
H_2O_2	0.79	0.88	0.83	0.83	0.05	0.06	0.10	0.07	0.84	0.94	0.92	0.90
KCl	1.58	1.64	0.89	1.37	1.73	1.25	2.20	1.73	3.31	2.89	3.09	3.10
IAA	1.02	0.94	1.10	1.02	0.15	1.02	0.03	0.40	1.17	1.96	1.13	1.42
IBA	2.79	2.13	3.13	2.68	0.25	0.35	0.44	0.35	3.03	2.48	3.56	3.02
SA	2.32	2.13	1.86	2.10	0.07	1.55	1.06	0.89	2.39	3.68	2.92	3.00
Bion	1.96	0.79	1.06	1.27	0.77	1.14	0.13	0.68	2.73	1.92	1.20	1.95
Control	0.84	0.84	0.84	0.84	0.10	0.10	0.10	0.10	0.94	0.94	0.94	0.94
Mean	1.61	1.34	1.39		0.45	0.78	0.58		2.06	2.12	1.97	2.05

* The tested concentrations of each compound were shown in Table (1).

Effect of soil inoculation with the vesicular arbuscular-mycorrhizal (VAM) fungi:

Data in **Table (5-a)** indicate that the % pre-emergence damping off (at seedling stage) and healthy plants (at maturity stage) only were significantly affected by the tested soil based VAM-preparations. The soil preparation containing G1+G2+G3+Multi-VAM completely suppressed incidence of pre-emergence (0.0%) and produced the highest % healthy plants (86.7%) compared with 23.3 & 36.7%, respectively in the control treatment. In this regard, the soil preparation contained G1 (*G. macrocarpum*) alone decreased % pre-emergence to 3.3% and increased % healthy plants to 76.7%. Thus, G1 alone was significantly better for decreasing pre-emergence and increasing healthy plants than the G2, G3 and Multi-VAM soil preparation each alone or in combination. On the contrary, soil preparation contained G2, G3, G1+G2, G1+G3 or G1+Multi-VAM had no significant effect on controlling incidence of pre-emergence (16.7-23.3%) compared with the control treatment (23.3%). Also, soil preparation contained G2 alone had no significant effect on % healthy plants (43.3%) compared with the control (36.7%).

Table (5-a): Effect inoculating pathogen-infested soil with different soil preparations of VAM fungi on the disease incidence caused by *M. phaseolina* on sesame plants.

VAM soil preparation	% Disease incidence			
	At seedling stage		At mature plant stage	
	% Pre-emergence	% Post-emergence	% Charcoal rot	% Healthy plants
G. macrocarpum [G1]	3.3	10.0	10.0	76.7
G. australe [G2]	23.3	16.7	16.7	43.3
Glomus sp. [G3]	20.0	13.3	13.3	53.3
Multi VAM	13.3	10.0	13.3	63.3
G1 + G2	20.0	16.7	16.7	46.7
G1 + G3	16.7	16.7	13.3	53.3
G1 + Multi VAM	16.7	13.3	10.0	60.0
G2 + G3	13.3	13.3	10.0	63.3
G2 + Multi VAM	10.0	16.7	13.3	60.0
G3 + Multi VAM	6.7	10.0	13.3	70.0
G1+G2 + G3 + Multi VAM	0.0	6.7	6.7	86.7
Control	23.3	20.0	20.0	36.7
LSD. At 5%	9.63	n.s.	n.s.	8.55

The data in **Table (5-b)** showed that adding the mixture of the axenic VAM cultures to the potted soil infested with *M. phaseolina* at rates of 1, 2, 4 or 8g/Kg soil significantly decreased incidence of pre-emergence (10.0-17.5%) compared with the control treatment (25.0%). The pre-emergence was decreased more

significantly at 4 than at 1, 2 or 8g. All tested levels had no significant effect on % post-emergence damping-off compared with control treatment. As for charcoal rot, the smaller levels i.e. 1 & 2g per Kg soil produced the highest significant decrease i.e. 7.5 & 2.5%, respectively. The larger levels (4 & 8g/Kg soil) had no significant effect in this respect (15.0 & 20.0%) compared with the control (22.5%). Applying VAM-mixture at rate of 2g/KG produced the highest increase in % healthy plants (70.0%). The % healthy plants were significantly decreased by increasing the added level of VAM-mixture up to 4 and 8g/Kg soil. The colonization of VAM fungi was detected in roots of both non-mycorrhizal **(Fig. 1-A & -B)** and mycorrhizal sesame plants **(Fig. 1-C)** grown in sterilized potted soil infested with the charcoal rot pathogen (*M. phaseolina*), respectively. The VAM colonization was more extensive in the VAM than non-VAM plants.

Table (5-b): Effect of soil inoculation with different levels of axenic cultures of vesicular arbuscular mycorrhizal [VAM] fungi on the disease incidence caused by *M. phaseolina* on sesame plants.

Inoculum levels (g/Kg soil)	% Disease incidence			
	At seedling stage		At maturity stage	
	% Pre-emergence	% Post-emergence	% Charcoal rot	% Healthy plants
1g	12.5	17.5	7.5	62.5
2g	12.5	15.0	2.5	70.0
4g	10.0	17.5	15.0	57.5
8g	17.5	20.0	20.0	42.5
Control	25.0	22.5	22.5	30.0
LSD. at 5%	7.31	n.s.	7.83	9.01

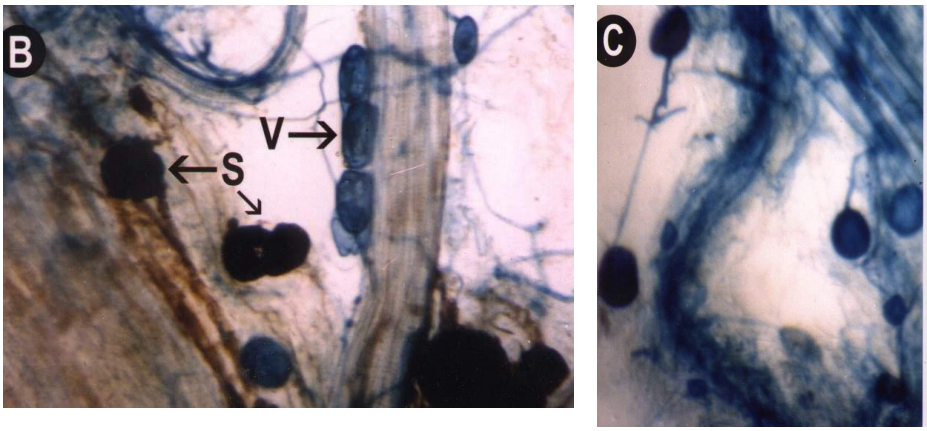

Fig. (1): Macerated tissues of non-mycorrhizal **(B)** and mycorrhizal **(C)** sesame plant roots grown in potted soil infested with the charcoal rot pathogen showed *M. phaseolina* sclerotia and vesicles of VAM fungi (arrows).

DISSCUSSION

Most tested antagonistic fungi and bacteria employed in the present study particularly *Trichoderma harzianum*, *Cheatomium bostrycoides* were significantly effective in suppressing disease incidence at seedling stage (pre- & post-emergence) and maturity stage (charcoal rot), consequently increased % healthy plants at the maturity stage. These results could be attributed to the *in vitro* antagonistic actions of the tested antagonistic microorganisms against *M. phaseolina* (**Khalifa, 2003 –** unpublished data). Also, the antagonistic microorganism(s) could suppress the activity of a plant pathogen through the enzymatic digestion of the pathogen cell walls **(Elad *et al.* 1983)** and/or production of inhibitory volatile substances **Sankar** and **Sharma (2001). Sankar** and **Sharma** stated that 2 out of 9 isolates of *T. viride* evaluated in preliminary tests showed superior performance against *M. phaseolina* (the causal of charcoal rot in maize) in the laboratory. However, all the nine isolates produced inhibitory volatile substances *in vitro*.

The present results indicated the filtered extracts of some plants controlled disease incidence better than the autoclaved extracts of the same plants but the opposite was noticed in some others. The efficiency of the tested plant extracts in controlling disease incidence might be depending on type of the active toxic substance(s) they have contained and to what extent they were affected by heating. The garlic extract, for example, might contain simple volatile toxic substances, which could be completely destroyed or broken to less toxic compounds by autoclaving. Reasonably the filtered extract of garlic was better in disease control than the autoclaved one. The cumin extract, as another example, might contained less toxic compound substances, which degraded by heat to more toxic compounds, then the autoclaved extract of cumin was better than the filtered one in disease

control. The equal efficiency of autoclaved and filtered extracts of rhubarb suggested that, rhubarb might contain thermostable toxic compound(s). These findings and explanations are in agreement with **Bashar (1991)**, **Osman (Nagwa) *et al*. (1996)**, **Baiuomy (1997)** and **Raja** and **Kurucheve (1999)**.

The herein results provide that soaking sesame seeds in solutions contained IBA at 100ppm or SA at 4mM completely suppressed incidence of charcoal rot and maximizing % healthy standing plants i.e. 100.0% and 96.7%, respectively. The promising effects of these chemical inducers could be attributed in part to their fungicidal properties as most tested chemical agents significantly decreased the *in vitro* linear growth of *phaseolina* and sclerotial production (**Khalifa, 2003** – Unpublished data). **Salama *et al*. (1985)** reported similar results. Also, the tested chemical agents might enhance systemic inducing resistance against infection with the charcoal rot pathogen. **Avdiushko *et al*. (1993)** mentioned that many plant enzymes are involved in defense reaction against plant pathogen. The oxidative enzymes such as peroxidase and polyphenol oxidase enhance formation of lignin and other oxidative phenols that contribute to formation of defense barriers for reinforcing the cell structure. The present results stated that the activities of the oxidative enzymes (peroxidase, polyphenol oxidase and catalase), the phenols and sugars contents were obviously higher in leaves of treated plants compared with control (untreated). These results are in agreement with many investigators (**Shahina Kalim *et. al*., 1999; Abdou *et al.*, 2001; Shalaby *et al.*, 2001**).

Among all tested soil preparations of the vesicular arbuscular mycorrhiza (VAM) fungi, the preparation contained G1+G2+G3+Multi-VAM led to complete suppression of pre-emergence caused by *M. phaseolina* and produced the highest % healthy plants at maturity stage. The soil preparation contained G2 alone has no significant effect compared with control treatment. The incidence of post-emergence (at seedling stage) and charcoal rot incidence (at mature stage) was not significantly affected by all tested soil preparations of the VAM fungi. Applying the mixture of the axinic VAM cultures at the lower rates (1 or 2g/Kg soil) gave the best disease control compared with the higher rates (4 and 8 g/Kg soil). In this point, **El-Fiki *et al.* (2001)** reported that increasing inocula rates of some isolates of VAM (grown in axenic cultures) resulted in growth retardation of maize plants. **Perrtin (1985)** reported that mycorrhizal association could improve or impair the health of plants. The protective effect had been demonstrated only for soil-borne diseases. The expression of this natural potential was related to the type of host, mycorrhizal fungi, pathogen and the soil conditions. The VAM fungi could protect plants against the invading pathogens by one or more of the following defence mechanisms. Increasing plant development and growth by improving P uptake **(Gracia and Ocampo, 1987).** Eliminating pathogens or reducing their effectiveness and improving host resistance through plant metabolism **(Schnobeck,**

1987). Production of phytoalexin **(Sundaresan *et al.*, 1993)**. Interacting directly with soil borne pathogens, or indirectly by stimulating other natural antagonistic **(Filion *et al.* (1999)**. The AM fungus could reduce pathogen population in soil and therefore posses a good biocontrol potential **(Mohan, 2000)**. In the present results, the VAMF colonization was detected in roots of both mycorrhizal and non-mycorrhizal sesame plants either in presence or absence of the charcoal rot pathogen (*M. phaseolina*). The sclerotia of *M. phaseolina* was nearly absent while VAMF colonization were noticed extensively in roots of sesame plants grown in presence of *M. phaseolina* and any VAM preparation (soil based or axenic culture). The presence of VAMF in roots of the non-mycorrhizal plants was reported also by **El-Fiki *et al.* (2001)** who could isolate and grow a VAMF in axenic culture from roots of maize plants grown in pathogen free sterilized sandy soil.

REFERENCES

Abdou, E.; H. M. Abd-Alla and A. A. Galal (2001): Survey of sesame root rot/wilt disease in Minia and their possible control by ascorbic and salicylic acids. Assiut Journal of Agricultural Sciences, l(23):135-152.

Allam, A. I. and S. P. Hollis (1972): Sulfide inhibition of oxidase in rice root. Phytopathology, 62: 634-639.

Avdiushko, S. A.; X. S. Ye and J. Kuc (1993): Detection of several enzmetic activities in leaf prints of cucumber plants. Physiological and Molecular Plant Pathology, 42: 441-454.

Baiuomy, M. A. M. (1997): Studies on the inhibitory activity of different plants against some causal pathogens. Ph.D., Fac. Agric., Al-Azhar Univ. p.168.

Bashar, M. A. (1991): Anti-fungal activity of Clematis gouriana against chickpea root pathogens. Bangladesh J. of Pl. Pathol., 7(1/2): 39-40.

Callan, N. W.; D. E. Mather and J. B. Miller (1990): Biopriming seed treatment for biological control of *Pythium ultimum* pre-emergence damping off in sh 2 sweet corn. Plant Dis., 74: 368-372.

Dinakaran, D. and T. Marimuthu (1997): Inhibition of *Macrophomina phaseolina* (Tassi.) Goid by mutants of *Trichoderma viride* Pers. ex Fr. Journal of Biological Control, 11(1/2):43-47.

Elad, Y.; R. Barak; I. Chet and Y. Henis (1983): Ultrastructural studies of the interaction between *Trichoderma* spp and plant pathogenic fungi. Phytopathologosche Z., 107(2): 168-175.

El-Fiki, A.I.I.; G.M.M. El-Habbaa; and Kh. E. Eid (2001): Successful growth and sporulation of the vesicular arbuscular mycorrhizal fungi in axenic cultures. Annals of Agric. Sci., Moshtohor, 39 (2): 933-952.

Filion, M.; C. Leclerc-Potvin; M. St-Arnaud; C. Hamel and S. H. Jabaji-Hare (1999): Use of quantitative molecular tools to study mycorrhizospheric interactions. Phtopathology, 89: S 25. Publication no.-P-1999-0178-AN. (c.f. meeting of Phytopathology, 89, (6), p: 525).

Goldschmidt, E. E.; R. Goren and S. P. Monselise (1968): The IAA oxidase system of citrus roots. Planta, 72: 213-222.

Gracia, G. J. M. and J. A. Ocampo (1987): Interactions between VAM and plant pathogenic organisms. Annals de Edafologia., Anales de Edafologia y, 46(9/10): 1233-1245. [c. f. Rev. Pl. Path., 67(12):5748].

Harman, G.E.; I. Chet and R. Baker (1980): *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *pythium* spp. or *Rhizotonia solani*. phytopathology, 70 (12): 1167-1172.

Khalifa, M. M. A. (1997): Studies of root-rot and wilt diseases of sesame plants. M.Sc.Thesis, Fac. Agric., Moshtohor, Zagazig Univ. Benha Branch, pp.158.

Khalifa, M. M. A. (2003): Pathological studies on charcoal-rot disease of sesame. Ph.D. Thesis, Fac. Agri., Moshtohor, Zagazig Uni, Benha Branch. pp 295.

Matta, A. and A. E. Dimond (1963): Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopath., 53: 547-587.

Maxwell, D. P. and D. F. Bateman (1967): Changes in the activities of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyle in relation to lesion maturation. Phytopathology, 57: 132-136.

Mohan, V. (2000): Endomycorrhizal interaction with rhizosphere and rhizoplane mycoflora of forest tree species in Indian arid zone. Indian Forester, 126(7):749-755.

Osman, Nagwa, A. I.; A. Hanafi, Awaref; A. A. El-Deeb and M. S. Abdel-Magid (1996): Effect of garlic extract and volatiles on root rot and pod rot diseases of peanut (*Arachis hypogaea* L.). Egypt J. Appl. Sci., 11(10): 162-175.

Park, J. L.; R. E. Rand; A. E. Joy and E. B. King (1991): Biological control of Pythium damping off and Aphanomyces root-rot of peas by application of *Pseudomonas cepacia* or *P. fluorescent* to seed. Plant Dis., 75: 987-992.

Perrtin, R. (1985): The ability of mycorrhizae to protect plants against disease: pamacea or chemero. Annules de Sciences forestieres, 42(4):453-469. [c. f. Rev. Pl. Path., 65(6):2881].

Phillips, J. M. and D. S. Hayman (1970): Improved features for clearing roots and staining parasitic and vesicular-arbuscular-mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55:158-161. (c.f. Fares, Clair, N. 1992).

Raja, J. and V. Kurucheve (1999): Fungicidal activity of plant and animal products. Annals of Agricultural Research, 20(1):113-115.

Salama, A. A. M.; I. M. K. Ismail and S. A. E. Ouf (1985): Soaking *Sclerotium cepivorum* in phenolic compounds and their effect on germination, growth and sclerotial formation. Bulletin of the Faculty of Sci., Cairo Univ., 53(1): 309-319.

Sankar, P. and R. C. Sharma (2001): Management of charcoal rot of maize with *Trichoderma viride*. Indian Phytopathology, 54(3): 390-391.

Schnobeck, F. (1987): Mycorrhiza and plant health: A contribution to biological protection of plants. Angewanddte Botanik, 61(112): 9. (c.f. Rev. Pl. Path., 66: 4093).

Shahina Kalim; Y. P. Luthra and S. K. Gandhi (1999): Copper induced effect on biochemical constituents in cowpea susceptible to *Rhizoctonia* species. Acta Phytopathologica et Entomologica Hungarica, 34(3):199-210.

Shalaby, I. M. S.; R. M. A. El-Ganainy; S. A. Botros and M. M. El-Gebally (2001): Efficacy of some natural and synthetic compounds against charcoal rot caused by *Macrophomina phaseolina* of sesame and sunflower plants. Assiut Journal of Agricultural Sciences,.32(5): 47-56.

Shalaby, S. I. M. and M. N. A. Saeed (2000): Biochemical defense mechanisms associated with the systemic induced resistance in sesame plants against Fusarium wilt disease. Zagazig J. Agric. Res. 27(1): 105-113.

Snell, F. D. and C. I. Snell (1953): Colorimetric Methods. Vol. III. D. Van Nostrand Co. Inc., Torento, N. Y., London, 606 pp.

Sundaresan, P.; N. U. Rájá and P. Gumaskaran (1993): Induction and accumulation of phytotoxins in cowpea roots infected with a mycorrhizal fungi *Glomus fasiculatus* and their resistance to Fusarium wilt disease. J. of Biosciences, 18(2): 219-301.

Sylvia, D.M. and Jarstfer, A.G. (1994): Production of inoculum and inoculation with arbuscular mycorrhyzal fungi. In: Robson, A.D., Abbott, L.K. and Malajczak, N. (eds.) Management of mycorrhyzas in agriculture, horticulture and forestry. Kluwer, Dordrecht, pp 231-238.

Thomas, W. and R. A. Dutcher (1924): The colorimetric determination of carbohydrates in plants by the picric acid reduction method. I. The estimation of reducing sugars and sucrose. J. Amer. Chem. Soc., 46: 1662-1669.

بعض الطرق التى يمكن استخدامها فى مكافحة مرض العفن الفحمي فى السمسم المتسبب عن الفطر ماكروفومينا فاسيولينا

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أظهرت الدراسة الحالية إمكانية مقاومة مرض العفن الفحمى فى السمسم بنجاح تحت ظروف الصوبة بطرق مختلفة حيث أمكن الحصول على أعلى نسبة مئوية من النباتات السليمة عند مرحلة النضج عن طريق معاملة البذور (قبل الزراعة) بأحد فطريات التضاد الحيوى التالية: تريكوديرما هارزيانوم (96.7%)، كيتوميوم بوسترايكويدس (90.0%)، عزلة رقم 5 من فطر تريكوديرما (83.3%) أو تريكوديرما هاماتوم (80.0%).

أمكن أيضا مقاومة المرض من خلال تحفيز المقاومة الجهازية فى النبات عن طريق نقع البذور فى المستخلصات المعقمة بالترشيح لنباتات الزعتر، الراوند أو الثوم (83.3-86.7% من النباتات السليمة) أو تلك المعقمة بالأوتوكلاف لنباتات الكمون أو النيم (86.7%). أو نقعها لمدة ليلة فى حامض السلسليك (4 تركيز جزء فى المليون) أو حامض البيوتيريك (تركيز 100 جزء فى المليون) إلى حدوث زيادة فى نسبة النباتات الناضجة السليمة إلى 96.7%، 100.0% على التوالى. وفى هذه المعاملات اختفى تماما العفن الفحمى. ويرجع التأثير لتلك المعاملات الى تحفيز إمكانية النبات على الدفاع الكيميوحيوية متمثلة فى زيادة نشاط وتكوين مادة مؤكسدة والمحتوى الأعلى من المركبات الفينولية والسكريات.

أظهرت النتائج أيضا أن إضافة تحضيرات تجارية محتوية على لقاح أنواع مختلفة من فطريات الميكوريزا الحويصلية الشجيرية (جنس جلومس) بمعدل 100/ اصيص إلى تربة الأصيص الملوثة بفطر ماكروفومينا فاسيولينا لم يكن لها تأثير معنوى فى تقليل نسبة موت البادرات بعد الإنبات أو خفض ظهور العفن الفحمى على النباتات الناضجة غير أنه قد أمكن الحصول على أعلى نسبة من النباتات السليمة فى مرحلة النضج باستخدام التحضير المحتوى على الأنواع الأربعة من الجنس جلومس (86.7%) يليه التحضير المحتوى على النوع 1 (76.7%) ثم المحتوى على النوعين 3، 4 (70.0%) عند مقارنتها بمعاملة الكنترول (36.7%).

أما إضافة مخلوط لقاح العزلات الأربعة من فطريات الميكوريزا الحويصلية الشجيرية المعزولة من نباتات مختلفة والمنماة على بيئة الشعير والرمل المعدلة بمعدل 2جم/كجم تربة (ملوثة بفطر العفن الفحمى) فقد رفعت من نسبة النباتات الناضجة السليمة إلى 70.0% مقارنة بمعاملة الكنترول (30.0%). أدى استخدام المعدلات المنخفضة من هذا المخلوط اللقاحى (1 و 2 جم/كجم تربة) إلى خفض معنوى فى نسبة النباتات الناضجة المصابة بالعفن الفحمى (2.5-7.5%) مقارنة بالكنترول (22.5%).