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TOWARDS AN INTEGRATED CONTROL OF SESAME (*SESAMUM INDICUM* L.) CHARCOAL ROT CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID

[41]

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ABSTRACT

Soil solarization in combination with fungal antagonists and soil amendments has been subjected to evaluation as a potential disease management strategy for the control of charcoal rot of sesame caused by *Macrophomina phaseolina* (Tassi) Goid. Solarization alone or in combination with *Trichoderma pseudokoningii* and *Emericella nidulans* singly or in mixed inocula reduces disease incidence from 30 % (control) to 80%, 91 %, 82 % and 85% respectively. It is noted that while pairing improved the biocontrols potentiality of *E. nidulans* by increasing the number of healthy plants in both unsolarized and solarized soils it leads to decrease in the biocontrol potentiality of *T. pseudokoningii.* On the other hand the combination of solarization with soil amendment with *Eucalyptus* powdered leaves showed a synergistic effect by increasing number of healthy plants from 65 % in amended unsolarized soil to 77 % in amended solarized soil.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. a soil borne fungus causes charcoal rot of roots and lower stem. The fungus can infect about 500 plant species in more than 100 families throughout the world **(Mihail & Taylor 1995 and Srivastava *et al* 2001).** It is of high incidence in Egypt especially during the hot period. In addition to root and stem root it causes in sesame (*Sesamum indicum* L.) early maturation, chlorosis and incomplete capsule filling **(Wyllie, 1988).** *M. phaseolina* survives as microsclerotia in the soil and infected plant debris. These microsclerotia serve as the primary source of inoculum and have been found to persist in the soil up to three years **(Cloud and Rupe, 1991)** but their survival is greatly reduced in wet soils.

Strategies of effective and economic disease management of charcoal rot are still inadequate. Management of charcoal rot requires integrated strategies that either reduce the population of microsclerotia in the soil or prevent infection. From an environmental point of view soil heating is a more acceptable method of enhancing efficacy of antagonistic strains of microbes against charcoal rot and many other plant pathogens. It can be accomplished in warm climates by solarization. This involves covering moistened field soil with transparent polyethylene sheets during the hot season causing an increase in soil temperature up to 45 ^{o}C. The resulting high soil temperature can reduce diseases caused by several soil borne pathogens including nematodes, fungi and bacteria **(Ramirez-Villapudua & Munnecke, 1988; Satour *et al* 1989; Osman, 1990; Sarhan, 1990; Ali *et al* 1990; Davis, 1991; Melero-Vara *et al* 1995; Chellemi *et al* 1997; Lodha *et al* 1997 and Pinkerton *et al* 2000).** Although soil temperatures attained by solarization may be sufficiently high to directly kill propagules of some pathogens present in the upper soil layers, the efficacy declines with soil depth **(Katan, 1981),** and therefore a combi-

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nation with other control measures is often necessary for improving the efficacy of soil solarization (**Washington *et al* 2003**). Improving of solarization can be accomplished by soil amendment with organic fertilizer or using antagonistic fungi.

The objective of the present investigation is to compare the efficacy of solarization alone and in combination with other means to control charcoal rot by *Macrophomina phaseolina*. These combinations comprised: the use of antagonistic fungi and amendment of soil with *Eucalyptus* leaves.

MATERIALS AND METHODS

Experimental design

An experiment consisted of two treatments namely, solarized (mulched with 0.9 mm thick transparent polyethylene sheets) and unmulched (exposed to direct sun-light) was conducted in the Botanical Garden of Faculty of Science, Suez Canal University at Ismailia in an artificially infested site with *M. phaseolina.* The site was divided into two plots ($10m^2$ each) in which both plots were further divided into five sections each measuring 1 x 2 m. All experiments were repeated twice in the two consecutive seasons 2003 & 2004 in which the effect of each of solarization, fungal antagonists, and soil amendment with *Eucalyptus* leaves alone and/ or in combination on the viability of inoculum of *M. phaseolina* were studied. *Eucalyptus* leaves were dried at 60 ^oC for 48 hr and powdered on the soil surface in the amount of 30 g m^2 then mixed with the soil by forking to a depth between 5 and 10 cm before solarization. The soil type is sandy with 55 percent sand, 16 percent silt & clay. The soil pH ranges from 7.6 to 7.9, electric conductivity ranged from 1.98 to 2.02 dSm^{-1} and organic matter ranges from 2.1 to 2.3 %.

Soil solarization and soil temperature

The plot was prepared by tilling and crumbling to a depth of 15 to 25cm and watered to field capacity. This was accomplished by covering moist soil with 0.9 mm thick transparent polyethylene sheets on 1st of July 2003, and the unmulched plot was left exposed to direct sun light. Edges of the polyethylene sheets were buried to 25 cm depth at the margins with special care to minimize the distance between the sheets and soil to prevent the formation of air pockets that retard the soil heating process. Five sections were solarized for 8 weeks and soil temperatures, minimum and maximum, were daily recorded for mulched and unmulched soil at the depths of 5 and 10 cm by using a soil thermometer.

Soil sampling and isolation of fungi

After solarization for eight weeks, samples were taken from the upper 20 cm of the soil profile with a sampling tube (ca 2.5 cm diameter). Five soil samples were collected at random from each section and kept in plastic bags to form composite. Total mycobiota was isolated using dilution plate method **(Johnson *et al* 1960)** in both mulched and unmulched plots. Czapek's agar supplemented with 0.5 % yeast extract (CYA), amended with rose bengal (1/15000) and chloramphenicol (50 ppm) was used for primary isolation. Twenty five plates were used for each sample. Plates were incubated at 28 ^oC for 10 days and developing fungi were counted. For maintaining cultures and for proper identification, pure cultures of isolated fungi were grown on standard media such as Vegetable Agar (V8), Oatmeal Agar (OA), Malt Extract Agar (MEA) Potato Dextrose Agar (PDA) and Potato Carrot Agar (PCA).

Taxonomic identification by morphology of fungal isolates was mainly based on the following identification keys: **Raper & Thom (1949) and Pitt** (**1980**) for *Penicillium*; **Raper & Fennell** (**1965**) for *Aspergillus*; **Ellis (1971 and 1976)** for dematiaceous hyphomycetes; **Booth (1971)** for *Fusarium*; **Arx (1981), Domsch *et al* (1980)** for miscellaneous fungi; **Arx *et al* (1986), Cannon (1986)** for *Chaetomium*. The systematic arrangement follows the latest system of classification appearing in the 9th edition of Anisworth & Bisby's Dictionary of the fungi (**Kirk *et al* 2001**).

Preparation of inocula

a- The pathogen

Sclerotia of *M. phaseolina* were initially isolated from naturally infected sesame plant. Infected stem of sesame plants were surface sterilized using 7 % sodium hypochlorite for three minutes then soaked in 55-75% ethanol for two minutes (**Royse & Ries, 1978 and Melgarejo *et al* 1985**). After rinsing with sterilized water, stem were sliced and transferred to plates of PDA, CYA amended with rose bengal and incubated at 30c for 7 days. Microsclerotia were collected from a culture of the pathogen grown on PDA for 10 days and kept at 7 ^oC till use. Inoculum for use in field

experiments was prepared by culturing the fungus in autoclaved barley grains (250 g of barley; 30 ml water) at 30 ºC for two weeks in 500 ml conical flask.

b- The antagonists

Preparation of Trichoderma and Emericella cultures.

The antagonistic abilities of various isolated taxa against *M. phaseolina* were characterized by the BioControl Index (BCI) values calculated according to **Leitgeb *et al*** (**2005**). Due to the high rate of recolonization of *T. pseudokoningii* **(Hermosa *et al* 2000)** and high antagonistic potentiality of *E. nidulans* they were selected for field studies. *T. pseudokoningii* and *E. nidulans* were grown on PDA for 10 days; spores of dried cultures were harvested by gentle scraping with a camel's hair brush from the dry agar surface into sterile vials containing surface sterilized, dry sesame seeds. Vials were shaken for 10 minutes to assure thorough dressing. Uncoated seeds were also used as control. Sections of both solarized and unsolarized soil were treated with fungal antagonists and *Eucalyptus* leaves as shown in **Figure (1).** These sections were planted by coated sesame seeds by spreading the seeds on soil surface at the rate of 500-600 seed/m^2 then forking to a depth of 2 to 3 cm.

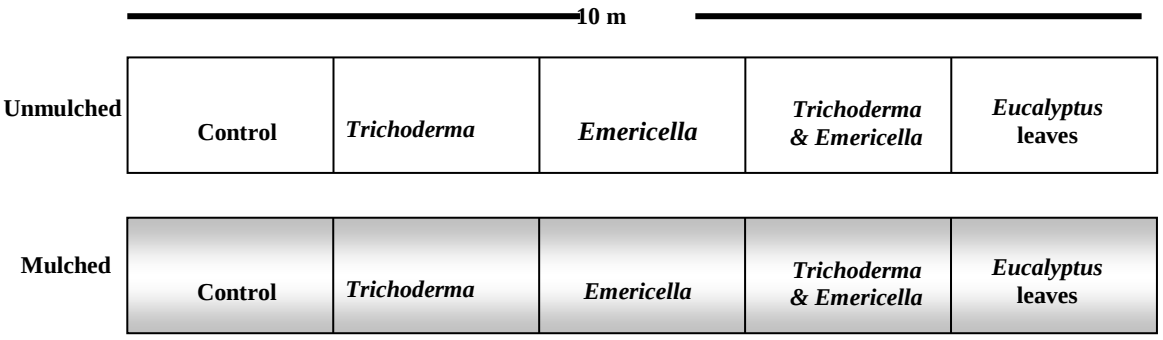

Figure 1. Experiment design and different treatments

RESULTS

Efficacy of solarization

Recorded data indicated that soil temperature during July-August elevated remarkably in solarized soil than in unsolarized at both depths **(Table 1).** Mulching increased average maximum soil temperature than unmulched one by 14.7 ºC and 6.2 ºC at 5 and 10 cm depths, respectively. At the depth of 5 cm, the mean of the maximum temperatures recorded in the solarized treatment was 48.2 + 0.7 ºC while in unsolarized treatment it was 37.4 + 0.9 ºC. While at the depth of 10 cm the mean of the maximum temperature in solarized soil was 40.4 + 0.5 ºC and it was 33.02 + 0.9 ºC in unsolarized soil with the maximum absolute temperature of 56.1 ºC in solarized and 41.2 ºC in unsolarized soil respectively.

Total mycobiota

The mycobiota of unmulched and mulched plots contained a total of 54 species (16621 isolates): Zygomycota (eight species, 3.89% of the total isolate number), teleomorphic Ascomycota (14 species, 8.76%), anamorphic (asexual) Ascomycota (24 species, 83.49%) and mitosporic fungi (8 species, 3.86 %) **(Table 2).**

Isolated species belonged to thirty-seven genera. The prevailing genera were *Aspergillus* (8 species including anamorph stages of one *Emericella*, two *Eurotium* and one *Neosartorya* species; 48.95%), *Chaetomium* (four species; 2.47%), *Penicillium* (four species including anamorph of one *Talaromyces* species; 1.1%) and *Fusarium* (three species, 6.9 %).

The most abundant species were: *Scopulariopsis brevicaulis* (26.26 % of the total isolate number), *Aspergillus versicolor* (25.53%), *A. terreus* (11.8 %), *A. flavus* (7.44 %) and *Fusarium oxysporum* (4.81 %). Forty-nine species were isolated from unsolarized soil, while thirty-eight were recovered from solarized plots. Thirty species were common for both unsolarized and solarized plots.

Table 1. Soil temperatures (°C) during solarization from 1st July to 25th August 2003 at the study area

Week	Depths (Cms)	Treatments							
		Mulched				Unmulched			
		Max.	Min.	Av.	*T.F.A.	Max.	Min.	Av.	*T.F.A.
First	5	43.1	36.6	41.2	6.5	39.2	31.9	33.8	7.3
	10	39.7	34.8	37.3	4.9	36.7	29.3	30.6	7.4
Second	5	45.6	30.5	41.7	15.1	39.6	31.2	35.1	8.4
	10	41.9	28.9	37.6	13	36.8	29	30.9	7.8
Third	5	49.2	44.3	45.2	4.9	40.2	32.6	36.9	7.6
	10	45	39	41.1	6	37.1	31.3	32.5	5.8
Fourth	5	53.4	47.9	48.6	5.5	40.7	31.9	38.2	8.8
	10	٤٧.7	39.9	43.5	7.8	37.4	30.6	33.1	6.8
Fifth	5	56.1	49.6	55.9	6.5	41.2	35.3	40.7	5.9
	10	44.7	39.3	41.1	5.4	37.4	32.1	34.5	5.3
Sixth	5	52.9	44.3	50.9	8.6	40.2	33	38.4	7.2
	10	43.2	39.6	40.7	3.6	36.9	29.9	33.8	7
Seventh	5	54.2	48.3	51.4	5.9	41	34.9	38.9	6.1
	10	42	38.1	41.6	3.9	38	30.3	34.7	7.7
Eighth	5	54.9	40.9	50.7	14	40.8	32.7	37.9	8.1
	10	46.1	37.9	40.9	8.2	36.5	29.6	34.1	6.9

***** Temperature fluctuation amplitude (TFA) is the difference between averages of minimum and maximum daily temperatures

Table 2. Total count (TC, colonies/ g dry soil), number of cases of isolation (NCI, out of 25 soil samples) and percentage frequency of fungal taxa recovered on Czapek's yeast extract agar at 28°C.

Species	Unsolarized			Solarized		
	TC	NC1	% F	TC	NC1	% F
Zygomycota						
Absidia glauca Hagem	0	0	0	137	7	28
Actinomucor elegans (Eidam) C.R. Benj. & Hesselt.	11	3	12	0	0	0
Cunninghamella echinulata (Thaxt.) Thaxt. Ex Blakeslee	18	3	12	0	0	0
Mucor circinelloid Tiegh.	21	5	20	9	2	8
M. racemosus Fresen.	67	10	40	0	0	0
Mycocladus corymbiferus (Cohn) J.H. Mirza	102	14	56	191	17	68
Rhizopus stolonifer var. *stolonifer* (Ehrenb.) Vuill.	13	3	12	37	5	20
Syncephalastrum racemosum Cohn ex J. Schröt.	41	7	28	0	0	0
Ascomycota (teleomorphic)						
Achaetomium macrosporum Rai, Wadhwani & J.P. Tewari	23	3	12	0	0	0
Byssochlamys nivea Westling	42	7	28	63	8	32
Chaetomium bostrychodes Zopf	38	4	16	0	0	0
Ch. globosum Kunze	99	19	76	119	16	64
Ch. gracile Udagawa	9	2	8	0	0	0
Ch. nigricolor L.M. Ames	59	6	24	87	8	32
Emericella nidulans (Eidam) Vuill.	117	16	64	339	19	76
Eurotium amstelodami L. Mangin	11	3	12	37	4	16
E. chevalieri L. Mangin	17	3	12	29	4	16
Gymnascella dankaliensis (Castell.) Currah	0	0	0	78	5	20
Microascus cinereus Curzi	54	3	12	145	7	28
M. trigonosporus C.W. Emmons & B.O. Dodge	32	4	16	0	0	0
Neosartorya fisherii (Wehmer) Malloch & Cain	0	0	0	27	2	8
Talaromyces flavus (Klöcker) Stolk & Samson	0	0	0	31	3	12

Table 2. cont.

Species	Unsolarized			Solarized		
	TC	NC1	% F	TC	NC1	% F
Ascomycota (anamorphic)*						
Acremonium implicatum (Gilman & Abbott) W. Gams	67	7	28	45	3	12
Alternaria alternata (Fr.) Keissl.	87	11	44	43	3	12
Aspergillus flavus Link	834	21	84	402	13	52
A. niger var. niger Tiegh.	78	14	64	41	7	28
A. terreus Thom	73	10	40	1889	21	84
A. versicolor (Vuill.) Tirab.	1234	23	92	3009	25	100
Bipolaris spicifera (Bainier) Subram.	13	2	8	0	0	0
B. indica J.N. Rai, Wadhwani & J.P. Tewari	27	5	20	0	0	0
Botrytis cinerea Pers.	19	4	16	0	0	0
Cephaliophora irregularis Thaxt.	58	8	32	32	3	12
Cladosporium herbarum (Pers.) Link	11	3	12	8	1	4
C. sphaerospermum Penz.	14	3	12	0	0	0
Curvularia oryzae Bugnic.	23	4	16	0	0	0
C. tuberculata B.L. Jain	33	4	16	0	0	0
Drechslera rostrata (Drechsler) Richardson & E.M. Fraser	31	4	16	0	0	0
Fusarium equiseti (Corda) Sacc.	18	2	8	0	0	0
F. oxysporum Schltdl.	129	15	60	670	17	68
F. solani (Mart.) Sacc.	97	12	48	233	14	56
Clonostachys rosea f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams	28	4	16	66	6	24
Paecilomyces varioti Bainier	17	2	8	31	3	12
Penicillium brevicompactum Dierckx	23	3	12	29	3	12
P. chrysogenum var. chrysogenum Thom	23	16	64	33	4	16
P. citrinum Thom	16	1	4	28	2	8
Scopulariopsis brevicaulis (Sacc.) Bainier	1189	18	72	3176	24	96
Mitosporic fungi						
Acrophialophora fusispora (S.B. Saksena) Samson	0	0	0	64	6	24
Epicoccum nigrum Link	3	1	4	0	0	0
Humicola fuscoatra Traaen	49	5	20	81	5	20
Macrophomina phaseolina (Tassi) Goid.	21	2	8	0	0	0
Microdochium dimerum (Penz.) Arx	36	4	16	0	0	0
Myrothecium roridum Tode	177	7	28	79	5	20
Stachybotrys chartarum (Ehrenb.) S. Hughes	8	2	8	9	1	4
Trichoderma pseudokoningii Rifai	83	10	40	21	5	20
Total	5293			11328		

* According to the system of **Kirk *et al* (2001).**

Effect of fungal antagonists and soil amendment on disease reduction

In *vitro* both of *T. pseudokoningii* and *E. nidulans* recorded a high percentage of frequency among all isolated taxa **(Table 3).** The growth of the pathogen, *M. phaseolina*, reached 4.5 cm in four days and by the 7th day of inoculation, the whole plate was covered. The data of Table 3 reveals the BCIs for the *in vitro* antagonism tests performed on two different media. Data clearly show that BioControl Index (BCI) of *T. pseudokoningii* on both media, PDA and CYA, is higher than that of *E. nidulans*.

In field studies, the count of healthy plants in percentage by using fungal candidates in unsolarized soil varies, while *T. pseudokoningii* revealed 83%$\pm$0.8 plant, *E. nidulans* showed 55%$\pm$0.5. In

case of mixed inocula (*T. pseudokoningii & E. nidulans)* the number of healthy plants was 75% ± 3.2.

In solarized soils, on the other hand, while the percentage of healthy plants recorded by using of *T. pseudokoningii* was 91%± 0.6, it was 82% ± 2.1 in case of using *E. nidulans*. By mixing of *T. pseudokoningii* and *E. nidulans* the number of healthy plants was 85% ± 2.5. In solarized soil, 77% ± 1.2 of the soil amendment treated plants were healthy in comparison with 65%± 0.7 in unsolarized treatments.

Table 3. In *vitro* (BCIs) for the two antagonists and in *vivo* the percentage of health plants with different treatments in both mulched and unmulched soil.

	Species	BCI (PDA)	BCI (CYA)
In *vitro*	*T. pseudokoningii*	75±0.7	71±0.4
	E. nidulans	62±0.8	58±0.3
		Healthy Plants %	
	Treatments	Unsolarized soil	Solarized soil
In *vivo*	*T. pseudokoningii*	83% ± 0.8	91%± 0.6
	E. nidulans	55% ± 0.5	82% ± 2.1
	Mixed inocula	75% ± 3.2	85% ± 2.5
	Soil amendment	65%± 0.7	77% ± 1.2

DISCUSSION

Soil solarization provided effective control of many soil borne diseases as well as charcoal rot of sesame. Soil solarization (8 weeks from July-August of soil mulching with transparent polyethylene sheets) reduced disease incidence throughout the cropping season. Our data on soil solarization, as a single control measure, clearly indicated that this approach apart from being feasible is very effective. The number of healthy plants significantly increased from 30 % in unsolarized soil up to 80 % in solarized soil. This level of increase for *M. phaseolina* is very much acceptable. Similar results of increase of disease control have also been reported in some other countries like: USA **(Stapleton, 1991),** occupied Palestine **(Grinstein and Ausher, 1991),** India **(Lodha, 1995; Lodha *et al* 1997), Pakistan (Ahmad *et al* 1996) and UK (Pinkerton *et al* 2000).**

Maximum temperatures obtained at the layer 5-1، cm of the mulched soil (57°C- 47.5°C) were in the range considered by many workers to be lethal to many soil fungi. **De Vay (1990); Stapleton (1990) and Keinath (1995)** reported that temperatures at 47°C or higher are lethal to many mesophilic fungi.

Soil solarization in combination with fungal antagonists has been subjected to control of charcoal rot of sesame caused by *M. phaseolina.* Such a combination might further enhance the long term supperssiveness of solarized soil. Whenever a pathogen is weakened by one treatment, a synergistic effect may be possible from double treatment. Such improvement in the antagonistic activity and accordingly the biocontrol potentiality might refer to a development of some sort of synergistic effect upon using single inocula plus soil solarization as mentioned by various investigators *viz*: **Elad *et al* 1981 and Ristaino *et al* 1991.**

The efficiency of solarization plus coating with pair inocula was tested in a trial to assess its efficiency to control charcoal rot under field conditions. The data of this experiment (mixing of *T. pseudokoningii* plus *E. nidulans*) showed that while pairing improved the biocontrol potentiality of *E. nidulans* by increasing the number of healthy plants in both unsolarized and solarized soils it leads to decrease in the biocontrol potentiality of *T. pseudokoningii* i.e. the number of healthy plants revealed by the pair is less than that obtained by *T. pseudokoningii* alone in unsolarized and solarized soils.

Where solarization plus soil amendments with *Eucalyptus* leaves were tested in a trial to manage charcoal rot of sesame under field conditions, the data showed that solarization improved the effect of soil amendment with *Eucalyptus* leaves by increasing the number of healthy plants. According to phytochemical investigations, the all promising extracts partaked in phenolic glycosides, sterols and/or triterpenes besides to pyrgallol tannins, saponins glycosides and alkaloids. As a bioactive agent against insect, nematode and microbial agents our results are in agreement with other investigations carried by **Sarvamangala & Govindaiah Datta (1993) and Johnson (2005).**

In view of the data obtained, it seems likely that solarization lie in its high efficacy and safety in the economical control of a wide array of soil borne pathogens. Such feasibility of solarization may be improved by integrated disease management with other control measures for controlling charcoal rot of sesame.

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