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EVALUATION OF SOME ESSENTIAL OILS AGAINST THE SEED- BORNE FUNGUS *Sclerotium rolfsii sacc.* FROM PEANUT SEEDS.

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ABSTRACT

Sixteen fungal isolates were recovered from infected peanut seeds on blotter and potato dextrose agar (PDA) medium. Among these isolates, *Sclerotium rolfsii* recorded the highest percentage on infected seeds. Seven essential oils of plant origin were evaluated for their action on the suppression of *S. rolfsii*. These oils were cinnamon oil, basil oil, lemon grass, tea oil, spearmint oil, mustard oil, and thyme oil. There was a reduction in the mycelium linear growth by increasing the oils concentrations. All the tested oils inhibited the sclerotial germination but at different concentrations. Higher concentrations of oils had an inhibitive effect while lower concentrations had a static action on the mycelial growth as well as sclerotial germination of *S. rolfsii*. Two concentrations for each of the 7 tested oils were tested for their effect on the development of the disease from infected seeds. Results revealed that basil and thyme oils at 2% caused 100% suppression of the fungal infection. Scanning electron microscopy of *S. rolfsii* treated with thyme oil showed that the volatile toxic compounds of the oil had a negative effect on the mycelium and the structure of sclerotia of seed-borne *S. rolfsii*.

Keywords: Seed-borne diseases, plant essential oils, *Sclerotium rolfsii*, scanning electron microscopy, peanut seeds.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) plays an important role in the economy of several provinces in Egypt. The long growing season, warm weather, and available moisture in Ismialia, Sharkia, and Behira governorates and El-Noubaria region make these areas favorable for peanut production. These conditions are also favorable for many fungal pathogens that reduce the peanut yield and profit. Stem-pod rot of peanut, caused by *Sclerotium rolfsii*, has been found in all peanut growing areas in Egypt. Peanut yield losses due to diseases can be as high as 25 to 80% (El-Wakil *et al.*, 1984) in Ismailia region, the largest peanut producing governorate in Egypt. Stem-pod rot is often a limiting factor for growing peanut, causing high annual yield losses. The postharvest use of chemicals including fungicides is restricted in most countries (Hayes and Laws,1991). Consumer demand for agricultural commodities without pesticide residues is high (Cutler & Cutler, 1999; Serrano *et al.*, 2005). Moreover, pesticides may also kill various beneficial organisms and their toxic forms may persist in soil (Hayes and Laws, 1991) and increase the incidence of pathogens resistance towards synthetic chemicals (Cakir *et al.*, 2005).

Thus, a new preservation technologies are needed, which have to be considered as human-safe and environmentally friendly (Duru *et al.*, 2003). Among the various alternatives, natural plant products, including essential oils that are biodegradable and eco-friendly, are catching the attention of scientists worldwide. Such products derived from higher plants are bio-efficacious, cost effective, environmentally safe, and can be ideal candidates for use as agrochemicals (Macias, *et al.*, 1997). Essential oils from a number of plants, including Eucalyptus and Cinnamon species have been reported to show activity against a wide array of plant pathogens (Hammer *et al.*, 1999 and Ramezani *et al.*, 2002). Singh *et al.* (1980) observed inhibitory effects of essential oils from *C. martinii, C. oliveri*, and *Trachysperumm ammi* on *Helminthosporium oryzae*, as well as inhibitory effects of the essential oils from rhizomes and leaves of *Zingiber chrysanthum* on plant pathogens such as *Alternaria* sp. and *Fusarium* sp. Application of the oil from *Cinnamomum camphora* at 4000 ppm (Mishra *et al.*, 1991) or the oil from *Cymbopogon citrates* at 1000 ppm (Mishra and Dubey, 1994) effectively controlled *Aspergillus flavus*, the causal agent of stored food rot. It was found that the oil from *C. citrates* was more effective than the synthetic fungicides, agrosan, thiride and bavistin, in the control of *A. flavus* (Mishra and Dubey, 1994). Essential oils of cinnamon and clove contain compounds such as cinnamaldehyde and eugenol, respectively, which have been tested on fresh fruits such as mandarin, kiwi and rambutan to control post-harvest diseases caused by fungi. Oils from *Eucalyptus globules* and *Ocimum canum* at 2000 ppm were effective in reducing mycelial growth and sclerotial production of *Sclerotium rolfsii* (Singh and Dwivedi, 1987). The essential oil from lemongrass was known to control a wide range of microorganisms including fungi such as *Trichoconiella padwickii* (Shetty *et.al.*, 1989).

The main objective of this investigation was to evaluate the effectiveness of a number of plant essential oils as an alternative control method against *Sclerotium rolfsii* as well as studying their effect on the structure and/or damage of *S. rolfsii* mycelium.

MATERIALS AND METHODS

Source of seed samples:

Samples of peanut seeds (cv. Giza 5) were collected from commercial production locations in Sharkia governorate. Seed sampling followed the procedures stated by the International Seed Testing Association (ISTA, 1999).

Source of plant essential oils:

Pure-grade (not containing synthetic chemicals and/or non-natural components) essential oils of basil (*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*), lemongrass *(Cymbopogon citrates)*, mustard (*Brassica juncea* L.), tea tree (*Melaleuca alternifolia)*, thyme (*Thymus capitatus*), and spearmint *(Mentha arvensis*) were obtained from the International Flavors and Plant Oils Inc., Giza, Egypt. These essential oils were stored in dark bottles at 4°C for further studies.

Frequency of seed-borne fungi associated with peanut seeds:

Detection of seed-borne fungi was carried out following the procedures published by ISTA (1999). One hundred seeds of each sample were tested using the standard blotter and agar plate methods as follows:

1- Standard blotter method.

In this method, 200 peanut seeds separated into two equal groups (100 seeds, each). Seeds were surface-sterilized in the first group and kept without sterilization in the second group following the procedure of ISTA (1999). For each group, five seeds were placed in 9-cm-diameter Petri dishes containing three layers of sterilized filter papers moistened with sterilized tap water (five seeds per dish and 5 dishes, as replicates, were used). The plates were incubated at 20±2°C for 7 days under cool white fluorescent light with alternating cycle of 12 h light and 12 h dark. The incubated seeds were examined after 7 days using a stereomicroscope (6-50X magnification) and a light microscope. The percentage of infected seeds was calculated and the associated fungi were isolated.

2- Agar plate method.

One hundred peanut seeds were surface sterilized with sodium hypochlorite (2.5%), then washed several times with sterilized water and dried between two folds of filter paper before putting on prepared potato dextrose agar (PDA) (5 seeds per plate) for a recovery of the associated fungi. The plates were incubated at 20±2°C for 4 days. The percentage of infected seeds was calculated and the associated fungi were isolated. The occurrence of different fungal species on the seeds was recorded.

Identification of the isolated fungi:

The isolated fungi were identified based on their habit characters under stereomicroscope and light microscope and according to Barnett and Hunter (1998), at the Seed Pathology Department, Plant Pathology Research Inst., ARC, Giza, Egypt.

Effect of plant essential oils on mycelial growth and sclerotia formation of *S. rolfsii in vitro:*

A mycelial disc (1-cm diameter) was taken from the periphery of an actively growing agar culture and placed at the centre of a 9-cm Petri dish containing 20 ml of PDA. Seven essential oils, namely cinnamon oil, basil oil, lemon grass, tea oil, spearmint oil, mustard oil, and thyme oil used at five concentrations (0.1, 0.5, 1.0, 1.5 and 2.0%) were added and mixed with the PDA medium before it was solidified. The dishes were quickly sealed with Parafilm and incubated at 25 °C. For each treatment, of each compound of the tested concentrations, eight replicate Petri dish cultures were used. Control treatments consisted of Petri dishes inoculated with the fungus but treated with distilled water instead of a volatile oil. After a -7-day-incubation at 25°C, the diameters of the fungal colonies and percentage of sclerotia formation were recorded.

Effect of plant essential oils on sclerotia germination of *S. rolfsii in vitro*

Sclerotia produced in the different treatments of the previous experiment were carefully collected under sterilized conditions and then transferred to new plates containing blank PDA medium. Twenty five sclerotia were randomly selected and placed in every Petri-dish. Same number of

sclerotia picked from the untreated plates served as control plates. Five replicate plates were made for each treatment and the control. All plates were then incubated at 25 °C for 7 days. The percentage of sclerotia germination was recorded after incubation period and also the type of activity of the volatile oil was classified as either germ static or germicidal.

Determination of the type of activity (fungistatic or fungicidal) of volatile oils against *S. rolfsii* growth *in vitro:*

The above-described experiment was repeated once more and treated cultures were observed 7 days of incubation at 25°C. If no mycelial growth was observed, then the Parafilm sealing was removed from the dishes and kept for an additional week under same conditions and the observed for the mycelia growth. A volatile oil was considered fungistatic when the mycelia grew during the additional incubation period and was considered fungicidal if no mycelial growth was detected.

Testing the most effective concentrations of volatile oils (as seed coating) for their efficacy in protecting peanut seeds against *S. rolfsii* infection:

Two hundred apparently healthy, surface-sterilized peanut seeds were coated with the most effective concentrations (1.5 and 2.0%) of essential oils tested after adding 1% Arabic gum solution as an adhesive material. Each oil was individually added to peanut seeds (cv.Giza-5). The treated seeds were left to dry at room temperature (25±5°C). Seven-day-old pure cultures of *S. rolfsii* was flooded with sterile distilled water (50 ml per plate), scraped with a spatula. The resulted fungal suspension was homogenized by blending in a kitchen blender for 2 minutes and the suspension was kept as a stock inoculum. Peanut seeds were dipped for 2 min. into *S. rolfsii* stock inoculum mixed with 1 ml of Arabic gum solution (1%) as an adhesive material. Then, the treated seeds were left to dry at room temperature (25±5°C) for one hour. Treated seeds were then placed in sterile Petri-dishes containing PDA medium (5 seeds per plate). Peanut seeds untreated with the essential oil but dipped into *S. rolfsii* suspension as described above were served as the control treatment. Twenty replicate plates were made for each treatment and the control. All plates were incubated at 25°C for 7 days percentage of seed infection was calculated according to ISTA (1999).

Scanning electron microscope study:

Specimens of *S. rolfsii* mycelium and sclerotia were taken at different stages of development, after 14 days of growing on PDA medium from plates previously treated with thyme essential oil (at .0.1%), whereas the untreated plates were used as control. Sections were made for the mature sclerotia by free hand. Samples were fixed in 2.5% glutraldehyde for 24 hours at 4°C, and then fixed in 1% osmium tetroxide for one hour at room temperature. The specimens were then dehydrated with acetone, critical point dried, and finally sputter coated with gold prior to the examination and photographing using a Joel T330 scanning electron microscope (Harley and Fergusen,1990) .

Statistical analysis:

The data were analyzed using MSTATE-C statistical package (A microcomputer program for the design, management and analysis of agronomic research experiments, Michigan State University, USA). All

multiple comparisons were first subjected to statistical analysis and significant differences among treatment means were determined with Tukey's studentized range test.

RESULTS

Frequency of seed-borne fungi associated with peanut seeds:

Six fungi have shown high frequency with blotter method using non-sterilized seeds. Those fungi were *S. rolfsii, Penicillium* sp., *Alternaria alternata, Aspergillus Flavus, Aspergillus* spp., and *Macrophomina phaseolina*. Their frequencies were 15, 11, 10, 11, 12, and 7%, respectively (Table 1). Also, other fungi such as *F. solani, F. moniliforme, F. semitectum*, and *Rhizoctonia solani* were recovered from the non-sterilized seeds but in less frequency (2, 3, 5, and 5%, respectively). However, sterilized seeds used in the blotter method were found infested in high frequency with three fungi, which are *S. rolfsii, A. alternata*, and *A. niger* with incidence of 20, 13, and 12%, respectively. Data in (Table 1) also, show that *S. rolfsii* was the most frequent fungus isolated from peanut seeds regardless of the method of isolation (blotter or agar method, whether on surface-sterilized or non-sterilized).

Table (1): Percentage of recovery of seed-borne fungi isolated from peanut seeds (sterilized and non-sterilized) using blotter and PDA medium

Fungi isolates	Blotter method*		Agar method (surface-sterilized)
	Non-sterilized seeds	Sterilized seeds	
Alternaria alternata	10.0	13.0	2.0
Aspergillus flavus	11.0	9.0	8.0
Aspergillus niger	6.0	12.0	7.0
Aspergillus spp.	12.0	11.0	11.0
Cladosporium sp.	4.0	0.0	4.0
Epicoccum sp.	9.0	5.0	3.0
Fusarium solani	2.0	1.0	0.0
Fusarium moniliforme	3.0	0.0	4.0
Fusarium oxysporum	0.0	5.0	8.0
Fusarium semitectum	5.0	9.0	9.0
Mucor sp.	0.0	7.0	2.0
Myrothecium sp.	0.0	8.0	8.0
Macrophomina phaseolina	7.0	0.0	0.0
Penicillium sp.	11.0	0.0	10.0
Rhizoctonia solani	5.0	0.0	11.0
Sclerotium rolfsii	15.0	20.0	13.0

* 100 seeds were examined for each method.

Effect of plant volatile oils (PEO) on mycelial linear growth (mm) of *S. rolfsii*:

Data shown in Table (2) indicate that, all concentrations (except the least one; 0.1%) of plant essential oils had a negative effect on *S. rolfsii* linear

growth and this effect increased by increasing the oil concentration. All the tested PEOs at 2% prevented *S. rolfsii* to grow (100% growth inhibition). Thyme, mustard, and lemongrass oils at 1.5%caused the highest fungal growth inhibition when compared with the rest of tested PEOs (Table 2). At 1.5% oil concentrations, Spearmint oil followed by tea tree oil were the least effective in this regard.

Table (2): Effect of plant essential oils (PEO) on mycelial linear growth (mm) of *S. rolfsii*

PEO	Mycelial linear growth (mm)									
	PEO Concentrations (%)									
	0.1	R[a]	0.5	R	1.0	R	1.5	R	2.0	R
Cinnamon oil	88.0 [b] a	2.0	56.3 c	33.7	44.4 bc	45.6	33.3 c	56.7	00.0 b	100.0
Basil oil	80.2 b	9.8	77.1 b	12.9	50.9 b	39.1	27.6 c	62.4	00.0 b	100.0
Lemongrass oil	86.1 a	3.9	51.6 c	38.4	45.1 bc	44.9	19.0 d	71.0	00.0 b	100.0
Tea tree oil	82.6 a	7.4	75.4 b	14.6	56.9 b	33.1	43.3 b	46.7	00.0 b	100.0
Spearmint oil	84.5 a	5.5	52.3 c	37.7	41.0 c	49.0	20.5 a	69.5	00.0 b	100.0
Mustard oil	75.9 c	14.1	50.0 c	40.0	35.0 d	55.0	17.2 d	72.8	00.0 b	100.0
Thyme oil	71.0 c	19.0	45.6 d	44.4	22.7 e	67.3	15.0 d	75.0	00.0 b	100.0
Control	90.0 a	0.0	90.0 a	0.0	90.0 a	0.0	90.0 a	0.0	90.0 a	0.0

[a] R= reduction in linear growth relative to the control
[b] Values followed by the same letter(s) within a column are not significantly different according to Tukey's studentized range test (P=0.05)

Effect of plant essential oils on sclerotia formation of *S. rolfsii* isolated from peanut seeds:

Results shown in Table (3) indicate that all the tested PEOs at the concentration of 1% or above had a significant inhibitory effect on the sclerotia formation of *S. rolfsii*. This effect increased by increasing the PEOs concentrations. Sclerotia formation was completely inhibited by all PEOs when used at 1.5 and 2.0%.

Table (3): Effect of plant essential oils (PEO) on sclerotia formation of *S. rolfsii* isolated from peanut seed

PEO	Sclerotia formation (%)				
	PEOs concentration (%)				
	0.1	0.5	1.0	1.5	2.0
Cinnamon oil	88.5 b[a]	80.1 b	66.6 b	0.0 b	0.0 b
Basil oil	100.0 a	65.3 c	25.9 d	0.0 b	0.0 b
Lemongrass oil	91.8 b	80.2 b	67.3 b	0.0 b	0.0 b
Tea tree oil	80.5 c	44.6 e	0.0 e	0.0 b	0.0 b
Spearmint oil	90.0 b	55.0 d	0.0 e	0.0 b	0.0 b
Mustard oil	100.0 a	70.5 c	0.0 e	0.0 b	0.0 b
Thyme oil	100.0 a	95.6 a	51.3 c	0.0 b	0.0 b
Control	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a

[a] Values followed by the same letter within a column are not significantly different according to Tukey's studentized range test (P=0.05)

Effect of plant essential oils on sclerotia germination of *S. rolfsii* isolated from peanut seed:

Data in Table (4) indicate that sclerotia germination of *S. rolfsii* were completely inhibited by all PEOs when used at 2% concentration, while at 1.5%, all except spearmint and mustard oils caused 100% inhibition of the sclerotia germination. At 0.1%, cinnamon oil was the most effective PEO causing 50% of sclerotia to germinate.

Table (4): Effect of plant essential oils (PEOs) on sclerotia germination of *S. rolfsii* isolated from peanut seed *in vitro*

PEOs	Sclerotia germination (%) PEOs concentration (%)				
	0.1	0.5	1.0	1.5	2.0
Cinnamon oil	50.0 d[a]	44.1 d	0.0 e	0.0 d	0.0 b
Basil oil	75.6 c	33.3 e	0.0 e	0.0 d	0.0 b
Lemongrass oil	25.0 c	10.5 f	0.0 e	0.0 d	0.0 b
Tea tree oil	100.0 a	80.5 b	55.5 c	0.0 d	0.0 b
Spearmint oil	70.5 b	33.6 e	44.5 d	20.1 c	0.0 b
Mustard oil	100.0 a	95.1 a	71.3 b	40.0 b	0.0 b
Thyme oil	90.5 b	66.6 c	0.0 e	0.0 d	0.0 b
Control	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a

[a] Values followed by the same letter within a column are not significantly different according to Tukey's studentized range test (P=0.05)

Detection of the type of action of the tested plant essential oils against mycelia growth and sclerotia of *S. rolfsii*:

In general, the higher concentrations (1.5 and 2%) of the tested PEOs had fungicidal action while their lower concentrations (0.1 and 0.5%) had fungistatic effect against the mycelia growth of *S. rolfsii* (Table 5-a). However, cinnamon, basil, and spearmint oils had a fungicidal effect against *S. rolfsii* mycelial growth at 1% while the other PEOs at this concentration had fungistatic effect (Table 5-a).On the other hand, all PEOs had germicidal effect against sclerocial germination when used at 1% or above and germstatic effect when used at 0.1% (Table 5-b).

Table (5-a): Detection of the type of action of the tested plant essential oils against *S. rolfsii* mycelia growth

Plant essential oils	Type of action against *S. rolfsii* mycelia growth									
	Fungicidal					Fungi-static				
	0.1	0.5	1.0	1.5	2.0	0.1	0.5	1.0	1.5	2.0
Cinnamon oil	-	-	+	+	+	+	+	-	-	-
Basil oil	-	-	+	+	+	+	+	-	-	-
Lemongrass oil	-	-	-	+	+	+	+	+	-	-
Tea tree oil	-	-	-	+	+	+	+	+	-	-
Spearmint oil	-	-	+	+	+	+	+	-	-	-
Mustard oil	-	-	-	+	+	+	+	+	-	-
Thyme oil	-	-	-	+	+	+	+	+	-	-
Control	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil

(+) Positive action. (-) Negative action.

Table (5-b): Detection of the type of action of the tested plant essential oils against sclerotia of *S. rolfsii*

Plant essential oils	Type of action against *S. rolfsii* sclerotial germination									
	Germicidal effect					Germstatic effect				
	0.1	0.5	1.0	1.5	2.0	0.1	0.5	1.0	1.5	2.0
Cinnamon oil	-	+	+	+	+	+	-	-	-	-
Basil oil	-	-	+	+	+	+	+	-	-	-
Lemongrass oil	-	-	+	+	+	+	+	-	-	-
Tea tree oil	-	-	+	+	+	+	+	-	-	-
Spearmint oil	-	+	+	+	+	+	-	-	-	-
Mustard oil	-	-	+	+	+	+	+	-	-	-
Thyme oil	-	-	+	+	+	+	+	-	-	-
Control	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil

(+) Positive action. (-) Negative action.

Efficacy of plant essential oils in protecting peanut seeds against *S. rolfsii* infection:

Data presented in Table (6) indicate that all tested essential oils at both concentrations significantly reduced the disease incidence on peanut seeds in comparison with the control. Basil and thyme oils caused 100% diseasecontrol when used at both concentrations (1.5 and 2%), as seed coat (Table 6)

Table (6): Effect of treating peanut seeds with plant essential oils (as coating) on the percentage of *S. rolfsii* seed infection

Treatment[a]	1.5% PEO		2% PEO	
	Disease incidence (%)	% Disease control efficacy [b]	Disease incidence (%)	% Disease control efficacy
Cinnamon oil	18.5 c[c]	81.5	12.5 c	87.5
Basil oil	0.0 e	100.0	0.0 e	100.0
Lemongrass oil	14.0 c	86.0	5.5 d	94.5
Tea tree oil	10.0 c	90.0	6.5 d	93.5
Spearmint oil	6.5 c	93.5	4.5 d	95.5
Mustard oil	7.5 d	92.5	3.5 d	96.5
Thyme oil	0.0 e	100.0	0.0 e	100.0
Control (1)	85.0 b	15.0	85.0 b	15.0
Control (2)	100.0 a	0.0	100.0 a	0.0

[a] 200 peanut seeds were used for each treatment using blotter method. Control (1) = Peanut seeds were coated with oil-free Arabic gum solution (2%) only as a carrier. Control (2) = Peanut seeds were treated with oil-free *S. rolfsii* suspension

[b] % Disease control efficacy was calculated in relative to the control treatment.

[c] Values followed by the same letter within a column are not significantly different according to Tukey's studentized range test (P=0.05).

Scanning electron microscope study:

Specimens of *S. rolfsli* mycelium and sclerotia were taken at different stages of development. Sections were taken on the mature sclerotia of the target fungus *S. rolfsii* by free hand section. The aim of this study was to show the effect of thyme essential oil on the mycelium structure and sclerotia of *S. rolfsii*. Fig. (1-b) illustrates an abnormal formation of the mycelium due to the toxic effects of the thyme volatile oil, whereas, Fig.(1-a), used as control treatment.

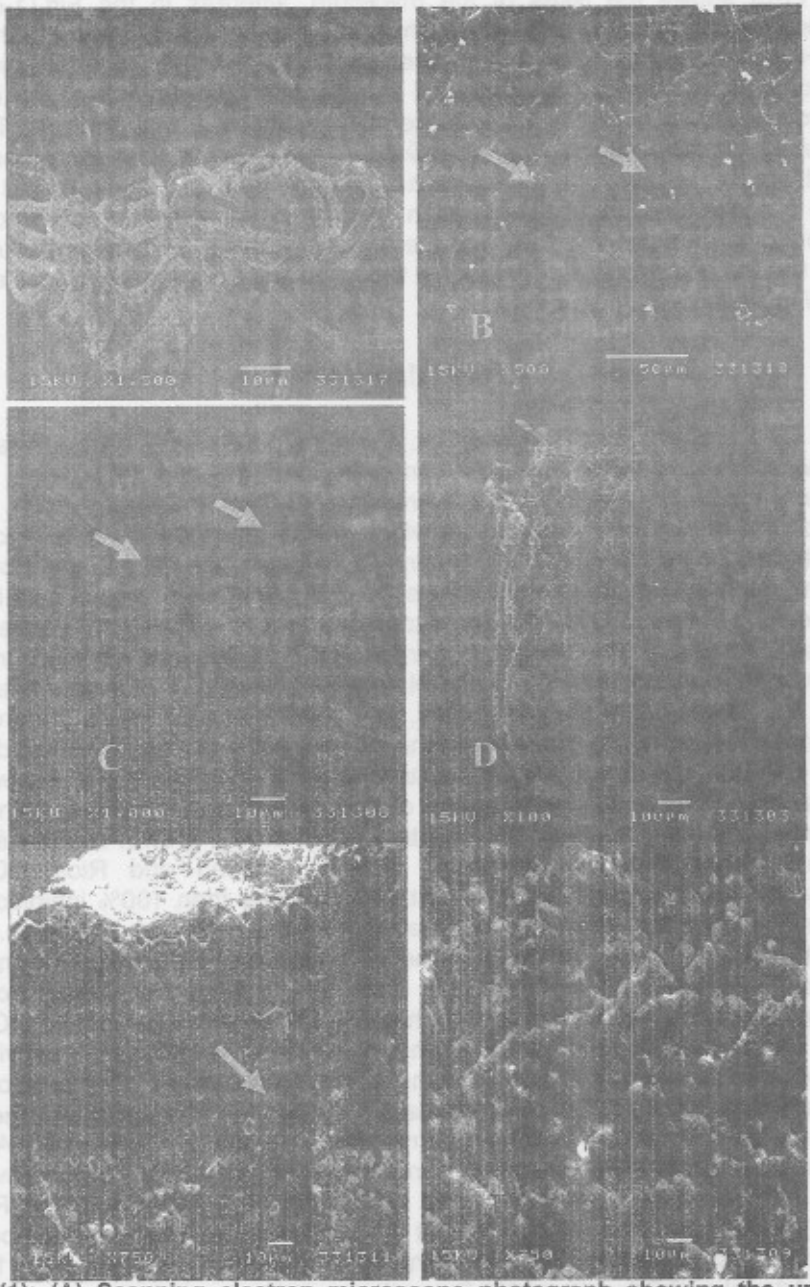

Fig.(1): (A) Scanning electron microscope photograph showing the untreated control of *S. rolfsii* mycelium (x1.500); (B) showed the action of thyme oil on *S. rolfsii* mycelium on PDA in the form of abnormal aggregations (x500) after14 days; (C) degradation of *S. rolfsii* mycelium (x1000); (D) Abnormal sclerotia due to the treatment with thyme oil (x100); (E) transverse section shows the action of thyme oil on upper and lower surfaces of sclerotia (x750), note the decline of the cell structure in the medulla; (F) Internal structure of sclerotia demonstrating the damaged inner cells (x750).

Fig. (1-c) shows the lyses of the mycelium, whereas in the Fig.(1-d), a general view of sclerotia of *S. rolfsii* which seem to be affected with the thyme oil. However, in Fig. (1-e&f), the free hand section of the sclerotia which collected from treated plates showed a noticeable degradation of the inner sclerotia cells as a result of the macerating action of the thyme oils. Thus, scanning electron microscope observation made during this study confirm that thyme essential volatile oils, as an example from the seven tested oils, gave a noticeable fungal deterioration with a different cell structure to the surrounding medulla (Fig.1 -F) and the observations on *S. rolfsii* mycelium of both treated and untreated ones show the lyses and degradation of treated *S. rolfsii* mycelium with thyme oil.

DISCUSSION

The present work revealed that, essential oils proved to be fungistatic at lower dosages, while at higher concentrations (1.5 and 2.0%, v/w), they became fungitoxic. Similar results were obtained by (Simi *et al.*, 2004), who found that the oil of *C. citratus* provided 100% inhibition of both mycelial growth and spore germination of *Didymella bryoniae*, the causal agent of the gummy stem blight of melon. Extracts from *C. citratus* were shown to have fungicidal potential against 10 dermatophytes and *A. fumigatus*. Tea tree oil has antibacterial and antifungal properties that have secured it a place in the commercial pharmaceutical market (Hammer *et al.* 1999). Essential oils are often fungistatic rather than fungicidal. This means that they stop the growth of fungi when they are exposed to the oil, but once the oil is removed the fungi can continue to grow. Our results in the following study are somewhat similar to these results with some of the tested oils. Clove and thyme essential oils have a significant inhibitory effect on the growth of *Penicillium digitatum*, confirming its antifungal activity (Nielsen and Rios, 2000). Moreover, even at concentrations that caused lower than 100% inhibition of mycelial growth, conidia have lost their pigmentation (became hyaline).This effect might decrease virulence of the pathogen; hence a decrease in the incidence of the disease. The strong antifungal activity of thyme and clove essential oils could be due to their high content of phenolic compounds. Cakir *et al.* (2005) established that a relationship exists between the high activity of the oils and presence of phenolic components. Harvey *et al.,* (2002) strongly indicate that the essential oil of mustard may successfully substitute methyl bromide soil fumigation to control some plant pathogens in nurseries. An *in vitro* toxic effect of AITC (the major component of this essential oil) on several fungi has also been reported (Arras and Piga, 1994 & Nielson and Rios, 2000). Recent studies suggested that the antifungal activity of cinnamon oil was probably due to the major component, cinnamaldehyde (Devi *et al.*, 1982 and Adegoke &Odesola.,1996).Several secondary metabolites, including methyl jasmonate (MJ), and exogenous phenolic compounds presented in Tea tree oil (TTO), have become well recognized as vital defensive compounds protecting plants from pathogen attack. The obtained data in our study are somewhat in agreement with the aforementioned results.

Microscopic observations using scanning electron microscope were carried out to determine the action of thyme oil on upper and lower surfaces of sclerotia, the depressions with a different cell structures to the medulla. Internal structures of sclerotia demonstrated damaged inner cells,also, the free hand section of the sclerotia which collected from treated plates showed a noticeable degradation of the inner sclerotia cells as a result of the macerating action of the thyme oils. Thus, scanning electron microscope observation made during this study confirm that thyme essential volatile oils, as an example from the seven tested oils, gave a noticeable fungal deterioration with a different cell structure to the surrounding medulla,also, the observations on *S. rolfsii* mycelium show that, lyses and degradation of treated *S. rolfsii* mycelium with thyme oil. The effect of this volatile oil also, is due to the inhibitive and toxic effect of the thyme oil on the mycelium and sclerotia of *S. rolfsii* (Singh and Dwivedi, 1987 and Hammer *et al.*, 1999).

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Conclusion

Harmful side effects of fungicides on plants, human and environment allover the world pushing the researchers and scientists to find a new, safe, and eco-friendly alternative compounds.One of the most important alternatives is the active ingredients of the natural plants essential volatile oils, which were evaluated in our study for their effect against a serious seed- and soil-borne pathogenic fungus (*S. rolfsii*). Further studies must be directed to this kind of work to decrease the environmental pollution due to the continuous usage of pesticides.

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تقييم بعض الزيوت العطرية لمقاومة الفطر سكليروشيم رولفزياي المحمول على بذور الفول السودانى.

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أمكن الحصول على ١٦ عزلة فطرية من بذور الفول السودانى وذلك بطريقتى ورق النشاف وبيئة الاجار المغذية وأظهر الفطر سكليروشيم رولفزياي أعلى نسبة مئوية للعزل من البذور المصابة. وأظهرت النتائج انه عند تقييم سبعة زيوت عطرية معملياً من حيث تأثيرها فى تثبيط الفطر سكليروشيم رولفزياي المعزول من بذور الفول السودانى (الصنف جيزة ٥) وجد ان لها تأثيرا مثبطا لنمو الميسيليوم الطولى (قطر المزرعة) والذى زاد بزيادة التركيز للزيوت الطيارة. وقد أدت جميع الزيوت الطيارة الى تثبيط إنبات الأجسام الحجرية للفطر والذى زادت درجته بزيادة تركيز الزيت . وقد تم تقييم اثنان من الزيوت السبعة تحت الدراسة وذلك من حيث تأثيرهما على حماية البذور من الإصابة بالفطر سكليروشيم رولفزياي ووجد أن زيت البازل (الريحان) وزيت الزعتر (عند تركيز ١.٥% لكل منهما) حققا حماية كاملة (١٠٠%) للبذور من الإصابة بالفطر المذكور. كما أظهرت الدراسة التى اجريت بالميكروسكوب الالكترونى الماسح ان زيت الزعتر كان له تأثيرا ساما مميتا على الميسليوم والاجسام الحجرية للفطر سكليروشيم رولفزياي المحمول على بذور الفول السودانى.

الكلمات الدالة: الزيوت العطرية ، سكليروشيم رولفزياي ، الميكروسكوب الالكترونى الماسح ، بذور الفول السودانى.

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