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IMPACT OF SOME ESSENTIAL OILS AND THEIR COMBINATIONS WITH VITAVAX-THIRAM FUNGICIDE ON CONTROLLING *SCLEROTIUM* ROOT-ROT OF SUGAR BEET

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ABSTRACT

Six different essential oils and their joint toxic effect with Vitavax-thiram were tested to control Sclerotium root-rot disease of sugar beet (*Beta vulgaris* L.) caused by *Sclerotium rolfsii* Sacc. *in vitro* all the tested oils exhibited effect on fungal growth of *S. rolfsii*. *Cuminum cyminum* oil was the most effective in inhibiting linear growth of *S. rolfsii*. The joint toxic effect between Vitavax-thiram and oils of *Eucalyptus globulus* and *C. cyminum* showed the highest effect in reducing the growth of *S. rolfsii*. Under greenhouse conditions, data indicted that oils have significant effect in improving the number of survived seedlings and reduced root-rot. Seed treatment with *C. cyminum* oil was more effective in controlling damping-off and root-rot diseases of sugar beet and increasing sucrose percentage, total soluble solids percentage (T.S.S%) and purity % of sugar beet plants sown in soil infested with *S. rolfsii*. Vitavax-thiram mixed with *E. globulus* or *C. cyminum* oils as a seed treatment were the most effective in controlling root-rot disease and increasing percentages of T.S.S., sucrose and purity. *C. cyminum* showed highest values in increasing activities of peroxidase and esterase enzymes. In the field, coating or soaking of sugar beet seeds with *C. cyminum, Nigella sativa, Allium sativum* and *Matricaria chamomilla* oils completely controlled root-rot infection, but *C. cyminum* gave the highest yield/plot in comparison with other oils. Vitavax-thiram combined with *E. glubulus* and *M. chamomilla* as a seed coating were completely effective in reducing sugar beet root rot, but *E. glubulus* and *A. sativum* as a seed soaking were the best treatments in controlling root rot disease. Analysis by gas chromatography-mass spectrometry (GC-MS) led to identification of 62 organic compounds from *C. cyminum* oil including hydrocarbons, alcohols, aldehyds and … etc.

Keywords: Sugar beet, Sclerotium root rot disease (*Sclerotium rolfsii*), essential oils, peroxidase, esterase, GC-MS, *Cuminum cyminum* oil.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops allover the world. In Egypt, has become recently one of the most economically important crops. This crop is liable to be attacked by certain soil-borne pathogens at all stages of growth causing pre- and post-emergence damping-off, as well as various degrees of root-rot. *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium solani* were considered among the most destructive diseases affecting yield crop in Egypt (El-Abyad *et al.*, 1992; El-Kazzaz *et al.*, 1999 and El-Kholi, 2000; Esh, 2000; Gouda, 2001 and El-Sayed, 2007). Neem oil was the most effective against *S. rolfsii* viability of sclerotia was only 8% following treatment with neem oil and 20% with blue gum leaf distillate compared with 71% in the untreated controls (Singh and Dwivedi, 1990). The essential oil which isolated from seed of *Cuminum cyminum* exhibited antifungal activity against *Aspergillus flavus* (Dwivedi and

Dubey, 1993). Some essential oils have an allelopathic on root-rot disease in sugar beet (Gouda, 2001; El-Sherbieny *et al.*, 2002 and Gouda *et al.*, 2009). They found that, oil of clove and cumin were superior to the other oils inhibiting linear growth of the tested fungi *in vitro* , green house and under natural infection in the field. *C. cyminum* was shown to be superior to all oils in controlling sugar beet root disease and increasing yield, sucrose%, total soluble solids % (T.S.S.%) and purity percentage in pot and field experiments. Polyphenol oxidase and peroxidase increased significantly in infected sugar beet roots than healthy plants. The highest amounts of the enzymes was produced in case of the infection with *S. rolfsii* followed by *Pythium debaryanum*, *R. solani, F. oxysporum* and the lowest was in case of *M. phaseolina* (Esh , 2000) . Peroxidase and esterase may play a role in the active defense mechanism of plant could be considered positive biochemical markers for *M. phaseolina* resistance in bean (Fayed *et al.*, 2003).

MATERIALS AND METHODS

The present study was carried out at Gemmeiza Research Station, Agricultural Research Center (ARC) during two successive seasons 2008/2009 and 2009/2010 to control Sclerotium root rot disease of sugar beet (*Beta vulgaris* L.) caused by *Sclerotium rolfsii* Sacc.

Six essential oils used in this study are shown in Table (1). They were purchased from Ghomhoriya Company for Medicine and Chemicals. Vitavax-200 (Vitavax-thiram) was used as a check for controlling the disease.

Table (1): List of plant essential oils tested.

English name	Scientific name	Family
Nigella	*Nigella sativa* L.	Ranunculaceae
Cumin	*Cuminum cyminum* L.	Umbelligerae
Garlic	*Allium sativum* L.	Lilaceae
Onion	*Allium cepa* L.	Lilaceae
Bluegume	*Eucalyptus globulus* L.	Myrtaceae
Chamomile	*Marticaria chamomilla*	Leguminasae

Laboratory experiments:

The isolated *S. rolfsii* was identified by Dept. of Mycology and Plant Dis. Survey, Plant Pathology Inst., A.R.C. according to Booth (1977) and Singh (1982). Six essential oils were compared *in vitro* using 100, 250, 500, 1000, 1500 and 2000 ppm with the same concentrations of Vitavax-thiram fungicide using PDA medium. Four Petri dishes of each concentration were inoculated at the center with 5 mm culture disc of *S. rolfsii* and incubated at (+1) 28^{o}C. Mycelial linear growth was measured daily. Percentage of inhibition (I%) in colony diameter was calculated using the formula of Vincent (1927).

$$I\% = \frac{\text{Fungal growth in control - fungal growth in treatment}}{\text{Fungal growth in control}} \times 100$$

IC_{25} of Vitavax-thiram was mixed with IC_{25} from every oil alone against *S. rolfsii in vitro.* Percentage of inhibition in colony diameter was calculated for each treatment.

Green house experiments:

Essential oils were evaluated for their efficiency against damping-off and root-rot diseases caused by *S. rolfsii* under greenhouse conditions. Seeds of sugar beet Kawmera cv. were soaked into a concentration of 2000 ppm of each oil under study for 8 hours before planting. Sugar beet seeds soaked only in water for 8 hrs served as a check treatment. Seeds were treated with Vitavax-thiram at the rate recommended dose (2 g/kg) and seeds were cultivated in *S. rolsfii* infested soil (15 seeds/pot). Three replicate pots (No. 35) were used and uninfected soil acted as control. Disease reading was taken 15, 45 and 150 days after planting for pre-, post-emergence damping off and root-rot, respectively. Chemical component i.e. total soluble solids (T.S.S.), sucrose percent and sugar purity were also estimated. T.S.S. was estimated in fresh roots using the hand refractometer according to McGinnis (1982). Sucrose percent was estimated according to A.O.A.C. (1990) by adding 173 ml 3% lead acetate to 26 g from the sample representing the interior of the roots. After filtration, sucrose percent was measured by the aid of sacarometer. Purity percent was calculated by dividing the sucrose percent by T.S.S.

Efficiency of Vitavax-thiram fungicide combined with the tested oils used as a soaking seeds for 8 hours. in controlling Sclerotium root rot of sugar beet Kawmera cv.in soil infested with *S. rolfsii* under greenhouse conditions. The combination consists of the quarter rates used individual treatments as mentioned before in greenhouse experiments (500 ppm for oil and 0.5 g/kg seed for fungicide). Disease reading was taken 15, 45 and 150 days after planting for pre, post-emergence damping-off and root-rot, respectively. Chemical component, i.e. T.S.S., sucrose percent and sugar purity were determined 150 days after planting.

Field experiment:

Field trials were carried out at Gemmiza Agricultural Research Station during 2008/2009 and 2009/2010 growing seasons. Seeds of sugar beet Kawmera variety were treated with essential oils or the tested combinations of Vitavax-thiram and oils in two methods i.e. seed soaking and coating with the same concentration using greenhouse experiments. At the end of experiments (200 days of sowing), roots were cut for estimating the disease incidence and severity in each treatment and healthy roots were recorded. Yield was also estimated per plot.

Assay of enzymes activity:

Enzymes were extracted from samples of infected and healthy roots grown in soil infested with *S. rolfsii* under greenhouse conditions from some treatments under study according to Maxwell and Batman (1976).

Enzymes activities were determined as follows:

i) Peroxidase activity was spectrophotometrically determined by measuring the oxidation of pyrogallol in the presence of H_2O_2 at wave length of 425 nm, according to Allam and Hollis (1972).

ii) Esterase activity was determined using spectrophotometer as described by Hobson (1963).

Gas chromatography mass spectrometry (GC-MS):

The essential oil of *Cuminum cyminum* was analyzed on a gas chromatograph (Agilent 6890N)-mass spectrometer (Agilent 5973N MSD) (GC-MS) equipped with a DB-5MS column (30 m x 0.25 μm film thickness, JO&(W Scientific, Falsom, CA). The oven temperature was programmed as: isothermal at 40^{o}C for 1 min, then raised to 250^{o}C at 6^{o}C/min and held at this temperature for 4 min. Helium was used as the carrier gas at the rate of 1.5 ml/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (280^{o}C). Ionization was obtained by electron impact (70 ev, source temperature 230^{o}C). Scan range was 25-800 amu. Retention times for comparison with authentic compounds were measured with a DB-1MS and a DB-FFAP column (30 m x 0.25 μm film thickness, J W (Scientific, Folsom, CA). Compounds were tentatively identified by comparison of mass spectra of each peak with those of authentic samples in the NIST MS library (Park *et al.*, 2007).

Statistical analysis:

The obtained data were subjected to analysis of variance (Steel and Torrie, 1960). Duncan's multiple range test (DMRT) was applied for comparing means (Duncan, 1955). IC_{25} and IC_{50} values were calculated by Probit analysis.

RESULTS

***In vitro* experiments:**

Data in Table (2) showed that, all the tested oils with all tested concentrations had a reductive effect on fungal growth of *S. rolfsii. C. cyminum* oil completely inhibited the growth of *S. rolfsii* at 2000 ppm concentration after Vitavax-thiram fungicide, it gave completely inhibited at 1500 and 2000 ppm concentrations. Results also revealed that *C. cyminum* oil was the highly effective in inhibiting the growth of *S. rolfsii* (70.22%). Essential oils of *M. chamomilla* (62.74%) and *A. sativum* (54.02%) were moderately toxic, followed by *N. sativa* 48.24%), *E. globulus* (47.45%) and *A. cepa* (46.66%) against the same fungus.

The fungitoxic effect of the combinations of Vitavax-thiram with plant oils were tested according to the results from Table (2). IC_{25} values for each of the tested plant oils combined with Vitavax-thiram were used in the examined mixture against *S. rolfsii*. According to the results of Table (3), combinations of Vitavax-thiram with *E. glabulus* and with *C. cyminum* were the most effective in inhibiting linear growth of *S. rolfsii*, hence it gave the highest values of inhibition percent i.e. 85.06% and 83.93%, respectively. While, the combination of Vitavax-thiram with *A. sativum* was the least effective (64.38%) compared with other combinations and control.

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Table (3): Percentage of inhibition (I%) of Vitavax-thiram combined with the tested oils against *S. rolfsii*.

Essential oils	*S. rolfsii*	
	L.G	I%
N. sativa L.	1.60 de	82.02 bc
C. cyminum L.	1.43 e	83.93 ab
A. sativum L.	3.17 b	64.38 e
A. cepa L.	2.83 c	68.20 d
E. globulus L.	1.33 e	85.06 a
M. chamomilla L.	1.73 d	80.56 c
Control	8.90 a	0.00 f

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

Green house experiments:

Data in Table (4) revealed that, seed treatment with *C. cyminum* oil was the most effective in controlling damping-off, hence, it gave the highest percentage of survival plants (71.11%) but Vitavax-thiram fungicide gave (84.45%), while *E. globulus* oil was the least effective in this respect (42.22%) compared with other treatments and the control (4.44%).

C. cyminum and *M. chamomilla* oils were the highly effective in reducing root-rot infection (5.56% and 6.67%, respectively) and showing the most effective in reducing disease severity (1..03% and 1.44%, respectively) compared with the fungicide didn't gave disease incidence of root-rot and gave the highest percentage of healthy plants x100%. On the other hand, *A. cepa* oil was the least effective oil in controlling root-rot disease of sugar beet, which, it gave 48.89% disease incidence and 14.44% disease severity compared with the control (100% disease incidence and 20.83% disease severity).

Data in Table (4) showed that, all the tested oils and Vitavax-thiram fungicide increased chemical components i.e. T.S.S. sucrose and purity percent compared with control*. C. cyminum* caused the highest degree of increasing T.S.S. (25.17%) and sucrose (19.37%), while *A. cepa* oil was the least effective in increasing chemical components compared with the control infected.

In general, T.S.S., sucrose and purity percentage were found to be increased by decreasing the disease incidence and disease severity of root-rot infected by *S. rolfsii* (Table 4).

Data in Table(5) show highly significant differences between treatments. with *E. globulus* + Vitavax-thiram and *C. cyminum* + Vitavax-thiram were the highly effective in controlling root-rot disease and gave the least disease severity. While combination of *c. cyminum* + Vitavax-thiram was the most effective in controlling damping-off disease, which gave the highest percent of survival plants (73.33%), followed by combination of *M. chamomilla* and *A. sativum* with Vitavax-thiram. On the other hand, seed treatment with *A. cepa* oil + Vitavax-thiram was the least effective in controlling damping-off and root-rot disease of sugar beet.

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Data in Table (5) indicated that, *C. cyminum* oil + Vitavax-thiram was the best treatment in increasing sucrose percentage (21.11%) and purity (82.57%), *E. globulus* + Vitavax-thiram was the best treatment in increasing T.S.S.% (26.67%). On the other hand, *A. cepa* oil + Vitavax-thiram was the least treatments in increasing T.S.S.% (20.73%), sucrose% (13.90%) and purity% (67.05%) compared with control and fungicide.

Data presented in Table (6) show the peroxidase and esterase activities of sugar beet roots grown in soil infested with *S. rolfsii*. Results indicated that seed treatment with *C. cyminum* gave the highest increase in peroxidase activity (11.763 U/g fresh weight), followed by Vitavax-thiram + *C. cyminum* oil (3.705 U/g). While Vitavax-thiram recorded the lowest increase in peroxidase activity (3.643 U/g) although it was higher than the infested control (1.302 U/g) in peroxidase activity.

Also, data in Table (6) showed that seed treatment with *C. cyminum* was the most effective in increasing the esterase activity, (3.612 U/g fresh weight) followed by Vitavax-thiram (2.591 U/g), while seed treatment with combination of Vitavax-thiram + *C. cyminum* oil gave the lowest increase in esterase activity (2.212 U/g) compared with the infected control (1.112 U/g).

Table (6): Effect of certain treatments on perxoidase and esterase activity of sugar beet roots grown in soil infested with *S. rolfsii*

Treatment	Peroxidase activity	Esterase activity
	Unit/gram fresh weight	
Vitavax-thiram	3.643	2.591
C. cyminum (oil)	11.763	3.612
Vitavax-thiram + *C. cyminum* oil	3.705	2.212
Infected control*	1.302	1.112
Uninfectd control**	3.120	1.312

*** Untreated seeds sown in soil infested with *S. rolfsii***
**** Untreated seeds sown in sterilized soil free of *S. rolfsii*.**

Field experiments:

Data in Table (7) revealed that coating the seeds with Vitavax-thiram fungicide and *C. cyminum* oil were superior in reducing root-rot disease (no infection was observed) and gave the highest yield per plot) (69 and 69 kg/plot during 2008/2009 season) and 73.67 and 73.33 kg/plot, during 2009/2010 season, respectively followed by *A. sativum, N. sativa* and *M. chamomilla*. *A. cepa* and *E. globulus* were the least effective in controlling root-rot disease of sugar beet compared with the control in both two seasons. These treatments were more effective during 2009/2010 season in controlling root-rot disease and in increase yield/plot than in 2008/2009 season.

In respect of the effect of seed soaking with the tested oils on controlling root-rot disease and yield, data presented in Table (8), revealed that there were significant effect for all tested oils on disease incidence, disease severity and yield/plot during the two successive seasons.

Also, data clarified that, seed soaked in *C. cyminum, N. sativa, A. sativum* and *M. chamomilla* were the best treatments (no. infection was observed) and gave the highest yield (71, 64, 64 and 49.33 kg/plot,

respectively during 2008/2009 season compared with control (21.77% disease incidence, 8.09% disease severity and 32 kg/plot). While, in 2009/2010 growing season there were no infection when the seed soaking in all the tested oils except *A. cepa*. But seed soaking in *C. cyminum* and *N. sativa* gave the highest yield per plot compared with the control.

In the second season was the best in increasing yield per plot of all treatments after treatment with Vitavax-thiram compared with the untreated control.

Generally, seed soaking was more effective than seed coating with all the tested oils used during the two seasons (Table 7 and 8).

Table (7): Evaluation of some oils used as seed coating on disease incidence, disease severity and yield/plot during 2008-2009 and 2009-2010 growing seasons.

Essential oils	2008-2009 season			2009-2010 season		
	Root-rot		Yield/	Root-rot		Yield/
	Disease incidence %	Disease severity %	Plot kg	Disease incidence %	Disease severity %	plot kg
N. sativa L.	0.00 d	0.0 c	56.00 b	0.00 c	0.00 b	61.00 c
C. cyminum L.	0.00 d	0.00 c	69.00 a	0.00 c	0.00 b	73.33 a
A. sativum L.	0.00 d	0.0 c	61.33 b	0.00 c	0.00 b	55.00 d
A. cepa L.	6.75 b	0.72 b	40.33 d	1.61 bc	0.16 b	43.00 e
E. globulus L.	4.06 c	1.04 b	36.00 de	2.76 b	0.27 b	40.33 e
M. chamomilla L.	0.0 d	0.00 c	46.67 c	0.00 c	0.00 b	43.67 e
Vitavax-thiram	0.00 d	0.00 c	69.00 a	0.00 c	0.00 b	73.67 a
Control	26.23 a	9.74 a	30.67 e	24.95 a	9.21 a	32.67 f

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT.

Table (8): Evaluation of some oils used as seed soaking on disease incidence, disease severity and yield/plot during 2008-2009 and 2009-2010 growing seasons.

Essential oils	2008-2009season			2009-2010 season		
	Root-rot		Yield/	Root-rot		Yield/
	Disease incidence %	Disease severity %	Plot kg	Disease incidence %	Disease severity %	plot kg
N. sativa L.	0.00 d	0.00 b	64.00 b	0.00 c	0.00 c	71.33 b
C. cyminum L.	0.0 d	0.00 b	71.00 a	0.00 c	0.00 c	74.33 a
A. sativum L.	0.00 d	0.00 b	64.00 b	0.00 c	0.00 c	60.67 c
A. cepa L.	3.52 b	0.35 b	48.67 c	6.32 b	1.18 b	44.67 e
E. globulus L.	1.67 c	0.17 b	47.00 c	0.00 c	0.00 c	53.00 d
M. chamomilla L.	0.00 d	0.00 b	49.33 c	0.00 c	0.00 c	57.66 cd
Vitavax-thiram	0.00 d	0.00 b	72.66 a	0.00 c	0.00 c	77.33 a
Control	21.77 a	8.09 a	32.00 d	20.24 a	8.96 a	35.00 f

Data presented in Table (9) showed that seed coating with *E. glubulus* or *M. chamomilla* combined with Vitavax-thiram were completely effective in reducing root-rot of sugar-beet, followed by *N. sativa* and *C. cyminum* combined with Vitavax-thiram. The best treatment in increasing yield per plot was Vitavax-thiram alone (67.67 kg) or combined with *C. cyminum* (64.67 kg), *N. sativa* (63 kg), *E. globulus* (62.67 kg) and *M. chamomilla* (58.33 kg)

compared with the control (35.67 kg). *A. cepa* and *A. sativum* combined with *Vitavax-thiram* were the least effective in controlling root-rot disease and in increasing yield/plot compared with the other treatments in the first season 2008/2009. These treatments were effective in reducing infection with root-rot disease and in increasing yield per plot during 2009/2010 season.

Table (9): Effect of Vitavax-thiram combined with the tested essential oils used as seed coating on root disease and yield/plot on sugar beet cultivar Kawmera grown in the field during 2008/2009 and 2009/2010 growing season.

Essential oils	2008/2009			2009/2010		
	Root-rot		Yield/Plot (kg)	Root-rot		Yield/Plot (kg)
	Disease incidence (%)	Disease severity (%)		Disease incidence (%)	Disease severity (%)	
N. sativa L.	1.33 bc	0.33 b	63.00 b	1.96 d	0.83 d	59.00 c
C. cyminum L.	2.66 bc	0.53 b	64.67 ab	2.90 c	1.09 c	60.67 b
A. sativum L.	2.78 bc	0.49 b	53.67 d	3.08 c	0.86 d	53.00 d
A. cepa L.	4.52 b	0.91 b	48.67 e	5.32 b	1.23 b	48.00 e
E. globulus L.	0.00 c	0.00 b	62.67 bc	1.12 de	0.22 e	61.83 b
M. chamomilla L.	0.00 c	0.00 b	58.33 c	0.00 f	0.00 f	58.07 c
Vitavax-thiram	0.00 c	0.00 b	67.67 a	0.00 f	0.00 f	65.67 a
Control	20.42 a	9.09 a	35.67 a	27.77 a	10.08 a	33.33 f

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT.

Data presented in Table (10) revealed that the effect of seed soaking with the tested oils combined with Vitavax-thiram on controlling root-rot disease and yield, there were significant effect for all tested combinations on disease incidence, disease severity and yield/plot during the two successive seasons 2008/2009 and 2009/2010.

Table (10): Effect of Vitavax-thiram combined with the tested essential oils used as seed soaking on root-rot disease and yield/plot on sugar beet cultivar Kawmera grown in the field during 2008/2009 and 2009/2010 growing season.

Essential oils	2008/2009			2009/2010		
	Root-rot		Yield/Plot (kg)	Root-rot		Yield/Plot (kg)
	Disease incidence (%)	Disease severity (%)		Disease incidence (%)	Disease severity (%)	
N. sativa L.	4.94 bc	1.14 bc	63.00 b	4.08 c	1.06 c	52.00 d
C. cyminum L.	1.95 cd	0.19 c	64.67 ab	1.77 d	0.23 e	61.00 c
A. sativum L.	0.00 d	0.00 c	53.67 d	0.98 e	0..20 e	63.33 b
A. cepa L.	8.41 b	2.07 b	48.67 e	7.00 b	1.95 b	41.83 f
E. globulus L.	0.00 d	0.00 c	62.67 bc	0.00 f	0.00 f	52.67 d
M. chamomilla L.	3.54 cd	0.85 bc	58.33 c	4.03 c	0.86 d	45.67 e
Vitavax-thiram	0.00 d	0.00 c	67.67 a	0.00 f	0.00 f	65.83 a
Control	18.46 a	7.87 a	35.67 f	18.82 a	9.35 a	34.00 g

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT.

Data presented in Table (10) showed that, seed soaking in combinations of Vitavax-thiram with *E. globulus, A. sativum* and *C. cyminum* were the best superior in reducing root-rot disease (no infection was observed) and gave the highest yield in the two seasons. While, *A. cepa* and *N. sativa* were the least effective in controlling root-rot disease of sugar beet compared with control in both two seasons.

Chemical components of *C. cyminum* oil:

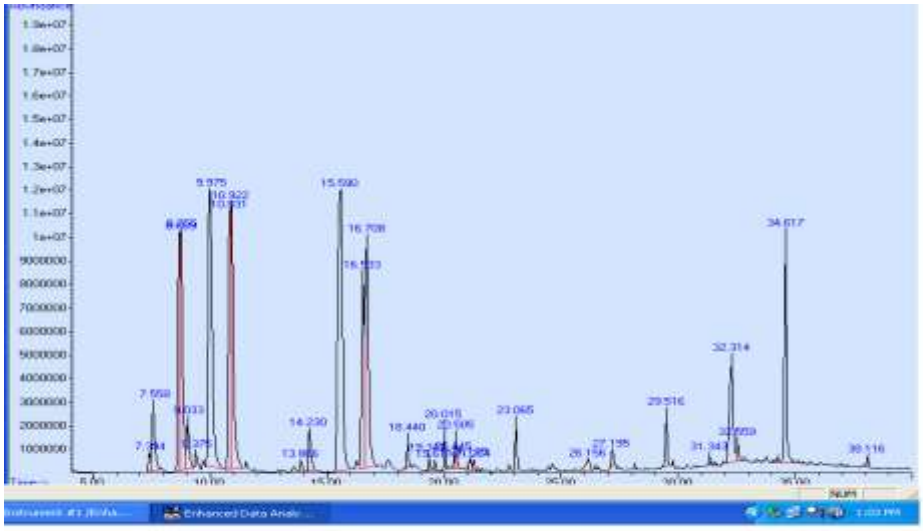
According to the results obtained from the previously mentioned tests *C. cyminum* was the best oil in controlling root rot disease and in increasing yield and chemical components in sugar beet roots. Therefore, *C. cyminum* oil was analyze by gas chromatography-mass spectrometry (GC-MS). Total ion chromatograms and chemical composition of *C. cyminum* oil are shown in Table (11) and Fig. (1). Analysis of GC-MS led to identification of 62 organic compounds from *C. cyminum* oil. Various compounds, including alcohol (such as propanol, 1-phenyl-1-butanol and alpha-n-propyl benzyl alcohol); aldehyds(1,3,3-trimethylcyclohex-1-ene-4-carboxaldehyde)and enzaldehyde, 4-(1-methyl); fatty acid (Hexadecanoic acid, octadecanoic acid and 9-ooctadecenoic acid) and hydrocarbons (hexadecane, heptadecane, octadecane, nonadecanen, ecosane n-heneicosane and n-tricosane) exist in *C. cyminum* oil (Table 11 and Fig. 1).

Table (11): Chemical components of the essential oil of *Cuminum cyminum L* determined by GC-MS methods.

Peak	RT(min)	Name of compound	Molecular formula
1	7.349	Bicyclo[3.1.0]hexa-2-ene,2-methyl-5-(1-methyleethyl)	C10 H16
2	7.349	Alpha thujene	C10 H16
3	7.560	Alpha -pinene	Unknown
4	7.560	Bicyclo[3.1.0]hept-2-ene, 2,6,6-trimethyl	C10-H16
6	7.560	Bicyclo[3,1,1]heptane,6,6,-dimethyl-2-methylen-eptan	C11 H18 O2
7	7.560	Bicyclo[3,1,0]hepta-2-enomythyl-5-(1-methylethyl)	C10H16
8	7.560	Beta-pinene6,6-dimethyl- 2-methylenebicyclo [3.1.1] heptane	C10H16
9	9.374	Phellandrene 1,3-cyclohexadiene ,2-methyl-5-diene	$C_{10}H_{16}$
10	9.694	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	$C_{10}H_{16}$
11	9.975	Benzene 1-methyl-2-(1-methylethyl)-	C10H14
12	9.975	1-Isopropyl-4-methylbenzene; 4-Isopropyltoluene	$C_{10}H_{14}$
13	10.833	Terpinene 4-methyl-1-(1-methylethyl)-1,3-cyclohexadiene	$C_{10}H_{16}$
14	10.925	Terpinene 4-methylene-1-(1-methylethyl)cyclohexene	$C_{10}H_{16}$
15	11.531	Cyclohexene, 1-methyl-4-(1-methylethylidene)	$C_{10}H_{16}$
16	13.574	Cyclohexanone, 5-methyl-2-(1-methylethenyl)-, trans-	$C_{10}H_{16}O$
17	13.866	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	C10H18O
18	14.232	1,3,3-trimethylcyclohex-1-ene-4-carboxaldehyde,	C10 H12 O
19	15.588	Propanal,2-methyl-3-phenyl	$C_{10}H_{14}O$
20	15.685	Benzaldehyde, 4-(1-methylethyl)-	$C_{10}H_{12}O$
21	16.252	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	$C_{10}H_{16}O$
22	16.532	1-phenyl-1-butanol	$C_{10}H_{14}O$
23	16.561	Alpha-n-propyl benzyl alcohol	$C_{10}H_{14}O$

Table (11):Cont.

Peak	RT(min)	Name of compound	Molecular formula
24	16.710	2,2-dimethyl-1-phenylpropan	C11H16O
25	16.738	Benzenemethanol, α-methyl-	$C_8H_{10}O$
26	17.637	1,4-Cyclohexadiene-1-methanol, 4-(1-methylethyl)-	$C_{10}H_{16}O$
27	18.438	Valencene Naphthalene, 1,2,3,5,6,7,8,8a-dimethyl1-7-(methylethyenyl)-	$C_{15}H_{24}$
28	19.347	Trans-caryophyllene bicyclo[7.2.0]undec-4-ene 4,11,11-trimethyl-8-methylene-	$C_{15}H_{24}$
29	19.611	bicyclo[3.1.1] hepta -2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)	C15 H24
30	20.017	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene	$C_{15}H_{24}$
31	20.446	2H-2,4a-methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl	$C_{15}H_{24}$
32	20.503	Zingiberene 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-	$C_{15}H_{24}$
33	21.035	Aromadendrene	$C_{15}H_{24}$
34	21.127	Beta-bisabolene yclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	$C_{15}H_{24}$
35	21.253	henol, 2,6-bis(1,1-dimethylethyl)-4-methyl	$C_{15}H_{24}O$
36	22.781	Caryophyllene oxide	$C_{15}H_{24}O$
37	22.815	Hexadecane	$C_{16}H_{34}$
38	23.067	Carotol 3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-	$C_{15}H_{26}O$
39	23.581	1,3-Benzodioxole, 4,5-dimethoxy-6-(2-propenyl)-	$C_{12}H_{14}O_4$
40	24.446	T-muurolol 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1r-(1α,4β,4aβ,8aβ)]-	$C_{15}H_{26}O$
41	24.623	Octadecyl acrylate	$C_{21}H_{40}O_2$
42	24.669	Heptadecane	$C_{17}H_{36}$
43	26.157	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	$C_{15}H_{24}$
44	24.460	Octadecane	$C_{18}H_{38}$
45	24.817	2,6,10,14-Tetramethylpentadecane	$C_{19}H_{40}$
46	25.996	tetrapentacontane,1,54-dibromo-	$C_{54}H_{108}Br_2$
47	27.192	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	$C_{13}H_{20}O$
48	27.307	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$
49	**27.678**	Dodecylcyclohexane	$C_{18}H_{36}$
50	28.159	Nonadecane	$C_{19}H_{40}$
51	29.515	Hexadecanoic acid	$C_{16}H_{32}O_2$
52	29.532	Dibutyl phthalate	$C_{16}H_{22}O_4$
53	29.784	Eicosane	$C_{20}H_{42}$
54	31.341	n-Heneicosane	$C_{21}H_{44}$
55	32.313	9-Octadecenoic acid	$C_{18}H_{34}O_2$
56	32.559	Octadecanoic acid	$C_{18}H_{36}O_2$
57	33.784	9,10-Anthracenedione, 1,4-diamino-2-methoxy-	$C_{15}H_{12}N_2O_3$
58	34.265	n-Tricosane	$C_{23}H_{48}$
59	34.619	9b,10-tetrahydro-8-methoxy-5-methylindeno[1,2-b]indol	Unknown
60	34.619	2-phenyl-4,7dimethyl-5-oxipyrano[4,3-b]pyridine	Unknown
61	34.871	Eicosane, 9-cyclohexyl-	$C_{26}H_{52}$
62	38.115	Cyclohexane, bromo	$C_6H_{11}Br$



RT(min)

Fig (1): Gas chromatogram tracing of commercial *C. cyminum* oil.

DISCUSSION

Sugar beet (*Beta vulgaris* L.) is one of the most important crops grown for sugar production allover the world. Trials were conducted to study the possibility of controlling sugar beet damping-off and root-rots caused by *S. rolfsii*. Concerning the effect of some essential oils on linear growth of *S. rolfsii* growing on PDA medium. The obtained data revealed that *C. cyminum* oil was the most effective in controlling linear growth of *S. rolfsii*, but *A. cepa* oil was the lowest effective *in vitro*. Also, seed treatment with *C. cyminum* oil was more effective in controlling damping-off and root-rots of sugar beet and increasing sucrose%, T.S.S.% and purity percentage in soil infested with *S. rolfsii* in pot experiment.

Seed soaking or coating with *C. cyminum* completely controlled root-rot infection and gave the highest yield per plot in field. These results are in accordance with those obtained by Dwivedi and Dubey (1993) and El-Shoraky (1998). Gouda (2001) reported that, oil of *C. cyminum* was superior to the other oils in inhibiting linear growth of *S. rolfsii in vitro*, while in greenhouse and field *Syzygium aromaticum* and *c. cyminum* oils were shown to be superior to all of the other oils in controlling sugar beet root diseases and increasing chemical components. El-Sherbieny *et al.* (2002) found that oils of thyme, cumin and mint gave the highest antifungal activity of *S. rolfsii*. The mycelial growth of *S. rolfsii* was completely inhibited with cumin oil and showed lower reduction effect on disease incidence than Rhizolex-thiram fungicide. Harmful activities of essential oils were found to be attributed to

their principle antifungal compounds such as cumin aldehyde of cumin (Grag and Siddique, 1993).

Generally, it could be concluded that some plant organs contain relatively high amounts of certain chemical compounds which showed inhibiting effect to soil born fungi. No doubt that antimicrobial agents involve numerous heterogenous group of biologically active ingredients i.e. alkaloids, essential oils, phenolic compounds, etc. Nanir and Kadu (1987); Agha (1992) and Abd El-Megid *et al.* (2003).

The present work indicated that Vitavax-thiram fungicide combined with essential oils was effective in inhibiting the linear growth of *S. rolfsii*. Results obtained revealed that, Vitavax-thiram combined with *E. globulus* oil followed by *C. cyminum* oil gave the highest inhibition percent of *S. rolfsii*. Combination of Vitavax-thiram with plant oils could successfully reduce damping-off and root-rots and increase sucrose percentage of sugarbeet root rot in greenhouse. These results are in agreement with those obtained by other investigators (Mohamed and Abo-Raya, 1993). They reported that Benlate and garlic extract combination was more effective in controlling tomato damping-off compared with each factor alone and increased stem length flowering and fruit-setting.

Studying the effect of certain treatments of peroxidase and esterase activities of sugarbeet infested with *S. rolfsii*. Results obtained indicated that all treatments increased peroxidase and esterase activity, but *C. cyminum* oil was the most effective in increasing activity of two enzymes compared with other treatments and control. Similar effects of some treatments were obtained by many other investigators (Xue *et al.*, 1998; Abo-Ellil *et al.*, 1998 and Metwally, 2004). Fayed *et al.* (2003) reported that these enzymes may play a role in the active defense mechanism of the plant.

These enzymes such as peroxidase played a role in oxidizing phenols to quinones that inhibit pathogens, the positive association between rapidity of the response of peroxidase activity, fungal infection and disease resistance was reported by Bi and Zhang (1993). Enhanced peroxidase activity is very often associated with resistance phenomena such as lignin production, or lignification (Hammerschmidt and Kuc, 1982).

Findings of this study suggest using essential oils in controlling sugar beet root disease rather than the hazardous fungicides that pollute the environment and affect drastically the public health. These results also meet the governmental recommendations of using natural products and giving up fungicidal application.

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تـأثير بعض الزيـوت النباتيـه و مخاليطهـا مـع المبيـد الفطـري فيتافـاكس-ثيـرام علـي مقاومـه العفن الاسكلروشيومى فى بنجر السكر.

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اختبرت ستة زيوت نباتية وكذلك التأثير المشترك لخلائط هذه الزيوت مع المبيد الفطرى فيتافاكس ـ ثيرام لمقاومة العفن الاسكلروشيومى فى بنجر السكر المتسبب عن الفطر سكليروشيم رولفسياى.

أوضحت نتائج المعمل أن كل الزيوت النباتية المستخدمة فعالة فى خفض النموالميسليومى للفطر ولكن زيت الكمون كان أكثرهم تأثيرا فى تنشيط النمو الميسليومى وعند دراسة التاثير المشترك وجد أن مخلوط مبيد فيتافاكس ثيرام مع كل من زيت الكافور وزيت الكمون أعطى أعلى تأثير فى خفض النمو الميسليومى لفطر سكليروشيم رولفسياى فى المعمل. تحت ظروف الصوبة أدى استخدام الزيوت النباتية إلى زيادة نسبة البادرات الحية وكذلك خفض الإصابة بمرض عفن الجذور ولكن معامله البذرة بزيت الكمون كان أكثر فعالية فى مقاومة كل من مرض موت البادرات وعفن الجذور فى بنجر السكر كما أدى لزيادة نسبة السكر والمواد الصلبة الذائبة تحت ظروف العدوى الصناعية بالفطر سكليروشيم رولفسياى. كانت أفضل المخاليط لمقاومة مرض عفن الجذور فى بنجر السكر هى مخاليط المبيد الفطرى فيتافاكس ـ ثيرام مع زيت الكافور وزيت الكمون وكذلك زيادة نسبة السكر والمواد الصلبة الذائبة ودرجة نقاوة السكر تحت ظروف العدوى الصناعية بالفطر. كما أدى زيت الكمون إلى زيادة النشاط الانزيمى لانزيمى البيروكسيديز والاستيريز أدى معاملة البذرة بزيوت الكمون وحبة البركة والثوم والبابونج سواء نقع أو تغليف إلى مقاومة كاملة لمرض عفن الجذور تحت ظروف الحقل ولكن زيت الكمون أعطى أعلى محصول مقارنة بالزيوت الأخرى. أما عند دراسة تأثير مخاليط الزيوت النباتية مع مبيد الفيتاماكس ـ ثيرام فى الحقل وجد أن تغليف البذرة بمخلوط المبيد الفطرى مع زيت الكافور والبابونج أعطى مقاومة كاملة لمرض عفن الجذور ولكن نقع البذرة فى مخلوط المبيد الفطرى مع زيوت الكافور والثوم كان الأفضل فى مقاومة مرض عفن الجذور فى بنجر السكر. وقد درس تأثير المبيد الفطرى فيتافاكس ـ ثيرام كمقارنة لتأثير تلك الزيوت على مقاومة العفن الاسكليروشيرمى فى بنجر السكر المتسبب عن الفطر سكليروشيم رولفسياى.

وعند التحليل الكروماتوجرافى باستخدام جهاز مطياف الكتلة لزيت الكمون تم تعريف 62 مركب عضوى تشمل مواد كربوهيدراتية وكحولات والدهيدات وغيرها من المواد العضوية الأخرى.

قام بتحكيم البحث

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Table (2): Effect of some essential oils on the linear growth of *S. rolfsii* in PDA medium.

Essential oils	Concentrations (ppm)												Mean		IC_{25} ppm	IC_{50} ppm
	100		250		500		1000		1500		2000					
	L.G	I%	L.G	I%	L.G	I%	L.G	I%	L.G	I%	L.G	I%	L.G	I%		
N. sativa L.	7.50 bc	15.06 f	6.10 cd	30.92 e	5.70 b	35.45 f	3.83 b	56.63 e	2.43 bc	72.48 e	1.87 b	78.82 e	4.57 b	48.24 e	220	800
C. cyminum L.	6.60 de	25.25 d	4.40 f	50.17 b	2.67 e	69.76 b	1.27 e	85.62 b	0.83 d	90.60 b	0.00 d	100.00 a	2.63 d	70.22 b	92	240
A. sativum L.	6.43 de	27.18 c	5.93 d	32.84 d	4.67 c	47.11 d	4.30 b	51.30 g	2.10 c	76.22 d	0.93 c	89.47 b	4.06 b	54.02 d	80	640
A. cepa L.	7.07 cd	19.93 e	6.67 bc	24.46 f	6.03 b	31.71 g	3.97 b	55.04 f	2.87 b	67.49 f	1.67 b	81.09 d	4.71 b	46.66 f	310	820
E. globulus L.	8.13 b	7.93 g	7.03 b	20.39 g	4.97 c	43.71 e	3.13 c	64.55 d	2.50 bc	71.69 e	2.07 b	76.56 f	4.64 b	47.45 ef	270	860
M. chamomilla L.	6.23 e	29.45 b	5.13 e	41.90 c	4.00 d	54.69 c	1.93 d	78.14 c	1.37 d	84.48 c	1.07 c	87.88 c	3.29 c	62.74 c	76	340
Vitavax-Thiram	1.80 f	79.61 a	1.30 g	85.28 a	1.03 f	88.33 a	0.80 e	90.94 a	0.00 e	100.00 a	0.00 d	100.00 a	0.82 e	90.71 a	3.50	15
Control	8.83 a	0.00 h	8.83 a	0.00 h	8.83 a	0.00 h	8.83 a	0.00 h	8.83 a	0.00 g	8.83 a	0.00 g	8.83 a	0.00 g	-	-

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT.
L.G=Liner growth

Table (4):Effect of some essential oils on damping-of, root-rot, T.S.S., sucrose and purity of sugar beet susceptible cultivar Kawmera under artificial infestation of *S. rolfsii* in a greenhouse during 2008/2009 season.

Essential oils	Damping-off		Survival	Root-rot		Healthy plants %	T.S.S. %	Sucrose %	Purity %
	Pre-emergence %	Post-emergence %	plants %	Disease incidence %	Disease severity %				
N. sativa L.	42.22 bc	6.66 cd	51.11 e	12.22 d	4.26 d	87.78 c	19.80 de	16.11 cd	81.36 a
C. cyminum L.	19.99 e	8.88 bc	71.11 c	5.65 e	1.03 ef	94.44 b	25.17 a	19.37 a	76.95 bc
A. sativum L.	37.78 cd	15.55 a	46.67 ef	42.22 c	9.63 c	57.78 d	20.37 d	13.28 e	65.19 d
A. cepa L.	31.11 d	11.11 abc	57.78 d	48.89 b	14.44 b	51.11 e	18.93 e	12.84 e	67.82 d
E. globulus L.	48.89 b	8.88 bc	42.22 f	11.11 d	2.07 e	88.89 c	22.60 c	18.03 b	79.78 ab
M. chamomilla L.	42.22 bc	11.11 abc	46.67 ef	6.67 e	1.44 e	93.33 b	23.03 c	15.64 d	67.91 d
Vitavax-thiram	13.33 e	2.22 de	84.45 b	0.00 f	0.00 f	100.0 a	24.53 ab	19.39 a	79.05 ab
Control infected	82.22 a	13.33 ab	4.44 g	100 a	20.83 a	0.00 f	8.87 f	3.92 f	45.21 e
Control uninfected	0.00 f	0.0 e	100.0 a	0.00 f	0.00	100.0 a	23.30 bc	17.22 bc	73.91 c

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT.

Table (5): Effect of Vitavax-thiram combined with some oils on damping-off, root-rot, and chemical component under artificial infestation of *S. rolfsii* in a greenhouse during 2009-2010 season.

Essential oils	Damping-off		Survival	Root-rot		Healthy	T.S.S.	Sucrose	Purity
	Pre-emergence %	Post-emergence %	plants %	Disease incidence %	Disease severity %	plants %	%	%	%
N. sativa L.	42.22 b	2.22 d	55.56 de	13.89 d	2.20c	86.11 d	21.47 cd	16.67 c	77.64 bc
C. cyminum L.	24.44 e	2.22 d	73.33 c	4.45 f	0.51 cd	95.56 b	25.57 ab	21.11 a	82.57 a
A. sativum L.	35.55 c	4.45 cd	60.00 d	17.78 c	4.26 b	82.22 e	21.37 d	16.23 c	75.95 cd
A. cepa L.	26.67 de	20.0 a	53.33 e	22.22 b	6.11 b	77.78 f	20.73 d	13.90 d	67.05 e
E. globulus L.	35.55 c	6.67 bcd	57.78 de	0.00 g	0.00 d	100 a	26.67 a	19.58 b	73.43 d
M. chamomilla L.	28.88 d	11.11 bc	60.00 d	8.52 e	1.65 cd	91.48 c	22.73 c	19.99 ab	79.15 b
Vitavax-thiram	13.33 f	4.44 cd	82.22 b	0.00 g	0.00 d	100 a	24.40 b	19.41 b	79.55 b
Control infected	82.22 a	13.33 b	4.44 f	100.0 a	22.22 a	0.00 g	8.70 e	4.69 e	53.09 f
Control uninfected	0.00 g	0.0 d	100 a	0.00 g	0.00 d	100 a	22.77 c	17.09 c	75.05 cd

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT.