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ISOLATION AND CHARACTERIZATION OF SOME PHENOLIC COMPOUNDS FROM *Ocimum basilicum* AND THEIR ANTIFUNGAL PROPERTIES AGAINST *Fusarium oxysporum*

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ABSTRACT

The fungicidal effect of four plants extracts namely *Chrysanthemum comarium, Hyoscyamum muticus, Ocimum basilicum* and *Solanum nigrum* were studied on growth and development of *Fusarium oxysporum* which cause serious diseases to members of most families. Among all species tested, *O. basilicum* extract was the most toxic to the tested fungus where its essential oil was more effective than petroleum ether, dichloromethane, chloroform and methanol extracts. They have EC_{50}'s as follows 723.6, 2331.8, 3539.9, 32200.3 and 70923.5 µg/ml, respectively. Aroma chemical constituents of *O. basilicum* leaves essential oil were identified by gas chromatography/mass spectroscopy (GC/MS). The major aroma constituents were eugenol (22%) which was the most effective and causes complete inhibition growth at all concentrations, followed by 1,8-cineol (4%) which showed no inhibitory effect and linalool (4%) was completely inhibited growth at concentration of 4000 µg/ml, as they appeared in (GC/MS).

Keywords: plant extracts, *Fusarium oxysporum*, basil, *Ocimum basilicum*, essential oil, aroma chemicals.

INTRODUCTION

Antimicrobial chemicals such as benzimidazoles, aromatic, hydrocarbons and sterol biosynthesis inhibitors are often used in controlling plant diseases in agriculture. However, there are problems against the effective use of these chemicals in areas where the fungi have developed resistance (Brent and Holloman, 1998). In order to overcome this problem, higher concentrations of these chemicals were used, but this increases the risk of high-level toxic residues in the products. In addition to the target pathogen, pesticides may also kill various beneficial organisms and their toxic forms may persist in soil (Hayes and Laws, 1991). Because of these problems there is a need to find natural substances alternatives to synthetic pesticides.

Among the various alternatives natural plant products that are biodegradable and eco-friendly are catching the attention of scientists worldwide. Such products, from higher plants and microbes are relatively broad-spectrum, bio-efficacious, economical and environmentally safe and can be ideal candidates for use as agrochemicals (Macias *et al.*, 1997 and Cutler, 1999). Among these, essential oils from a number of aromatic and medicinal plants have been reported to show activity against a wide range of plant pathogenic fungi (Rice, 1995). These are relatively safe to the users and to the environment (Wilson *et al.*, 1997)

The aim of the present study was as follow: (a) examine the activity of ethanolic extracts of *Chrysanthemum comarium, Hyoscyamum muticus, Ocimum basilicum* and *Solanum nigrum* against *Fusarium oxysporum*, (b) evaluate the antifungal activity of the essential oil, petroleum ether, dichloromethane, chloroform and methanol extracts of *Ocimum basilicum* (c) purification, separation and identification of aromatic chemical compounds in the essential oil of *Ocimum basilicum* which were identified by gas chromatography / mass spectroscopy (GC/MS).

MATERIALS AND METHODS

1. Plant materials:

Basil (*Ocimum basilicum*) and mandeliya (*Chrysanthemum comarium*) were collected from Beni Suief governorate during summer 2005 and 10th of Ramadan city during spring 2005, respectively. They were identified by the Flora and Classification Research Department, Agricultural Research Center.

Black night shade (*Solanum nigrum*), and Egyptian henbane (*Hyoscyamum muticus*) plants were purchased from a local market.

2. Preparation of the crude extracts:

After the plant parts had been dried under room temperature (30 ± 2°C), the dried materials of tested plants were grounded separately then, extracted with ethanol (95 %) using the rate of 5 ml/gm plant material in a soxhlet apparatus for 10 hours. The filtrates were evaporated by a rotary evaporator under vacuum at 45 °C temperature. The crude extracts were weighed and redissolved in 10 ml ethanol and kept in a refrigerator until use (Yossry *et al.*, 1998).

3. Extraction of promising plants:

Basil leaves, flowers, seeds and stems were extracted consecutively at room temperature with four solvents varied in their polarities, petroleum ether (60-80) °C, dichloromethane, chloroform and methanol. Each extract was prepared by extracting 100 gm of powdered plant material two times in the same solvent (20 % w/v), on a shaker at 200 rpm for 48 hours in dark glass bottles at room temperature. The extracts were combined, filtered and dried over anhydrous sodium sulfate. The filtrates were taken to dryness in vacuum at 40°C. The crude extracts were weighed and redissolved in 10 ml ethanol and kept in a refrigerator until use (Radwan and El-Sheikh, 2006).

4. Extraction of essential oils from fresh plant leaves by hydrodistillation:

Hydrodistillation was carried out by applying batches (20 % w/v) of basil fresh plant leaves to Clevenger-type apparatus for 4 hrs. The solution obtained was saturated with sodium chloride and extracted by fractioning with diethyl ether. The ether extract was dried by using anhydrous sodium sulfate, filtered and evaporated to remove the solvent. The essential oil was collected and stored in the freezer at -20°C until use. (Cakir *et al.*, 2005).

5. Antifungal activity assays:

Antifungal activity was studied by using an in vitro contact assay which produces hyphal growth inhibition (Cakir *et al.*, 2004). Briefly potato dextrose agar (PDA) plates were prepared using 9 cm diameter glass Petri dishes. Calculated the amount of each antifungal was added to the medium after sterilization (at 45 – 50 °C) to obtain the desired concentration of each antifungal.

To test the antifungal activity of basil oil, sterile Petri dishes containing the oil/Tween 80 emulsifier (80/20 v/v) diluted in PDA medium were prepared. Tween 80 alone as a control was added to PDA medium. Discs (5 mm diameter) of the test species were cut from a 1– week – old cultures of *Fusarium oxysporum* on PDA plates and place mycelial surface down, on opposite edges of the test plates on the center of dishes. The plates were then incubated at 28 ± 2 °C.

The extension – diameter (mm) of hyphae from the centers of the dishes sides was measured by caliper every 48 hrs for 12 days. Mean of fungal growth measurements were calculated from 4 replicates of each antifungal compound. PDA plates treated with distilled water without any extracts were used as negative control.

The percentage of growth inhibition was calculated using the following formula:

$$\%.of\ Inhibition = \frac{C-T}{C} x100$$

Where:

C : is an average of 4 replicates of hyphal extension (mm) of controls.

T: is an average of 4 replicates of hyphal extension (mm) of plates treated with either essential oil or crude extract solution.

The corrected percentage of growth inhibition was used to calculated the LC_{50} and LC_{90} values according to *Finney* (1971). Toxicity index was calculated according to *Sun* (1950).

6. Determination of the minimum inhibitory concentrations (MICs) using a broth dilution micro-method:

Initial emulsions of compounds were prepared at 1000 ppm were applied in sterile distilled water with Tween 80. Twofold serial dilutions of the stock solution in broth medium (100 µl of potato dextrose broth) were prepared on a Nunclon (8 x 12) microtiter plate (96 wells). Then 1 µl of the fungal suspension (in sterile distilled water) adjusted to 10^7 spores/ml was added to each well. Microtiter plates were then incubated for 2 days at 28°C. MICs were the determined as the lowest concentrations preventing visible growth (Sirot, 1990).

7. Separation and identification of aroma chemical compounds in the essential oils:

Separation and identification of the aroma chemical compounds of the essential oils were carried out by using GC-MS Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973 (Lee *et al.*, 2005).

A fused silica capillary column (HP-5MS), 5% phenyl polysiloxane as non polar stationary phase (30 m x 0.25 mm x i.d) and 0.25 µl film thickness. Operating conditions were as follows:

- Injection port temperature: 250°C.
- Carrier gas: helium at a flow rate of 1.0 ml/min pulsed split less mode
- Column temperature was maintained at 80°C, for 3 min, then programmed at 80°C/min to 260°C and held for 18 min.
- Total analysis time was 41 min.
- A 1 µl volume was injected split less.
- Mass spectrometric detector (MSD) was operated in electron impact ionization mode with an ionizing energy of 70 Ev, scanning from m/z 50 to 500.
- The ion source temperature was 230°C and the quadrapole temperature 150°C.
- The electron multiplier voltage (EM voltage) was maintained 1100 V above auto tune, and solvent delay of 3 min was employed.
- The instrument was manually tuned using perfluorotributylamine (PFTBA).

8. Chemical analysis:

The promising compounds of highly biological activity were analyzed by using different tools of spectroscopy. IR spectra were recorded (KBr) on Pye-Unicam SP/2000 spectrophotometer and Maltston-1000 series FTIR spectrometer using wafer technique. The ^{1}H-NMR spectrum was determined on Brucker AC-200, Varian Unity 300, Varian VXR400, Varian Nova 500, using TMS as internal stander. All chemical shifts were measured in δ-scale.

The mass spectra were measured by Shimadzu single focusing mass spectrometer, Micromass Autospec and Hewlett-Packard 5890 series II GC interfaced to VG-Trio 1000 mass spectrometer. Elemental analyses were determined by Perkin-Elmer 2400 CHN, elemental analyzer.

RESULTS AND DISCUSSION

1. Effectiveness of crude plants extract:

The four plants listed in table (1) were extracted by using ethanol 95%. The ethanolic extract gives gradually effects according to incubation periods (2, 6 and 8 days) and concentration. Effectiveness of the tested extracts was regularly increased with an increase in the concentration. In general, after 8 days incubation, the lowest inhibitory effect was occurred to the radial growth of the tested fungus by ethanolic extract of *Solanum nigrum.* On the other hand the inhibitory effect of the *Hyoscyamum muticus* ethanolic extract was inversely proportional with the concentration at the same period of time.

Ocimum basilicum ethanoilc extract was the most effective one followed by *Chrysanthemum comarium* with EC_{50}'s 2160 and 3400 ppm, respectively. Also basil extract recorded reduction growth percentage (68.1%) more than *Chrysanthemum comarium* (53.1%)

Table (1): Effect of different concentrations of some plant extracts on the percentage of reduction growth in *F. oxysporum* after different incubation periods at 28°C.

Concentrations (µg\ml)	Plant extracts	Days of incubation											
		Chrysanthemum coronarium			*Hysocyamum muticus*			*Ocimum basilicum*			*Solanum nigrum*		
		2	6	8	2	6	8	2	6	8	2	6	8
250	% of radial growth inhibition	-	2.9	5.2	3.9	4.9	21.1	2.5	8.9	4.7	9.1	16.0	9.8
500		12.7	8.7	11.7	5.12	5.2	12.3	21.8	21.7	12.2	12.6	18.3	11.2
1000		27.3	20.5	22.3	6.4	5.4	6.0	62.4	40.3	27.0	16.7	20.0	12.2
2000		47.5	38.5	37.0	7.9	5.6	3.0	92.0	62.4	47.6	21.6	21.9	13.2
4000		62.0	59.2	53.1	10.6	6.1	(-)	100	79.8	68.1	24.9	23.9	14.6
Slope		0.94	0.97	0.86	0.23	0.08	-	1.86	0.93	0.95	0.33	0.15	0.11
EC_{50}		2200	2900	3400	16165	$9x10^5$	-	820	1380	2160	40800	$9749x10^3$	$3x10^5$
Toxicity Index		37.2	47.59	63.5	5.07	0.02	-	100	100	100	2.01	0.014	$72x10^{-6}$

(-) Stimulation of fungal growth (radial growth of treatments larger than control)

2. Effectiveness of promising plant extracts:

The obtained extracts from flowers, seeds and stems of the basil plant with different solvents (petroleum ether, dichloromethane, chloroform and methanol) hadn't any fungicidal activity while the leaves extracts for the same solvents had a potential effect.

The effect of different concentrations of *O. basilicum* extracts using petroleum ether, dichloromethane, chloroform and methanol inhibited the growth of F. oxysporum in vitro shown in table (2). Radial growth inhibitions were directly proportional with the concentrations of basil leaves extracts with different solvents. The EC_{50} values (µg/ml) could be arranged descendingly as follows: 70359, 32201.3, 3539.9 and 2331.8 ppm for methanol, chloroform, dichloromethane, and petroleum ether, respectively. In dose response study, *F. oxysporum* is less inhibited by low concentrations of basil leaves extracts.

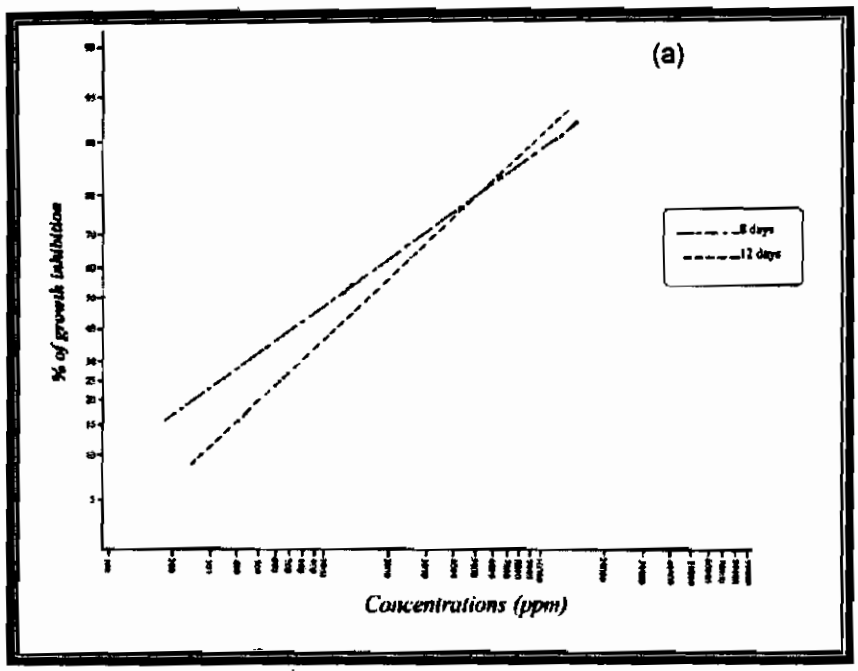
However, higher concentrations exhibited marked inhibition. The response *of F. oxysporum* to petroleum ether compared with the other extracts used (Figure 1a-e).

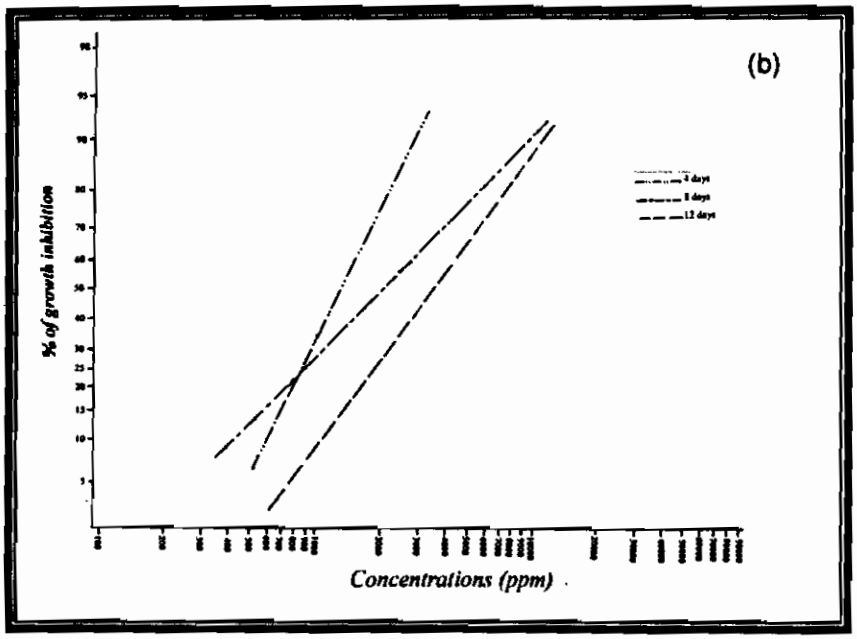
3. Fungicidal effect of basil essential oil:

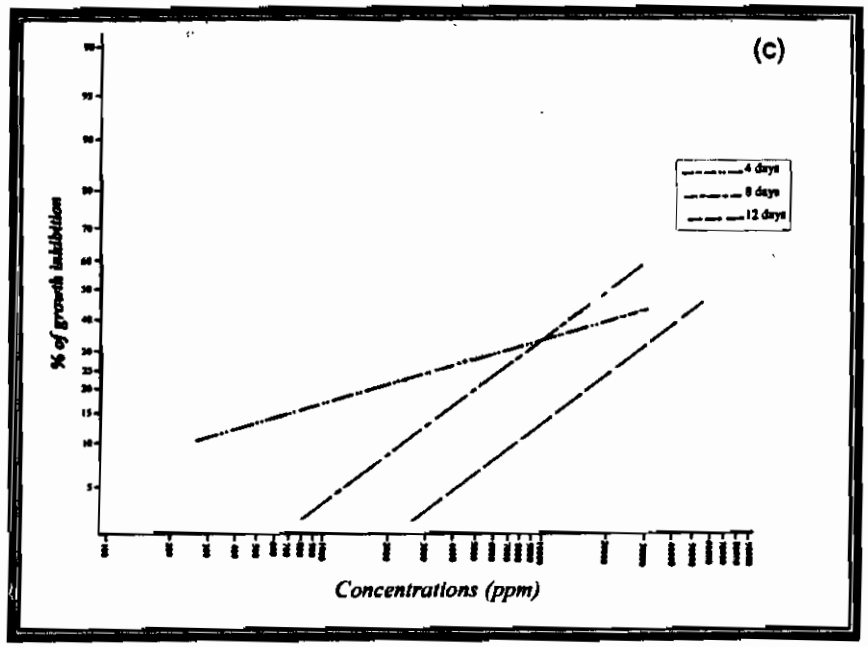
Data illustrated in table (3) indicated that the fungicidal effects were due to the presence of antifungal substances in essential oil extracted from fresh leaves of *O. basilicum*. The essential oil revealed highly toxic against *F. oxysporum*. With EC_{50} value was 723.6 ppm, while it was 2331.8 ppm for petroleum ether extract of dry leaves.

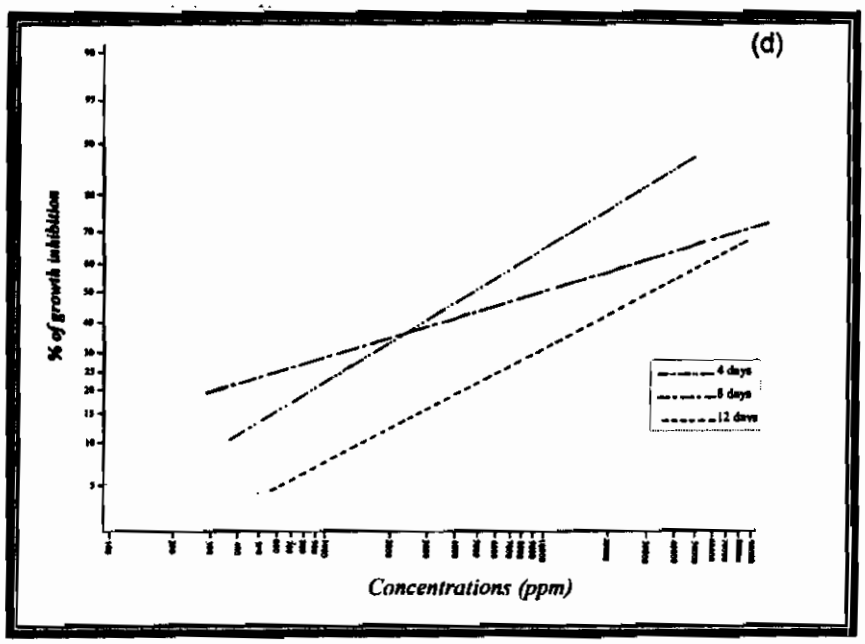
Data summarized in table (4) indicated that the essential oil of fresh leaves was more effective than petroleum ether, dichloromethane, chloroform and finally methanol extracts of dry leaves, respectively. Also, the highest toxicity index was (100 %) for the essential oil followed by petroleum ether, dichloromethane, chloroform and methanol extracts with toxicity indexes 31.03, 20.44, 2.25 and 1.03%, respectively. On the other hand the relatively potency of the essential oil was more effective than methanol extract by 97.2 folds where, the petroleum ether, dichloromethane and chloroform extracts had relative potencies than methanol extract with 30.17, 19.88 and 2.18 folds, respectively. The last results were agreed with that obtained by Montes-Belmont and Carvajal, (1998), who found that the *Aspergillus fluvas* fungus was totally inhibited by the essential oil extracted from *Ocimum basiliucm* in the maize grain protection. Those effects were probably related to the volatility of the perspective active principle.

Also, Basilico and Basilico, (1999), found that, the spice essential oil obtained from basil was completely effective initially (7 days) against *Aspergillus ochraceus* NRRL 3174 at the concentration 1000 ppm, but permitted the mould growth afterwards.


(a)
% of growth inhibition
Concentrations (ppm)
8 days
12 days


(b)
% of growth inhibition
Concentrations (ppm)


(c)
% of growth inhibition
4 days
8 days
12 days
Concentrations (ppm)


(d)
% of growth inhibition
4 days
8 days
12 days
Concentrations (ppm)

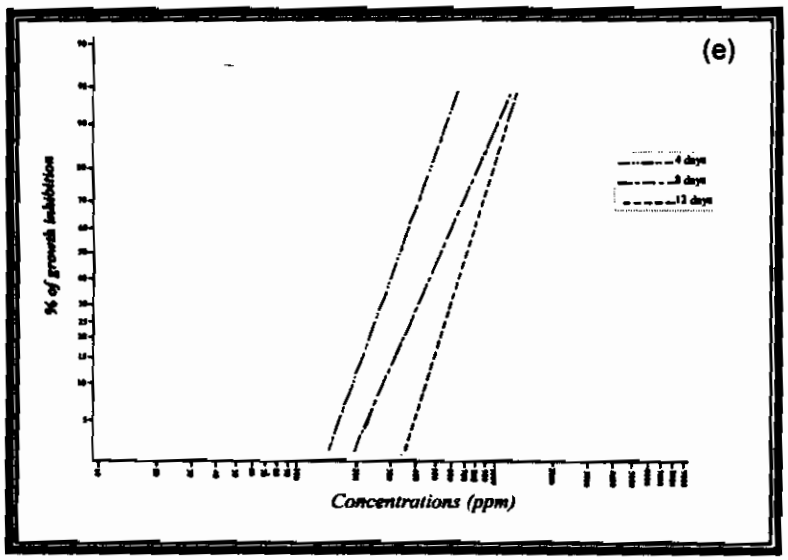


Fig. (1): Ldp lines of: (a) petroleum ether (60-80)°C, (b) dichloromethane, (c) chloroform, (d) methanol dry leaves extracts and (e) essential oil extracted form fresh leaves extracts of *O. basilicum* against *F. oxysporum* at different time interval.

These results were in harmony with; Nwachukwu and Umechuruba (2001), illustrated that, the leaf extract of basil has some fungicidal properties that inhibit the growth of seed borne fungi including *Fusarium moniliforme, Aspergillus niger, Aspergillus flavus* and *Botryodiplodia btheobromae*. The crude extracts were more effective than the aqueous extracts. Bansal and Gupata (2000), who found that, *Ocimum basilicum* was toxic to *Fusarium oxysporum*, inhibiting mycelial growth and spore germination at five concentrations (20, 40, 60, 80 and 100%) but did not exhibit complete inhibition even at 100% concentration. Finally, Rai, *et al.*, (1998), found reported that, the essential oil prepared from *Ocimum basilicum* was found to be active against all *Fusarium* species (*Fusarium solani, Fusarium oxysporum, Fusarium pallidoroseum, Fusarium chlamydosporum* and *Fusarium acuminatum). Fusarium oxysporum* was, among all *Fusarium* species tested, found to be the least sensitive fungus.

Finally, Soliman and Badeaa, (2002), reported that the essential oil of basil caused complete growth inhibition of four fungi; *Aspergillus flavus, A. parasiticus, A. ochraceaus* and *Fusarium moniliforme* at 3000 ppm. The high antifungal activity of the essential oil was due to the phenolic compound present in the essential oil that have been known to possess antimicrobial activity and some are classified as "Generally Recognized as Safe" (GRAS) substances and therefore can be used to prevent post-harvest growth of native and contaminant (Ponce, *et al.*, 2003). The exact cause-effect relation for the mode of action of phenolic compounds has not been determined yet, but Davdison (1993) indicated that, it may deactivate essential enzymes, reacting with the cell membrane or disturbing material functionality.

Table (2): Evaluation the effectiveness of *O. basilicum* leave extracts with different polarity solvents upon the linear growth inhibition at different incubation periods

Solvent	Time (days)		Leave extracts concentrations in ppm. (µg/ml)							EC_{10}	EC_{50}	EC_{90}	Slope
			500	1000	2000	4000	6000	8000	10000				
Petroleum ether	4	% of growth inhibition	-	51.7	100	100	100	100	100	864.1	995.3	1146.3	-
	8		32.3	46.9	61.8	75.1	81.6	85.5	88.1	109.7	1152.4	12101.1	0.7
	12		12.2	26.2	45.4	65.8	76.1	82.3	86.3	423.9	2331.8	12827.5	0.92
Dichloro-methane	4		5.2	31.8	74.8	96.5	99.3	100	100	611.2	1333.2	2907.9	2.0
	8		12.1	26.9	48.2	70.0	80.5	86.4	90	446.2	2114.1	10015.9	0.95
	12		1.4	8.2	26.5	55.3	71.9	81.5	87.3	1102.8	3539.9	11362	1.3
Chloroform	4		13.3	21.7	53.0	46.1	54.1	59.6	63.8	362.6	4880.5	65696.2	0.6
	8		22.8	27.8	34.0	40.6	44.6	47.5	49.8	64.5	10217.9	1.62×10^6	0.32
	12		4.1	7.3	12.2	19.1	24.1	28.0	31.2	1508.4	32201.3	6.87×10^5	0.52
Methanol	4		13.1	16.7	21.2	26.5	29.8	32.3	34.2	275.5	52843.6	1.01×10^7	0.29
	8		1.02	3.7	8.3	16.2	22.6	27.9	32.3	2399.1	22151.9	2.05×10^5	0.71
	12		(-)	(-)	(-)	4.3	7.0	9.6	12.1	8281.5	70359.5	5.98×10^5	0.75

(-) Stimulation of fungal growth (radial growth of treatments larger than control)

Table (3): Inhibition of mycelial growth at different concentrations of the essential oil extracted from *O. basilicum* fresh leaves on *F. oxysporum* after different incubation times.

Time (days)		Essential oil concentrations in ppm. (µg/ml)							EC_{10}	EC_{90}	EC_{90}	Slope
		150	200	300	400	600	800	1000				
4	% of growth inhibition	2.5	10	36.7	63.0	89.9	97.4	100	200.1	347.0	601.8	2.9
8		0.6	2.4	11.8	26.6	56.5	76.5	87.6	285.4	551.9	1066.9	2.4
12		0	0	0.5	4.2	29.2	61.5	82.8	466.8	723.6	1121.8	3.6

Table (4): LC_{50}'s, toxicity indexes and relative potency of different basil extracts against *F. oxysporum* at the end of incubation.

Extract	LC_{50}(ppm)	Toxicity index (%)	Relative Potency (fold)
Petroleum ether dry leaves extract	2331.8	31.03	30.17
Dichloromethane dry leaves extract	3539.9	20.44	19.88
Chloroform dry leaves extract	32201.3	2.25	2.18
Methanol dry leaves extract	70359.5	1.03	1.0
Essential oil from fresh leaves	723.6	100	97.2

4. The fungicidal effect of the promising identified compounds:

The remarkable activities of essential oil constituents linalool, eugenol and 1,8-cineol were illustrated (Table 5). Eugenol at any used concentration caused complete inhibition to the radial growth of the tested fungus even after 12 days of incubation. On the other hand, linalool gave complete inhibition to the radial growth of fungus after 12 days at 4000 µg/ml where its EC_{50} was 2059 µg/ml. But, 1.8-cineol has not any fungicidal effect against the tested fungus.

Table (5): Effect of active compounds isolated from essential oil extracted from *O. basilicum* fresh leaves on the linear growth inhibition of *F. oxysporum* at different incubation periods.

Comp.	Time (days)	Concentrations in ppm. (µg/ml)						EC_{10}	EC_{50}	EC_{90}	Slope
		125	250	500	1000	2000	4000				
Linalool	4	1.1	7.0	25.4	56.1	83.4	100	294.99	877.6	2611.1	1.26
	8	0.02	0.5	6.0	30.0	69.4	93.8	662.4	1438.4	3123.6	1.7
	12	0	0	0	0.02	43.9	99.9	1611.4	2059.6	2632.8	1.73
Eugenol	4	16.35	100	100	100	100	100	-	-	-	-
	8	6.04	100	100	100	100	100	-	-	-	-
	12	9.6	100	100	100	100	100	-	-	-	-
1,8-cineol	4	6.3	5.2	4.3	3.5	2.8	2.3	-	-	-	-
	8	(-)	(-)	(-)	(-)	(-)	2.5	-	-	-	-
	12	0	0	0	0	0	0	-	-	-	-

(-) Stimulation of fungal growth (radial growth of treatments larger than control)

5. Minimum inhibitory concentrations (MICs) of the identified compounds:

The results represented in table (6) showed that; the lowest MIC was that of eugenol (200 µg/ml) against the fungus tested. The MIC of linalool was 1000 ppm and while the 1,8-cineol compound had not any inhibitory effect even at 4000 ppm against the fungus tested.

Table (6): Minimum inhibitory concentrations (MICs) of the promising identified compounds against F. oxysporum.

Compound	Minimum Inhibitory Concentration (ppm)
1,8-cineol	> 4000
Eugenol	≤ 200
Linalool	≤ 1000

This means that eugenol was the most effective component. This result was disagreed with those reported by Beatriz, *et al.*, (2001) who found that, the inhibitory effect of eugenol appeared to be more pronounced since at 100 mg/ml. The activity of eugenol was stronger than that of thymol at a concentration 200 mg/ml. At a concentration of 200 mg/ml, eugenol gives complete fungal growth inhibition. It was observed at a concentration of 200 mg/ml eugenol completely inhibited the growth of strains of *Penicillium citrinum*.

Also Karapinar (1990) concluded that, the antifungal activity eugenol which is active components of commonly used spices was studied against two strains of *Aspergillus parasiticus*. Concentration of 300 µg/ml, eugenol inhibited the growth of both strains of *A. parasiticus* NRRL 299.

6. Chemical identification of the active separated compounds:

The separation and identification of the aroma chemical compounds of the essential oil was carried out by using GC/MS. For essential oil of basil fresh leaves, eugenol was the major compound of 22 % (Labra, *et al.*, 2004), while linalool and 1,8-cineol were minor compounds 4% and 2%, respectively.

Table (7): Major components of basil essential oil separated by gas chromatography and identified by comparison with wiley mass spectra library data version 7n.1.

Compound	Basil oil	
	RT	Area %
1, 8 – cineol	4.93	4.0
Linalool	5.95	4.0
Eugenol	8.5	22.0

The chemical structure of a promising compound isolated from basil was confirmed by different tools of spectroscopy:

1- Correct elemental analysis:

	C	H	O
Experimental	72.9	7.39	-
Calculated	73.08	7.31	19.61

The experimental percentage of carbon and hydrogen elements were 72.9% and 7.39% while the calculated percentages were 73.08% and 7.31%, respectively which gave a structural formula $C_{12}H_{16}O_2$.

2-The IR spectra:

The IR spectra showed an absorption bands in the region of wavelength (cm^{-1}) 2925 – 2980 ($\upsilon_{CH\ aliphatic}$), 2980 – 3075 ($\upsilon_{CH\ aromatic}$) and 3450 ($\upsilon_{phenolic}$).

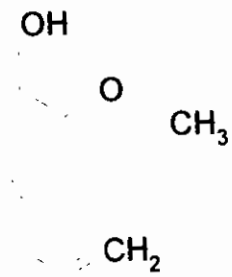
3-The ^1H – NMR spectra was displayed at δ (ppm):

The promising compound showed a signal as follows:

3.3 (d, 2H, – CH_2 –), 3.8 (s, 3H, O – CH_3), 5 (d, 2H, = CH_2), 6 (m, 1H, – CH =), 6.8 – 7 (m, 3H, Ar – H) and 6.8 (s, 1H, – OH).

4-The mass spectra:

Fragmentation peaks at m/z (abundance %): 164 (M^+, 100), 149 (36), 133 (23), 103 (30), 77 (22) and 55 (15).



Structure of Eugenol

From the above data, we can conclude that the isolated compound of highly biological activity was eugenol which compared with Wiley data library of MS-GC instrument.

REFERENCES

Bansal, R. K. and Gupata, R. K. (2000). Evaluation of plant extracts against *Fusarium oxysporum*, wilt pathogen of fenugreek. Indian Phytopath. 53(1): 107 – 108.

Basilico, M. A and Basilico, J. C. (1999). Inhibitory effects of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxine A production. Letters in Appl. Microb. 29: 238 – 241.

Beatriz, I. V.; Fente, C.; Franco, C. M.; Vazquez M. J. and Cepeda, A. (2001). Inhibitory effects of eugenol and thymol on *Penicillium citrinum* strains in culture media and cheese. Inter. J. of Food Microb. 67: 157–163.

Brent, K. J. and Holloman, D. W., (1998). Fungicide resistant: the assessment to risk. FRAC, Global Crop Protection Federation; Brussels, Monograph No. 2, PP. 1 – 48.

Cakir, A. ; Kordali, S.; Kilic, H and Kaya, E (2005). Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse. Bioch. System. and Ecol.. 33: 245–256

Cakir, A; Kordali, S.; Zengin, H.; Izumi, S; Hirata, T. (2004). Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifloium* and *Hypericum heterophyllum*. Flav. Frag. J. 19(1): 62 – 68.

Cutler, H. G., (1999). Allelopathy in the biological control of plant disease. ed. Recent Advances in Allelopathy. Vol. 1. A science for the future. Servicio de Publicationes, Universidad de Cadiz, Spain, PP. 397 – 414.

Davidson, P. M. (1993). Parabens and Phenolic Compounds. In: P. M. Davidson, & Branen (Eds.). Antimicrob. in Foods. pp. 263 – 305. New York, Marcel Dekker, Inc.

Finney, D.J. (1971). Probit Analysis (3rd ed. Cambridge Univ. Press, London).

Hays, W. J.; Laws, E. R. (1991). Handbook of pesticides toxicology. Vol (1). Academic press, New York. PP. 55 – 56.

Karapinar M. (1990). Inhibitory effects of anethole and eugenol on the growth and toxin production of *Aspergillus parasiticus*. International J. Food Microb. 10(3-4): 193-199.

Labra M.; Miele M.; Ledda B.; Grassi F.; Mazzei M. and Sala F. (2004). Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Sci.. 167:725–731

Lee, S.; Umano, K.; Shibamoto, T. and Lee, K. (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem. 91:131–137

Macias, F. A.; Castellano, D.; Oliva, R. M.; Cross, P.; Torres, A. (1997). Potential use of allelopathic agents as natural agrochemicals. Brighton Crop Prot. Conf. – weeds : 33 – 38.

Montes-Belmont, R. and Carvajal, M. (1998). Contorl of *Aspergillus flavus* in Maize with plant essential oils and other components. J. of Food Prot. 61(6): 616-619.

Nwachukwu, E. O and Umechuruba, C. I. (2001). Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seed, seed germination and seedling emergence. J. Appl. Sci. Environ. Mgt. 5(1): 29 -32.

Ponce, A. G.; Fritz, R.; del Vàllé, C. and Roura, S. I. (2003). Antimicrobial activity of essential oils on the native microflora of organic swiss chard. Lebensm Wiss u Technol. 36: 679 – 684.

Radwan M. M Eman. and El-Sheikh Y. W. A. (2006). Toxicological and Biological effect of *Calendula officinalis* extracts on the cotton leaf worm, *Spodoptera littoralis* (Bosid.). Monsoura Univ. J. Agric. Sci. 31(2): 1045 – 1060.

Rai, M. K.; Qureshi, S. and Pandey A. K. (1998). In vitro susceptibility of opportunistic *Fusarium* spp. To essential oils. Mycoses 42: 97 – 101.

Rice, E. L., (1995). Biological control of weeds and plant diseases. University of Oklahoma Press, Norman, 439 P.

Sirot, J. (1990). Bactèriologie Mèdicale, 2nd Ed., Edits. L. Le Minor and M. Vèron, pp. 303, Paris.

Soliman, K. M. and Badeaa, R. I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chem. Toxic. 40: 1669-1675.

Sun, Y. P. (1950): Toxicity Index and improved method of comparing the relative toxicity of insecticides. J. Econ. Entom., 43: 45 – 53.

Wilson, C. L.; Solar, J. M.; Ghaouth, A. and Wisniewski, M. E. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against Botrytis cinerea. Plant Disease. 81 : 204 – 210.

Yossry, A. Amani, Abd El-All, S. A. and El – Tmery, S. M. (1998). Fungitoxic properties of some plant extracts against growth of soil born disease fungi. Ann. of Agric. Sci. Cairo 3 : 891 – 909.

فصل وتعريف بعض المركبات الفينولية من نبات الريحان وتأثيرها الإبـــادي علـــى فطر الفيوزاريم أوكسيزبورم

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تم دراسة التأثير الإبادي لمستخلصات نباتات المندليه، السكران المـــصري، الريحـــان وعنب الديب على فطر الفيوزاريم أوكسيزبورم المسبب للعديد من الأمراض في العائلات النباتيـــة المختلفة. من خلال المستخلصات التي تم دراستها وجد أن المستخلص الخام لنبات الريحان كان له أعلى تأثير على الفطر من مستخلصات النباتات الأخرى.

وكان الزيت العطري للأوراق المستخلصة بطريقة التقطير البخاري أعلى تـــأثيرا مـــن مستخلص الإيثير البترولي (٦٠-٨٠°م) ثنائي كلور الميثان والكلوروفورم والميثـــانول لـــلأوراق وكانت قيم التأثير النصفي الإبادي(EC_{50}) كما يلي: ٧٢٣,٦ ، ٢٣٣١,٨ ، ٣٥٣٩,٣ ، ٣٢٢٠٠,٣ و ٧٠٩٢٣,٥ جزء في المليون على التوالي.

وقد تم التعرف على الزيت العطري لأوراق الريحان بواسطة جهاز الفـــصل الغـــازي الكروماتوجرافي ذو مطياف الكتلة (GC/MS) وكانت نسبة مركب الـــــEugenol هـــي ٢٢٪ وأعطى تأثيرا إباديا تاما عند التركيزات الآتية ٢٥٠ ، ٥٠٠ ، ١٠٠٠ ، ٢٠٠٠ ، ٤٠٠٠ جزء في المليون (ppm) وكانت نسبة مركب 1.8-cineol هي ٤٪ حيث لم يكن له أي تأثير إبادي عنـــد أي تركيز بينما كانت نسبة المركب Linalool هي ٤٪، كان له تأثير إبادي كامـــل علـــى فطـــر الفيوزاريم أوكسيزبورم عند تركيز ٤٠٠٠ جزء في المليون طبقا لظهور هذه المركبات في التحليل الكروماتوجرافي على الترتيب.