

Antifungal activity of some Naturally Occurring Compounds against Economically Important Phytopathogenic Fungi

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Abstract: This study was undertaken to investigate the antifungal activities of naturally occurring compounds namely; thymol, eugenol, methyl cinnamate, linalool and 1,8-cineol against some economically important phytopathogenic fungi in the Egyptian environment. The LC₂₅ and LC₅₀'s of the tested compounds against *R. solani*, *S. rolfisii*, *B. cinerea*, *F. oxysporum* and *A. solani* were determined according to the relationship drawn between the logarithm of concentration and the percent of growth inhibition (ldp lines), and the toxicity indexes and relative potencies were calculated. Thymol was the most effective compound followed by eugenol, methyl cinnamate and linalool against *R. solani*, *F. oxysporum* and *B. cinerea* whereas the thymol followed by methyl cinnamate, eugenol and linalool were the most effective compounds against *S. rolfisii* and *A. solani* descendingly. The LC₅₀'s of thymol against *S. rolfisii*, *R. solani*, *B. cinerea*, *F. oxysporum* and *A. solani* were 107.39, 59.28, 46.60, 81.89 and 156.41 µg/ml, respectively where the LC₅₀'s of eugenol were 254.47, 95.58, 270.47, 214.74 and 414.13 µg/ml and the LC₅₀'s of methyl cinnamate were 216.3, 102.78, 288.55, 290.66 and 192.37 µg/ml, respectively. The 1,8-cineol compound didn't show any fungicidal activity.

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1. Introduction:

Food safety is an increasingly important public health issue. Nearly, 30% people in the world suffer from food borne diseases every year caused by microbes (Burt, 2004; Shephard, 2008). Most diseases in plants are caused by various pathogens including fungi, nematodes, bacteria, and viruses (Montesinos, 2003). Fungi are the major pathogens and a source of many diseases of plants. Pathogenic fungi also could decrease the growth of many economically important crops (Fletcher et al., 2006).

Moulds or microscopic filamentous fungi are ubiquitous microorganisms with a great capacity to colonize many kinds of substrates and to proliferate under extreme environmental conditions (Singh et al., 1991; Nickelsen and Jakobsen, 1997). Poor storage management can lead to rapid deterioration in nutritional quality of food commodities with production of volatile metabolites giving off-odors. Moulds also produce mycotoxins that can be teratogenic, carcinogenic or cause feed refusal and emesis (Yu et al., 2003; Magan et al., 2004). Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals may cause toxic residues in treated products (Barnard et al., 1997; Isman, 2000). Synthetic pesticides can also cause environmental pollution owing to their slow biodegradation (Barnard et al., 1997; Misra and

Pavlostathis, 1997). In addition, the risk of developing the resistance by microorganisms and the high cost-benefit ratio are other disadvantages of synthetic pesticide usage (Brent and Hollomon, 1998).

In the few last years, there has been target interest in biologically active compounds isolated from plant species for elimination of different fungi on the plants and food products, because they are safe substances for human and environment (Romanazzi et al., 2007; Kumar et al., 2008). Essential oils are complex multicomponent mixtures of fragrant volatile substances, monoterpenes, sesquiterpenes, aromatic compounds and their derivatives that plants usually synthesize in response to stress conditions and produce antibacterial, antiviral and antifungal effects (Lovkov et al., 2001). The antifungal activity of essential oils against a large number of phytopathogenic fungi under *in vitro* conditions is well documented (Bouchra et al., 2003; Boyraz and Ozcan 2006; Viuda-Martos et al., 2007). But, a few studies on the efficacy of essential oils and their constituents to control and maintain quality of postharvest pathogens of some fruit and vegetables such as strawberry, apple, cherry tomato, table grape were investigated (Reddy et al., 1998; Martinez-Romero et al., 2004; Valero et al., 2006; Guillén et al., 2007; Lee et al., 2007; Tripathi et al.,

2008). The objective of this study is to determine the effect of thymol, eugenol, methyl cinnamate, linalool and 1,8-cineol on the growth of *Sclerotium rolfsii*, *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Alternaria solani*, which are common phytopathogenic fungi in Egyptian.

2. Material and Methods:

1. Fungal Cultures:

The fungal isolates were kindly supplied by Plant Pathology Institute, Agricultural Research Center. They were as follows; *Sclerotium rolfsii*, *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Alternaria solani*.

2. Chemical Compounds:

The oil components, thymol, eugenol, methyl cinnamate, linalool and 1,8-cineol were purchased from Merck Company, Germany.

3. Antifungal activity of essential oils on mycelial growth in vitro conditions:

The antifungal compounds were prepared in dimethyl sulfoxide (DMSO) and tested for mycelial growth inhibition activity against 5 fungal isolates *Sclerotium rolfsii*, *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Alternaria solani* using the food poison technique. A potato dextrose agar medium was used as the basal medium for all test fungi. To test the antifungal activities of the selected compounds, sterile Petri dishes containing the compound dissolved in DMSO/Tween 80 emulsifier (80/20 v/v) diluted in PDA medium were prepared. Tween 80 alone as a control was added to PDA medium as an emulsifier control. Plates containing media mixed with DMSO (0.1% by volume) were included as a solvent control. Also, DMSO and Tween 80 (4/1 v/v) were added to PDA medium as a solvent/emulsifier control. Finally, PDA plates treated with distilled water without any extracts were served as a negative control.

Agar disks (5 mm in diameter) of the tested fungi were cut from completely grown cultures and placed at the center of the plates containing antifungal substances of the used concentration in ppm ($\mu\text{g/ml}$) (Joong – Hyeop *et al.*, 2005).

Four replicates of each concentration (50, 100, 150, 200 and 250 ppm for thymol, eugenol and methyl cinnamate compounds) (500, 1000, 2000, 4000 and 8000 ppm for linalool and 1,8-cineol compounds) of each fungus were incubated at 28 °C for all tested fungi. Radial growth was measured from the centers of the dishes sides by caliper and the mean was calculated of two perpendicular colony diameters in each replicate. Inhibition of growth was calculated in relation to the growth in the control, according to the equation (Sztejnberg *et al.*, 1983):

$$\% \text{ of inhibition} = 1 - \left(\frac{\text{Diameter of treated colony}}{\text{Diameter of control colony}} \right) \times 100$$

The corrected percentage of growth inhibition was used to calculate the EC_{50} values according to Finney (1971). The toxicity lines were drawn for evaluating EC_{10} , EC_{25} , EC_{50} and EC_{90} and slope for every treatment. The toxicity index and relative potency were calculated according to Sun (1950).

$$\text{Toxicity Index} = \frac{\text{LC}_{50} \text{ of the most effective compound}}{\text{LC}_{50} \text{ of the other compound}} \times 100 = \dots\%$$

$$\text{Relative Potency} = \frac{\text{LC}_{50} \text{ of the least effective compound}}{\text{LC}_{50} \text{ of the other compounds}} = \dots \text{Fold}$$

4. Determination of the minimum inhibitory concentrations (MICs):

Emulsions of compounds were prepared at 500 ppm in DMSO 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) was added to each well. Microtiter plates were then incubated for 2 days at 28°C. Then, the MICs were determined as the lowest concentrations preventing visible growth (Sirot, 1990).

3. Results:

I. Antifungal activities of tested compounds against:

(a) *Sclerotium rolfsii*:

The results of antifungal assays of some naturally occurring compounds under studying upon *S. rolfsii* are given in table (1). The percentage of growth inhibition at the concentration 50 $\mu\text{g/ml}$ of thymol was 10.47%; at the concentrations 100, 150, 200 and 250 $\mu\text{g/ml}$ the percentages of growth inhibition were 45.34, 70.85, 84.64 and 91.73%, respectively. The 50 $\mu\text{g/ml}$ of eugenol showed 12.43% growth inhibition, the 100, 150, 200 and 250 $\mu\text{g/ml}$ recorded 25.39, 35.39, 43.22 and 49.5% growth inhibition, respectively. The concentration of 50 $\mu\text{g/ml}$ of methyl cinnamate had 0.95% growth inhibition, the 100, 150, 200 and 250 $\mu\text{g/ml}$ had 10.81, 27.87, 45 and 59.17% growth inhibition percentage. The 500 $\mu\text{g/ml}$ concentration of linalool gave 12.71% growth inhibition; the 2000 $\mu\text{g/ml}$ gave 93.29% and the 8000 $\mu\text{g/ml}$ gave complete growth inhibition. After that, the 500 $\mu\text{g/ml}$ of 1,8-cineol showed only 1.24% growth inhibition, the 4000 $\mu\text{g/ml}$ showed 2.58% and the 8000 $\mu\text{g/ml}$ showed only 4.99% growth inhibition.

Generally, the five tested compounds showed variable fungicidal activity on the growth of *S. rolfsii*. From the attained results; it was found that, the LC_{25} - LC_{50} 's of thymol, methyl cinnamate, eugenol, linalool and 1,8-cineol were 71.22 - 107.39, 142.01 - 216.3, 98.31 - 254.47, 638.72 - 910.25 and 640981 - 4.35×10^9 $\mu\text{g/ml}$, respectively.

The toxicity indexes were 49.65, 42.2, 11.8 and 0.0008 % for methyl cinnamate, eugenol, linalool and

1,8-cineol, respectively when compared with the most effective compound (thymol) which had the 100% toxicity index. While the relative potencies of the tested compounds when compared with the least effective one (1,8-cineol) were 125076.8, 62098.94, 52784.22 and 14756.39 folds than 1,8-cineol for thymol, methyl cinnamate, eugenol and linalool, respectively.

The Ldp-lines of the five tested compounds against *S. rolfssii* were plotted in figure (1). The slope of each line was calculated separately. The highest slope value was for linalool (4.38). The lowest slope

value was (0.51) for 1,8-cineol. The slope values for thymol, eugenol and methyl cinnamate were 3.78, 1.63 and 3.69, respectively. It was found that all the slope values were higher than 1. It means that this applied compound is slightly toxic and had high antifungal activity.

It can be concluded that compounds can be arranged descendingly according to their effectiveness upon *S. rolfssii* as follows;

**thymol > methyl cinnamate > eugenol
> linalool > 1,8-cineol.**

Table (1): Effect of the selected compounds on *Sclerotium rolfssii*.

Compounds tested	Concentrations in ppm ($\mu\text{g/ml}$)					LC ₂₅	LC ₅₀	Slope	Toxicity Index	Relative Potency
	A	B	C	D	E					
Thymol*	10.47	45.34	70.85	84.64	91.73	71.22	107.39	3.78	100	125076.8
Eugenol*	12.43	25.39	35.39	43.22	49.50	98.31	254.47	1.63	42.2	52784.22
Methyl cinnamate*	0.95	10.81	27.87	45	59.17	142.01	216.30	3.69	49.65	62.098.94
Linalool**	12.71	57.1	93.29	99.75	100	638.72	910.25	4.38	11.8	14756.39
1,8-Cineol**	1.24	1.8	2.58	3.62	4.99	640981	$\frac{1.34}{\times 10^7}$	0.51	0.0008	1

A, B, C, D and E concentrations are:

* 50, 100, 150, 200 and 250 $\mu\text{g/ml}$.

** 500, 1000, 2000, 4000 and 8000 $\mu\text{g/ml}$.

(b) *Rhizoctonia solani*:

The antifungal activity of selected compounds upon *R. solani* is given in table (2). The percentage of inhibition of thymol at the concentration 50 $\mu\text{g/ml}$ is 35.95%, at 100 $\mu\text{g/ml}$ was 86.51% and at 150, 200 and 250 $\mu\text{g/ml}$ were 97.47, 99.49 and 99.88% growth inhibition, respectively. For eugenol compound, the 50 $\mu\text{g/ml}$ concentration had 11.84% growth inhibition, the 100 $\mu\text{g/ml}$ gave 53.29% and the concentrations 150, 200 and 250 $\mu\text{g/ml}$ had 79.48, 91.11 and 96.04% linear growth inhibition, respectively. When using methyl cinnamate compound, the lowest concentration, 50 $\mu\text{g/ml}$, gave 12.19% growth inhibition, approximately the middle concentration, 150 $\mu\text{g/ml}$, gave 72.97% while the highest concentration, 250 $\mu\text{g/ml}$, gave 92.47% growth inhibition. After that, for linalool compound, the 500 $\mu\text{g/ml}$ concentration gave percentage of growth inhibition 44.61%, the 2000 $\mu\text{g/ml}$ gave 99.97% and the concentrations 4000 and 8000 $\mu\text{g/ml}$ gave complete linear growth inhibition. Finally, for cineol compound, the concentration of 500 $\mu\text{g/ml}$ gave approximately no antifungal activity, it gave only 0.27% growth inhibition, the 4000 $\mu\text{g/ml}$ showed 5.65%, while the 8000 $\mu\text{g/ml}$ showed 34.64% growth inhibition.

In general, it can be noticed that the high concentrations of the tested compound had high

percentage of linear growth inhibition and vice versa. The LC₂₅'s for the tested compounds thymol, eugenol, methyl cinnamate, linalool and 1,8-cineol were 43.08, 66.06, 67.75, 416.78 and 5778.15 $\mu\text{g/ml}$, respectively. The LC₅₀'s for the tested compounds thymol, eugenol, methyl cinnamate, linalool and 1,8-cineol were 59.28, 95.58, 102.78, 523.47 and 12669.8 $\mu\text{g/ml}$, respectively. From the LC₅₀'s of the selected compounds we attained the toxicity indexes in reference to the highest effective compound and the relative potency in reference to the lowest effective compound.

The toxicity indexes of the tested effective compound thymol which had recorded the highest toxicity index 100%. The relative potencies of the selected compounds, thymol, eugenol, methyl cinnamate and linalool were 213.73, 132.56, 123.27 and 24.2 fold, respectively when compared with the lowest effective compound 1,8-cineol which recorded the highest LC₅₀ value.

The Ldp-lines of the five tested compounds against *R. solani* were presented in figure (2). The highest slope value was for linalool compound (6.81). Then the lowest slope was 1,8-cineol compound (1.98). The slope values for thymol, eugenol and methyl cinnamate compounds were 4.87, 4.21 and 3.73, respectively.

It can be concluded that compounds can be arranged descendingly according to their effectiveness upon *R. solani* as follows;

thymol > eugenol > methyl cinnamate > linalool > 1,8-cineol.

Table (2): Effect of the selected compounds on *Rhizoctonia solani*.

Compounds tested	Concentrations in ppm ($\mu\text{g/ml}$)					LC ₂₅	LC ₅₀	Slope	Toxicity Index	Relative Potency
	A	B	C	D	E					
Thymol*	35.95	86.53	97.47	99.49	99.88	43.08	59.28	4.87	100	213.73
Eugenol*	11.84	53.29	79.48	91.11	96.04	66.06	95.58	4.21	62.02	132.56
Methyl cinnamate*	12.19	48.23	72.97	85.93	92.47	67.75	102.78	3.73	57.68	123.27
Linalool**	44.61	97.2	99.97	100	100	416.78	523.47	6.81	11.33	24.2
1,8-Cineol**	0.27	1.53	5.65	16.1	34.64	5778.15	12669.8	1.98	0.468	1

A, B, C, D and E concentrations are:

* 50, 100, 150, 200 and 250 $\mu\text{g/ml}$.

** 500, 1000, 2000, 4000 and 8000 $\mu\text{g/ml}$.

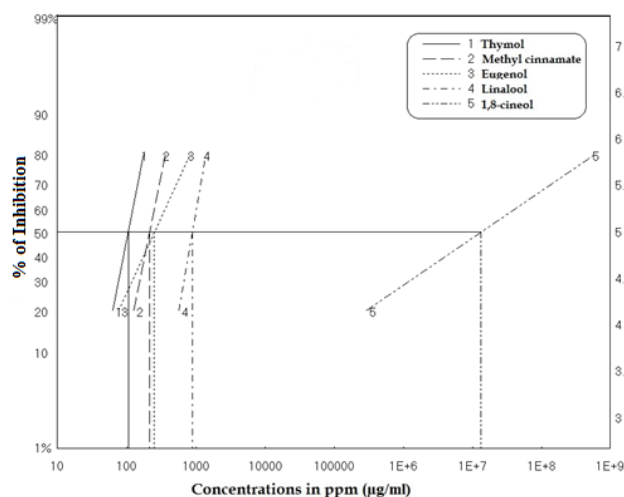


Fig.(1): Dose response curves of the selected compounds against *S. rolfsii*

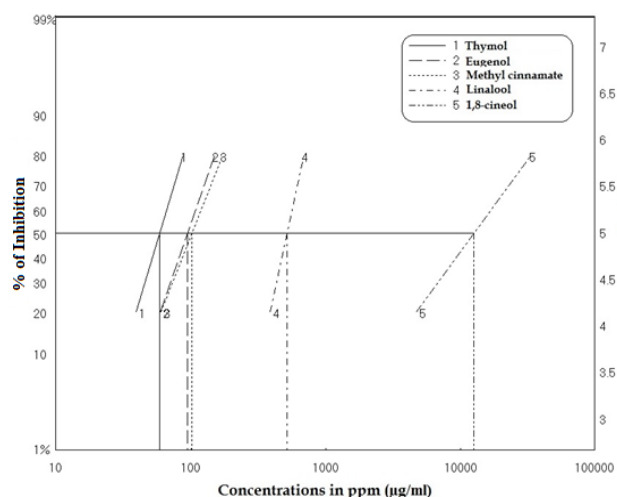


Fig.(2): Dose response curves of the selected compounds against *R. solani*.

(c) *Botrytis cinerea*:

The antifungal activity of the tested compounds upon *B. cinerea* after 6 days of incubation is given in table (3). From the obtained results, the inhibition percentage when using thymol compound at 50 $\mu\text{g/ml}$ was 53.34%, at 250 $\mu\text{g/ml}$ was 97.69% and concentrations 100, 150 and 200 $\mu\text{g/ml}$ gave 81.84, 91.81 and 95.85% growth inhibition, respectively. In case of using eugenol, 50 $\mu\text{g/ml}$ concentration gave 10.46% growth inhibition; 250 $\mu\text{g/ml}$ gave 47.66% while the concentration 100, 150 and 200 $\mu\text{g/ml}$ gave 22.95, 33.04 and 41.1% growth inhibition, respectively. For methyl cinnamate, the concentrations 50, 100 and 150 $\mu\text{g/ml}$ gave no growth inhibition while the 200 and 250 $\mu\text{g/ml}$ concentrations had 4.53 and 27.04% growth inhibition. For the lowest effective compound,

linalool, the 500 $\mu\text{g/ml}$ concentration gave 11.66%; the 8000 $\mu\text{g/ml}$ gave complete growth inhibition whereas 4000 $\mu\text{g/ml}$ concentration gave 85.1% growth inhibition. The 1,8-cineol compound showed no antifungal activity at all the tested concentrations.

From the LC₅₀ values of the tested natural occurring compounds given in the table we concluded that the most effective compound was thymol followed by eugenol, methyl cinnamate and finally linalool where the 1,8-cineol compound showed no antifungal activity on the tested fungus. The LC₅₀'s were 46.06, 270.47, 283.55 and 1048.2 $\mu\text{g/ml}$ for thymol, eugenol, methyl cinnamate and linalool, respectively.

From the LC₅₀'s of the selected compounds the toxicity indexes of the tested compounds, eugenol, methyl cinnamate and linalool were 17.23, 16.43 and

4.45%, respectively when comparing with the highest effective compound thymol which had recorded the highest toxicity index 100%. The relative potencies of the selected compounds, thymol, eugenol and methyl cinnamate were 22.49, 3.88 and 3.7 folds, respectively when compared with the lowest effective compound linalool which recorded the highest LC₅₀ value since 1,8-cineol showed no antifungal activity against *B. cinerea* at this time period.

The Ldp-lines of the selected compounds were plotted on a logarithmic paper (log concentration) in relation to percentage of fungal

growth inhibition as given in figure (3). The highest slope was for methyl cinnamate (11.18) and then after, when using linalool (3.71). The lowest slope was for eugenol compound (1.71), after that, the slope of thymol (2.74).

It can be concluded that compounds can be arranged descendingly according to their effectiveness upon *B. cinerea* after 6 days of incubation as follows;

**thymol > eugenol > methyl cinnamate
> linalool.**

Table (3): Effect of the selected compounds on *Botrytis cinerea*.

Compounds tested	Concentrations in ppm (µg/ml)					LC ₂₅	LC ₅₀	Slope	Toxicity Index	Relative Potency
	A	B	C	D	E					
Thymol*	53.34	81.84	91.81	95.85	97.69	26.45	46.60	2.74	100	22.49
Eugenol*	10.46	22.95	33.04	41.1	47.66	109.29	270.47	1.71	17.23	3.88
Methyl cinnamate*	0	0	0	4.53	27.04	246.77	283.55	11.18	16.43	3.7
Linalool**	11.66	46.98	85.1	98.38	100	689.6	1048.2	3.71	4.45	1
1,8-Cineol**	0	0	0	0	0	-	-	-	-	-

A, B, C, D and E concentrations are:

* 50, 100, 150, 200 and 250 µg/ml.

** 500, 1000, 2000, 4000 and 8000 µg/ml.

(d) *Fusarium oxysporum*:

The antifungal activity of the tested compounds upon *F. oxysporum* after 6 days of incubation is given in table (4). From the obtained results, the inhibition percentage when using thymol compound after 6 days of incubation at 50 µg/ml was 16.93%, at 250 µg/ml was 98.41% and concentrations 100, 150 and 200 µg/ml gave 65.08, 87.98 and 95.82% growth inhibition, respectively. In case of using eugenol, 50 µg/ml concentration gave 3.91% growth inhibition; 250 µg/ml gave 57.29% while the concentration 100, 150 and 200 µg/ml gave 17.78, 33.23 and 46.57% growth inhibition, respectively. For methyl cinnamate, the lowest concentration (50 µg/ml) gave 0.06% growth inhibition, approximately middle concentration (150 µg/ml) had 11.14% linear growth inhibition while the highest concentration (250 µg/ml) had 39.05% growth inhibition. For the lowest effective compound, linalool 500 µg/ml concentration gave 1.36%; the 8000 µg/ml gave complete growth inhibition whereas 4000 µg/ml concentration gave 80.64% growth inhibition. The 1,8-cineol compound showed no antifungal activity for concentrations 500, 1000, 2000 and 4000 µg/ml and gave only 10.67% growth inhibition at 8000 µg/ml concentration.

From the LC₅₀ values of the tested natural occurring compounds given in the table we concluded that the most effective compound was thymol followed by eugenol, methyl cinnamate and finally linalool where the 1,8-cineol compound showed no antifungal activity on the tested fungus. The LC₅₀'s were 81.89, 214.74, 290.66 and 1357.4 µg/ml for thymol, eugenol, methyl cinnamate and linalool, respectively.

According to the LC₅₀ values, the toxicity indexes of the tested compounds, eugenol, methyl cinnamate and linalool were 38.14, 28.16 and 6.03%, respectively when comparing with the highest effective compound thymol which had recorded the highest toxicity index 100%. The relative potencies of the selected compounds, thymol, eugenol and methyl cinnamate were 16.58, 6.32 and 4.67 folds, respectively when compared with the lowest effective linalool compound.

The Ldp-lines of the selected compounds were plotted on a logarithmic paper (log concentration) in relation to percentage of fungal growth inhibition as given in figure (4). The highest slope was for linalool compound (5.14) and then after, when using thymol (4.47). The lowest slope was for eugenol

compound (2.78), after that, the slope of methyl cinnamate (4.24).

It can be concluded that compounds can be arranged descendingly according to their

effectiveness upon *F. oxysporum* as follows; thymol > eugenol > methyl cinnamate > linalool.

Table (4): Effect of the selected compounds on *Fusarium oxysporum* after 6 days of incubation.

Compounds tested	Concentrations in ppm ($\mu\text{g/ml}$)					LC ₂₅	LC ₅₀	Slope	Toxicity Index	Relative Potency
	A	B	C	D	E					
Thymol*	16.93	65.08	87.98	95.82	98.41	57.84	81.89	4.47	100	16.58
Eugenol*	3.91	17.78	33.23	46.57	57.29	122.90	214.74	2.78	38.14	6.32
Methyl cinnamate*	0.06	2.5	11.14	24.54	39.05	201.58	290.66	4.24	28.16	4.67
Linalool**	1.36	24.76	80.64	99.21	100	1003.42	1357.4	5.14	6.03	1
1,8-Cineol**	0.0	0.0	0.0	0.0	10.67	-	-	-	-	-

A, B, C, D and E concentrations are:

* 50, 100, 150, 200 and 250 $\mu\text{g/ml}$.

** 500, 1000, 2000, 4000 and 8000 $\mu\text{g/ml}$.

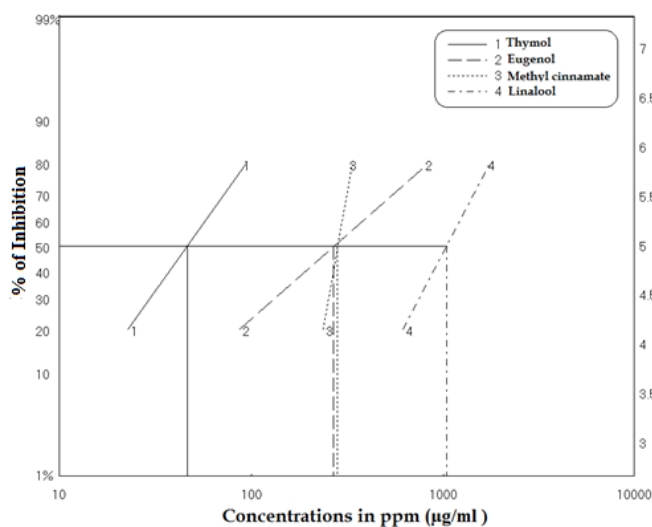


Fig.(3): Dose response curves of the selected compounds against *B. cinerea*

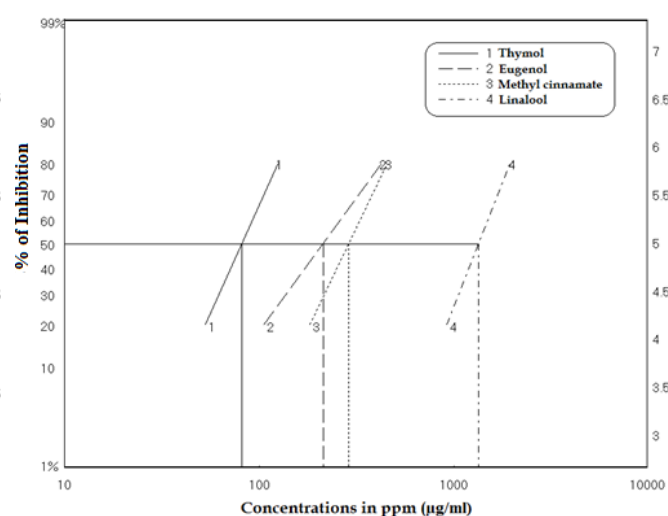


Fig.(4): Dose response curves of the selected compounds against *F. oxysporum*

(e) *Alternaria solani*:

The antifungal activity of tested compounds upon *A. solani* after 10 days of incubations is given in table (5). From the obtained results, the inhibition percentage at 50 $\mu\text{g/ml}$ of thymol was 3.37%, at 150 $\mu\text{g/ml}$ was 47.32% and at 250 $\mu\text{g/ml}$ concentration gave 77.43% growth inhibition. The 50 $\mu\text{g/ml}$ concentration of eugenol gave 0.29% growth inhibition; 150 $\mu\text{g/ml}$ gave 9.28% while the concentration 250 $\mu\text{g/ml}$ gave 25.52% growth inhibition. The lowest concentration (50 $\mu\text{g/ml}$) of methyl cinnamate didn't give any growth inhibition, approximately middle concentration (150 $\mu\text{g/ml}$) had 6.94% linear growth inhibition percentage while the highest concentration (250 $\mu\text{g/ml}$) had 94.04% growth

inhibition. The 500 $\mu\text{g/ml}$ concentration of linalool gave 5.57%; the 8000 $\mu\text{g/ml}$ gave 35.61% whereas 4000 $\mu\text{g/ml}$ concentration gave 16.32% growth inhibition. Finally, for the fourth time in a row the low concentrations of 1,8-cineol results in higher percentage of linear growth than the high ones, the 500 $\mu\text{g/ml}$ concentrations of 1,8-cineol showed only 6.46% growth inhibition, the 2000 $\mu\text{g/ml}$ showed 4.39% and the 8000 $\mu\text{g/ml}$ showed 2.88% growth inhibition.

From the LC₅₀'s of the selected compounds the toxicity indexes of the tested compounds, eugenol, methyl cinnamate and linalool were 37.77, 81.31 and 0.85%, respectively when comparing with the highest effective compound thymol which had recorded the

highest toxicity index 100%. The relative potencies of the selected compounds, thymol, eugenol and methyl cinnamate were 117.88, 44.52 and 95.85 folds, respectively when compared with the lowest effective compound linalool which recorded the highest LC₅₀.

The Ldp-lines of the selected compounds were plotted on a logarithmic paper (log concentration) in relation to percentage of fungal growth inhibition as given in figure (5). The highest slope was for methyl

cinnamate (13.7) and then after, when using thymol (3.7). The lowest slope was for linalool compound (1.02), after that, the slope of eugenol (3).

It can be concluded that compounds can be arranged descendingly according to their effectiveness upon *A. solani* after 10 days of incubation as follows;

thymol > methyl cinnamate > eugenol > linalool.

Table (5): Effect of the selected compounds on *Alternaria solani* after 10 days of incubation.

Compounds tested	Concentrations in ppm (µg/ml)					LC ₂₅	LC ₅₀	Slope	Toxicity Index	Relative Potency
	A	B	C	D	E					
Thymol*	3.37	23.62	47.32	65.35	77.43	102.78	156.41	3.70	100	117.88
Eugenol*	0.29	3.21	9.28	17.13	25.52	246.88	414.13	3	37.77	44.52
Methyl cinnamate*	0	0	6.94	59.13	94.04	171.76	192.37	13.7	81.31	95.85
Linalool**	5.57	9.9	16.32	24.98	35.61	4006.3	18438.1	1.02	0.85	1
1,8-Cineol**	6.46	5.34	4.39	3.58	2.88	-	-	-0.32	-	-

A, B, C, D and E concentrations are:

* 50, 100, 150, 200 and 250 µg/ml.

** 500, 1000, 2000, 4000 and 8000 µg/ml.

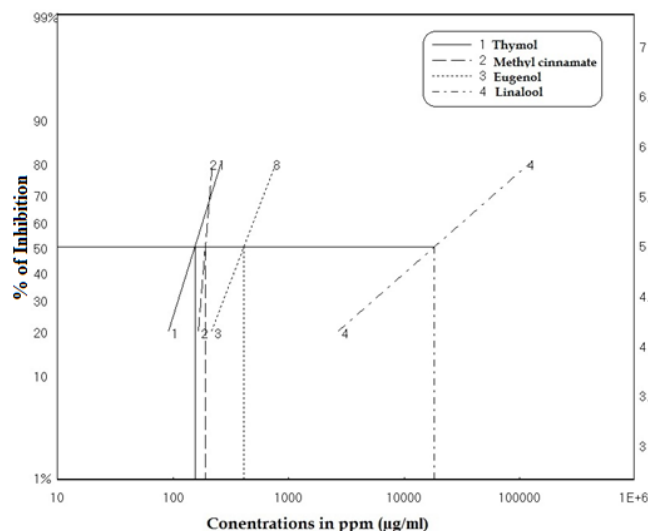


Fig.(5): Ldp lines of the selected compounds against *A. solani*

II. Determination of the Minimum Inhibitory Concentration (MIC's):

From the results represented in table (6); the lowest MIC's were that of thymol against the five tested fungi namely, *S. rolfisii*, *R. solani*, *F. oxysporum*, *B. cinerea* and *A. solani*. The MIC's of thymol were 100µg/ml against four of the five fungi tested except for *A. solani* where the MIC was 150 µg/ml. The MIC's of eugenol were 150 µg/ml for *S.*

rolfisii and *R. solani*; 200 µg/ml for *F. oxysporum* and 250 µg/ml for both *B. cinerea* and *A. solani*.

The MIC of methyl cinnamate was 150 µg/ml for *R. solani*; 200 µg/ml for *S. rolfisii* and *A. solani*; whereas they were 250 µg/ml for both *F. oxysporum* and *B. cinerea*.

While the MIC's of linalool was 2000 µg/ml for *F. oxysporum*; 1000 µg/ml for *S. rolfisii*, *R. solani* and

A. solani; also the MIC of linalool against *B. cinerea* was 500 µg/ml.

In contrast, the 1,8-cineol compound had not any inhibitory effect even at 8000 µg/ml against the five fungal strains tested.

Table (6): Minimum inhibitory concentrations (MICs) of thymol, eugenol, methyl-cinnamate, linalool and 1,8-cineol compounds against tested fungi.

Fungi \ Comp.	Thymol	Eugenol	Methyl cinnamate	Linalool	1,8-cineol
<i>S. rolfssii</i>	≤ 100	≤ 150	≤ 200	≤ 1000	> 8000
<i>R. solani</i>	≤ 100	≤ 150	≤ 150	≤ 1000	> 8000
<i>B. cinerea</i>	≤ 100	> 250	≤ 250	≤ 500	> 8000
<i>F. oxysporum</i>	≤ 100	≤ 200	≤ 250	≤ 2000	> 8000
<i>A. solani</i>	≤ 150	≤ 250	≤ 200	≤ 1000	> 8000

4. Discussion:

The presence and growth of microorganisms in food may cause spoilage and result a reduction in quality and quantity (Soliman and Badeaa, 2002). One of the two mechanisms determining how food-borne diseases are primarily caused, is by infection as a consequence of consuming foods contaminated with the growth of pathogenic microorganisms, such as bacteria, mould, viruses and parasites (Vattem *et al.*, 2004). In addition to passive transfer of food pathogens, active growth may also occur in foods, for instance because of improper storage, which leads to marked increases in poses important public health and economic concerns for the human society (Celiktas *et al.*, 2007).

Recently, several investigations have been conducted into the antifungal actions of essential oils against phytopathogenic fungi (Shimoni *et al.*, 1993; Zygadlo *et al.*, 1994; Prudent *et al.*, 1995; Zygadlo *et al.*, 1995; Carta *et al.*, 1996 and Bishop and Thornton 1997).

Accordingly, an investigation was carried out to elucidate the effect of five naturally occurring compounds namely eugenol, thymol, methyl cinnamate, linalool and 1,8-cineol on some phytopathogenic fungi. From our obtained results we can conclude that thymol was the most effective compound against all the fungi under studying. This was in harmony with that obtained by Tsao and Zhou, 2000 who found that thymol was the most potent inhibitor of *B. cinerea* and *M. fructicola*. It prevented completely the spore germination and mycelial growth of *B. cinerea* and *M. fructicola* at 100 µg/ml. Even at 10 µg/ml medium (0.25 mg/petri dish), it exhibited 82% inhibition at 48 h against *B. cinerea*, and 57% inhibition against *M. fructicola*.

Biological activity of natural compounds in relation to their chemical structure:

In the antimicrobial action of essential oil components, the lipophilic character of their

hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance. The activity rank of essential oil components is as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons. The highest activity was reported for phenols – thymol, carvacrol and eugenol, which is explained by the acidic nature of the hydroxyl group, forming a hydrogen bond with an enzyme active center (Kalembe and Kunicka 2003). Therefore, essential oils with phenols as main compounds express the highest activity against microorganisms, and their activity spectrum is the broadest.

The antifungal activity of 47 kinds of essential oils and several related compounds were examined against seven fungi. The results suggest that secondary alcohols (e.g., 2-octanol, L-menthol, borneol) and tertiary alcohols (e.g., linalool) possess a markedly lower antifungal activity as compared to primary alcohols such as cinnamyl alcohol, geraniol, and citronellol. The antifungal activity of eugenol (4-allyl-guaiacol), a phenolic compound, was found to be 8-10 times higher than that of guaiacol (o-methoxyphenol) and 3-4 times higher than that of creosol (4-methylguaiacol). From the molecular structure, it is clear that the addition of alkyl or alkenyl groups to the benzene ring of either phenol or guaiacol enhanced the antimicrobial activity. The activity of these phenolic compounds appeared to depend on the size of the added alkyl or alkenyl group, where the larger the size of the alkyl or alkenyl group, the stronger the antimicrobial activity (Kurita *et al.*, 1981, Knobloch *et al.*, 1989 and Pelczar *et al.*, 1993). Because alkyl or alkenyl groups are hydrophobic, these results indicate that hydrophobicity above a minimum extent was required for phenolic compounds to show a potent antimicrobial effect.

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.

Conclusion

According to the hypothesis relationship between chemical structure of natural compound and antifungal activity; thymol and eugenol (phenolic compound) were the most predominant antifungal activity towards the tested fungi followed by methyl cinnamate (as a part of keto compound) where the lowest antifungal activity were linalool and 1,8-cineol (tertiary alcohol and ether).

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