

**COMPARATIVE STUDIES ON THE TOXICITY AND
BIOCHEMICAL EFFICACY OF NATURAL PLANT
OILS AGAINST *Aphis craccivora* KOCH (HEMIPTERA)**

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ABSTRACT

Environmental friendly organic natural botanical extracts (essential oils) of Lupine, *Lupinus termis* Forsk (Fabacea), Marjoram, *Majorana hortensis* L. (Lamiaceae), Anise, *Pimpinella anisum* (Umbelliferae), Orange oil. *Citrus vulgaris* (Rutaceae) and Olive oil, *Olea europaea* L (Oleaceae) were tested to evaluate their toxic effect on both laboratory and field strains of *Aphis craccivora*, Koch. The results showed that the field strain was more resistant to all compounds than that of the laboratory strain. On the other hand, the biochemical activity of the detoxification enzymes; MFO, alkaline Phosphatase, β and α -esterase was also investigated and showed fluctuated results according to the source of strain and compound used.

INTRODUCTION

Essential oils of botanical origin and their major components, often their various monoterpenoids have attracted attention in recent years as potential pest control agents. Due to their insecticidal, repellent and/or antifeedant properties (Amos *et al.*, 1974; Grundy and Still, 1985; Shaaya *et al.*, 1997; Lee *et al.*, 2003; Ketoh *et al.*, 2005, Rozman *et al.*, 2007; Cosimi, *et al.*, 2009 and Shehawy, 2010). Also, some natural plant compounds used in the control of insect pests are known to influence the enzymatic profiles (Nathan, *et al.*, 2005). Cytochrome P450 monooxygenases (CYPs) and esterases (ESTs) are two major detoxifying enzymes in most organisms. At least one of them is involved in detoxification of insecticides in insects (Bull, 1981). In insects, the diverse functions of P450 enzymes range from synthesis and degradation of ecdysteroids and juvenile hormones to the xenobiotics metabolism (Feyereisen, 2005). Alkaline phosphatase (ALP) is a brush border membrane marker enzyme and is especially active in tissues with active membrane transport, such as intestinal epithelial cells, Malpighian tubules (Etebari and Matindoost, 2004b) and hemolymph (Etebari *et al.*, 2007).

The present investigation aims to investigate the five essential oils with possible insecticidal activity for their toxic efficacy against the laboratory and field strain of cowpea aphid, *Aphis craccivora* Koch and to study the relationship between the efficacy of the tested compounds and some biochemical aspects; *i.e.* Mixed Function Oxidase, Alkaline Phosphatase and non specific esterases activities on aphid species under this study.

MATERIALS AND METHODS

All experiments were carried out at department of Sucking and Piercing insects, plant protection research institute, Agricultural research center (ARC), Giza, Egypt.

Insect bioassay methods

In order to evaluate the toxicity of plant oils as natural products against laboratory strain of *A. craccivora* and field strain that collected from Faba bean plant in Monufia, Governorate. Leaf-dip technique was applied under laboratory conditions as described below.

a. leaf dip technique:

The method described by Harlow and Lampert, (1990), was adopted by the 7 different concentration; 375, 750, 1500, 3000, 4500, 6000 and 7500 ppm, to evaluate the efficiency of the different plant oils used against *A. craccivora* and used to draw the dosage mortality regression line (Ldp line). Ten replicates with 10 apterous adults for each concentration, plant leaf was dipped in water dilution of toxicant for 10 second, the plant leaf was gently agitated for 10 seconds in the toxicant solution then dried in the dry air. While in the control, the leaves dipped in water according to the same technique. The treated plant leaf was put in Petri dish under laboratory conditions. Mortality counts were taken after 24 hours of treatment. Aphids responding to touch with brush were considered alive.

b. Data analysis:

Mortality data were corrected according to Abbott's formula (1925), plotted on log dosage paper and regression line were fitted according to Finney (1971), Sun (1950), described the toxicity index as a mean for comparing the relative susceptibility of the tested insecticides. He proposed the following equation in calculating the toxicity index values:

$$\text{Sun's toxicity index} = \frac{\text{LC}_{50} \text{ of standard material}}{\text{LC}_{50} \text{ of tested material}} \times 100$$

Detoxification enzyme assays:

Effect of essential oils on the activities of four detoxification enzymes [mixed function oxidase (MFO), alpha esterases (α -esterases), beta esterases (β -esterases), and alkaline phosphatase (ALP)] were measured.

A. craccivora were treated topically with LC_{50} of test essential oils for 24hrs. Then, preserved in refrigerator until analysis, after that, the specimen homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 8000 r. p. m. for 15 minutes at 5°C , and the supernatants were used directly to determine the activity mixed function oxidase MFO, alkaline phosphates, beta esterase and alpha esterase. P-nitroanisole o-demthylation was assayed to determine MFO activity according to the method of Hansen and Hodgson (1971) with slight modification. α -esterases and β -esterases were determined according to Van Asperen (1962) using α -naphthyl acetate or β -naphthyl acetate as substrates. ALP was determined according to the method described by Powel and Smith (1954) using disodium phenyl phosphate as substrate.

RESULTS AND DISCUSSION

1. Toxicological assay by leaf dip-technique:

Mortality percentages of laboratory *A. craccivora* strain after 24 hours of exposure to essential oils increased with the increasing oil concentration. *L. termis* extract was able to induce 92% mortality within 24 hours of exposure (Figure 1) followed by *O. europaea* (78%), *P. anisum* (62 %), *M. hortansis* oil (61.0%), *C. vulgaris* induced the lowest mortality percent 48%) at 7500 ppm. On the other hand, mortality percentages of field *A. craccivora* strain after 24 hours of exposure to essential oils also increased with the increasing oil concentration. *L. termis* oil was able to induce 80% mortality within 24 hours of exposure (Figure 2) followed by *O. europaea* extract (76%), *P. anisum* (52 %), *M. hortansis* (50.0%), *C. vulgaris* induced the lowest mortality percent 42%) at 7500 ppm in laboratory strain. The results are in agreement with those obtained by Khalequzzaman and Nahar (2008), they decided that Azadirachtin as a natural plant origin insecticide proved to be the most toxic having LC₅₀ as 0.41 µg cm⁻² for *A. craccivora*, 0.34 µg cm⁻² for *A. gossypii* and 0.44 µg cm⁻² for both *M. persicae* and *L. erysimi*.

Effect of botanical insecticides on laboratory and field strains of *A. craccivora* on the bases of LC₅₀, toxicity index and potency level values.

The data obtained in Table (1) showed that the LC₅₀ values of different concentrations of plant extracts; *L. termis*, *O. europaea*, *P. anisum*, *M. hortansis* and *C. vulgaris* were 2180.911, 3263.641, 5893.508, 6776.757 and 11530.0874 respectively, in laboratory strain Table (1). Whereas, it was 4247.082, 4767.84, 7787.493, 8299.527 and 11648.06, respectively in the field strain Table (2).

On the bases of toxicity index at LC₅₀ level results indicated that *L. termis* extract was distinctly potent 100%, while ethanolic *C. vulgaris* was the least effective one and recorded 18.915% as toxic as *L. termis* in case of testing these phytochemical oils against laboratory strain of *A. craccivora* (Table 1). Also, the toxicity index LC₅₀ level indicated that *L. termis* extract was distinctly potent 100%, while ethanolic *C. vulgaris* was the least effective one and record 36.46% as toxic as *L. termis* in case of testing these phytochemical oils against field strain of *A. craccivora* (Table 2).

Regarding, the relative potency levels at LC₅₀ values expressed as number of folds as shown in Table (1), it was obvious that *L. termis*, *O. europaea*, *P. anisum*, *C. vulgaris* and *M. hortansis* at LC₅₀ level were 5.29, 3.53, 1.95 and 1.70 times more effective than *C. vulgaris*, respectively in the laboratory strain (Table . 1), while in the field strain it was 2.74, 2.44, 1.5 and 1.09 times more effective than *C. vulgaris*, respectively (Table . 2). The present results are in agreement with those of Shehawy (2007) who decided that, the most toxic extracts according to LC₅₀ value against *Aphis craccivora* were ethanolic extracts of *L. termis* (LC₅₀ 2071.61 ppm), *Z. officinale* (LC₅₀ 2828.868 ppm), *P. nigrum* (LC₅₀ 3550.541 ppm), *T. foenum graecum* (LC₅₀ 3876.341 ppm) and *A. maritima* (LC₅₀ 4968.637 ppm).

Comparison on basis of the slope of toxicity lines

In the laboratory strain of *A. craccivora* it was found that, *L. termis* had the steepest toxicity line, followed by *O. europaea*, *P. anisum*, followed by *C. vulgaris*, while *M. hortensis* had the flattest lines. And it was clear similarity between *Lupinus termis* oil and *Olea europaea* regard their mode of action and rate of effectiveness in spite of remarkable differences in the potency of these compounds. On the other hand, *P. anisum* and *C. vulgaris* represents another type of mode of action and rate of effectiveness. Also, *M. hortensis* represents another type of mode of action and rate of effectiveness against laboratory strain of *A. craccivora* (Fig. 1). While, in the field strain of *A. craccivora* it was found that clear similarity among *L. termis*, *O. europaea* and *M. hortensis* regarding their mode of action and rate of effectiveness in spite of remarkable differences in the potency of these compounds against *A. craccivora*. On the other hand, *C. vulgaris* and *P. anisum* represents another type of mode of action and rate of effectiveness against field strain of *A. craccivora* (Fig. 2). The obtained results are in harmony with those obtained by Abbassy *et al.* (2009) who studied that the essential oil extracted from leaves of *M. hortensis* Moench (Lamiaceae) against *Aphis fabae* L. (Hemiptera: Aphididae).

Table (1): LC₅₀ values of *Lupinus termis*, *Olea europaea*, *Pimpinella anisum*, *Majorana hortensis* and *Citrus vulgaris* against Laboratory strain of cowpea aphid, *Aphis craccivora* Koch.

Compound	LC ₅₀	Upper limit	Lower limit	Slope	Potency Level	Toxicity Index
<i>Lupinus termis</i>	2180.911	2900.47	1608.518	1.737	5.29	100
<i>Olea europaea</i>	3263.641	3772.835	2844.589	1.734	3.53	66.824
<i>Pimpinella anisum</i>	5893.508	7360.75	4933.401	1.549	1.95	37.005
<i>Majorana hortensis</i>	6776.757	9142.139	5412.603	1.281	1.70	32.182
<i>Citrus vulgaris</i>	11530.09	17005.45	8885.269	1.614	1.0	18.915

Table (2): LC₅₀ values of *Lupinus termis*, *Olea europaea*, *Pimpinella anisum*, *Majorana hortensis* and *Citrus vulgaris* against field strain of cowpea aphid, *Aphis craccivora* Koch.

Compound	LC ₅₀	Upper limit	Lower limit	Slope	Potency Level	Toxicity Index
<i>Lupinus termis</i>	4247.082	5826.78	3301.987	1.938	2.74	100
<i>Olea europaea</i>	4767.84	6686.016	3718.196	1.94	2.44	89.08
<i>Pimpinella anisum</i>	7787.493	9990.949	6461.881	1.755	1.5	54.54
<i>Majorana hortensis</i>	8299.527	10716.2	6880.346	1.847	1.09	51.17
<i>Citrus vulgaris</i>	11648.06	17237.96	9146.757	2.021	1.0	36.46

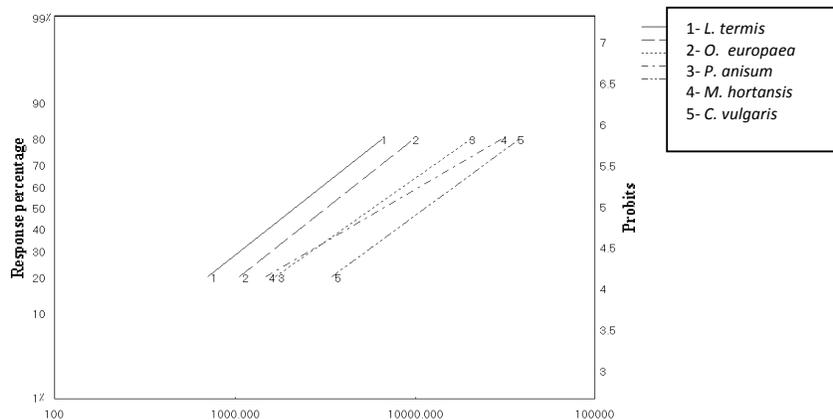


Fig. (1): semi-log of LC_{50} curve slope for *Lupinus termis*, *Olea europaea*, *Pimpinella anisum*, *Majorana hortansis* and *Citrus vulgaris* against Laboratory strain of cowpea aphid, *Aphis craccivora* Koch.

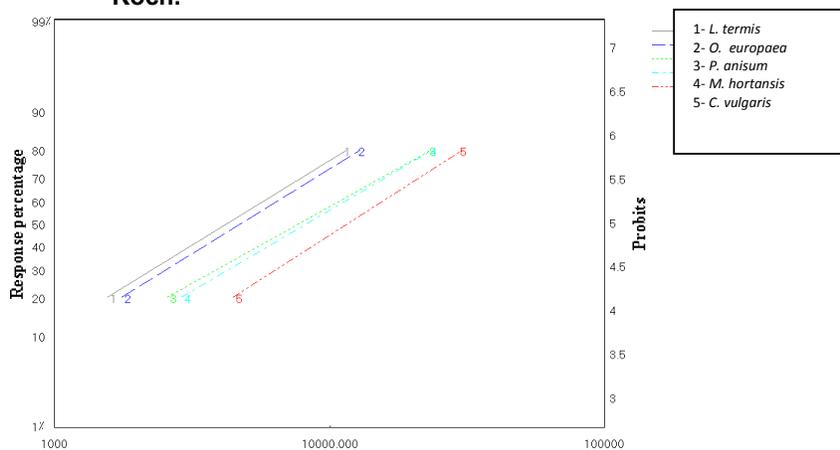


Fig. (2): semi-log of LC_{50} curve slope for *Lupinus termis*, *Olea europaea*, *Pimpinella anisum*, *Majorana hortansis* and *Citrus vulgaris* against field strain of cowpea aphid, *Aphis craccivora* Koch.

2. Enzymatic activity in the laboratory and field strains of *Aphis craccivora*

The activities of the tested hydrolyzing enzymes i.e., Mixed function oxidase, alpha and beta-esterases and alkaline phosphatase were estimated in both field colonies of *A. craccivora* and Laboratory strain.

The obtained results of detoxification enzymes showed that, the different insecticidal effects of essential oils corresponding to different botanical families and mode of action on laboratory and field strain of *A. craccivora*

The activity of mixed function oxidase.

The biochemical assays of mixed function oxidase (MFO) activity in *A. craccivora* are shown in Table (3). In case of laboratory strain which treated with LC₅₀ of *C. vulgaris*, *M. hortansis*, *P. anisum*, *L. termis* and *O. europaea*, the biochemical activity of MFO were 276.66, 267.33, 253.33, 477.0 and 510.0 n mole substrate hydrolyzed min/mg protein, respectively Table (3). The results showed that the activities of MFO in laboratory strain were elevated by all botanical insecticides except *P. anisum* reduced the activity of MFO.

On the other hand, the biochemical activity of MFO of field strain collected from Monufia and treated with LC₅₀ of botanicals mentioned before were 626.66, 916.33 704.33, 819.66, 795.66 and 782.66 n mole substrate hydrolyzed min/mg protein, respectively, (Table . 4). The results showed that the activities of MFO in field strain were elevated by all botanical insecticides.

One group linked herbivore feeding on plant material protected by chemical defenses with P-450 detoxification in larval tobacco hornworms. The induction in P-450 after initial nicotine ingestion allowed the larval tobacco hornworms to increase feeding on the toxic plant tissues (Snyder and Glendinning, 1996). Herbivores generate enzymes that counter and reduce the effectiveness of numerous toxic secondary metabolic products produced by plants. One such enzyme group, mixed function oxidases (MFOs), detoxify harmful plant compounds by catalyzing oxidative reactions (Feyereisen, 1999)

Enzymatic activity of alpha and beta-esterases:

The biochemical assay of alpha-esterase activity on *A. craccivora* are shown in Table (3&4), In case of laboratory strain which treated with LC₅₀ of *C. vulgaris*, *M. hortansis*, *P. anisum*, *L. termis* and *O. europaea*, the biochemical activity of alpha-esterase were 24.0, 11.53, 12.07, 13.17 and 19.0 mg α naphthyl acetate released/min/mg protein, respectively. While, the biochemical activity of Alpha-esterase were 20.1, 22.00, 19.33, 27.66, 22.66 and 22.53 mg α naphthyl acetate released/min/mg protein, respectively, in case of field strain of *A. craccivora*. Generally, the reduction in the activities of α -esterase was showed in *C. vulgaris*, *M. hortansis*, *P. anisum* and *O. europaea* whereas it increased by *L. termis* in laboratory strain of *A. craccivora*, while it was reduced in all treatments in field strain.

On the other hand, The biochemical assay of beta-esterase activity on *A. craccivora* are shown in Table (3&4), In case of laboratory strain which treated with LC₅₀ of *C. vulgaris*, *M. hortansis*, *P. anisum*, *L. termis* and *O. europaea*, the biochemical activity of β -esterases were 53.3, 11.73, 22.0, 18.0 and 17.83 mg β naphthyl acetate released/min/mg protein, respectively, While, in case of field strain of *A. craccivora*; the biochemical activity of Beta-esterase were 62.33, 82.33, 55.0, 73.33, 47.66 and 38.17 mg β naphthyl acetate released/min/mg protein, respectively. Generally it was found that the

activities of β -esterase laboratory strain was elevated by all botanical insecticides whereas in field strain it was reduced by all botanical oils except *C. vulgaris* elevated β -esterases. Terriere, (1984) stated that such increase in enzyme activities has been shown to protect insects from insecticide poisoning as part of defense mechanism. Saleem *et al.*, (1998) reported that the increased activities of esterase enzymes of *T. castaneum* adults after Cypermethrin treatment may be due to decreased body weight defend against insecticide stress conditions and or increase the energy production. Esterase's takes part in different biological processes such as regulation of hormone, digestion, reproduction, insecticide resistance etc. (Lima-catelani *et al.* 2004). When organisms were treated with the insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase may in turn result sudden death of the organisms, probably due to low production of esterase or lack of gene that produce these isozymes (Tsakas and Krimbas 1970)

Variation in the enzyme activity may be used as an alternative biomarker of environmentally induced stress, but, from the present study, it was difficult to represent any straight forward conclusion regarding the susceptibility levels of insecticides against esterases and need further investigation as other pesticide inhibiting enzymes as like glutathion tranferase, monoxigenase may contribute to become resistant (Abdur Rashid 2012).

Alkaline Phosphatase activity:

The biochemical assay of alkaline phosphatase activity in laboratory strain of *A. craccivora* treated with LC_{50} of *C. vulgaris*, *M. hortansis*, *P. anisum*, *L. termis* and *O. europaea*, were 2.43, 2.03, 2.35, 2.73 and 3.23 $\mu \times 10^3$ /mg protein respectively, while, the Alkaline Phosphatase activity of field strain were 1.55, 1.77, 1.67, 1.67, 1.68 and 1.51 $\mu \times 10^3$ /mg protein respectively.

Generally, increase in the activities of ALP was showed by *C. vulgaris*, *M. hortansis*, *P. anisum*, *O. europaea* and *L. termis* in laboratory strain of *A. craccivora*, while in field strain it was reduced *C. vulgaris*, *M. hortansis*, *P. anisum* treatments in contrast it increased in *O. europaea* and *L. termis* treatment. Miao, (2002) and Zera and Zhao, (2004) stated that different stress, disease and toxic chemicals causes considerable decrease in the activity of ALP. The newly emerged adults had significantly stimulated ALP activity by all extracts (Ghoneim *et al.* 2014).

Table (3):Biochemical assays of detoxification enzymes activities in laboratory strain of *Aphis craccivora* exposed to different Phytochemicals by leaf-dip technique.

Compound	M FO (n mole substrate oxidized/min/ mg protein)	α -esterase (ug α - naphthyl acetate released /min/mg protein)	β -esterase (ug β - naphthyl acetate released /min/mg protein)	Alkaline Phosphatase $U \times 10^3$ /mg protein
<i>Citrus vulgaris</i>	626.66±35.5	20.1±2.0	62.33±3.5	1.55±0.09
<i>Majorana hortensis</i>	916.33±51.2	22.0±1.5	82.33±8.2	1.77±0.07
<i>Pimpinella anisum</i>	704.33±41.6	19.33±1.8	55.0±2.6	1.67±0.07
<i>Lupinus termis</i>	819.66±30.7	27.66±1.2	73.33±3.3	1.67±0.06
<i>Olea europaea</i>	795.66±48.4	22.66±1.4	47.66±3.7	1.68±0.12
Control	782.66±33.2	22.53±1.5	38.17±2.9	1.51±0.10

Table (4):Biochemical assays of detoxification enzymes activities in field strain of *Aphis craccivora* exposed to different Phytochemicals by leaf-dip technique.

Compound	M FO (n mole substrate oxidized/min/ mg protein)	α -esterase (ug α - naphthyl acetate released /min/mg protein)	β -esterase (ug β - naphthyl acetate released /min/mg protein)	Alkaline Phosphatase $U \times 10^3$ /mg protein
<i>Citrus vulgaris</i>	276.66±18.5	24.0±2.5	53.3±3.3	2.43±0.08
<i>Majorana hortensis</i>	267.33±17.1	11.53±1.0	11.73±0.8	2.03±0.08
<i>Pimpinella anisum</i>	253.33±19.6	12.07±1.5	22.0±1.2	2.35±0.10
<i>Lupinus termis</i>	477.0±20.8	13.17±1.4	18.0±1.5	2.73±0.08
<i>Olea europaea</i>	510.0±28.1	19.0±2.0	17.83±0.7	3.23±0.08
Control	782.66±33.2	22.53±1.5	38.17±2.9	1.51±0.10

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دراسات مقارنة علي السمية والنظم البيوكيميائية للزيوت النباتية ضد حشرة مَن اللوبيا

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معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة- مصر

تمت الدراسة لمقارنة سمية خمس أنواع من الزيوت النباتية تشمل زيت الترمس و البردقوش و البنسون و الزيتون و البرتقال علي كل من السلالة المعملية و السلالة الحقلية لحشرة من اللوبيا أظهرت النتائج ان كل المركبات لها تأثير سام و تختلف السمية باختلاف التركيز ونوع النبات كما أشارت النتائج الى ان السلالة الحقلية اكثر مقاومة لهذه المركبات عن السلالة المعملية. علي الصعيد الاخر أظهرت النتائج ايضا ان هناك اختلاف لنشاط بعض الانزيمات المسئولة عن تحطيم المركبات السامة التي تشمل انزيمات (الاكسده) و كذلك انزيم فوسفاتيز القاعدي والفا و بيتا استيريزس وترجع هذه الاختلافات للمركب الذي تعرضت له الحشرة وكذلك السلالة سواء كانت سلالة الحشرة معملية او حقلية.