INFLUENCE OF CERTAIN VOLATILE PLANT OILS ON BIOLOGICAL AND BIOCHEMICAL ASPECTS OF PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* (L.)

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INTRODUCTION

Callosobruchus chinensis is one of the most serious pests which cause great damage to several pulse crops during storage (Singh *et al.*, 1977). To protect different stored crops from insect attack, there are natural and non- insecticidal extracts. The plant derivatives are desirable because they don't contaminate environment and are safe for users and consumers (Finney, 1990). In the search for alternatives to commercial insecticides and fumigants, essential oils extracted from plants have been investigated (Regnault and Hamraoui, 1994; Namrata *et al.*, 1997; Ketoh *et al.*, 2000; Adhikari *et al.*, 2002 and Park *et al.*, 2003).

Since proteins are the most complex and at the same time the most characteristic chemical compounds in insect body, the effects of volatile oils poisoning on proteins synthesis and enzymes activities were evaluated on different insects (Mostafa and El Sherif, 1995; El- Naggar and Abdel- Fattah, 1999 and Mohammed *et al.*, 2003). They are presented as chief structural elements of cuticle, muscles, glands, building tissues and as functional molecules like enzymes (Agosin, 1978). The present study was carried out to evaluate the insecticidal effects of peppermint, eucalyptus, vetiver and chamomile oils against *C. chinensis* which is a major pest of stored pulses in Egypt. The study also deals with the biochemical effect of the tested oils on total protein contents and the activities of some enzymes involved in emerged adult females.

MATERIAL AND METHODS

Stock cultures

Pulse beetle adults of *C. chinensis* (obtained from the Stored Product Department, Ministry of Agriculture, Dokki, Giza, Egypt) were reared on cowpea seeds, *Vigna unguiculata* (L.) at 30 ± 2 °C and 70 ± 5% R.H.

Tested volatile oils and the application

The oils tested in the present study were peppermint oil (*Mentha piperita* L.); eucalyptus oil (*Eucalyptus globules*); vetiver oil (*Vetivera zizanioides*) ; and chamomile oil (*Anthemis nobilis* L.). Tested oils were obtained from the Egyptian Sugar and Distillation Company (Food flavors and essences Factory at Giza Governorate). Peppermint, eucalyptus, vetiver leaves and chamomile flowers were extracted according to the methods described by A. O. A. C. (1980).

The volatile plant oils were applied to cowpea seeds according to the method described by Pereira (1983). Diluted oil of peppermint, eucalyptus, vetiver and chamomile were separately mixed with cowpea seeds at dose rates of 1.0, 1.5, 2.0 and 2.5 ml/kg. The oils were diluted with acetone solvent. Conical flasks containing the seeds and oil were shaken manually until the seeds were coated. After shaking, seeds were taken out and air dried for one hour to evaporate the acetone. Control seeds were treated in the same manner with acetone only. Samples of 20 gm of treated or control seeds were put in plastic cups (replicated four times), five pairs of newly emerged adults were transferred to each cup, covered with muslin cloth and kept in the incubator at the prementioned conditions.

The insecticidal effect of each oil was investigated to determine adult mortality, female fecundity, egg hatchability and F1 adult emergence.

Adult mortality was recorded at the 3rd and 5th day of treatment. The number of eggs laid was recorded and percentage of reduction in egg laying was estimated. Egg hatchability was also calculated according to Pereira (1983), where the hatched eggs were recognized by their morphology, since they became opaque as a function of the residues discharged by the larvae during their penetration into seeds. Emerged adults were counted and separated to evaluate the biochemical effects of plant oils. Percentage of reduction in adult emergence was calculated for each oil treatment and control.

Biochemical estimation

Females emerged from untreated or treated seeds with 1.0 and 1.5 ml/kg of different plant oils were homogenized for five minutes in a cold glass homogenizer containing 3 ml phosphate buffered saline (PH 7) with trace of saturated phenylthiourea (PTU) according to the method of Sammour *et al.* (1986). Insect suspension was centrifuged at 14000 rpm for 60 min at 4°C. The clear supernatants were used for direct estimation of total protein according to the method of Bradford (1976) using crystalline bovine serum albumin as standard. The activity of alkaline phosphatase enzyme (ALP) was analyzed colorimetrically following the method of Wilkinson (1969), aspartate aminotransferase (AST) was estimated according to Bergmeyer *et. al.*, 1986 and Tyrosinase activity (Duckworth and Coleman, 1970). The enzyme activity was expressed as IU of product per gm tissue. Adenosine triphosphatase (ATPase) was assayed according to Haber and Loeb (1984), the enzyme activity was expressed as μg inorganic phosphate per min. per gm tissue.

Statistical analysis

Results were subjected to one way analysis of variance using statistical software "SPSS" version 11.5 (2002). Differences were considered statistically significant if the p value <0.05.

RESULTS AND DISCUSSION

The insecticidal effects of the tested volatile plant oils against *C. chinensis*

The obtained results are graphically illustrated in Figs. (1 and 2) which show that adult mortality was directly proportional to the oil rates and also with time after treatment. When cowpea seeds were treated with peppermint oil (2.0 and 2.5 ml oil/kg) and eucalyptus (2.5 ml oil/kg), complete mortality (100%) occurred after 3 days of treatment compared with 0% mortality in control (Fig. 1). On the other hand, chamomile and vetiver oils applied on cowpea seeds at dose 2.0 ml oil/kg caused the least mortality percentage (32.5 and 52.5%) respectively, which increased significantly at the 5^{th} day after treatment to reach 80% compared with 12.5% in the control (Fig. 2). Adedire and Lajide (1999) stated that the volatile plant oils caused mortality due to the strong choky odors which may have exerted a toxic effect by disrupting normal respiration activity of weevils, thereby resulting in asphyxiation and subsequent death. Shaaya *et al.* (1997) recorded the highest mortality against four stored product insects after 3 hours of exposure to vapor of peppermint oil in a fumigant chamber. Also, Magd El- Din (2003) recorded that eucalyptus oil caused 100% mortality of *C. chinensis* adults after 96 hours of treatment with 4000 ppm.

The results present in Table (1) indicated that the lowest average number of eggs (38.5) was on seeds treated with peppermint oil at dose 1.0 ml/kg compared to (65.5 eggs) in the control. The reduction percentage in egg laying was 41.22% compared to their corresponding chamomile (28.6%), vetiver (7.63%) and eucalyptus (13.74%) (Fig. 3). Dose 2.0 ml/kg peppermint oil completely inhibit the egg laying as compared to the other oils and the reduction reached 100%.

Increasing of eucalyptus dose (2.5 ml/kg) inhibited egg laying compared to vetiver (95.4%) and chamomile (90.4%) (Fig. 3).

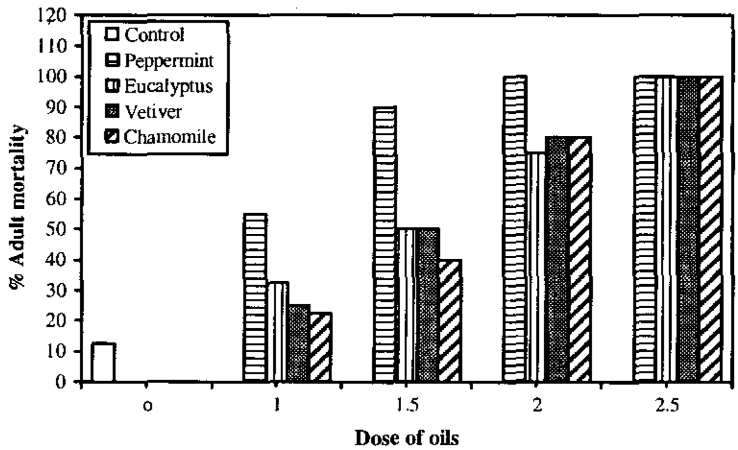

Fig. (1): The percentages adult mortality of *C. chinensis* after 3 days of treatment with the tested volatile plant oils.

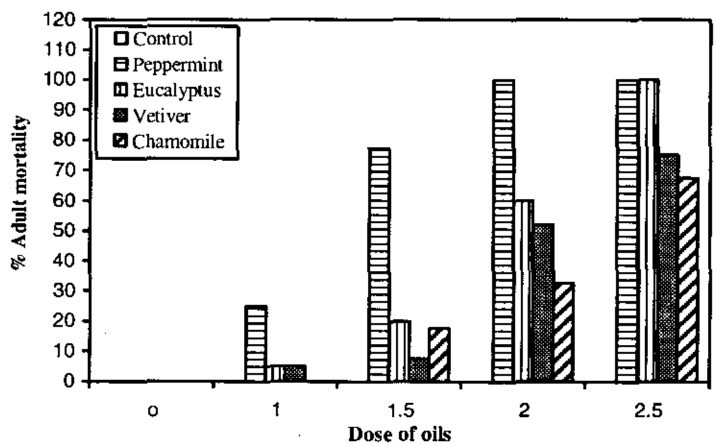

Fig. (2): The percentages adult mortality of *C. chinensis* after 5 days of treatment with the tested volatile plant.

Results given in Table (1) revealed that the egg hatchability was prevented completely (0%) at doses (2.0 and 2.5 ml/kg) of peppermint and eucalyptus at (2.5

ml/kg) compared to 89.31% in the control. Peppermint was effective in reducing number of emerged adults, by increasing the dose, the emergence was affected. The average of adult emergence was 20.75 and 5.25 at doses of (1.0 and 1.5 ml/kg). At dose of 2.0 ml/kg seeds, the percent reduction was 100% as compared to chamomile (51.34%); vetiver (46.86%) and eucalyptus (37.08%), (Fig. 4). Raja *et al.* (2001) reported that vapor of volatile oil derived from peppermint (*Mentha piperita)* probably diffuse into eggs of *Callosobruchus maculates* and affected the physiological and biochemical processes associated with embryonic development.

TABLE (I)

Effect of tested volatile oils on fecundidty, egg hatchability and adult emergence of *C. chinensis.*

Volatile plant oils	Dose (ml /kg)	Mean no. of laid eggs/ female ±S.E	% Hatch-ability	Mean no. of emerged adults ±S.E	% Emergence
Control	0	65.5±2.2	89.31	47.25±1.57	72.14
Peppermint	1.0	38.5±1.55	87.01	20.75±1.49	53.90
	1.5	11.5±0.95	73.91	5.25±0.75	45.60
	2.0	0	0	0	0
	2.5	0	0	0	0
Eucalyptus	1.0	56.5±2.0	83.18	30.25±1.54	53.54
	1.5	54.0±1.8	89.35	27.0±1.84	50.0
	2.0	32.75±3.3	72.27	14.86±1.22	45.39
	2.5	0	0	0	0
Vetiver	1.0	60.5±1.10	78.38	31.5±1.32	52.07
	1.5	49.25±2.56	81.21	24.62±0.95	50.0
	2.0	15.0±1.77	63.33	5.75±0.75	38.33
	2.5	3.0±0.71	33.33	0	0
Chamomile	1.0	46.75±2.14	70.43	27.25±1.35	36.90
	1.5	40.5±3.30	68.52	18.25±1.25	37.65
	2.0	23.5±1.55	63.83	8.25±2.26	35.10
	2.5	6.25±1.44	64.0	2.0±0.41	32.0

From the aforementioned data, it could be concluded that peppermint oil showed the most potential effect, whereas, eucalyptus, vetiver and chamomile oils offered good protection for seeds against *C. chinensis* at high doses.

Several authors have previously reported the ovicidal activity of many plant oils on bruchid pests, Carlos and Cardona (1981) reported that oil can penetrate minute opening of the egg surface and stop the biological activity. Don-Pedro (1989) recorded the ovicidal action of the groundnut oil against *C. maculates,* he also suggested that egg mortality was caused by the physical properties of oil coating, blocking respiration rather than specific chemical effect. Also, Credland (1992) explained the ovicidal effect on terms of asphyxiation of the developing insects as a result of occluding of the funnel structure at the posterior pole of *Callosobruchus* spp. eggs which may be the major route of gaseous exchange.

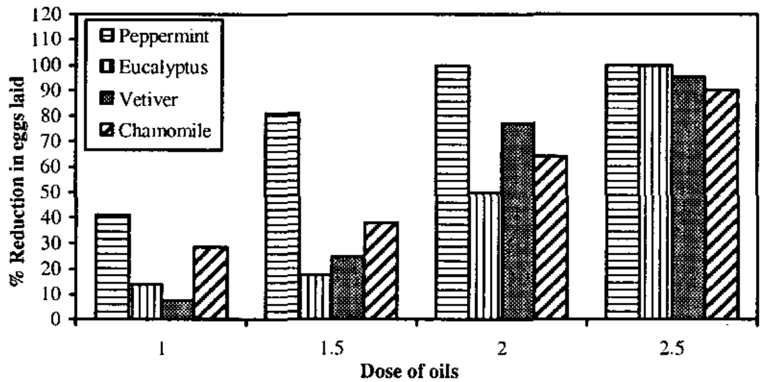

Fig. (3): The percentages reduction of *C. chinensis* eggs laid on treated cowpea with the tested volatile plant oils.

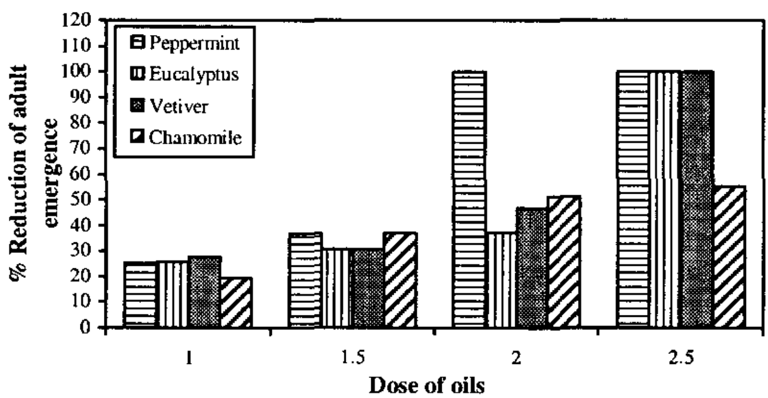

Fig. (4): The percentages reduction of adult emergence of *C.chinensis* from cowpea treated with the tested volatile plant oils.

The biochemical effects of the tested volatile plant oils

The level of total protein content and the activity of different enzymes in adult females (0- 24 hr old) of *C. chinensis* emerged from untreated and treated seeds with 1.0 and 1.5 ml/kg of each oil were illustrated in Table (2).

a. Total protein content

The results in Table (2) showed that the total protein contents of bodies in untreated adult females was 3.56 mg/gm tissue, while the protein contents in the whole body tissue of adult females treated with peppermint, eucalyptus, vetiver and chamomile oils (1.0 ml/kg) were increased significantly (5.61, 6.2, 7.45 and 7.2 mg/gm tissue), respectively. While at the higher dose of oils (1.5 ml/kg), the levels of protein contents were decreased significantly after treatments with peppermint, vetiver and chamomile oils (1.95, 1.22 and 1.28 mg/gm tissue) respectively, and non-significantly with eucalyptus (3.32 mg/gm tissue) as compared to the control (3.56 mg/gm tissue).

TABLE (II)

Total protein contents in *C. chinensis* adult females emerged from cowpea treated with the testedvolatile plant oils.

Volatile plant oils	Dose (ml /kg)	Mean total protein (mg/g tissue) ±S.E
Control	0	3.56±0.13
Peppermint	1.0	5.61±0.13
	1.5	1.95±0.07
Eucalyptus	1.0	6.2±0.12
	1.5	3.32±0.11
Vetiver	1.0	7.45±0.19
	1.5	1.22±0.11
Chamomile	1.0	7.2±0.12
	1.5	1.28±0.10

The higher protein contents at the low dose of oils may be an attempt by the insects to detoxify the oil, where the ingredients of the oil at low dose may act in stimulatory mode with low toxic components, whereas higher dose abolished this influence allowing the toxicants inducing their inhibitory actions. Proteins help to synthesis microsomal detoxifying enzyme (Ishaaya, 1993). The present results are in agreement with results of Mohammed *et al.* (2003).

b. Enzymes activity

The present investigation deals with some metabolic enzymes which are involved in intermediary metabolism and so may be expected to have their highest rates of activity during the development of the reproductive system and gametes in newly emerged adults females.

The effect of the different volatile oils on the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), tyrosinase and adenosine triphosphatase (ATPase) enzymes were illustrated in Figures (5, 6, 7 and 8). Results indicated that the enzymes activities were increased at the lower dose of different oil treatments (1 ml/kg), while they decreased at the high dose (1.5 ml/kg) as compared with the control.

Fig. (5) showed that significant increase in ALP activity occurred (2.03, 3.77, 2.30 and 1.84I U/g) as compared to the control (1.50 IU/g) at the lower dose of peppermint, eucalyptus, vetiver and chamomile (1.0 ml/kg), respectively. The enzyme activity was decreased significantly to reach 1.0, 0.61, 0.73 and 0.87 IU/g, respectively, as compared to the control (1.50 IU/g) at the higher dose of latter oils (1.5 ml/kg). Similar results were obtained by Saleem *et al.* (1998) after treatment of *Tribolium castaneum* by synthetic pyrethroid insecticide (cypermethrin).

The present results referred to a significant increase in the AST activity in adult females emerged from cowpea treated with peppermint, eucalyptus, vetiver, and chamomile oils at dose of 1.0 ml/kg (64.86, 77.32, 61.946 and 54.28 IU/g tissue, respectively when compared with the control (17.63 IU/g tissue). However, at the high dose of the same oils, results showed, respectively decreased activity (35.49, 27.43, 26.06 and 20.97 IU/g tissue) (Fig. 6). During maturation of oocytes, the female specific protein synthesis proceeds at a high rate and the Transaminases decrease.

Living organisms require a continual input of free energy for three major purposes: (i) the performance of mechanical work in muscle contraction and other cellular movements (ii) the active transport of molecules and ions (iii) synthesis of macromolecules and other biomolecules from simple precursors (Agosin, 1978). ATPase is a special carrier of free energy in most processes. In biological system ATPase serves as the principle immediate donor of free energy rather than as a long-term storage of energy.

Regarding the effect of the applied oils on ATPase activity of female *C. chinensis* as shown in Fig. (7), an enzymatic activity increased significantly at the low dose of the different volatile oils, while at the high dose the activity was decreased but non-significant as compared with the control. Present result agree with that obtained by Salama *et al.* (2000) worked on *Ceratitis capitata* and recorded the increase in ATPase activity for production and yield of lactate. The lactate accumulated in the flight muscles and then cause the inability of motion.

Figure (8) showed that Tyrosine enzyme activity in untreated females was 62.46 IU/g , while in females emerged from treated seeds with peppermint, eucalyptus, vetiver and chamomile (at dose 1.0 ml/kg) the enzyme activity significantly increased (132.06, 160.4, 115.16 and 141.60 IU/g, respectively). Meanwhile, enzyme activity was decreased at high dose of the tested oils: 52.98, 72.32, 24.67 and 17.19 IU/g, respectively. This may be due to interference of oil ingredients with the enzyme substrate (Gluck, 1992).

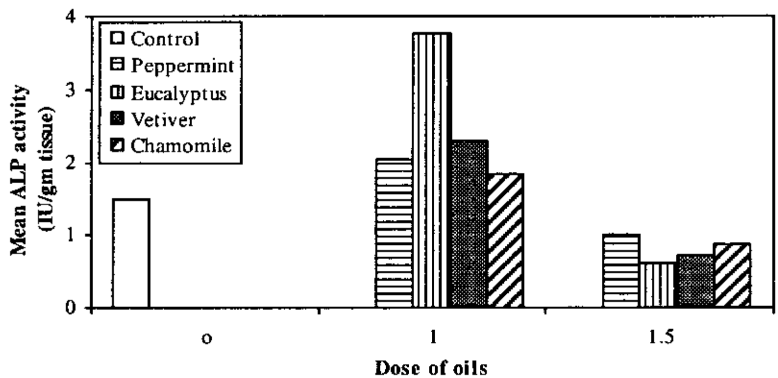

Fig. (5): Pattern of ALP activity in *C. chinensis* females emerged from cowpea treated with the tested volatile plant oils at doses 1.0 and 1.5 ml /kg.

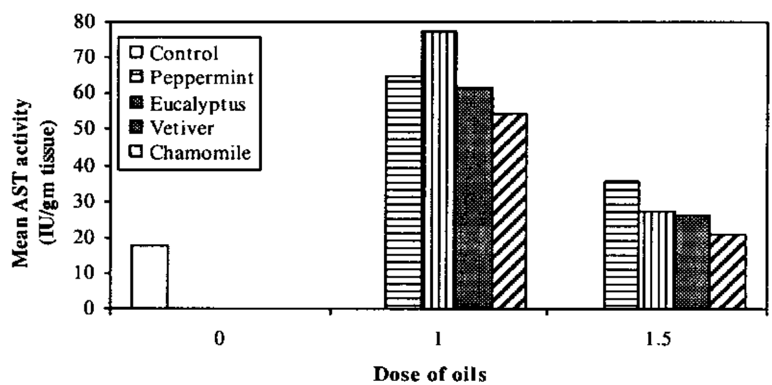

Fig. (6): Pattern of AST activity in *C. chinensis* females emerged from cowpea treated with the tested volatile plant oils at doses 1.0 and 1.5 ml /kg.

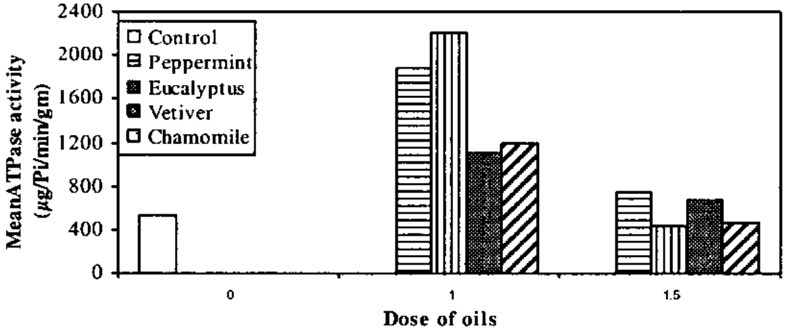

Fig. (7): Pattern of ATPase activity in *C. chinensis* females emerged from cowpea treated with the tested plant volatile oils at doses 1.0 and 1.5ml /kg seeds.

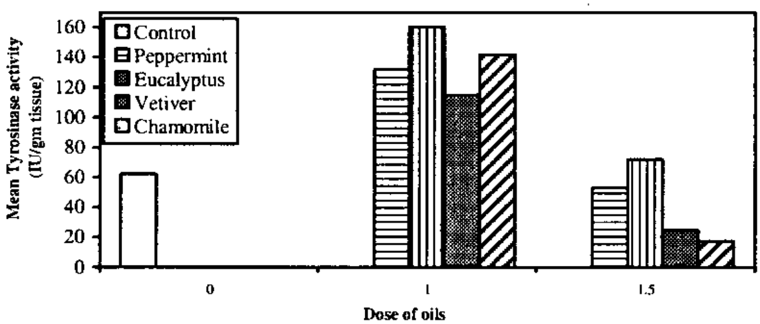

Fig. (8): Pattern of Tyrosinase activity in *C. chinensis* females emerged from cowpea treated with the tested plant volatile oils at doses 1.0 and 1.5ml /kg seeds.

It could be concluded that plant derivatives might be useful as insecticide agents for commercial use, increasing benefit for agricultural sectors in developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazards.

SUMMARY

The efficacy of four volatile plant oils, i.e., peppermint, vetiver, chamomile and eucalyptus was investigated as cowpea seed protectants against *C. chinensis*. Peppermint oil was effective inducing the highest percentage of mortality. All the tested oils caused reduction in oviposition and inhibited the adult emergence. All

extracts increased the protein content, alkaline phosphatase, aspartate aminotransferase, tyrosinase and adenosine triphosphatase enzymes in emerged adult females at low dose of oils. By increasing the dose, the total protein and all enzyme activities were decreased.

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