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Biochemical Influences of Some Volatile Oils on Potato Tuber Moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae)

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Abstract: The vapours of ten volatile oils (citronella, eucalyptus, clove, lemon grass, dill, geranium, celery, lemon, cardamon and coriander) were used to study their effect on total protein, albumin and alkaline phosphatase, which are thought to be related to reproductive capacity of potato tuber moth *Phthorimaea operculella*. The results showed, clearly, that the biochemical responses were variable according to the type of oil and sex. Lemon had no effect in most cases. It only decreased females' total protein and alkaline phosphatase activity. All the remaining oils exerted effects, particularly six oils (clover, eucalyptus, citronella, dill, lemon grass and celery), generally, reduced the majority of the tested biochemical parameters in both males and females.
Total proteins and albumin were reduced more by the tested oils than alkaline phosphatase, which increased in some cases, especially in males.

Key words: Essential oil, volatile oils, *Phthorimaea operculella*, protein, albumin, alkaline phosphatase.

Introduction

The potato tuber worm, *Phthorimaea operculella* Zeller is a destructive pest of potato plants and tubers. Recently, the volatile oils as natural soft control agents are considered as insecticides in field IPM (Integrated Pest Management). According to Fahmy *et al.* (1993), the volatile oils are extracted from aromatic plants, and the majority of them can evaporate under normal conditions without leaving residues. The hydrocarbons and terpenoids are the effective components in these oils. The ability of some volatile oils to control some insects has been discussed by many authors (Ahmed and Eapen, 1986; El-Nahal *et al.*, 1989; Sawires *et al.*, 1995; El-Shazly *et al.*, 1998).

Although the mode of action of oils is not well known, it is generally believed that they provoke the death of insect eggs or larvae by suffocation or through a fumigation effect (Schoonhoven, 1978), or in some cases, through antifeedant or repellent activity (Alford *et al.*, 1987). The recent reports indicated that some of volatile oils affect reproduction of *P. operculella* (Haiba, 1996), and fecundity, fertility and ovarian amino acids of the female *Musca domestica* when the oils are applied on the larvae (Shoukry, 1997).

The present investigation aims to study the effect of ten volatile oils on some biochemical aspects, i.e., total proteins, albumin and alkaline phosphatase which are thought to be related to reproductive activity of *P. operculella* treated for 24 hr as newly emerged adults.

Materials and Methods

The potato tuber moth *P. operculella* was reared in Laboratory of Entomology, National Center for Radiation Research and Technology at 29±1°C and 50-65% relative humidity (Haiba, 1990). The larvae were fed on potato tubers (*Solanum tubersum*), while adults were fed 5% sugar solution.

The tested volatile oils were obtained from Research Sector of Food Extracts and Volatile Oils factories, and Sugar and Integrated Industries Company, Giza. The ten volatile oils are illustrated in Table 1 and they were used in their pure form as fumigants.

Table 1: The tested volatile oils and their plant sources.

Volatile oils	Plant sources
Cardamon	*Elettaria cardamonum* fruits
Citronella	*Cymbopogon winterianus* leaves
Eucalyptus	*Eucalyptus globulus* leaves
Clove	*Eugenia caryophyllus* flower buds
Lemon grass	*Cymbopogon citrates* leaves
Dill	*Anethum graveolens* herb and fruits
Coriander	*Coriandrum sativum* fruits
Lemon	*Citrus aurantifolia* fruit peels
Geranium	*Pelargonium graveolens* herbs
Celery	*Apium graveolens* herbs and fruits

Each 25 of newly emerged moths were confined in chimney glass cages, about 10 cm in diameter and 16 cm height, the lower rim of which rests on the bottom of a petri-dish lined with a disc of filter paper and the upper rim covered with muslin secured by a rubber band. The cage was provided with tube (1x3 inches) containing cotton pieces soaked with the tested oil (0.1 ml), and covered with porous cover allowing oil vapour distribution. This amount of tested oil (0.1 ml) was chosen because it enables all the treated males and females to live more than 24 hr (Haiba, 1996). Control moths received no vapours. After the exposure to each of the tested oils vapours for 24 hr, moths were counted, weighed and refrigerated till used for analysis.

Each 20 moths were homogenized in distilled water at 5°C using Teflon tissue grinder for 5 min. Centrifugation was done at 3500 rpm for 10 min using a refrigerated centrifuge (5°C). The resultant supernatant was used for analysis. Alkaline phosphatase (Alkpase) activity was measured using an optimized standard method according to recommendations of Deuche Gesellschaft für Klinische Chemie (DGKC) (1972), and expressed as U (unit)x10^3/g.b.wt., where one unit of alkaline phosphatase will hydrolyze 1 μmole of P-nitrophenyl phosphate per min at 37°C. Total protein was estimated according to the method of Bradford (1976), and expressed as mg protein/g b.wt., while albumin content was evaluated using bromocresol green (BCG) at pH 4.2 as described by a kit of bio Mérieux (69280 Marcy-Vetoile/France) and expressed as mg albumin/g b.wt. The colour produced was read by a spectrophotometer (Spectronic 1201, Milton Roy Co., USA). Ultimately, data obtained were statistically analyzed using t-test according to procedures outlined by Snedecor and Cochran (1980).

Results and Discussion

The data in Table 2 and 3 show total protein, albumin and alkpase activity of female and male, respectively, potato

Table 2: Total protein, albumin and alkpase activity of female potato tuber moth, *P. operculella* Zeller exposed for 24 hr immediately after emergence, to vapours of volatile oils.

Volatile oils	Total protein (mg/g b.wt.)		Albumin (mg/g b.wt.)		Alkpase (Ux10^3/g b.wt.)	
	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
Control	79.00±1.95	-	46.25±0.88	-	1264±26.5	-
Citronella	90.15±1.50*	+ 18.77	16.60±1.44*	- 64.12	651±24.0*	- 48.50
Eucalyptus	95.25±0.80*	+ 25.50	26.54±1.44*	- 42.62	888±37.0*	- 29.75
Clove	47.00±3.65*	- 38.08	23.21±0.71*	- 49.82	908±1.15*	- 28.09
Lemon grass	49.20±1.80*	- 35.18	25.48±1.35*	- 44.91	944±1.73*	- 25.31
Dill	45.50±1.55*	- 40.05	24.39±0.68*	- 47.26	979±21.93*	- 22.54
Coriander	76.60±2.65NS	+ 0.92	38.78±0.88*	- 16.15	1192±30.59*	- 5.69
Lemon	58.55±2.20*	- 22.86	43.31±1.72NS	- 6.36	967±81.00*	- 23.50
Geranium	51.50±1.75*	- 32.15	21.49±1.22*	- 53.54	902±63.00*	- 28.63
Celery	51.30±1.70*	- 32.41	25.77±0.77*	- 44.28	1217±15.00NS	- 3.72
Cardamon	54.95±2.25*	- 27.60	22.67±0.62*	- 50.98	1312±73.00NS	+ 3.8

* Significant at 5% level NS: non-significant

Table 3: Total protein, albumin and alkpase activity of male potato tuber moth, *P. operculella* Zeller exposed for 24 hr immediately after emergence, to vapours of volatile oils.

Volatile oils	Total protein (mg/g b.wt.)		Albumin (mg/g b.wt.)		Alkpase (Ux10^3/g b.wt.)	
	Mean±SD	% Change	Mean±SD	% Change	Mean±SD	% Change
Control	85.00±3.15	-	38.95±0.36	-	787±10.70	-
Citronella	56.35±3.30*	-34.10	23.76±0.78*	-38.99	485±21.00*	- 38.37
Eucalyptus	92.60±0.60*	+8.30	26.77±1.55*	-31.27	360±20.00*	- 54.25
Clove	54.85±2.70*	-35.85	13.73±2.32*	-64.75	1268±103.00*	+ 61.12
Lemon grass	73.05±6.03*	-14.56	34.11±0.57*	-12.43	860±47.92NS	+ 9.28
Dill	64.10±2.15*	-25.03	31.99±0.45*	-17.87	845±66.39NS	+ 7.37
Coriander	76.20±2.65*	-10.88	38.12±1.27NS	-2.13	1055±47.92*	+ 34.05
Lemon	80.55±2.20NS	-5.79	41.77±2.94NS	+7.24	856±94.00NS	+ 8.77
Geranium	95.20±3.90*	+11.35	46.31±2.54	+18.9	688±45.00*	- 12.58
Celery	74.25±3.85*	-13.16	16.86±1.44	-56.71	777±96.00NS	- 1.27
Cardamon	84.65±2.95NS	-0.99	35.16±0.84*	-7.79	842±19.00*	+ 6.99

NS: non-significant *: Significant at level 5%

tuber moth *P. operculella*

Total protein: The results indicated that eucalyptus significantly increased total proteins of females and males as compared to control by 25.5 and 8.3%, respectively. Also, citronella significantly increased females total proteins by 18.77%, while it was 11.35% in males after treatment with geranium. Coriander had no significant effect on females, and lemon and cardamon in the case of males. The remainder oils decreased total proteins especially dill in female and clove in male ones. The reduction was 40.05 and 35.85%, respectively (Table 2 and 3).

Albumin: The data revealed that most of the oils significantly reduced albumin, especially citronella, which reduced albumin in females by 64.12% as compared to control (Table 2) and clove which also reduced it by 64.75% in case of males (Table 3). Geranium is the only cause which increased albumin. It increased in males by 18.9% as compared to control. Lemon in the case of females and males and coriander in that of males caused non-significant changes.

Alkpase: The results showed that all the treatments reduced alkpase activity in females, with the exception of cardamon, which caused non-significant increase, especially in the case of citronella which caused 48.5% reduction as compared to control. The effect on males alkpase activity was variable. Lemon grass, lemon, dill and celery caused non-significant changes. Clove, coriander and cardamon caused significant increase by 61.12, 34.05 and 6.99%, respectively as compared to control. While alkpase activity was reduced by 54.55, 38.37 and 12.58% in case of eucalyptus, citronella and geranium, respectively, as compared to control.

At least six oils (clover, eucalyptus, citronella, dill, lemon grass and celery), generally, reduced the majority of tested biochemical parameters in both males and females with few exceptions such as celery which reduced total protein, albumin and female alkaline phosphatase, but insignificantly decreased male alkaline phosphatase. Some oils such as geranium decreased all the parameters of females, but increased mostly those of males. Others affected a specific biochemical constituent in both males and females such as eucalyptus which increased total protein in both sexes.

The results indicated that the biochemical responses were variable according to type of oil and sex. This variation was also achieved by Moustafa *et al.* (2001). He used citrus oils extracted from closely related plants (lemon, naring, orange, limonene and mandarin) to study their effect on nutrient content, digestive enzymes and other parameters of *Pectinophora gossypiela* larvae. Although the hydrocarbons and terpenoids are the effective components in these oils, yet more chemical studies, followed by studies concerning the living organisms, might explain such differences in their action. It was also observed that lemon had no effect in most cases. It only decreased females total protein and alkaline phosphatase. Some reports indicated that lemon had no toxicity to the adults of *Callosbruchs chinensis* ,*C. maculatus* and *P. operculella,* but had some effects on oviposition (Mahgoub and Zewar, 1989; Risha *et al.*, 1993; Haiba, 1996). Other studies revealed lemon of moderate toxicity to rice weevil, *Sitophilus oryzae* (Su *et al.*, 1972).

The significance of studying proteins not only because they are the major cell components which play the most important role in all biological processes including reproduction, but also because the reduced level of them due to suppression in the availability of dietary proteins is one of the mechanisms of action of such natural products as suggested by Feeny (1970). Some reports indicated that plant extracts reduce protein level

(Abo El-Ghar *et al.*, 1995). While petroleum ether rextracts of total oils of the santonic plant, *Artimisia obsinthium* insignificantly increased total proteins of *P. operculella* (Aly, 1996). She added that the insignificant increase in protein biosynthesis in adults may be due to the increase of body metabolism which resulted from the interaction between these compounds and the detoxifying enzymes such as carboxyl esterase and glutathion-S-transferase (Ishaaya, 1993). Quadri and Narsaiah (1978) demonstrated that injection of *Periplaneta americana* nymphs with azadirachtin which is the most active component of the Indian neem tree, caused reduction in albumin, globulin, DNA and RNA levels in the haemolymph. Shoukry (1997) found that the amino acids as groups and fractions have great changes in *Musca domestica* females after treatment with *Matricaria chamomilla* and *Clerodendron inerme* oil.

Alkpase activity, in the present study, was relatively less reduced by the volatile oils than total protein and albumin especially in males, where there were increased levels after treatment with clove, dill, coriander and cardamon. The importance of this enzyme refers to its relation to gamete maturation (Rousell, 1971). Abdel-Hafez *et al.* (1982) reported that *Spodoptera littoralis* adults exhibited higher level of alkaline phosphatase activity in both sexes than acid phosphatase. Ayyanger and Roa (1990) injected azadirachtin into the sixth instar of *S. litura* larvae. This reduced the level of alkpase activity. On the other hand, the recorded higher level of this enzyme than control especially in males after the treatment with some oils is not understood, but it might be a defense mechanism, since it is known that phosphatases were implicated in resistance to some insecticides. They hydrolases which can metabolize such xenobiotics, or they might be liberated by lysosomes of the damaged cells due to insecticide treatment (Rayender, 1986).

The effect of ten tested oils on total proteins, albumin and alkaline phosphatase activity might explain, partially, malformation in ovary of *P. operculella*, and high severe effect after exposure to the oils which led to decrease in fecundity and fertility and adults failed to mate as reported by some authors (Haiba, 1996).

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