

FUMIGANT TOXICITY OF ESSENTIAL OIL FROM *CARUM COPTICUM* AGAINST INDIAN MEAL MOTH, *PLODIA INTERPUNCTELLA*

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Abstract: Fumigant toxicity of the essential oil derived from *Carum copticum* C.B. Clarke (*Apiaceae*) were assessed against growth stages of Indian meal moth, *Plodia interpunctella* (Hubner) (*Lepidoptera: Pyralidae*). Seeds of the plant were collected from Tehran, Iran and hydrodistilled to extract their essential oil. The essential oil was analyzed by GC and GC-MS. Thymol (64.51%), γ -terpinene (17.52%) and *p*-cymene (16.16%) were the main components among the eight constituents characterized in the oil, representing 98.19% of the total components detected.

All bioassay tests were conducted at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH and photoperiod of 16:8 h (light:dark). After preliminary dose-setting experiments, LC_{50} and LC_{90} values of four growth stages including eggs, larvae, pupae and adults were calculated. Results showed that the toxicity on the growth stages of Indian meal moth was differed markedly. Adult insects were about 500 times ($LC_{50} = 257.83 \mu\text{l/m}^3$ air and $LC_{90} = 598.94 \mu\text{l/m}^3$ air) more susceptible than other growth stages. Moreover, last instar larvae ($LC_{50} = 91.36 \mu\text{l/l}$ air and $LC_{90} = 213.79 \mu\text{l/l}$ air) and pupae ($LC_{50} = 105.69 \mu\text{l/l}$ air and $LC_{90} = 203.24 \mu\text{l/l}$ air) were significantly more susceptible than eggs ($LC_{50} = 184.61 \mu\text{l/l}$ air and $LC_{90} = 435.32 \mu\text{l/l}$ air). These findings indicated that essential oil from seeds of *C. copticum* could have potential of practical value for application in management of the Indian meal moth in storage.

Key words: *Carum copticum*, Indian meal moth, essential oil, fumigant toxicity

INTRODUCTION

Indian meal moth, *P. interpunctella*, is distributed world-wide and is a serious stored-product pest of grain and seeds (Lecato 1976; Cuperus *et al.* 1990; Doud and Phillips 2000; Nansen *et al.* 2004) as well as flour and other milled products (Lecato 1976). They attack a wide variety of dried fruits and nuts (Johnson *et al.* 1992; Shojaad-

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dini *et al.* 2005). In recent years, it was considered as the most important pest of stored pistachios in Iran which cause severe qualitative and quantitative losses in this fruit crop (Shojaaddini *et al.* 2005). Larvae are able to penetrate and infest a wide range of packaged foods (Cline 1978), and can have a great economic impact due to direct product loss and indirect factors such as the cost of pest control and loss of sales from consumer complaints (Sauer and Shelton 2002).

Current stored-product pest management has relied on the use of chemicals. However, chemical control methods using fumigants are restricted because of development of pest resistance, health hazards and risk of environmental contamination. Therefore, many studies such as low temperature storage and heat treatment (Na and Ryoo 2000; Sauer and Shelton 2002), pheromone-baited traps (Mullen and Arbogast 1979) as well as change in photoperiod (Shojaaddini *et al.* 2005) and other non-chemical control methods for Indian meal moth infestations are being suggested as alternatives for fumigants. Recently, there has been a growing interest in research concerning the possible use of natural products as an alternative to chemical treatment and fumigation. The fumigant toxicity of a large number of essential oils and their components from aromatic plants has been evaluated on the major stored-product insects such as *Sitophilus oryzae* L., *Rhizopertha dominica* F., *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* F. and *Ephestia cautella* Zell. (Poplawski *et al.* 2000; Kim and Ahn 2001; Lee *et al.* 2001; Nansen and Phillips 2004). In organic agriculture, compounds extracted from plants can be used as biopesticides to kill or repel pest insects (Singh and Upadhyay 1993).

Carum copticum is popularly known as ajowan; it is an annual herbaceous plant bearing feathery leaves and white flowers grow in compound umbels. When the seeds are ripe, they are dried and threshed. Ajowan is widely distributed plant throughout India (Thangam and Dhananjayan 2003). It is indigenous to southern India and it is cultivated in various areas such as Europe, Egypt, Pakistan, Afghanistan (Gersbach and Reddy 2002) and in Iran. Ajowan fruits are an important commercial product for the food/flavoring industry and they accumulate up to 5% essential oil in compartments referred to as canals or vittae (Minija and Thoppil 2002). Composition of the essential oil has been analyzed extensively (Tecele and Abegaz 1983; Gersbach and Reddy 2002; Minija and Thoppil 2002). The essential oil is considered for its antimicrobial and insecticidal activity. Rani and Khullar (2004) showed antimicrobial activity of ajowan essential oil against multi-drug resistant *Salmonella typhi* (Schroeter). Fumigant toxicity of the essential oil has been studied on three stored-product beetles including, *Callosobruchus maculatus* F., *Sitophilus oryzae* L., and *T. castaneum* (Sahaf *et al.* 2007).

In the present study, chemical constituents of the essential oil from *C. copticum* were analyzed by GC and GC-MS. In addition, fumigant toxicity of the oil was assessed against eggs, larvae, pupae and adults of *P. interpunctella*.

MATERIALS AND METHODS

Extraction of the essential oil

Seeds of *C. copticum* were collected from the fields of Tarbiat Modares University, Tehran, Iran. Essential oil was extracted from dried seeds with a Clevenger-type apparatus (Cavalcanti *et al.* 2004; Negahban *et al.* 2007) where the seeds were subjected to hydrodistillation. Conditions of extraction were: 50 g of air-dried seeds; 1:10 plant ma-

terial: water volume ratio and 4 h distillation time. The resulting oil was desiccated over anhydrous sodium sulfate (10 min) and immediately placed into sealed plastic tubes (Negahban *et al.* 2006). Extracted oil was stored in cold dark in a refrigerator at 4°C.

Analysis of the essential oil

Gas chromatographic analysis was carried out with a Shimadzu GC-9A (Kyoto, Japan) with helium as a carrier gas and equipped with a DB-5 capillary column (30 m × 0.32 mm i.d × 0.25 µm film thickness). The column temperature was kept at 60°C for 3 min and then heated to 210°C at a rate of 3°C/min and held constant at this temperature for 8.5 min. Injector and flame ionization detector (FID) temperatures were 300°C and 280°C, respectively. The GC mass analysis was performed on a Varian 3400 equipped with a DB-5 column with the same characteristics as the one used in GC. The transfer line temperature was 260°C. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40–300 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. Identification of the constituents of the oil was made by comparison of their mass spectra and retention indices (RI) with those published in the literature (Adams 1995) and presented in the MS computer library.

Insect Culture

Indian meal moth was taken from a pistachio warehouse from Kerman province, Iran. Cultures were reared at 25 ± 1°C, 65 ± 5% RH and photoperiod of 16L: 8D in growth chamber (Binder 240L). Larvae were fed on pistachio kernels with initial moisture content at 5 ± 0.1% in 2100 ml hyaline plastic containers. Each container covered with a fine plastic mesh in its cap for good ventilation (Locatelli and Limonta 1998; Shojaaddini *et al.* 2005). In the bioassay experiments, uniformed insects; eggs (< 24 h old), fifth instar larvae (< 1 day old), pupae (1–5 days old) and adults (< 48 h old) were used.

Bioassays

Essential oil was applied on 2 cm diameter round filter papers inside the lids of glass jars, using a micro-sampler. No material was applied in control jars. In order to avoid loss of essential oil through evaporation, lids were sealed up using laboratory film (Parafilm). After dose-setting experiments, final doses with exposure time of 24 h were used. After exposure time, the mortality for larvae and adults were recorded 72 h after treatment, but for eggs and pupae, they were taken out of jars and were kept in clean plastic petri-dishes and final mortality counts were made one week later. A stereomicroscope was used to examine the eggs. All experimental procedures were carried out at 25 ± 1°C, 65 ± 5% RH and 16L: 8D. For each growth stages, ten homogenous insects were used in each replication and each test were replicated four times.

Statistical analysis

The data were corrected for the mortalities in controls (Abbott 1925) and were subjected to probit analysis to estimate LC₅₀ and LC₉₀ values (Finney 1971) using SAS statistical program (SAS Institute 1997).

RESULTS

Chemical composition of *C. copticum*

Eight compounds were identified in the oil of *C. copticum* representing 99.16% of the total oil, with thymol (64.51%), γ -terpinene (17.52%) and *p*-cymene (16.16%) as the major constituents (Table 1).

Table 1. Chemical composition of the essential oil from *Carum copticum* (Tehran, Iran)

| Compounds | Retention Index | Percentage |
|---------------------|-----------------|------------|
| thymol | 1332.84 | 64.51 |
| γ -terpinene | 1055.83 | 17.52 |
| <i>p</i> -cymene | 1026.59 | 16.16 |
| β -pinene | 975.57 | 0.39 |
| myrcene | 986.80 | 0.33 |
| α -thujene | 928.98 | 0.17 |
| α -pinene | 938.19 | 0.06 |
| sabinene | 972.16 | 0.02 |
| Other compounds | | 0.84 |

Fumigant toxicity

Values of LC_{50} in these experiments showed that the growth stages of Indian meal moth influenced by *C. copticum* essential oil were significantly different. Adult insects were about 500 times ($LC_{50} = 257.83 \mu\text{l/m}^3$ air and $LC_{90} = 598.94 \mu\text{l/m}^3$ air) more susceptible than those of other growth stages (Table 2, Fig. 1) with LC_{50} and LC_{90} values of 257.83 and 598.94 $\mu\text{l/m}^3$ air respectively. Adults in high concentrations, showed excitation by rapid moving of their wings and some were rotating the last segments of their abdomens mostly in females. Then they showed paralysis and were killed between 2–5 h after inhalation of the essential oil.

Table 2. LC_{50} and LC_{90} values of *Carum copticum* essential oil on growth stages of *Plodia interpunctella*

| Growth Stages | Intercept (\pm SE) | Slope (\pm SE) | LC_{50} (95% fiducial limits) | LC_{90} (95% fiducial limits) |
|---------------|-----------------------|-------------------|--|--|
| Eggs | -7.79 (\pm 1.42) | 3.44 (\pm 62) | 184.61 (155.31 – 217.81) ($\mu\text{l/l}$ air) | 435.32 (336.46 – 715.38) ($\mu\text{l/l}$ air) |
| Larvae | -6.80 (\pm .94) | 3.47 (\pm .47) | 91.36 (79.89 – 104.62) ($\mu\text{l/l}$ air) | 213.79 (172.09 – 304.27) ($\mu\text{l/l}$ air) |
| Pupae | -9.13 (\pm 1.55) | 4.51 (\pm .74) | 105.69 (91.67 – 118.04) ($\mu\text{l/l}$ air) | 203.24 (173.28 – 268.84) ($\mu\text{l/l}$ air) |
| Adults | -8.44 (\pm 1.54) | 3.50 (\pm .63) | 257.83 (221.35 – 297.08) ($\mu\text{l/m}^3$ air) | 598.94 (469.91 – 964.66) ($\mu\text{l/m}^3$ air) |

In the developmental stages, last instar larvae was the most susceptible to the essential oil ($LC_{50} = 91.36 \mu\text{l/l}$ air and $LC_{90} = 213.79 \mu\text{l/l}$ air). In higher concentrations, due to acute toxicity of the fumigant, treated larvae turned brown and did not move 24 h after treatment. In mid concentrations, survived larvae showed simultaneous browning for about 48–72 h after exposure and finally they were desiccated before dying. At low concentrations, the larvae showed no visible acute symptoms but all of them even those treated by the lowest concentration, failed to feeding and pupation due to sub-lethal effects of the essential oil.

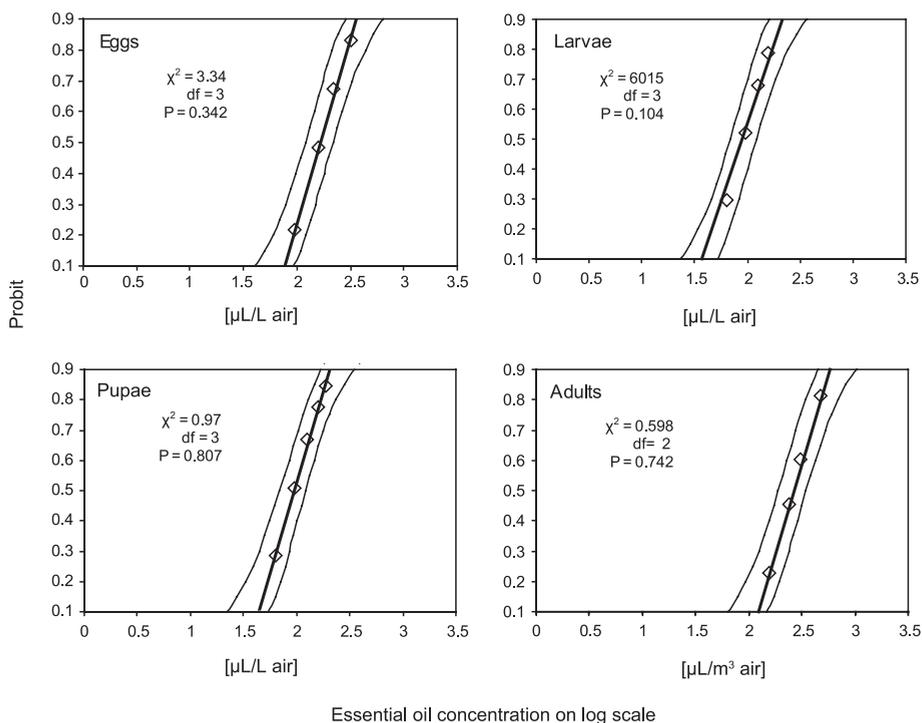


Fig. 1. Regression line plots with 95% fiducial limits for different concentrations of *Carum copticum* essential oil on *Plodia interpunctella* 24 h after exposure

LC_{50} and LC_{90} values of pupae were found to be 105.69 and 203.24 $\mu\text{l/l}$ air, respectively. Died pupae compared with healthy ones did not turn dark and remained bright brownish because they were killed before their histogenesis stage was completed. Based on the overlap of the 95% fiducial limits of LC_{50} values, the susceptibility of larvae and pupae, to the oil was not significantly different (Table 2, Fig. 2). However, they were more susceptible than those of the eggs (Fig. 2). The results showed that LC_{50} and LC_{90} values for the eggs were to be 184.61 and 435.32 $\mu\text{l/l}$ air respectively. Died eggs showed brown color and consequently did not hatch.

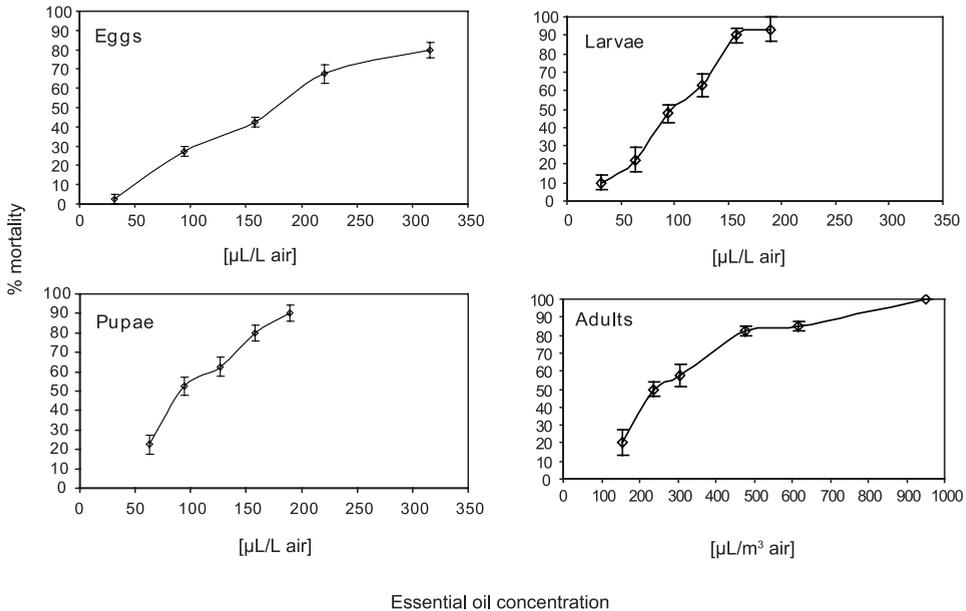


Fig. 2. Percentage mortality of *Plodia interpunctella* exposed to various concentrations of the *Carum copticum* essential oil. Vertical bars indicate standard error of the mean

DISCUSSION

Many plant species produce various chemical compounds that could be repellent or deterrent or even toxic for plant feeding insects. Some of these compounds are also toxic to the plant itself, and therefore they are stored in special organs such as flowers and seeds. These chemical weapons are aimed directly against the plant feeding animal.

Plant defense compounds belong to a wide variety of chemical compound groups like phenolics, terpenes, alkaloids and glucosinolates. The composition of ajowan essential oil has been reported to contain up to 55% of the aromatic monoterpene thymol, and there are significant amounts of the thymol precursors *p*-cymene and γ -terpinene. The comparison between the results of GC analysis to the other reports shows that ajowan from different regions of the world have contained different amounts of constituents. Carvacrol (54.4%), γ -terpinene (28.3%) and *p*-cymene (15.4%) are major components of African ajowan (Teclé and Abegaz 1983) whereas Minija and Thoppil (2002) showed that ajowan seeds from south India have contained 97.9% thymol. However, the Iranian ajowan in the present study contained 64.51% thymol, 17.52% γ -terpinene and 16.16% *p*-cymene.

Among the major monoterpenoids of essential oil, it was reported that γ -terpinene and thymol are the most active constituents against adults of the rice weevil, *S. oryzae* (Erlér 2007). The rapid action against Indian meal moth may be indicative of a neurotoxic mode of action. There is evidence for the octopaminergic system as a target for some monoterpenoids (Enan 2001). However, it is necessary to elucidate

whether the toxic action of thymol and γ -terpinene is mediated through octopamine. The octopamine is a multifunctional, naturally occurring biogenic amine that plays a key role as a neurotransmitter, neurohormone and neuromodulator in invertebrate systems such as in insects (Evans 1980; Orchard 1982).

Our results clearly indicate variations in the activity of ajowan essential oil regarding the growth stage of Indian meal moth. The great promise is that the essential oil of *C. copticum* proved to be a highly toxic fumigant even at low concentrations and to be an active control agent against *P. interpunctella* adults. A concentration of 598 $\mu\text{l}/\text{m}^3$ air with exposure time of 24 h was enough to get 90% adult mortality, compared with the recommended concentration of methyl bromide of 30–50 g/m^3 . The essential oil investigated in this study is used as pharmaceutical (Thangam and Dhananjayan 2003) and in flavoring (Sahaf *et al.* 2007) and is therefore considered to be less harmful to humans than most conventional insecticides. Furthermore, studies have shown that it is readily biodegradable and less detrimental to non-target organisms than conventional chemical pesticides (Tunc *et al.* 2000). However, the possibility of employing this natural fumigant in the management of Indian meal moth is plausible, but is worthy of further investigation. Future research should focus on residues on target commodity and the influence of any residues on product acceptability.

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POLISH SUMMARY

TOKSYCZNOŚĆ PAR OLEJKU ETERYCZNEGO Z *CARUM COPTICUM* W ZWALCZANIU MKLIKA RODZYNKOWCA, *PLODIA INTERPUNCTELLA*

Oceniano toksyczność oparów olejku eterycznego otrzymanego z *Carum copticum* C.B. Clarke (*Apiaceae*) w stosunku do mklika rodzynekowca, *Plodia interpunctella* (Hübner) (*Lepidoptera: Pyralidae*) w różnych stadiach rozwoju. Nasiona rośliny zabrano w Teheranie (Iran), a następnie destylowano z parą wodną w celu ekstrakcji olejku eterycznego. Olejek analizowano za pomocą GC i GC-MS. Spośród ośmiu komponentów, głównymi składnikami olejku były tymol (64.51%), γ -terpinen (17.52%) i *p*-cymen (16.16%) stanowiąc 98.19% całości.

Wszystkie testy biologiczne wykonano w temperaturze $25 \pm 1^\circ\text{C}$ i przy względnej wilgotności powietrza $\text{RH} = 65 \pm 5\%$ oraz fotoperiodzie 16:8 h (dzień: noc). Po wstępnych eksperymentach w celu ustalenia dawek, wyliczono wartości LC_{50} i LC_{90} dla czterech stadiów rozwojowych, mianowicie jaj, larw, poczwerek i dorosłych osobników. Rezultaty wykazały wyraźne różnice w toksyczności w zależności od stadium rozwojowego. Dorosłe owady były o około 500 razy ($\text{LC}_{50} = 257.83 \mu\text{L}/\text{m}^3$ powietrza a $\text{LC}_{90} = 598.94 \mu\text{L}/\text{m}^3$ powietrza) podatne niż inne stadia. Ponadto, larwy ostatniej fazy ($\text{LC}_{50} = 91.36 \mu\text{L}/\text{m}^3$ powietrza i $\text{LC}_{90} = 213.79 \mu\text{L}/\text{m}^3$ powietrza) i poczwarki ($\text{LC}_{50} = 105.60 \mu\text{L}/\text{m}^3$ powietrza i $\text{LC}_{90} = 203.24 \mu\text{L}/\text{m}^3$ powietrza) były bardziej wrażliwe niż jaja ($\text{LC}_{50} = 184.61 \mu\text{L}/\text{m}^3$ powietrza i $\text{LC}_{90} = 435.32 \mu\text{L}/\text{m}^3$ powietrza). Powyższe badania wskazują, że olejek eteryczny z nasion *C. copticum* może mieć potencjalne, praktyczne zastosowanie w ochronie magazynów żywności przed mklikiem rodzynekowcem.