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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 ± 1°C, 65±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 µl/m^3 air and LC_{90} = 598.94 µl/m^3 air) more susceptible than other growth stages. Moreover, last instar larvae (LC_{50} = 91.36 µl/l air and LC_{90} = 213.79 µl/l air) and pupae (LC_{50} = 105.69 µl/l air and LC_{90} = 203.24 µl/l air) were significantly more susceptible than eggs (LC_{50} = 184.61 µl/l air and LC_{90} = 435.32 µl/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 (Tecle 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 240 L). 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 LC_{50} and LC_{90} 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
p-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 μl/m^3 air and LC_{90} = 598.94 μl/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 μl/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 (± SE)	Slope (± SE)	LC_{50} (95% fiducial limits)	LC_{90} (95% fiducial limits)
Eggs	–7.79 (±1.42)	3.44 (± 62)	184.61 (155.31 – 217.81) (μl/l air)	435.32 (336.46 – 715.38) (μl/l air)
Larvae	–6.80 (±.94)	3.47 (±.47)	91.36 (79.89 – 104.62) (μl/l air)	213.79 (172.09 – 304.27) (μl/l air)
Pupae	–9.13 (±1.55)	4.51 (±.74)	105.69 (91.67 – 118.04) (μl/l air)	203.24 (173.28 – 268.84) (μl/l air)
Adults	–8.44 (±1.54)	3.50 (±.63)	257.83 (221.35 – 297.08) (μl/m^3 air)	598.94 (469.91 – 964.66) (μl/m^3 air)

In the developmental stages, last instar larvae was the most susceptible to the essential oil (LC_{50} = 91.36 µl/l air and LC_{90} = 213.79 µl/l air). In higher concentrations, due to acute toxicity of the fumigant, treated larvae turned brown and did not move 24 h after teatment. 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.

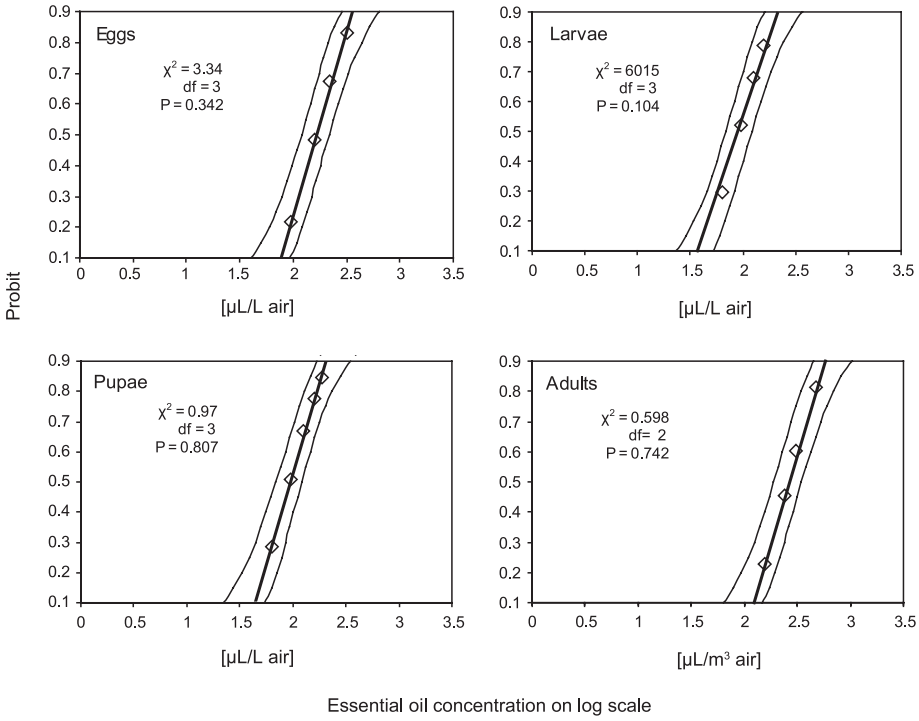

Fig. 1. Regression line plots with 95% fiducial limits for different concentrations of *Carum copticum* essential oil on *Plodia interpunctella* 24 h after exposure

LC_{50} and LC_{90} values of pupae were found to be 105.69 and 203.24 µl/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 limitsof 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 µl/l air respectively. Died eggs showed brown color and consequently did not hatch.

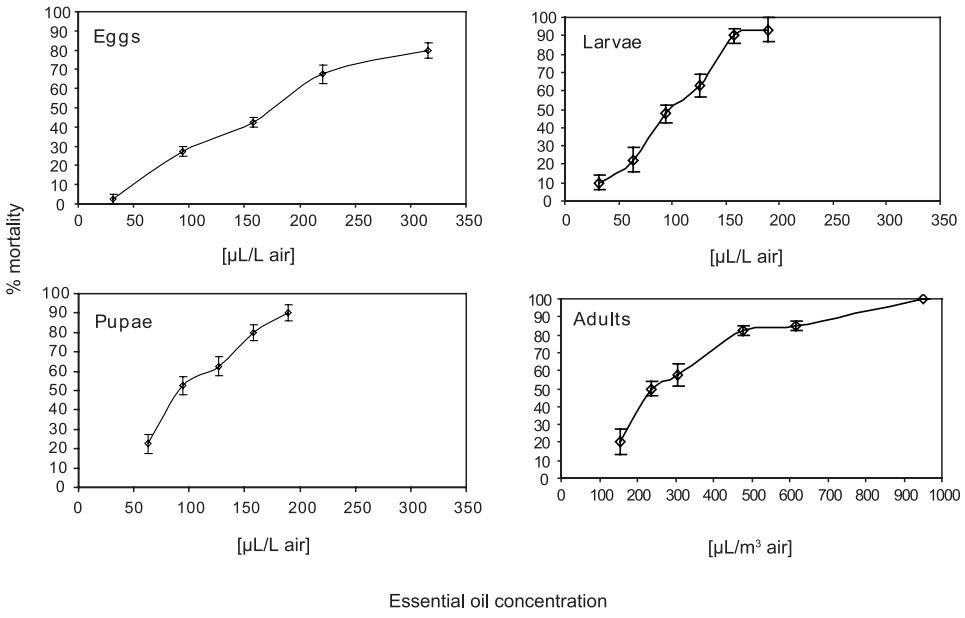

Fig. 2. Percentage mortality of *Plodia interpunctella* exposed to various concentrations of the *Carum copticum* essential oil. Vertical bars indicate standard error of the mean

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 (Tecle 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* (Erler 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 monotrpinoids (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 µl/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.

REFERENCES

Abbott W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Adams R.P. 1995. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Carol Stream, IL, USA, 469 pp.

Cavalcanti E.S.B., Morais S.M., Lima M.A.A., Santana E.W.P. 2004. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. Mem. Inst. Oswaldo Cruz, Rio de Janeiro 99: 541–544.

Cline L.D. 1978. Penetration of seven common flexible packing materials by larvae and adults of eleven species of stored-product insects. J. Econ. Entomol. 71: 726–729.

Cuperus G.W., Noyes R.T., Fargo W.S., Clary B.L., Arnold D.C., Anderson K. 1990. Management practices in a high-risk stored-wheat system in Oklahoma. Am. Entomol. 36: 129–134.

Doud C.W., Phillips T.W. 2000. Activity of *Plodia interpunctella* (Lepidoptera:Pyralidae) in and around flour mills. J. Econ. Entomol. 93: 1842–1847.

Enan E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. Part C. 130: 325–337.

Erler F. 2007. Fumigant activity of monoterpenoides against the rice weevil, *Sitophilus oryzae.* p. 281–285. In: Working Group "Integrated Protection of Stored Products" (S. Navarro, C. Adler, J. Riudavets, V. Stejskal, eds.). 20–23 September 2005, IOBC/WPRS, Prague, Czech Republic, Bull. 30 (2).

Evans P.D. 1980. Biogenic amines in the insect nervous system. Adv. Insect Physiol. 15: 317–473.

Finney D.J. 1971. Probit analysis, 3rd Edition. Cambridge University Press, London, 333 pp.

Gersbach P.V., Reddy N. 2002. Non-invasive localization of thymol accumulation in *Carum copticum* (Apiaceae) fruits by chemical shift selective magnetic resonance imaging. Ann. Bot. 90: 253–257.

Johnson J.A., Wofford P.L., Whitehand L.C. 1992. Effect of diet and temperature on development rates, survival, and reproduction of the Indian meal moth (Lepidoptera: Pyralidae). J. Econ. Entomol. 85: 561–566.

Kim D.H., Ahn Y.J. 2001. Contact and fumigant activities of constituents of *Foeniculum vulgare* fruit against three coleopteran stored-product insects. Pest Manag. Sci. 57: 301–306.

Lecato G.L. 1976. Yield, development, and weight of *Cadra cautella* (Walker) and *Plodia interpunctella* (Hübner) on twenty-one diets derived from natural products. J. Stored Prod. Res. 12: 43–47.

Lee S.E., Lee B.H., Choi W.S., Park B.S., Kim J.G., Campbell B.C. 2001. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus orysae* (L). Pest Manag. Sci. 57: 548–553.

Locatelli D.P., Limonta L. 1998. Development of *Ephestia kuehniella*, *Plodia interpunctella* and *Cotcyra cephalonia* (Lepidoptera: Pyralidae) on kernels and whole meal flours of *Fagopyrum esculentum* and *Triticum aestivum*. J. Stored Prod. Res. 34: 269–276.

Minija J., Thoppil J.E. 2002. Essential oil composition of *Trachyspermum ammi* (L) Sprague from South India. Indian J. Pharmaceut. Sci. 64: 250–251.

Mullen M.A., Arbogast R.T. 1979. Time-temperature-mortality relationships for various stored product insect eggs and chilling times for selected commodities. J. Econ. Entomol. 72: 476–478.

Na J.H., Ryoo M.I. 2000. The influence of temperature on development of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried vegetable commodities. J. Stored Prod. Res. 36: 125–129.

Nansen C., Phillips T.W. 2004. Attractancy and toxicity of an attracticide for Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). J. Econ. Entomol. 97: 703–710.

Nansen C., Phillips T.W., Parajulee M.N., FranquiRivera R.A. 2004. Comparison of direct and indirect sampling procedures *Plodia interpunctella* in a corn storage facility. J. Stored Prod. Res. 40: 151–168.

Negahban M., Moharramipour S., Sefidkon F. 2006. Insecticidal activity and chemical composition of *Artemisia sieberi* Besser essential oil from Karaj, Iran. J. Asia-Pacific Entomol. 9: 61–66.

Negahban M., Moharramipour S., Sefidkon F. 2007. Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. J. Stored Prod. Res. 43: 123–128.

Orchard I. 1982. Octopamine in insects: neurotransmitter, neurohormone and neuromodulator. Can. J. Zool. 60: 659–669.

Popławski J., Łozowicka B., Dubis A., Lachowska B., Winiecki Z., Nawrot J. 2000. Feeding-deterrent activity of α-asarone isomers against some stored Coleoptera. Pest Manag. Sci. 56: 560–564.

Rani P., Khullar N. 2004. Antimicrobial evaluation of some medicinal plants for their antienteric potential against multi-drug resistant *Salmonella typhi*. Phytother. Res. 18: 670–673.

Sahaf B.Z., Moharramipour S., Meshkatalsadat M.H. 2007. Chemical constituents and fumigant toxicity of essential oil from *Carum copticum* against two stored product beetles. Insect Sci. 14: 213–218.

Sauer J.A., Shelton M.D. 2002. High temperature controlled atmosphere for post-harvest control of Indian meal moth (Lepidoptera: Pyralidae) on preserved flowers. J. Stored Prod. Res. 95: 1074–1078.

Shaaya E., Kostjukovski M., Eilberg J., Sukprakarn C. 1997. Plant oils as fumigants and contact insecticides for the control of stored- product insects. J. Stored Prod. Res. 33: 7–15.

Shojaaddini M., Farshbaf Pour Abad R., Haddad Irani Nejad K., Mohammadi S.A. 2005. Effects of different photoperiods on some biological parameters of Indian meal moth *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) on fried and unfried pistachio cultivars. Turkish J. Entomol. 29: 279–287.

Singh G., Upadhyay R.K. 1993. Essential oils: a potent source of natural pesticides. J. Sci. Indust. Res. 52: 676–683.

Thangam C., Dhananjayan R. 2003. Antiinflammatory potential of the seeds of *Carum copticum* Linn. Indian J. Pharmacol. 35: 388–391.

Tunc I., Berger B.M., Erler E., Dagli F. 2000. Ovicidal activity of essential oils from five plants against two stored-product insects. J. Stored Prod. Res. 36: 161–168.

POLISH SUMMARY

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

Oceniano toksyczność oparów olejku eterycznego otrzymywanego z *Carum copticum* C.B. Clarke (*Apiaceae*) w stosunku do mklika rodzynkowca, *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 ± 1°C i przy względnej wilgotności powietrza RH = 65±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, poczwarek i dorosłych osobników. Rezultaty wykazały wyraźne różnice w toksyczności w zależności od stadium rozwojowego. Dorosłe owady były o około 500 razy (LC_{50} = 257.83 μL/m^3 powietrza a LC_{90} = 598.94 μL/m^3 powietrza) podatne niż inne stadia. Ponadto, larwy ostatniej fazy (LC_{50} = 91.36 μL/m^3 powietrza i LC_{90} = 213.79 μL/m^3 powietrza) i poczwarki (LC_{50} = 105.60 μL/m^3 powietrza i LC_{90} = 203.24 μL/m^3 powietrza) były bardziej wrażliwe niż jaja (LC_{50} = 184.61 μL/m^3 powietrza i LC_{90} = 435.32 μL/m^3 powietrza). Powyższe badania wskazują, że olejek eteryczny z nasion *C. copticum* może mieć potencjalne, praktyczne zastosowanie w ochronie magazynów żywności przed mklikiem rodzynkowcem.