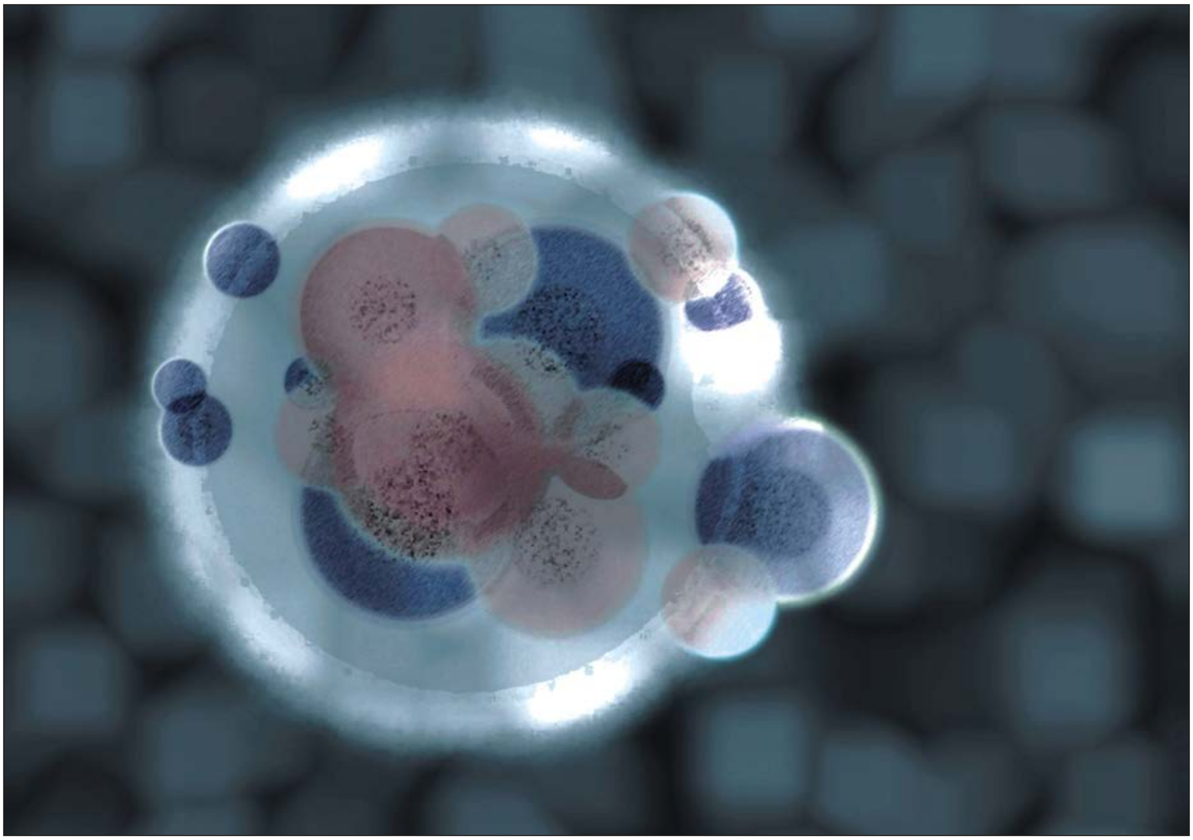

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 9 (1): 92-95, 2009
ISSN 1727-3048

Fumigant Toxicity of *Ziziphora clinopodioides* (Boiss.) (Lamiaceae) Against Adults and Eggs of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae)

[1]F.A. Lolestani and [2]N. Shayesteh
[1]Department of Entomology, College of Agriculture, Urmia University, P.O. Box 57135-165, Urmia, Iran
[2]Department of Plant Protection, College of Agriculture, Islamic Azad University,
Branch of Mahabad, P.O. Box 57135-165, Mahabad, Iran

Abstract: The insecticidal and ovicidal effects of essential oil extracted from *Ziziphora clinopodioides* (Boiss.) (Lamiaceae) were tested on adults and eggs of *Callosobruchus maculatus* (Fab.). Oil concentrations of 9, 12.5, 17.6, 24.5 and 34.2 μl L^{-1} air were tested on adults while concentrations of 3.5, 5.8, 9.7, 16.1 and 26.7 μl L^{-1} air were tested on eggs. Adults and eggs were exposed for 24, 48 and 72 h. After each exposure, insecticide effect was estimated by counting the number of dead adults of *C. maculatus* while ovicidal effect was estimated by counting the number of unhatched eggs. Results showed that the oil had high fumigant action against adults and eggs, the adults being more susceptible than the eggs. After 72 h of exposure to an oil concentration of 34.2 μl L^{-1} air , the adult mortality was 94.65% while the egg mortality was 61.10% for an oil concentration of 26.7 μl L^{-1} air. The lowest values after 72 h were observed on adults of *C. maculatus* (Fab.) (4.01). The LC_{50} amount for eggs at this time was 16.98 μl L^{-1} air. Progeny was reduced by 57.76% after a 72 h exposure of oil at a concentration of 34.2 μl L^{-1} air. Fumigant effects of this essential oil were considered to warrant further research into their potential for commercial use.

Key words: *Callosobruchus maculatus* (Fab.), essential oil, fumigant toxicity, *Ziziphora clinopodioides*

INTRODUCTION

Pest control in many storage systems depends on fumigation with either methyl bromide or phosphine. The use of methyl bromide is being restricted because of its potential to damage the ozone layer. The future use of phosphine could be threatened by the development of resistant strains (Bell and Wilson, 1995). Essential oils are potential alternatives to current stored-grain fumigants because of their low toxicity to warm-blooded mammals and their high volatility (Marcus and Lichtenstein, 1979; Shaaya *et al*., 1991, 1997). Plant extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects in insects (Isman, 2006). Earlier studies have assessed fumigant activity of essential oils on adults and larvae and recently researchers have described the contact and fumigant toxicity of essential oils or their major components against eggs of stored-product insects (Huang *et al*., 1997, 2000; Tunc *et al*., 2000). The most promising botanical groups for pest control are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, Aristolochiaceae and Malvaceae (Schmutterer, 1990). *Ziziphora clinopodioides*, common Persian name "Kakuti", is widespread all over Iran and includes nine subspecies native to Iran. The composition, anti bacterial and anti oxidant activity of the essential oil and various extracts of *Z. clinopodioides* were already reported by Ozturk and Ercisli (2007) and Salehi *et al*. (2005). In this study, the toxicity of essential oil extracted from *Z. clinopodioides* was tested against the adults and eggs of *Callosobruchus maculatus* (Fab.). In our best knowledge, no referenced data are available about the effect of the essential oil of this plant on storage pest especially on bruchid beetles.

MATERIALS AND METHODS

Insect culture: The initial stock culture of *C. maculatus* was obtained from the Department of Plant Protection, College of Agriculture, Urmia University, Urmia, Iran. Cowpea grains of the variety Kamran were cleaned and disinfested by storage at -12°C for a week. *C. maculatus* was reared on cowpea seeds. The cultures were maintained in a growth chamber set at 27±1°C and 65±5% of relative humidity (r.h). Adult insects and eggs, both less than 24 h old were used for the toxicity tests. All experiments were carried out under the same conditions at the continues darkness.

Corresponding Author: F.A. Lolestani, Department of Entomology, College of Agriculture, Urmia University, P.O. Box 57135-165, Urmia, Iran Tel: +98 441 277 9552 Fax: +98 441 277 99558

Source and preparation of oil: A sufficient quantity of *Z. clinopodioides*, extracted by steam distillation was obtained from Golbahar Co., Urmia, West Azabaijan, Iran.

Experimental technique: This study was conducted in 2008. To determine the fumigant toxicity of the essential oil extracted from *Z. clinopodioides*, glass jars of 500 mL capacity with screwed lids, were used as exposure chambers. The substances were applied with a micro pipette on to filter paper attached to the lower surface of the lid of the jars, which were then tightly sealed. Untreated filter papers (without oil) were used in the control jars. Exposures doses for adults and eggs were as follow: 9, 12.5, 17.6, 24.5 and 34.2 µl L^{-1} air for adults and 3.5, 5.8, 9.7, 16.1 and 26.7 µl L^{-1} air for eggs. Mentioned doses were supplied on the basis of preliminary tests (Robertson and Preisler, 1992). Thirty adults of *C. maculatus* were introduced in small tubes (2 cm diameter, 6 cm high) containing 30 cowpea seeds and secured with nylon mesh. The tubes were placed the bottom of the glass jars. For ovicidal test, 30 cowpea seeds with one egg on each seed were placed in small tubes (30 eggs in each jar). All experiments carried out in 27±1°C and 65±5% r.h. After exposure for 24, 48 or 72 h, the tubes containing insects were taken out of the jars. Insect mortality was assessed by counting insects that showed count no leg or antennal movements. For ovicidial test, unhatched eggs after 7 days were counted as dead. All adults (dead and alive) were removed from the tubes and the tubes were left in the growth chamber at the same conditions for a further 35 days to assess progeny production. Progeny production was assessed from eggs layed by the adults used in the experiments. No mortalities of both adults and eggs were observed in untreated controls except than 2.5% for 72 h, in *C. maculatus* for adults. Each concentration and control was replicated four times for every experiment.

Statistical analysis: Experiments were carried out adopting a factorial design. The mortality counts were corrected by using Abbott's (1925) formula. Percentage of reduction in progeny production was determined by the [(No. progeny in control-No. progeny in treatment)/ No. progeny in control×100] formula (Aldryhim, 1990). The data were analyzed using Analysis of Variance (ANOVA). The Duncan's Multiple Range Test was used at p = 0.05 to identify differences among multiple means (SAS, 2000). To equalize variances, mortality percentage of adults and eggs and percentage of reduction in progeny production were transformed using the square root of the arcsin. Probit analysis (SPSS, 1999) was used to estimate LC_{50} and LC_{95} values.

RESULTS AND DISCUSSION

In all cases, a direct relationship between concentrations and mortalities was observed. Results showed that the oil was relatively more toxic against adults of *C. maculatus*. Adult mortality above 90% was observed when insects were exposed to oil at a concentration of 34.2 µl L^{-1} air for 72 h (Table 2). The highest egg mortality (61.1%) was obtained after 72 h of exposure with an oil concentration of 26.7 µl L^{-1} air (Table 3). The lowest concentration (9 µl L^{-1} air) of the oil caused 71.78% mortality in adults of *C. maculatus* (Fab.) after 72 h exposure, (Table 2), but the mortality eggs of *C. maculatus* (Fab.) at the lowest concentration (3.5 µl L^{-1} air) of the oil was 22.21% after 72 h of exposure (Table 3).

All main effects and associated interactions were significant at the $p<0.0001$ level for adults (rate: df = 4. 59; F = 15.30; exposure: df = 2. 59; F = 67.94; rate× exposure: df = 8. 59; F = 0.26) and eggs (rate : df = 4. 59: F = 72.37; exposure: df = 2. 59; F = 59.83; rate × exposure: df = 8. 59; F = 1.38) of *C. maculatus*. The parameters of the probit analysis, LC_{50} and LC_{95} are given in Table 1. Probit analysis showed that adults of *C. maculatus* were more susceptible (LC_{50} = 4.01 µl L^{-1} air, LC_{95} = 39.90 µl L^{-1} air) to *Z. clinopodioides* oil after 72 h than their eggs (LC_{50} = 16.98 µl L^{-1} air, LC_{95} = 436.60 µl L^{-1} air).

The highest and lowest progeny reduction observed in adults of *C. maculatus* treated with the essential oil of *Z. clinopodioides* after 72 and 24 h were 57.76% for an oil concentration of 34.2 µl L^{-1} air and 11.67% for an oil concentration of 9 µl L^{-1} air , respectively (Table 4). For the percentage of progeny reduction of *C. maculatus*, all main effects as well as all associated interaction were significant at the $p<0.0001$ (rate: df = 4. 59; F = 16.07 ; exposure: df = 8.59; F = 18.98; rate×exposure: df = 8.59; F = 0.31).

The results showed a high-mortality rate in adults of *C. maculatus* a compared to eggs. In present experiment, adults of *C. maculatus* showed 94.65% mortality 72 h after treatment with the essential oil of *Z. clinopodioides* at a concentration of 34.2 µl L^{-1} air. In contrast egg mortality was 61.10% after exposure of the essential oil of *Z. clinopodioides* at a concentration of 26.7 µl L^{-1} air for 72 h. Keita *et al*. (2001) also reported a higher susceptibility of adults of *C. maculatus* as compared to eggs. At a dose of 25 µl L^{-1} air, 80% mortality was recorded for *Ocimum gratissimum* (Lamiaceae), but the egg hatch rate was reduced to 3% with *Ocimum basilicum* and 15% with *O. gratissimum* using a concentration of 30 µl L^{-1} air. In all cases, considerable differences in

Table 1: Probit analysis for fumigant toxicity of *Z. clinopodioides* against adults and eggs of *C. maculatus*

	LC_{50} (µl L^{-1}air)	LC_{95} (µl L^{-1} air)	Probit parameters±SE: Intercept	Slope	p- value	Chi-square
Adult	4.01	39.9	4.00±0.39	0.32±1.64	0.81	0.96
Egg	16.98	463.60	3.59±0.21	1.14±0.20	0.90	0.57

Table 2: Mean mortality (%±SE) of *C. maculatus* (Fab.) adult treated with *Z. clinopodioides* oil at after 24, 48 and 72 h of exposure

	Dose (µl L^{-1}air)				
Hour	9	12.5	17.6	24.5	34.2
24	37.49±6.57h	47.49±2.84fgh	55.83±2.09fg	59.99±5.27ef	64.16±1.59def
48	40.83±6.99gh	49.99±7.20fgh	57.49±4.16efg	59.99±4.71ef	75.83±4.58bcd
72	71.78±7.16cde	79.48±3.69bc	86.34±2.43bc	88.00±2.20ab	94.65±2.16a

Means followed by the same letter(s) in the row are not significantly different (Duncan's Multiple Range Test at p = 0.05)

Table 3: Mean mortality (%±SE) of *C. maculatus* (Fab.) eggs treated with *Z. clinopodioides* oil at after 24, 48 and 72 h of exposure

	Dose (µl L^{-1} air)				
Hour	3.5	5.8	9.7	16.1	26.7
24	12.03±0.92g	15.73±0.92fg	22.21±1.5ef	26.84±1.77de	35.18±3.20c
48	15.73±0.92fg	19.44±3.81f	35.17±2.39c	37.03±3.02c	46.29±4.40b
72	22.21±1.51ef	30.55±1.77cd	37.95±3.16c	46.29±2.39b	61.10±2.39a

Means followed by the same letter(s) in the row are not significantly different (Duncan's Multiple Range Test at p = 0.05)

Table 4: Mean percentage of reduction (%±SE) in progeny production (F_1) of *C. maculatus* (Fab.) treated with *Z. clinopodioides* oil at after 24, 48 and 72 h of exposure

	Dose (µl L^{-1} air)				
Hour	9	12.5	17.6	24.5	34.2
24	11/67±4/07h	25/6±2/23efg	25/6±2/23efg	30/98±5/62cdefg	32/41±3/45bcdef
48	21/80±1/71g	27/66±1/9defg	33/9±3/16bcdef	36/46±4/07bcde	42/66±3/99abc
72	24/99±5/04fg	33/9±4/46bcdef	40/08±2/6abcd	44/21±5/96ab	57/76±5/27a

Means followed by the same letter(s) in the row are not significantly different (Duncan's Multiple Range Test at p = 0.05)

mortality of adults and eggs to essential oil were observed with different concentrations and times. For instance in present study, for adults at 34.2 µl L^{-1} air, the mortality after 24, 48 and 72 h was 64.16, 75.83 and 94.65% (Table 2). For eggs, at the highest concentration 26.7 µl L^{-1} air, mortality after 24, 48 and 72 h was 35.18, 46.29 and 61.10% (Table 3). There are many reviews dealing with the use of plant products in general, against insect pest of stored products (Isman, 2006). Studies have not been reported previously concerning the activity of *Z. clinopodioides* as a fumigant on storage pests. The fumigant activity of essential oils from other species of Lamiaceae has been evaluated against a number of stored product insects. The essential oil of *O. basilicum*, at 90 µL , 96% mortality on *C. maculatus* (Fab.) was observed (Keita *et al*., 2000). It has been reported that SEM76 and ZP51, essential oils from plants belonging to Lamiaceae, were relatively effective against several stored product insects (Shaaya *et al*., 1991, 1997). The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they have fumigant activity that might be of importance for controlling stored-product insects (Regnault-Roger and Hamraoui, 1995). The main constituents of essential oil extracted from *Z. clinopodioides* are reported oxygenated monoterpenes (94.3%) were the predominant fraction of the oil with pulegon (65.2%), isomenthone (11.9%), 1, 8-cineole (7.8%) and piperitenone (6.5%) as the main constituents (Salehi *et al*., 2005). Antibacterial activity of the oil and also its two main components (pulegone and 1, 8-cineole) were tested against seven bacteria (Salehi *et al*., 2005). 1, 8-cineol from *Ocimum kenyense* (Ayobangira) (Obeng-Ofori *et al*., 1997) is toxic and repellent against some stored product beetles.

As a result, it may be possible to use this essential oil commodities packed in non-absorbent materials or for disinfestations where higher standards of workers and environmental safety are sought. In future, the main components of essential oils that have insecticidal activity may be formulated and used in field levels.

REFERENCES

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

Aldryhim, Y.N., 1990. Efficacy of the amorphous silica dust, dryacide against *Tribolium confusum* DuV. and *Sitophilus granarius* (L.) (Coleoptera: Tenebrionidae and Curculionidae). J. Stored Prod. Res., 26: 207-210.

Bell, C.H. and S.M. Wilson, 1995. Phosphine tolerance and resistance in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). J. Stored Prod. Res., 31: 199-205.

Huang, Y., J.M.W.L. Tan, R.M. Kini and S.H. Ho, 1997. Toxicity and antifeedant action of nutmeg oil against *T. castaneum* (Herbst) and *Sitophilus Zeamais* Motsch. J. Stored Prod. Res., 33: 289-298.

Huang, Y., S.L. Lam and S.H. Ho, 2000. Bioactivities of essential oil from *Elletaia cardamomum* (L.) Maton. to *Sitophilus zeamais* Motschusky and *Tribolium castaneum* (Herbst). J. Stored Prod. Res., 36: 107-117.

Isman, M.B., 2006. The roll of botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol., 51: 45-66.

Keita, S.M., C. Vincent, J.P. Schmit, S. Ramaswamy and A. Belanger, 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res., 36: 355-364.

Keita, S., V. Charles, P. Jeen, T. John and B. Andre, 2001. Efficacy of essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculates* (fab.). J. Stored Prod. Res., 37: 339-349.

Marcus, C. and P. Lichtenstein, 1979. Biologically active components of anise: Toxicity and interaction with insecticides in insects. J. Agri. Food. Chemis., 27: 1217-1223.

Obeng-Ofori, D., C.H. Reichmuth, J. Bekele and A. Hassanali, 1997. Biological activity of 1, 8-cineol a major component of essential oil of *Ocimum kenyense* (Ayobangria) against stored product beetles. J. Applied Entomol., 121: 237-243.

Ozturk, S. and S. Ercisli, 2007. Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. J. Food Control, 18: 535-540.

Regnault-Roger, C. and A. Hamraoui, 1995. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera) a bruchid of kidney bean (*Phaseolus vulgaris* L.). J. Stored Prod. Res., 31: 291-299.

Salehi, P., A. Sonboli, F. Eftekhar, S. Nejad-Ebrahimi and M. Yousefzadi, 2005. Essential oil composition, antibacterial and antioxidant activity of the oil and various extracts of *Ziziphora clinopodioides* subsp. rigida (Boiss.) RECH. f. From Iran. Biol. Pharm. Bull., 28: 1892-1896.

SAS, 2000. The SAS System for Windows. Version 7, SAS Institute, Cary, NC.

Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachata indica*. Ann. Rev. Entomol., 35: 271-297.

Shaaya, E., U. Ravid, N. Paster, B. Juven, U. Zisman and V. Pissarev, 1991. Fumigant toxicity of essential oils against four major stored-product insects. J. Chem. Ecol., 17: 499-504.

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

SPSS, 1999. SPSS for Windows User,s Guide Release 10. SPSS Inc, Chicago.

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