Molluscicidal Activity and Biochemical Effects of Certain Monoterpenoids against Land Snails

Hussein, H. I., E. H. Eshra and Y. Abo Bakr
Department of Agricultural Animal Pests, Plant Protection Research Institution, Sabahia, 21616, Baccous, Alexandria, Egypt.

ABSTRACT

The monoterpenoid compounds fenchone, fenchol and thymol were tested for their molluscicidal activity against two terrestrial snails *Eobania vermiculata* (Müller) and *Theba pisana* (Müller). Fenchone exhibited molluscicidal activity against both of tested snails *T. pisana* and *E. vermiculata* with LD_{50} values of 527.93 and 552.38 µg/g body weight, respectively. Fenchol was active against *E. vermiculata* with LD_{50} 664.22 µg/g body weight, while thymol exhibited molluscicidal action against *T. pisana* (LD_{50} = 516.15 µg/g body weight). Sub-lethal doses of fenchone caused marked decrease in protein level of the two snail species. Aspartate transaminase (AST) and alanin transaminase (ALT) activities were stimulated by the most tested doses of fenchone. Response of ALT toward fenchone treatments was more sensitive than AST. The two tested enzymes of *E. vermiculata* were more responsive than those of *T. pisana* to fenchone treatments.

INTRODUCTON

Terrestrial gastropod mollusk species are currently constitute some of t the most significant threats to sustainable agriculture in many parts of the world (Barker, 2002). Land Snails are important pests of a wide range of agricultural and horticultural crops in temperate and humid habitats world-wide (Speiser and Kistler, 2002). They attack leaves, roots, buds, flowers, fruits and even the trunk of trees causing great damage to the cultivated plants (Abdallah *et al.*, 1998). Damage caused by snails is due to feeding and to contamination with their bodies, faeces or slime, leading to deterioration of the product quality beside the financial loss (Iglesias *et al.*, 2003). In addition, rotting agents such as bacteria, viruses and fungi usually establish themselves at places where snails have fed, so that fruits and vegetables in storage suffer further damage (Bundy, 1967). In Egypt, the importance of land snails as pest organisms has drastically increased in the past few decades (Kassab and Daoud, 1964; El-Okda, 1980; Abdallah *et al.*, 1992; Abo Bakr, 1997; and Eshra, 2004). Synthetic molluscicides are the main mollusks control method, but environmental hazards of these chemicals (Buchs *et al.*, 1989) directed researchers to find out environmentally safer alternative control tools. The development of biopesticides as a possible substitute for chemical pesticides is gaining wide attention. Although large numbers of phytochemicals have already

been isolated and shown to have molluscicidal activity against aquatic snails (Mott, 1987; Thiilborg *et al.*, 1993; Lemmich *et al.*, 1995; Ekabo *et al.*, 1996), little work has been carried out to detect natural molluscicides against terrestrial mollusks and few number of natural compounds of plant origin were proved to be biologically active against terrestrial gastropod species (Hussein *et al.*, 1994; Hussein and El-Wakil, 1996; Hussein, 2005; El-Zemity, 2006). Monoterpenoids have been used for the purpose of pest control such as insecticides (Rice and Coats, 1994), fungicides (Vaughn and Spencer, 1994), acaricides (Ellis and Baxendale, 1997), nematicides (Sangwan *et al.*, 1990) and molluscicides (El-Zemity *et al.*, 2001) The present work is devoted to evaluate the molluscicidal activity of certain monoterpenoid compounds against two terrestrial snails and their biochemical impacts.

Key words: Monoterpenoids, fenchone, fenchol, thymol, molluscicidal activity, land snails.

MATERIALS AND METHODS

1- Snails

Adult terrestrial herbivorous snails *Theba pisana* (Müller) and *Eobania vermiculata* (Müller) (family: Helicidae) were collected from pesticide-free garden in Noubaria. Snails were fully acclimated to laboratory conditions prior to testing.

2- Chemicals

Three monoterpenoid compounds, fenchone, fenchol (Fig.1-a and b) and thymol (Fig.1-c) were tested for their molluscicidal activity against terrestrial snails. Fenchone and fenchol were obtained from Fluka AG, while thymol was obtained from Chem Sevice.

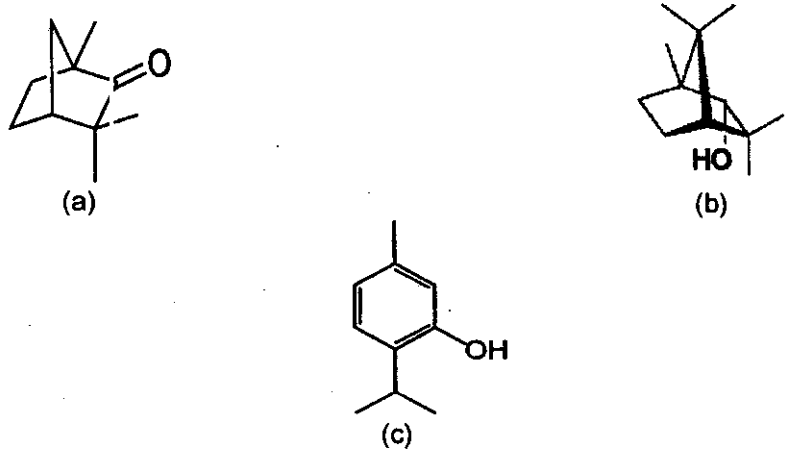


Fig (1): Chemical structure of tested monoterpenoids (a) fenchone, (b) fenchol and (c) thymol.

2-1- Fenchone: 1, 3, 3- trimethyl-2-norcamphone. It is isomeric with camphor, but is distinguished from it by its greater resistance to oxidation. Its d- form occurs in fennel oil and the l- form in oil of thuja.

2-2- Fenchol (fenchyl alcohol): 1, 3, 3-trymethyl-2- norbornanol. It is an isomer of borneol and is extensively used in perfumery.

2-3- Thymol: It is a hydroxyl derivative of 1-methyl-4- isopropyl-benzene (p-cymene). Its chemical name is 5-methyl-2-(1-methyl-ethyl) phenol. It is used as antiseptic and is found in many essential oils, e.g. oil of thyme and of oil of ajowan

3- Bioassay

Stock solutions of the tested compounds were prepared by dissolving the compound in dimethylsulfoxide (DMSO), and then were diluted with water. Tween 80® was added to prevent precipitation. Concentrated stocks were diluted with water to obtain the lower doses. Control snails were treated with the solvent. Three replicates were used for each dose with 10 snails each. Tested dose was gently applied on the surface of the snail's mantle collar using a micropipette as the method described by Hussein *et al.,* (1994). Snails were fed on lettuce *ad libitum.* Dead snails were detected 24 hr after treatment by loss of response to a thin stainless steel needle

(WHO, 1965). Toxicity values were determined by probit analysis (Finney, 1971).

4- Sub-lethal treatments

Snails were treated with1/2, 1/5 and 1/10 of LD_{50} values of molluscicidal active compound(s). After 24 hr of treatment, snails' tissues were prepared for biochemical tests.

5- Sample preparation for biochemical tests

Shells of snails were removed and tissues were homogenized in 10 folds (w/v) of distilled water by using glass homogenizer. Homogenates were centrifuged at 5000 rpm for 30 minutes using a cooling centrifuge at 4 °C. The supernatant was used as a source of protein and enzyme assay.

6- Determination of protein content

Estimation of protein concentration has been carried out according to the method of Lowery *et al.* (1951) using bovine serum albumin as standard and absorbance was measured at 750 nm.

7- Aspartate transaminase (AST) and alanin transaminase (ALT) assay

In vivo effects of molluscicidal active compound(s) against AST and ALT were studied according to the method described by Reitman and Frankel (1957) using Diamond Diagnostics kit. Absorbance was measured at 546 nm.

RESULTS AND DISCUSSION

1- Molluscicidal activity

The molluscicidal activity results of the tested monoterpenoid compounds are presented in Table (1). The results show that fenchone has molluscicidal activity against both of tested snails *T. pisana* and *E. vermiculata* with LD_{50} values of 527.93 and 552.38 µg/g body weight, respectively. Although, fenchol did not exihibit molluscicidal activity against *T. pisana*, it was active against *E. vermiculata* with LD_{50} 664.22 µg/g body weight. In contrary, thymol exhibited molluscicidal action against *T. pisana* (LD_{50}=516.15 µg/g body weight), while it was inactive against *E. vermiculata*. Our findings are confirmed by those obtained by El-Zemity and Radwan (2001), who reported the molluscicidal activity of major constituents of some essential oils including thymol and other monoterpenoids. They also observed that mono- and bicyclic ketones were

more toxic than their analogues of alcohols. The results of the present study support this observation whereas; fenchone the bicyclic monoterpenoid ketone was more potent than its alcoholic analogue, fenchol against both tested snails. Although *E. vermiculata* and *T. pisana* belong to the same family, Helicidae and subfamily, Helicinae (Godan, 1983), species specificity was observed in particular with fenchol and thymol. Many authors reported considerable species specificity in terrestrial mollusks to different compounds (El-Zemity and Radwan, 1999; El-Sebaii, 2006; and Youssef, 2006). Fenchone was tested as a semiochemical against terrestrial slugs (Dodds, 1996; Dodds *et al.*, 1996), however, there is no trials on the molluscicidal action of fenchone or fenchol against terrestrial snails have been reported. The toxic action of the tested monoterpenoids against land snails is rapid whereas the toxicity symptoms appeared within 1 hr post- treatment. Excessive mucous secretion was observed and snails retracted their bodies inside the shell and killed in this position within 24 hr. These symptoms are similar to those described by El-Zemity and Radwan (2001) when they used some essential oils and their major chemical constituents against *T. pisana* snail.

2- Biochemical effects

It is important to study the influence of toxic compounds on the biochemical systems of the target pest. Sub-lethal doses 1/10, 1/5 and 1/2 of fenchone LD_{50} altered protein level as presented in Table (2). Marked decrease of *T. pisana* protein levels was observed due to 52.79 and 105.58 µg/g body weight treatments that caused 43 and 30 reduction percentages respectively compared to control. Also, *E. vermiculata* protein level was affected; doses of 110.46 and 276.19 µg/g body weight reduced the protein level by 50 and 47% respectively. Many authors reported the reduction of mollusks' protein level as a result of toxicosis by different toxicants either synthetic or natural (Adewunmi *et al.*, 1988; Mohammed *et al.*, 1981; Chaudhary and Lomte, 1990; El-Wakil and Radwan, 1991 and Abd-ElNaby, 2007).

Transaminases (aminotransferases) constitute a group of enzymes that catalyzes the interconversion of amino acid in α- keto acid by transferring amino group (Moss and Henderson, 1998). Aspartate transaminase (AST) and alanin transaminase (ALT) are enzymes located in molluscan digestive gland (hepatopancreas). The transaminases are good indicators of tissue lesion. Results of *in vivo* effect of fenchone on *E. vermiculata* and *T. pisana* AST and ALT activities are illustrated in Figures (2-5). Marked increases were observed in *E. vermiculata* AST activity (Fig.2) reached more than 3 folds compared to control with 1/5 and 1/2 of

fenchone LD_{50}, while *T. pisana* AST did not affected by treatments (Fig.3) except the lower dose, 1/10 of LD_{50} which caused 60 % elevation of

enzyme activity. Dramatic increases of *E. vermiculata* ALT activity (Fig.4) exceeded 10 folds compared to control due to the treatments with 1/5 and 1/2 of fenchone LD_{50}. Also, *T.pisana* ALT activity has been induced (Fig.5) and reached levels up to 5 folds than control. It was observed that as the concentration of fenchone increased, the activity of ALT increased in both tested snails. It is clear that ALT was more sensitive than AST to fenchone treatments either that of *T. pisana* or *E. vermiculata*. In addition, it was observable that AST and ALT of *E. vermiculata* were more responsive than those of *T. pisana* to fenchone treatments. Elevation of AST and ALT activities in terrestrial snails treated with compounds possess molluscicidal properties has been reported (El-Wakil and Radwan, 1991; Abdallah *et al.*, 1998). Results of fenchone *in vivo* effect indicate the lesion occurred in digestive gland (hepatopancreas) function due to sub-lethal treatments of fenchone.

ACKNOWLEDGEMENT

Authors acknowledge Dr. Hamdy Kotb, Mr. Ahmed Bedair and Mr. Gaber Mamdouh, Plant Protection Research Station, for their help during this work.

REFERENCES

Abdallah, E.A.M.; F.A. Kassem and E.A. Kadous (1992). Laboratory and field evaluation of local bait formulations of certain pesticides against mollusca species. J. Pest Cont. Environ. Sci., 4 (2): 179-192.

Abdallah, E. A. M., H. B. El-Wakil, F. A. Kassem, E. I. El-Agamy and Y. Abo Bakr (1998). Impact of aldicarb and metaldehyde exposure on different molluscan enzyme activities and stress protein response. 7th Conf. Agric. Ain Shams Univ. Cairo, Dec.15-17. Ann. Agric Sci., 3: 1089 -1102.

Abd-ElNaby, Shimaa S. (2007). Toxicity of formulations and their effects on the enzyme activities of some terrestrial snails. M.Sc. Thesis, Faculty of Agric. (Saba Basha), Alex. Univ., Egypt.

Abo Bakr, Y. (1997). Toxicological and environmental studies on some terrestrial gastropods. M. Sc. Thesis, Faculty of Agric., Alex. Univ., Egypt.

Adewunmi, C. O., O. S. Awe and K. S. Adesina (1988). Enhanced potency of a molluscicidal glycoside isolated from *Tetrapleura tetraptera* on *B. glabrata*. Planta Medica, 54(6): 550-551.

Barker, G. M. (2002). Molluscs as crop pests. CAB Inter. pp.439.

Buchs, W., U. Heimbach, and E. Czarnecki (1989). Effects of snail baits on non-target carabid beetles. In Slugs and Snails in World Agriculture (ed. Henderson, I. F.), BCPC Monograph, 41: 245-252.

Bundy, J. W. (1967). The significance of pest and disease damage in the production and marketing of processed vegetables. Proc. Brit. Insect. Funic. Conf. Brighton, U.K., 2: 534-544.

Chaudhari, T. R and V. S. Lomte (1990). Pesticidal impact on protein metabolism in digestive gland of the snail *Bellamya (Viviparas) bengalensis*. Environ. Ecol., 8(1A):189-191.

Dodds, C. J. (1996). The control of slug damage using plant-derived repellents and antifeedants. In: Slug and snail pests in agriculture. BCPC Monograph, 66: 335-340.

Dodds, C. J., M. G. Ford, I.F. Hederson, L. D. Leake, A. P. Marten, J. A. Pickett, L. J. Wadhams and P. Watson (1996). Slug chemical ecology: electrophysiological and behavioural studies. In: Slug and snail pests in agriculture. BCPC Monograph, 66: 73-81.

Ekabo, O. A., N. R. Fransworth, T. O. Hendrson, G. Mao and R. Mukherjee (1996). Antifungal and molluscicidal saponins from *Serjania salzmanniana*. J. of Natural Products, 59: 431- 435.

Ellis, M. D. and F.P. Baxendale (1997). Toxicity of seven monoterpenoids to trcheal mites (Acari: Tarsonemidae) and their honey bee (Hymenoptera: aphidae) hosts when applied as fumigants. J. Econ. Entomol., 90(5):1087-1091.

El-Okda, M. M. K. (1980). Land snails of economic importance on vegetable crops at Alexandria and neighboring regions. Agric. Res. Rev., 58 (1): 79 – 86.

El-Sebaii, M. A. M. (2006). Molluscicidal effect of certain plnt crude extract against land snails. Alex. Sci Exch. J., 27(4):274-280.

El-Wakil, H. B and M. A. Radwan (1991). Biochemical studies on the terrestrial snail *Eobania vermiculata* (Müller) treated with some pesticides. J. Environ. Sci. Health, B26 (5&6): 479-489.

El-Zemity, S. R. (2006). Synthesis and molluscicidal activity of novel N-methyl carbamate derivatives based on naturally occurring monoterpenoides. J. Appl. Sci. Res., 2(2):86-90.

El-Zemity, S. R. and M. A. Radwan (2001). Molluscicidal and antifeedant activity of some essential oils and their major chemical constituents

against *Theba pisana* snails. Arab Univ. J. Agric. Sci. Ain Shams Univ. Cairo, 9(1):483 - 493.

El-Zemity, S. R., Sh. A. Mohamed, M. A. Radwan and Sh. M.Sherby (2001). Molluscicidal efficacy and repellency of some naturally occurring monoterpenoids against the land snail *Helix aspersa*, Muller (Mollusca : Pulmonata). Annals Agric. Sci. Ain Shams Univ., 46(1): 339-346.

El-Zemity, S. R. and M. A. Radwan (1999). Synthesis and structure activity relation ships for anticipated molluscicidal activity of some 2-amino-5-substituted pyridine derivatives. Pestic. Sci., 55:1203-1209.

Eshra, E.H. (2004). Studies on terrestrial mollusks at some governorates of West Delta with special reference to its integrated management. Ph.D. Thesis, Faculty of Agric., Al-Azhar Univ., Egypt.

Finney, D. J. (1971). Probit analysis. 3rd Ed. Cambridge Univ.Press, London, pp.318.

Godan, D. (1983). Pest slugs and snails, biology and control. Springer Verlag. Berlin pp.445.

Hussein, H. I. (2005). Composition of essential oils isolated from three plant species and their molluscicidal activity against *Theba pisana* snails. J. Pest Cont. Environ. Sci., 13(2): 15-24.

Hussein, H. I. and H. B. El-Wakil (1996). A pioneer molluscicidal and antifeeding agent from *Calotropis procera* extract, against land snails. J. Pestic. Manag. Environ., 1: 110-116.

Hussein, H. I., A. Kamel, M. Abou-Zeid, A. H. El-Sebae and M. A. Saleh (1994). Uscharin, the most potent molluscicidal compound tested against land snails. J. Chem. Ecol., 20:135-140.

Iglesias, J., J. Castillejo and R . Castro (2003). The effects of repeated applications of the molluscicide metaldehyde and the biocontral nematode *Phasmarhabditis hermaphrodita* on mollusks, earth worms, nematodes, acarids and collembolans: a two-years study in north–west Spain. Pest Manag. Sci., 59: 1217-1224.

Kassab, A. and H. Daoud (1964). Notes on biology and control of land snails of economic importance in the U. A. R. Agric. Res. Rev., 42: 77-98.

Lemmich, E. C. Cornett, P. Furu, C. L. Jorstian, A. D. Knudsen, C. E. Olsen, A. Salih and S. T. Thiiborg (1995). Molluscicidal saponins from *Catunaregam nilotica*. Phytochemistry, 39:63-68.

Lowery, O. M., N. J. Rosebrough, A. L. Farr and R. J. Randoll (1951). Protein measurement with follin phenol reagent. J. Biol. Chem., 193: 256-257.

Mohamed, A. M., S. El-Fiki, H. B. El-Wakil and M. F. El-Sawy (1981). Effect of prolonged exposure of *Biomphalaria alexandrina* snails to low concentrations of some molluscicides II: On total tissue proteins, carbohydrates and lipids. J. Egypt. Soc. Parasit., II: 459-468.

Moss, D. W. and A. R. Henderson (1998). Enzymas. In: Burtis, C. A. and E.R. Ashwood (eds), Tietz Fundamentos de Quimica Clinica, 4th ed., Guanbara Koogan Rio de Janeiro, pp 275-325.

Mott, K. E. (1987). Plant molluscicides (Chichester: Wiley), pp. 326.

Reitman, S. and S. Frankel (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic trnsaminase. Am. J. Clin. Path., 28:56-63.

Rice, P. J. and J. R. Coats (1994). Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae) and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol., 87(5): 1172-1179.

Speiser, B and C. Kistler (2002). Field tests with a molluscicide containing iron phosphate. Crop Protection, 21: 389 - 394.

Sangwan, N. K., B. S. Verma, K. K. Verma and K. S. Dhindsa (1990). Nematicidal activity of some essential plant oils. Pestic. Sci., 28:331-335.

Thiilborg, S. T., S. B. Chistensen, C. Cornett, C. E. Olsen andE. Lemmich (1993). Molluscicidal saponins from *Phytolocca dodecandra*. Phytochemistry, 32:1167-1171.

Vaughn, S. F. and G. F. Spencer (1994). Antifungal activity of natural compounds against thiabedazole-resistant *Fusarium sambucinum* strains. J. Agric. Food Chem., 42:200-203.

WHO (1965). Molluscicidal screening and evaluation. Bull., 38: 507-581.

Youssef, H. A. (2006). Molluscicidal and biochemical activity of some crude plant extracts against three types of land snails. J. Agric. Sci. Mansoura Univ., 31 (10): 6735-6742.

Table (1): Median lethal dose (LD_{50})* values of monoterpenoids against land snails.

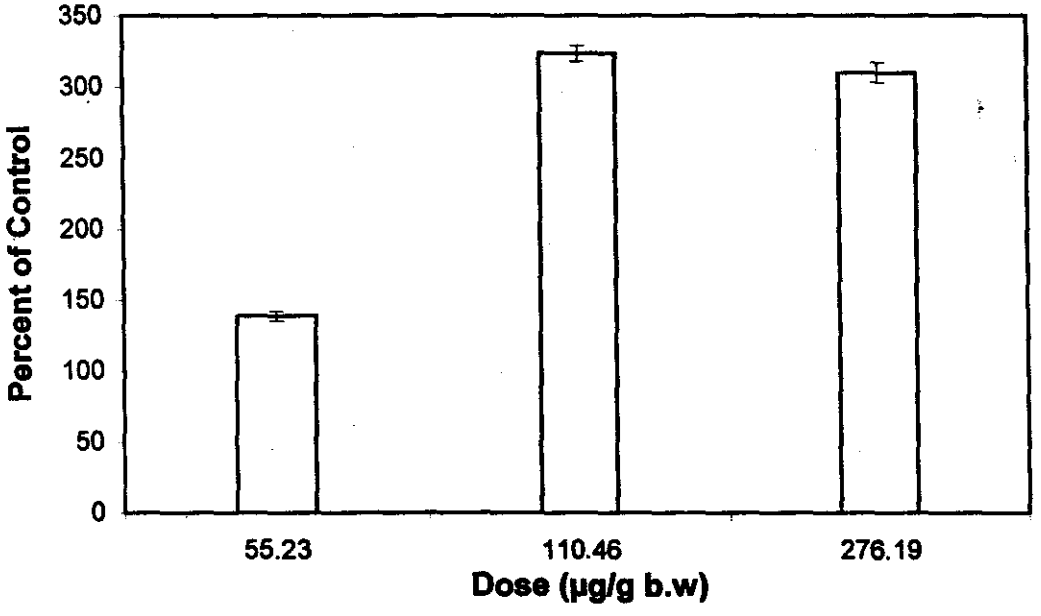
Compound	Experimental Snail	
	Theba pisana	*Eobania vermiculata*
Fenchone	527.93	552.38
Fenchol	>1000	664.22
Thymol	516.15	>1000

*LD_{50} values are expressed as µg/g body weight.

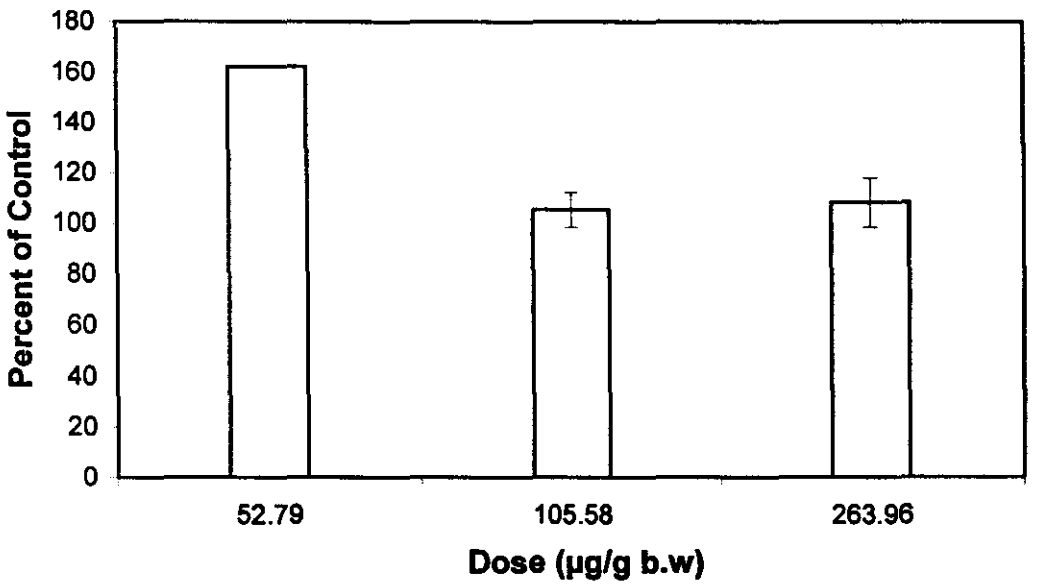
Table (2): Effect of fenchone on snails' protein concentration level.

Theba pisana			*Eobania vermiculata*		
Dose (µg/g)	Mean* ± SD	% Control	Dose (µg/g)	Mean* ± SD	%Contro
Control	9.30 ± 0.0	-	Control	8.98 ± 0.14	-
52.79	5.31 ± 0.044	57.1	55.24	9.09 ± 0.03	101
105.58	6.49 ± 0.070	69.8	110.46	4.46 ± 0.13	49.7
263.96	7.20 ± 0.131	77.4	276.19	4.79 ± 0.1	53.3

*Values of mean protein concentration are expressed as mg protein ml^{-1}.



Fig(2): Effect of fenchone on *Eobania vermiculata* AST activity.



Fig(3): Effect of fenchone on *Theba pisana* AST activity.

الملخص العربي

النشاط الإبادي والتأثيرات البيوكيميائية لبعض التربينات الأحادية ضد القواقع الأرضية

حمدي إبراهيم حسين, السيد حسن عشرة, ياسر أبو بكر

معهد بحوث وقاية النباتات- الصبحية- الإسكندرية.

تم إختبار النشاط الإبادي لثلاث مركبات من التربينات الأحادية هي الفينكون و الفينكول والثيمول ضد نوعين من القواقع الأرضية وهما القوقع البني إيوبانيا فيرميكيولاتا والقوقع الأبيض ثيبا بيسانا. أظهرت النتائج أن لمركب الفينكون تأثير إبادي ضد نوعي القواقع تحت الإختبار وكانت الجرعة القاتلة لنصف القواقع المختبرة هي ٥٢٧,٩٣ و ٥٥٢,٣٨ ميكروجرام/جرام من وزن جسم القوقع مع كل من القوقع الأبيض و القوقع البني على الترتيب. كما إتضح من النتائج أن مركب الفينكول أظهر تأثير إبادي ضد القوقع البني فقط في حين أن مركب الثيمول أبدى فاعلية إبادية ضد القوقع الأبيض. و أظهر إختبار الجرعات المنخفضة لمركب الفينكون انخفاض واضح لمستوى البروتين في كل من نوعي القواقع المختبرة. كما أحدثت هذه الجرعات إرتفاع واضح في نشاط إنزيمي أسبارتيت ترانس أمينيز و ألانين ترانس أمينيز مقارنة بالمجموعة الضابطة (الكنترول) مما يدل على حدوث خلل في الغدة الهاضمة.