

## **Molluscicidal activity of some naturally occurring compounds and their blends against land snails.**

**By**

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### **ABSTRACT**

A laboratory screening test had been conducted to throw light on the molluscicidal activity of some plant essential oils components and their blends against the white garden, *Theba pisana* and the brown garden, *Helix aspersa* terrestrial snails. The role of piperonyl butoxide in synergizing the toxicity of the tested chemicals was also evaluated. The results showed that thymol proved to be the most effective one of the tested products against the two tested snails, followed by eugenol and pulegone. *T. pisana* snails were more susceptible to the tested chemicals than *H. aspersa*. The synergist, piperonyl butoxide enhanced the toxicity of some of the tested chemicals, particularly against *T. pisana*.

**Keywords:** Molluscicidal activity, natural products, plant essential oils components, *Theba pisana*, *Helix aspersa*, terrestrial snails.

## INTRODUCTION

Land snails are becoming a serious agricultural animal pest in Egypt especially in the northern coastal areas. Such animals were recorded with a relatively high population density on the majority of economic crops cultivated in the northern newly reclaimed desert lands due to the availability of favourable weather conditions (mainly, temperature and relative humidity), sandy soils and manuring. They attack numerous orchard trees, field and vegetable crops as well as ornamental plants, causing great damage to all plant parts including fruits (El-Okda, 1980; Godan, 1983 and Nakhla *et al.*, 1993).

The control of terrestrial gastropod species by chemical means is very limited to the use of the commercial products containing either metaldehyde or carbamates. One of the main problems associated with the use of these synthetic compounds is their harmful effect against non-target organisms including mammals and wildlife (Homeida and Cooke, 1982 and Smith *et al.*, 1988). Currently attention is being drawn to the use of natural plant products in the field of molluscan control because they are inexpensive and environmentally safe as an alternative approach (Marston and Hostettmann, 1985).

Extensive screening of plant molluscicides have been carried out during recent years in developing agents to control aquatic gastropod species (Ekabo *et al.*, 1996 and Singh *et al.*, 1998), whereas few attempts have been successful in discovering non-hazardous molluscicide of natural origin against land gastropod species (Hagin and Bobnick, 1991 and Hussein *et al.*, 1994). Therefore, the present work was planned to study the molluscicidal activity of some naturally occurring compounds and their blends against the two terrestrial snails, *Theba pisana* and *Helix aspersa*, in an attempt to find out a new natural

molluscicide. Synergism of these chemicals using the synergist, piperonyl butoxide, was also evaluated under laboratory screening conditions.

## MATERIALS AND METHODS

**Test animals:** Specimens of the herbivorous snails, *Theba pisana* (Muller) and *Helix aspersa* (Muller) were collected during autumn 1999, from untreated nursery plants and farms in Alexandria Governorate, Egypt. The snail species used in these studies were selected on the basis of their geographical distribution and economic importance. They identified according to the key given by Godan (1983). Adult animals with a similar shell size of approximately 14 mm and 24 mm length for *T. pisana* and *H. aspersa*, respectively, were chosen and allowed to acclimatize to the laboratory conditions for three weeks and were fed on bran bait *ad libitum*.

**Test chemicals:** Ten commercially available plant oils components (Fig.1) and two of their blends were used in this study. The used components are: benzyl alcohol (99 %), cinnamyl alcohol (98%), cinnamyl aldehyde (99%),  $\alpha$ -terpineol (98%), thymol (98%), eugenol (99%), anethole (99%), anisole (99%), pulegone (98%), phenylethylpropionate (98%), 3-blend (eugenol +  $\alpha$ -terpineol + cinnamyl aldehyde) and 5-blend (eugenol +  $\alpha$ -terpineol + cinnamyl aldehyde + thymole + anisole) and all were supplied by Ecosmart company. Pure methiocarb (4-methylthio-3,5-dimethylphenyl *N*-methyl-carbamate) was used as reference,

**Laboratory testing:** Stock solutions of the tested chemicals or their blends were prepared in dimethyl sulfoxide (DMSO), which was found to cause little distress to slugs and has been shown to be the most appropriate solvent for the topical application

(Young and Wilkins, 1989). The same solvent was then used to achieve the desired tested concentrations.

The molluscicidal toxicity of the tested components against the two terrestrial snails was performed according to the method published by El-Zemity and Radwan (1999). Preliminary experiments were carried out to establish the effective range of the tested components. Six different concentrations, ranged from 2.5 to 25 g/liter for each chemical alone or chemical + the synergist, piperonyl butoxide at the ratio of 1:2, respectively, were prepared. It was observed that there was no mortality up to 750 µg/snail of piperonyl butoxide alone. Three replicates (10 animals each) were kept in 0.5 liter glass jars covered with cloth netting and secured with a rubber band to prevent snails from escaping. Control snails were treated with DMSO. The tested dose was gently applied on the surface of the snail body inside the shell and/ or the internal wall of the shell aperture with the aid of an Eppendorf micropipet containing 30 µl in the case of *H. aspersa* and 5 µl in the case of *T. pisana*. Snails were provided with lettuce leaves to feed on every 24 hr. after treatment. Dead animals were detected 24, 48, and 72 hr after treatment by loss of response to touch by a thin stainless steel needle according to the WHO procedure (Anonymous, 1965).

**Statistical procedure:** Percentage mortality was corrected using Abbott's formula (Abbott, 1925). Toxicity parameters for each treatment were computed according to the probit-analysis method by Finney (1971).

## RESULTS AND DISCUSSION

The comparative results of the contact molluscicidal activity of the tested naturally occurring compounds and their

blends has been evaluated against *T. pisana* and *H. aspersa* snails (Table 1) compared with methiocarb as a standard.

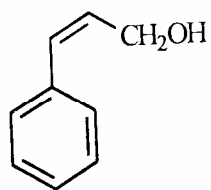
The results presented in Table (1) showed that there are some promising components which could be developed or used as building block for synthesizing a new potent and safe molluscicide. In addition, the molluscicidal activity of the components such as  $\alpha$ -terpineol and benzyl alcohol was shown to be moderately effective while the rest of the screening components were less effective.

Thymol is known to be biotoxic against the aquatic *Biomphalaria glabrata* snails (Marston and Hostettmann, 1985). Also, in the present study, thymol was found to be the most effective compound among the tested chemicals against either *T. pisana* or *H. aspersa* snails followed by eugenol and pulegone. The LD<sub>50</sub> values were 120.61, 125.82 and 361.79  $\mu\text{g}/\text{snail}$ , respectively, against *T. pisana*. The corresponding LD<sub>50</sub> values against *H. aspersa* were 344.56, 617.03 and 613.50  $\mu\text{g}/\text{snail}$ , respectively.

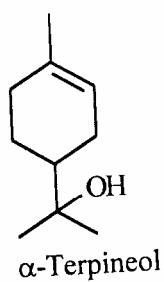
Variation or modification of the chemical structure proved to have a significant impact on the molluscicidal potency of the screened products against the land snails, *T. pisana* and *H. aspersa*. The structure of thymol proved to be most favorable among the compared active ingredients (Fig. 1). However, the structure of pulegone did not favour the molluscicidal potency.

Several studies showed considerable species specificity in terrestrial snails to different compounds (Crowell, 1967; Radwan 1993 and El-Zemity and Radwan 1999). Similarly, our studies on *H. aspersa* and *T. pisana* snails demonstrate that *T. pisana* is more susceptible to the tested chemicals than *H. aspersa*.

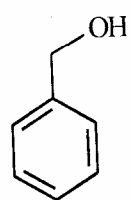
*S.R.El-Zemity*



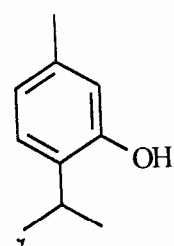
Cinnamyl alcohol



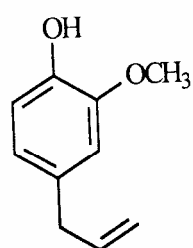
$\alpha$ -Terpineol



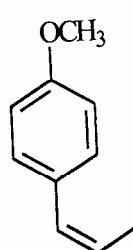
Benzyl alcohol



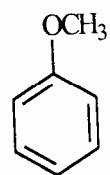
Thymol



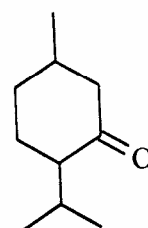
Eugenol



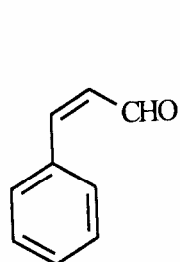
Anethole



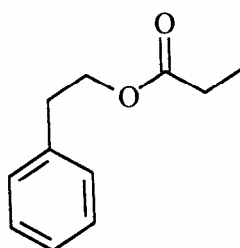
Anisole



Pulegone



Cinnamyl aldehyde



Phenethylpropionate

**Fig 1.** Chemical structures of the tested components

Mixing of the tested chemicals as represented in 3- and 5-blends enhanced the molluscicidal activity as demonstrated by LD<sub>50</sub> values of 240.74 and 251.43 ug/snail, respectively against *T. pisana*. On the other hand, The two blends were synergized by piperonyl butoxide and the synergistic ratio (SR) was pronounced in the case of the 3-blend.

Piperonyl butoxide usually exert its synergistic action with synthetic pesticides and/ or natural products by inhibiting the mixed function oxidase activities which detoxify xenobiotics (Metcalf, 1967 and Matsumura, 1985). Besides, it may increase the penetration of the pesticide resulting in a high titer at the active site of action (Singh *et al.*, 1998). In the present study, synergistic action of piperonyl butoxide with the tested naturally occurring components may be due to the inhibition of microsomal oxidases and thus reducing the detoxification rate of the tested chemicals *in vivo* resulting in extending the effective dose at the active site.

Synergism expressed as synergistic ratio (SR) was measured according to the equation by El-Sebae *et al.* (1964) and Hewlett (1969). The results showed that piperonyl butoxide synergized some of the tested chemicals against *T. pisana* and *H. aspersa* snails, with SR values varied from 1.3 to 5.33 fold against *T. pisana* and from 1.33 to > 1.7 against *H. aspersa*, depending on the chemical structure. Mixing the chemical(s) or their blends with the synergist, piperonyl butoxide improved the molluscicidal activity compared to the chemical or the blend alone. Benzyl alcohol, thymol, cinnamyl alcohol, and the 3-blend were the most synergized compounds against *T. pisana* snails. This suggests that the mixed-function oxidases play a role in detoxifying these chemicals.

Table 1: Toxic effect of certain naturally occurring components and their blends alone and in combination with piperonyl butoxide (PB)  
1: 2 against *Theba pisana* and *Helix aspersa* snails after 48 h.

| Material(s)               | <i>T. pisana</i><br>LD <sub>50</sub> (ug/ snail) | synergistic<br>ratio <sup>b</sup> | slope &<br>variance | <i>H. aspersa</i><br>LD <sub>50</sub> (ug/ snail) | synergistic<br>ratio <sup>b</sup> | slope &<br>variance |
|---------------------------|--------------------------------------------------|-----------------------------------|---------------------|---------------------------------------------------|-----------------------------------|---------------------|
| Benzyl alcohol            | 447.71(418.13-479.37) <sup>a</sup>               |                                   | 4.39 ± 0.138        | > 750                                             |                                   |                     |
| Benzyl alcohol + PB       | 83.93(72.97-96.53)                               | 5.33                              | 1.97 ± 0.037        | 442.15 (380.76-513.49) <sup>a</sup>               | > 1.70                            | 1.77 ± 0.061        |
| α-Terpineol               | 417.52(398.12-437.85)                            |                                   | 8.08 ± 0.422        | > 750                                             |                                   |                     |
| α-Terpineol + PB          | 157.49(135.51-183.12)                            | 2.65                              | 1.74 ± 0.029        | > 750                                             |                                   |                     |
| Cinnamyl alcohol          | 549.52(507.22-595.38)                            |                                   | 3.83 ± 0.139        | > 750                                             |                                   |                     |
| Cinnamyl alcohol + PB     | 160.14(127.94-200.75)                            | 3.43                              | 1.39 ± 0.033        | 344.56 (313.79-378.33)                            |                                   | 3.12 ± 0.076        |
| Thymol                    | 120.61(92.94-156.35)                             |                                   | 2.44 ± 0.093        | 258.13 (200.67-313.74)                            | 1.33                              | 1.26 ± 0.054        |
| Thymol + PB               | 34.96(27.16-44.88)                               | 3.45                              | 1.69 ± 0.042        | 617.03 (516.13-737.96)                            |                                   | 1.83 ± 0.068        |
| Eugenol                   | 125.82(116.71-135.65)                            |                                   | 4.04 ± 0.073        | 378.12 (298.18-479.42)                            | 1.63                              | 1.08 ± 0.054        |
| Eugenol + PB              | 50.13(37.51-66.81)                               | 2.51                              | 1.14 ± 0.031        | > 750                                             |                                   |                     |
| Anisole                   | > 750                                            |                                   |                     | > 750                                             |                                   |                     |
| Anisole + PB              | > 750                                            |                                   | 2.15 ± 0.079        | > 750                                             |                                   |                     |
| T-Anethole                | 668.97(567.03-789.53)                            |                                   | 1.73 ± 0.062        | > 750                                             |                                   |                     |
| T-Anethole + PB           | 514.72(436.91-606.54)                            | 1.30                              |                     | > 750                                             |                                   |                     |
| Phenethyl propionate      | > 750                                            |                                   |                     | NT                                                |                                   |                     |
| Phenethyl propionate + PB | 602.14 (532.57-680.91)                           | > 1.24                            | 2.64 ± 0.09         | 613.50 (525.94-715.85)                            |                                   | 2.12 ± 0.075        |
| Pulegone                  | 361.79(323.19-404.98)                            |                                   | 2.43 ± 0.066        | 421.22 (370.74-478.60)                            | 1.46                              | 2.08 ± 0.064        |
| Pulegone + PB             | 256.49(224.65-292.79)                            | 1.41                              | 2.50 ± 0.066        | 598.86 (550.84-651.09)                            |                                   | 3.93 ± 0.165        |
| Cinnamyl aldehyde         | 582.73 (526.89-644.54)                           |                                   | 3.14 ± 0.11         | NT                                                |                                   |                     |
| Cinnamyl aldehyde + PB    | 230.24 (184.25-287.49)                           | 2.53                              | 1.58 ± 0.057        | > 750                                             |                                   |                     |
| 3-blend                   | 240.74(210.74-274.93)                            |                                   | 2.62 ± 0.068        | 487.11 (396.16-599.17)                            | > 1.54                            | 1.32 ± 0.057        |
| 3-blend + PB              | 71.30(61.28-82.92)                               | 3.38                              | 1.87 ± 0.037        | > 750                                             |                                   |                     |
| 5-blend                   | 251.43(226.83-278.64)                            |                                   | 3.41 ± 0.082        | > 750                                             |                                   |                     |
| 5-blend + PB              | 189.90(163.09-221.03)                            | 1.32                              | 1.86 ± 0.027        | 210.72 (189.20-244.90)                            |                                   | 1.19 ± 0.055        |
| Methiocarb <sup>c</sup>   | 107.34(87.83-124.35)                             |                                   | 2.43 ± 0.062        |                                                   |                                   |                     |

<sup>a</sup> 95% confidence limits in parenthesis; <sup>b</sup> Synergistic ratio (LD<sub>50</sub> of chemical / LD<sub>50</sub> of chemical + PB); <sup>c</sup> Methiocarb was used as standard molluscicides; NT not tested.



In general, it could be concluded that, three of the tested components; thymol, eugenol and pulegone could be developed or used as building block for synthesizing a new potent and safe molluscicide against *T. pisana* and *H. aspersa* snails. In addition, the molluscicidal activity of thymol, eugenol and benzyl alcohol were more effective than the standard molluscicide, methiocarb, when mixed at the ratio of 1:2 with piperonyl butoxide against *T. pisana* snails. The data reveals positive potential for these safer chemicals plant origin in the programme of pest control as successful alternatives to the conventional synthetic compounds.

## REFERENCES

- Abbott, W. S.; (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**: 265-67.
- Anonymous.;(1965). Molluscicidal screening and evaluation. *WHO Bull.*, **38**: 507-81.
- Crowell, H. H.; (1967). Slug and snail control with experimental poison baits. *J. Econ. Entomol.*, **60**: 1048-1050.
- Ekabo, O. A., Fransworth, N. R.; Henderson, T. O.; Mao, G. and Mukherjee, R.; (1996). Antifungal and molluscicidal of saponins from *Serjania salzmanniana*. *J. Natural products*. **59**: 431-435.
- El-Okda, M. M. K.; (1980). Land snails of economic importance on vegetable crops at Alexandria and neighbouring regions. *Agric. Res. Rev.*, **58** (1): 79-86.

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- El-Sebae, A. H., Metcalf, R. L. and Fukuto, T. R.; (1964). Carbamate insecticides: Synergism by organothiocyanates. *J. Econ. Entomol.*, **57** (4): 478-482.
- El-Zemity, S. R. and Radwan, M. A.; (1999). Synthesis and structure-activity realtionships for anticipated molluscicidal activity of some 2-amino-5-substituted pyridine derivatives. *Pestic. Sci.*, **55**: 1203-1209.
- Finney, D. J.; (1971). Probit analysis, 3 *rd* Ed. *Cambridge Univ. Press, London* 318.
- Godan, D.; (1983). Pest slugs and snails, biology and control . *Springer- Verlag, Berlin*, 191-92.
- Hagin, R. D. and Bobnick, S. J.; (1991). Isolation and identification of a slug-specific molluscicide from quackgrass (*Agropyron repens* L. Beavy). *J. Agric. Food Chem.*; **39**: 192-196.
- Hewlett, P. S.; (1969). The potentiation between thanite and arprocarb in their action on houseflies. *Ann. Appl. Biol.*, **63**: 277-481.
- Homeida, A. M. and Cooke, R. G.; (1982). Pharmaological aspects of metaldehyde poisoning in mice. *J. Vet. Pharmacol. Ther.* **5**: 77-82.
- Hussein, H. I., Kamel, A. Abou-Zeid, M., El-Sebae, A. H. & Salah, M. A.; (1994). Uscharin, the most potent molluscicidal compound tested against land snails. *J. Chem. Ecol.*, **20**: 135-40.

*J. Pest Cont. & Environ. Sci.* **9**(3): 39-50 (2001)

Marston, A. and Hostettmann, K.; (1985). Plant molluscicides. *Phytochemistry*, **24**: 639-652.

Matsumura, F.; (1985). *Toxicology of insecticides*. Plenum Press, New York and London, , pp. 413.

Metcalf, R. L.; (1967). Mode of action of insecticide synergists. *Ann. Res. Entomol.*, **12**: 229-256.

Nakhla, J. M.; Tadros, A. W.; Abdel-Hafez, A. A. and Hashem, A. G.; (1993). Survey and monitoring of land snails in pear Orchards at the northern reclaimed lands. *Alex. Sci. Exch.*, **14** (3): 43-57.

Radwan, M. A.; (1993). A technique for testing the efficacy of molluscicidal baits against land snails. *Com. In Sci. & Dev. Res.*, **651**: 17-26.

Singh, K.; Singh, A. and Singh, D. K.; (1998). The use of piperonyl butoxide and MGK-264 to improve the efficacy of some plant-derived molluscicides. *Pestic. Sci.*, **54**: 145-149.

Smith, G., Hardy, A. R., Brown, P. M., Fletcher, M. R., Stanely, P. I., Westlake, G. E. and Lloyd, G. A.; (1988). Assessment of the hazard to wildlife from the use of methiocarb to protect ripening cherries. *ADAS Tolworth Lab., Hook Ries South, Surbiton KT67NF, United Kingdom*.

Young, A. G. & Wilkins, R. M.; (1989). A new technique for assessing the contact toxicity of molluscicides to slugs. *J. Moll. Stud.*, **55**: 533-36.

## الملخص العربى

النشاط الالادى لبعض المركبات الطبيعية ومخاليطها ضد القواقع الارضية

سعد رشاد الزميتى

قسم كيمياء المبيدات- كلية الزراعة (الشاطبى)- جامعة الاسكندرية

تم عمل اختبار معملى لمعرفة كفاءة بعض مكونات الزيوت النباتية ومخاليطها ضد القواقع الارضية تيبا بيدانا (قوقع الحديقة الابيض) والقوقع هيلكس اسبرسا (قوقع الحديقة البنى) ، كذلك درس فى هذا البحث دور مركب الببرونيل بيتوكسيد فى تنشيط السمية للمكونات المختبرة وحددت قيم التنشيط. أوضحت النتائج أن مركب الثيمول هو أكثر المركبات نشاطا ضد النوعين من القواقع التى تم عليها الاختبار ثم جاء بعد ذلك مركب اليوجينول ثم البلجون . القوقع الارضى تيبا بيدانا كان أكثر حساسية للمكونات المختبرة عن قوقع الهليكس اسبرسا. المنشط ببيرونيل بيتوكسيد رفع سمية بعض المكونات المختبرة وبخاصة ضد قوقع التيبا بيدانا.