Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

The synergistic effect of piperonyl butoxide on the molluscicidal potential of monoterpenes and phenylpropenes against *Theba pisana*

Samir A.M. Abdelgaleil*, Mona M.G. Saad

Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt

Vol. 58, No. 4: 381–386, 2018

DOI: 10.24425/jppr.2018.124649

Received: May 7, 2018
Accepted: November 23, 2018

*Corresponding address:
samirabdelgaleil@gmail.com

Abstract

The molluscicidal activity of six monoterpenes and two phenylpropenes against *Theba pisana* adults was evaluated using fumigation and direct contact methods. In the fumigant toxicity assay, (-)-citronellal showed the highest toxicity with LC_{50} value of 7.79 µl · l^{-1} air after 24 h of treatment, followed by (-)-terpinen-4-ol (LC_{50} = 12.06 µl · l^{-1}), (-)-menthone (LC_{50} = 12.28 µl · l^{-1} air) and p-cymene (LC_{50} = 16.07 µl · l^{-1} air). Eugenol and *trans*-cinnamaldehyde were the most potent contact toxicants against *T. pisana*. Their LD_{50} values were 0.18 and 0.29 mg · snail^{-1} after 24 h of treatment, respectively. These two compounds were more toxic than a reference molluscicide, methomyl. In contrast, α-terpinene and (-)-citronellal were the least toxic compounds. In another experiment, the synergistic effect of piperonyl butoxide (PBO) on tested monoterpenes and phenylpropenes by topical application was examined. The results showed that the toxicity of the tested compounds was increased when mixed with PBO at a ratio [compound/PBO (1 : 2)] except for α-pinene and (-)-terpinen-4-ol in which the toxicity of binary mixtures was less than for single compounds. The synergistic effect of PBO improved with increased exposure time. The highest synergistic effect was observed with (-)-menthone and α-terpinene with synergistic ratios of 9.25 and 4.37, respectively. Monoterpenes and phenylpropenes and their mixtures with PBO described herein merit further studies as potential *T. pisana* control agents.

Keywords: molluscicidal activity, monoterpenes, phenylpropenes, piperonyl butoxide, synergistic effect, *Theba pisana*

Introduction

In the last few decades, synthetic molluscicides have been widely used to control harmful terrestrial and aquatic snails. However, the continuous application of the limited number of synthetic molluscicides available has led to serious environmental hazards (Rao and Singh 2001). Therefore, there is a crucial need to develop new, convenient and safer alternatives to synthetic molluscicides. Plant secondary metabolites, such as essential oils and their major constituents (monoterpenes and phenylpropenes) are among the most promising alternatives in this regard. These botanical products with potential molluscicidal activity have the advantage of providing novel modes of action and offering new leads for the design of target-specific molecules (Isman 2008).

Monoterpenes and phenylpropenes are two groups of volatile plant secondary metabolites. They are the main constituents of many plant essential oils, and have been shown to possess a wide spectrum of biological activities, including insecticidal, herbicidal, antimicrobial and molluscicidal properties (Isman 2000; Pasay *et al.* 2010).

Piperonyl butoxide (PBO) is a semi-synthetic methylenedioxyphenyl compound obtained from natural safrole (Tozzi 1998). Piperonyl butoxide is not a pesticide but when mixed with pesticides it improves

their effectiveness, so it is called a pesticide synergist. This compound has been used in pesticide products since the 1950s, particularly with natural pyrethrins or man-made pyrethroids. It increases pesticide absorption and/or inhibits some enzymes which break down the pesticides, such as mixed function oxidase (Metcalf 1967; Singh *et al.* 1998). This combination enhances the pesticide activities, allows for the use of smaller amounts of pesticides and decreases the number of pesticide applications. The synergistic effect of PBO on the toxicity of several natural products, such as essential oils (Yadav *et al.* 2009), azadirachtin (Singh *et al.* 1998) and monoterpenes (Radwan and El-Zemity 2007) has been reported against different pests.

The white garden snail, *Theba pisana* Muller (Mollusca: Gastropoda: Helicidae), is a destructive agricultural animal pest of several economic crops, including fruit trees, vegetables and ornamental plants (Barker 2002). This snail is distributed in all African and European countries of the Mediterranean Basin (CABI, 2017). Few studies have been reported on the molluscicidal activity of monoterpenes and phenylpropenes against *T. pisana* (El-Zemity 2001; Radwan and El-Zemity 2007; Abdelgaleil 2010). Therefore, the aim of this study was to evaluate the fumigant and contact toxicities of six monoterpenes and two phenylpropenes as new, ecologically safe, alternative molluscicides on *T. pisana*. The synergistic effect of PBO on the contact toxicity of these compounds was also examined.

Materials and Methods

Test animal

Adults of white garden snails (16 ± 0.5 mm shell diam.), *T. pisana* (Muller), were collected in April 2016 from the Faculty of Agriculture Garden, Alexandria, Egypt. The snails were kept under laboratory conditions at 26 ± 2°C in ventilated glass jars for 2 weeks before bioassay and were fed a diet of fresh lettuce leaves (*Lactuca sativa* L.).

Chemicals

Three monoterpene hydrocarbons [p-cymene (99%), α-pinene (98%) and α-terpinene (85%)], three oxygenated monoterpenes [(-)-citronellal (95%), (-)-menthone (90%) and (-)-terpinen-4-ol (95%)], and two phenylpropenes [*trans*-cinnamaldehyde (99%) and eugenol (99%)] were used in this study. The tested compounds were purchased from Sigma-Aldrich Chemical Co., Steinheim, Germany. The chemical structures of test monoterpenes and phenylpropenes are presented in Figure 1.

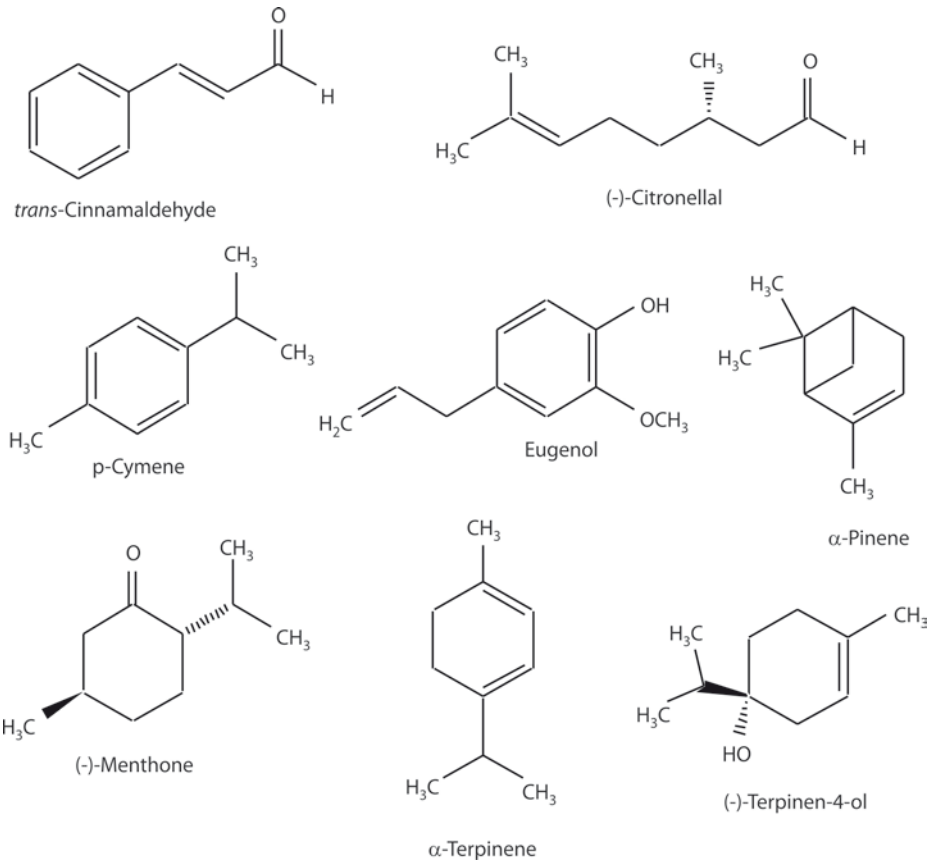

Fig. 1. Chemical structures of monoterpenes and phenylpropenes

Methomyl (99%) and PBP (95%) were supplied by Kafr-Elzayat Pesticides and Chemicals Co., Egypt. All chemicals were of the highest grade commercially available.

Fumigant toxicity bioassay on *Theba pisana*

Glass jars (1 liter capacity) were used as fumigant chambers to test the toxicity of monoterpene and phenylpropene vapors against adults of *T. pisana*. The tested compounds were applied to pieces of Whatman no. 1 filter paper (3 × 3 cm) attached to the undersurface of the screw caps of the glass jars. The final tested concentrations were 0.5, 1, 2.5, 5, 10, 20, 40, 60, 80 and 100 µl · l^{-1}. Similar units but without tested compounds were used and served as the control treatment. For each treatment, four replicates with five snails each were maintained. The snails were fed fresh lettuce leaves during the experiment. Mortality was determined after 24 h of exposure. Test snails were considered dead if no response was observed after being touched with a thin needle (WHO 1965). Values of LC_{50} and LC_{95} expressed as µl · l^{-1} air for each compound were calculated from log-concentration mortality regression lines (Finney 1971).

Contact toxicity and synergistic bioassays on *Theba pisana*

The contact toxicity of monoterpenes and phenylpropenes was evaluated on adult snails of *T. pisana* as described by Hussein *et al.* (1994) with slight modifications. Stock solutions of monoterpenes, phenylpropenes, binary mixtures [compound/PBO (1 : 2)] and methomyl, a reference molluscicide, were prepared in dimethyl sulfoxide (DMSO). The snails were treated with doses of 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.8 and 1 mg · snail^{-1}. These doses, in 5 µl DMSO solution, were gently applied to the surface of the snail body inside the shell using a micropipette. Four replicates (five snails in each) of each concentration were used. Control snails were treated with the same volumes of DMSO. The treated snails were transferred to 0.3 l glass jars. The jars were covered with cheesecloth fastened with rubber bands to prevent the escape of snails and to ensure proper ventilation. The snails were fed fresh lettuce leaves during the experiment. The mortality percentages were recorded after 24 and 48 h of treatment. Values of LD_{50} and LD_{95} were calculated as described previously.

Statistical analysis

The mortality of each concentration in fumigant toxicity assay was calculated after 24 h of treatment as the mean of four replicates, while the mortality of each dose in contact toxicity assay was calculated after 24 and 48 h. The mortality percentages were subjected to probit analysis (Finney 1971) to obtain the LC_{50}, LC_{95}, LD_{50} and LD_{95} values, using SPSS 12.0 (SPSS, Chicago, IL, USA). Values of LC_{50}, LC_{95}, LD_{50} and LD_{95} were considered significantly different if the 95% confidence limits did not overlap.

Results and Discussion

Fumigant toxicity of monoterpenes and phenylpropenes

The results of fumigant toxicity assay showed that monoterpenes were more toxic than phenylpropenes to adults of *T. pisana*. The tested monoterpenes had remarkable fumigant toxicity based on their LC_{50} values (Table 1). The oxygenated opened ring monoterpene (-)-citronellal caused the highest fumigant toxicity

Table 1. Fumigant toxicity of monoterpenes and phenylpropenes against the adults of *Theba pisana* after 24 h of treatment

Compound	LC_{50} [µl · l^{-1} air] (95% CL)	LC_{95} [µl · l^{-1} air] (95% CL)	Slope[a] ± SE	(χ^2)[b]
trans-Cinnamaldehyde	>100	–	–	–
(-)-Citronellal	7.79 (5.06–10.19)	91.45 (60.67–187.3)	3.37 ± 0.25	12.13
p-Cymene	16.07 (11.09–23.17)	49.42 (31.44–149.9)	1.54 ± 0.23	1.00
Eugenol	>100	–	–	–
(-)-Menthone	12.28 (10.17–14.79)	24.02 (19.56–40.04)	5.34 ±0 .46	5.63
α-Pinene	35.55 (33.27–37.79)	68.30 (61.06–80.11)	5.80 ± 0.58	0.70
α-Terpinene	35.08 (29.70–40.14)	139.4 (107.9–209.0)	2.75 ± 0.35	0.71
(-)-Terpinen-4-ol	12.06 (9.75–14.60)	131.0 (84.35–258.0)	1.59 ± 0.18	0.32

LC_{50} – the concentration causing 50% mortality; LC_{95} – the concentration causing 95% mortality; CL – confidence limits
[a] Slope of the concentration – mortality regression line ± standard error
[b] Chi square value

(LC_{50} = 7.79 µl · l^{-1} air), followed by (-)-terpinen-4-ol (LC_{50} = 12.06 µl · l^{-1} air) and (-)-menthone (LC_{50} = = 12.28 µl · l^{-1} air). At the applied concentrations, phenylpropenes exhibited weak fumigant toxicity as their LC_{50} values were higher than 100 µl · l^{-1}. To the best of our knowledge, this is the first report on the fumigant toxicity of tested phenylpropenes and monoterpenes against *T. pisana*. However, other monoterpenes, such as (L)-fenchone, myrcene, 1-8-cineole have been shown to possess higher fumigant toxicity against this snail than the tested compounds (Abdelgaleil 2010). In addition, the essential oils of *Mentha microphylla*, *Schinus terebenthifolius*, *Lantana camara*, *Citrus reticulate* and *Eucalyptus camaldulensis* containing some of the tested monoterpenes as major constituents were found to have remarkable fumigant toxicity against *T. pisana* adults (El-Aswad and Abdelgaleil 2008).

Contact toxicity of monoterpenes and phenylpropenes

The tested monoterpenes and phenylpropenes showed variable levels of contact toxicity against *T. pisana*. Unlike the fumigant toxicity, the phenylpropenes (eugenol and trans-cinnemaldehyde) were the most potent compounds with LD_{50} values of 0.18 and 0.29 mg · $snail^{-1}$ after 24 h of treatment, respectively (Table 2). Similarly, p-cymene, (-)-terpinen-4-ol and α-pinene showed pronounced contact toxicity. These five compounds were more toxic than a reference molluscicide, methomyl, after 24 h of treatment. In general, the tested compounds showed higher contact toxicity after 48 h than after 24 h of treatment except α-terpinene and p-cymene which had similar toxicity after both exposure times (Tables 2 and 3). This contact molluscicidal activity of monoterpenes and phenylpropenes is supported by earlier studies in which other monoterpenes showed contact toxicity against the adults of *T. pisana* (Radwan and El-Zemity 2007; Abdelgaleil 2010). Also, essential oils (*E. camaldulensis*, *Lavandula dentata*, *Ruta chalepensis*, *M. microphylla* and *L. camara*) were described to possess contact toxicity against adults of this snail (Hussein 2005; Abdelgaleil and Badawy 2006).

Synergistic effect of PBO on the toxicity of monoterpenes and phenylpropenes

The results of the synergistic assay revealed that all binary mixtures [compound/PBO (1 : 2)] were synergized except for α-pinene and (-)-terpinen-4-ol (Tables 2 and 3). α-Terpinene/PBO mixture showed the highest toxicity against *T. pisana*, followed by eugenol/PBO mixture after 24 h of treatment, while (-)-menthone/PBO

Table 2. Contact toxicity of monoterpenes and phenylpropenes and their mixtures with piperonyl butoxide (1 : 2) against the adults of *Theba pisana* after 24 h of treatment

Monoterpene	LD_{50} [mg · $snail^{-1}$] (95% CL)	LD_{95} [mg · $snail^{-1}$] (95% CL)	Slope[a] ± SE	(χ^2)[b]	Synergistic ratio[c]
trans-Cinnamaldehyde	0.29 (0.22–0.34)	1.60 (1.15 –2.88)	2.20 ± 0.34	1.01	
trans-Cinnamaldehyde + PBO (1 : 2)	0.20 (0.17–0.22)	0.69 (0.55–0.96)	3.00 ± 0.31	0.44	1.45
(-)-Citronellal	0.46 (0.44–0.52)	1.54 (1.18–2.35)	3.17 ± 0.41	0.04	
(-)-Citronellal + PBO (1 : 2)	0.33 (0.26–0.44)	3.67 (1.80–15.14)	1.57 ± 0.27	0.35	1.39
p-Cymene	0.30 (0.17–0.40)	6.12 (2.61–65.60)	1.25 ± 0.31	0.07	
p-Cymene + PBO (1 : 2)	0.17 (0.13–0.24)	4.76 (1.96–25.25)	1.14 ± 0.18	0.02	1.76
Eugenol	0.18 (0.15–0.20)	0.51 (0.43–0.66)	3.53 ± 0.34	0.07	
Eugenol + PBO (1 : 2)	0.09 (0.08–0.10	0.29 (0.24–0.37)	2.22 ± 0.29	2.23	2.0
(-)-Menthone	0.60 (0.53–0.67)	2.49 (1.84–3.84)	2.68 ± 0.32	1.98	
(-)-Menthone + PBO (1 : 2)	0.50 (0.45–0.54)	1.34 (1.15–1.66)	3.82 ± 0.35	2.13	1.2
α-Pinene	0.33 (0.28–0.36)	0.98 (0.81–0.1.30)	3.40 ± 0.06	0.06	
α-Pinene + PBO (1 : 2)	0.63 (0.55–0.74)	2.70 (1.91–4.79)	2.59 ± 0.33	0.00	0.52
α-Terpinene	0.92 (0.69–1.60)	14.44 (5.09–211.7)	1.37 ± 0.31	1.12	
α-Terpinene + PBO (1 : 2)	0.32 (0.29–0.37)	1.12 (0.85–1.70)	3.04 ± 0.34	0.82	2.88
(-)-Terpinen-4-ol	0.33 (0.26–0.41)	3.22 (2.07–6.43)	1.66 ± 0.20	0.24	
(-)-Terpinen-4-ol + PBO (1 : 2)	>1.0	–	–	–	–
Methomyl	0.42 (0.32–0.67)	7.29 (2.70–72.40)	1.32 ± 0.27	0.15	

LD_{50} – the dose causing 50% mortality; LD_{95} – the dose causing 95% mortality; CL – confidence limits; PBO – piperonyl butoxide

[a] Slope of the concentration – mortality regression line ± standard error

[b] Chi square value

[c] Synergistic ratio = LD_{50} (compound alone)/LD_{50} (compound + PBO)

Table 3. Contact toxicity of monoterpenes and phenylpropenes and their mixtures with piperonyl butoxide (1 : 2) against the adults of *Theba pisana* after 48 h of treatment

Monoterpene	LD_{50} [mg · snail^{-1}] (95% CL)	LD_{95} [mg · snail^{-1}] (95% CL)	Slope[a] ± SE	(χ^2)[b]	Synergistic ratio[c]
trans-Cinnamaldehyde	0.14 (0.13–0.16)	1.01 (0.64–2.49)	1.61 ± 0.29	1.97	
trans-Cinnamaldehyde + PBO (1 : 2)	0.09 (0.06–0.13)	0.55 (0.45–0.73)	2.82 ± 0.23	3.46	1.56
(-)-Citronellal	0.34 (0.28–0.44)	2.82 (1.63–10.06)	1.85 ± 0.38	1.68	
(-)-Citronellal + PBO (1 : 2)	0.17 (0.14–0.24)	1.93 (0.93–8.46)	1.57 ± 0.27	0.01	2.0
p-Cymene	0.30 (0.17–0.40)	6.12 (2.61–65.60)	1.25 ± 0.31	0.07	
p-Cymene + PBO (1 : 2)	0.15 (0.12–0.18)	2.07 (1.70–4.97)	1.43 ± 0.18	0.82	2.0
Eugenol	0.12 (0.09–0.14)	0.57 (0.44–0.88)	2.39 ± 0.31	1.20	
Eugenol + PBO (1 : 2)	0.09 (0.08–0.10	0.29 (0.24–0.37)	2.22 ± 0.29	2.23	1.33
(-)-Menthone	0.37 (0.33–0.40)	0.85 (0.75–0.99)	4.50 ± 0.39	3.79	
(-)-Menthone + PBO (1 : 2)	0.04 (0.03–0.06)	2.90 (0.99–3.14)	0.99 ± 0.18	0.99	9.25
α-Pinene	0.30 (0.26–0.34)	1.01 (0.82–0.1.39)	3.14 ± 0.39	0.01	
α-Pinene + PBO (1 : 2)	0.57 (0.50–0.67)	2.56 (1.80–4.52)	2.53 ± 0.33	1.12	0.53
α-Terpinene	0.92 (0.69–1.60)	14.44 (5.09–211.7)	1.37 ± 0.31	1.12	
α-Terpinene + PBO (1 : 2)	0.21 (0.18–0.23)	0.65 (0.53–0.87)	3.31 ± 0.32	0.30	4.38
(-)-Terpinen-4-ol	0.14 (0.11–0.17)	0.86 (0.61–1.51)	2.09 ± 0.29	1.91	
(-)-Terpinen-4-ol + PBO (1 : 2)	>1.0	–	–	–	–
Methomyl	0.06 (0.04–0.08)	0.25 (0.20–0.37)	2.64 ± 0.49	1.38	

LD_{50} – the dose causing 50% mortality; LD_{95} – the dose causing 95% mortality; CL – confidence limits
[a] Slope of the concentration – mortality regression line ± standard error
[b] Chi square value
[c] Synergistic ratio = LD_{50} (compound alone)/LD_{50} (compound + PBO)

revealed the highest toxicity, followed by α-terpinene/PBO after 48 h of treatment. It was also noticed that the synergistic effect of BPO on the toxicity of compounds increased with increased exposure time. For example, the toxicity of (-)-menthone/PBO mixture increased 1.2 fold after 24 h and 9.25 fold after 48 h of treatment. Similarly, the toxicity of α-terpinene/PBO mixture increased 2.88 fold after 24 h and 4.38 fold after 48 h of treatment.

Piperonyl butoxide is used as a synergist to inhibit the activity of mixed function oxidases, which are involved in detoxification of some natural and synthetic toxicants. Therefore, the enhancement of contact toxicity of tested monoterpenes and phenylpropenes against *T. pisana* may be attributed to the inhibitory effect of PBO on mixed function oxidases. Radwan and El-Zemity (2007) reported that PBO improved the molluscicidal activity of monoterpenes against *T. pisana* with the synergistic ratio ranging between 1.3 and 5.3 after 48 h of treatment. Also, the synergistic effect of PBO was observed on the toxicity of β-citronellol and carvacrol towards *Biomphalaria alexandrina* and *Helix aspersa* snails (El-Zemity *et al.* 2001a, b). On the other hand, the toxicity of α-pinene and (-)-terpinen-4-ol was decreased when mixed with PBO. This may be attributed to the fact that the presence of PBO inhibits its conversion of these two compounds to more toxic forms inside the body of a snail. A similar finding was observed by Rao and Singh (2001) on the toxicity of *Cedrus deodara* oil mixtures with PBO and MGK-264.

In conclusion, the tested monoterpenes [(-)-citronellal, p-cymene, (-)-menthone and (-)-terpinen-4-ol)] showed promising fumigant toxicity, while all monoterpenes and phenylpropenes exhibited remarkable contact toxicity against the adults of *T. pisana*. In addition, PBO improved the contact toxicity of the tested compounds. Therefore, the results of the current study recommend that the tested monoterpenes and phenylpropenes are promising candidates for preparing new formulations with possible application for controlling *T. pisana*.

References

Abdelgaleil S.A.M., Badawy M.E.I. 2006. Acaricidal and molluscicidal potential of three essential oils isolated from Egyptian plants. Journal of Pest Control and Environmental Science 14 (1): 35–46. DOI: 10.1007/s10493-010-9363-y

Abdelgaleil S.A.M. 2010. Molluscicidal and insecticidal potential of monoterpenes on the white garden snail, *Theba pisana* (Muller) and the cotton leafworm, *Spodoptera littoralis* (Boisduval). Applied Entomology and Zoology 45 (3): 425–433. DOI: https://doi.org/10.1303/aez.2010.425

Barker G.M. 2002. Mollusks as Crop Pests. CABI Publishing, Wallingford, Oxon, UK, 441 pp.

CABI. 2017. Centre for Agriculture and Bioscience International. Invasive Species Compendium: https://www.cabi.org/isc/datasheet/62094

El-Aswad A.F., Abdelgaleil S.A.M. 2008. Insecticidal, antifeedant and molluscicidal potential of essential oils extracted from Egyptian plants. Journal of Egyptian Society of Toxicology 38 (1): 81–91.

El-Zemity S.R., Mohamed S.A., Radwan M.A., Sherby S.M. 2001a. Molluscicidal activity of some essential oils and their major chemical constituents against *Biomphalaria alexandrina* snails. Alexandria Journal of Pharmaceutical Science 15 (1): 67–70.

El-Zemity S.R., Mohamed S.A., Radwan M.A., Sherby S.M. 2001b. Molluscicidal efficacy and repellency of some naturally occurring monoterpenoids against the land snail, *Helix aspersa* Muller (Mollusca: Pulmonoata). Annals Agricultural Science Ain Shams University Cairo 46 (1): 339–346.

El-Zemity S.R. 2001. Molluscicidal activity of some naturally occurring compounds and their blends against land snails. Journal of Pest Control and Environmental Science 9 (1): 39–50.

Finney D.J. 1971. Probit Analysis. 3rd ed. Cambridge University Press, London, 318 pp.

Hussein H.I. 2005. Composition of essential oils isolated from three plant species and their molluscicidal activity against *Theba pisana* snails. Journal of Pest Control and Environmental Science 13 (1): 15–24.

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. Journal of Chemical Ecology 20 (1): 135–140. DOI: 10.1007/BF02065996

Isman B.M. 2008. Perspective botanical insecticides: for richer, for poorer. Pest Management Science 64 (1): 8–11. DOI: https://doi.org/10.1002/ps.1470

Isman M.B. 2000. Plant essential oils for pest and disease management. Crop Protection 19 (8–10): 603–608. DOI: https://doi.org/10.1016/S0261-2194(00)00079-X

Metcalf R.L. 1967. Mode of action of insecticide synergists. Annual Review of Entomology 12: 229–256.

Pasay C., Mounsey K., Stevenson G., Davis R., Arlian L., Morgan M., Vyszenski-Moher D., Andrews K., McCarthy J. 2010. Acaricidal activity of eugenol based compounds against scabies mites. PLoS ONE 5: e12079.

Radwan M.A., El-Zemity S.R. 2007. Naturally occurring compounds for control of harmful snails. Pakistan Journal of Zoology 39 (5): 339–344.

Rao I.G., Singh D.K. 2001. Combinations of *Azadirachta indica* and *Cedrus deodara* oil, with piperonyl butoxide, MGK-264 and *Embelia ribes* against *Lymnaea acuminata*. Chemosphere 44 (8): 1691–1695. DOI: https://doi.org/10.1016/S0045-6535(00)00563-4

Singh K., Singh A., Singh D.K. 1998. The use of piperonyl butoxide and MGK-264 to improve the efficacy of some plant-derived molluscicides. Pesticide Science 54 (2): 145–149. DOI: 10.1002/(SICI)1096-9063(1998100)54:2<145::AID-PS789>3.0.CO;2-3

Tozzi A. 1998. A brief history of the development of piperonyl butoxide as an insecticide synergist. p. 1–5. In: "Piperonyl Butoxide: the Insecticide Synergist" (D.G. Jones, ed.) Academic Press, San Diego, 323 pp.

WHO. 1965. Molluscicidal screening and evaluation. WHO Bulletin 38: 507–581.

Yadav S., Mittal P.K., Saxena P.N., Singh R.K. 2009. Effect of synergist piperonyl butoxide (PBO) on the toxicity of some essential oils against mosquito larvae. The Journal of Communicable Diseases 41 (1): 33–38.