Naturally Occurring Compounds for Control of Harmful Snails

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Abstract.- Ten naturally occurring compounds have been tested for molluscicidal activity against the fresh water snail, *Biomphalaria alexandrina* and the white garden snail, *Theba pisana*. The role of the synergist, piperonyl butoxide (PB) in improving the efficacy of these chemicals was also investigated. The results showed that thymol was the most effective against *B. alexandrina* snails, followed by *trans*-anethole, pulegone and cinnamyl aldehyde. In case of *T. pisana* snails, thymol was found to be the most effective compound followed by eugenol and pulegone. *B. alexandrina* snails were more susceptible to the tested chemicals than *T. pisana*. PB enhanced the toxicity of some of the tested chemicals, particularly against *B. alexandrina*. The toxic effect of these chemicals alone against the two tested snails was less active than the standard molluscicides. However, the mixture of thymol, eugenol or benzyl alcohol with PB notably increased the molluscicidal activity over methiocarb against *T. pisana* snails.

Key words: Molluscicidal activity, natural products, *Biomphalaria alexandrina, Theba pisana*, fresh water snails, terrestrial snails.

INTRODUCTION

Snails are of great concern in agriculture, medical and veterinary practices due to their damage in agriculture, horticulture and forestry as well as their main role as intermediate hosts for the trematodes causing schistosomiasis and fascioliasis in humans and domestic animals (Godan, 1983). Biomphalaria alexandrina is the intermediate host of the blood fluke, Schistosoma mansoni which transmits the endemic human bilharziasis in Egypt (Webbe and El-Hak, 1990). The land snail, Theba pisana is becoming a serious agricultural animal pest, especially in the northern coastal areas of Egypt, causing damage to a wide range of plants including ornamental flowers, shrubs, vegetables, citrus, almond, olive trees and grapevines (El-Okda, 1980; Nakhla et al., 1993).

The control of molluscan pests using synthetic molluscicides is still considered to be the most effective measures. However, these synthetic compounds may lead to problems of toxicity to nontarget organisms in addition to deleterious long-term Copyright 2007 Zoological Society of Pakistan.

effects to the environment (Homeida and Cooke, 1982; Sabry, 1983; Awad, 1995; Smith *et al.*, 1988). Therefore, alternative environmentally friendly measures or compounds for effective molluscan control need to be developed. Currently attention is being drawn to the use of natural plant products for 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 (Lemma, 1970; Adewunmi and Sofowora, 1980; El-Sawy et al., 1981; Kady et al., 1986; Ekabo et al., 1996; Singh et al., 1998; El-Din, 2006), whereas few attempts have been successful discovering non-hazardous molluscicide of in natural origin against land snail species (Hussien et al., 1994; Rao and Singh, 2002; Chaieb et al., 2005). In the present study we tested the molluscicidal activity of some naturally occurring compounds against the two snails. B. alexandrina and T. pisana. in an attempt to find out molluscicide of plant origin. Synergism of these chemicals using the synergist, piperonyl butoxide (PB) was also

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evaluated under laboratory screening conditions.

MATERIALS AND METHODS

Snails

Biomphalaria alexandrina, intermediate hosts for schistosomiasis (Bilharziasis) of human disease, $8mm \pm 2mm$ diameter were collected from fresh water ponds at Kafr El-Dwar, Behera Governorate, Egypt during May, 2006, and were maintained in glass aquaria oxygenated for one hour daily, one week before testing.

Theba pisana (Muller), herbivorous snails, were collected during autumn 2006, 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 length were chosen and allowed to acclimatize to the laboratory conditions for three weeks and were fed on bran bait *ad libitum*.

Chemicals

Ten commercially available naturally occurring compounds namely: Benzyl alcohol (99%), cinnamyl alcohol (98%), cinnamyl aldehyde (99%), α-terpineol (98%), thymol (98%), eugenol (99%), *trans*-anethole (99%), anisole (99%), pulegone (98%) and phenylethylpropionate (98%) and the synergist, piperonyl butoxide (α -[2-(2butoxyethoxy) ethoxy] -4,5- methylenedioxy-2propyltoluene), all were purchased from Aldrich Chemical Company, UK. Niclosamide (2.5-Dichloro-4-nitrosalicylanilide) supplied by Bayer AG Leverkusen, Germany and copper sulfate (Pro Analysis grade, purity 99%) obtained from Merck, Darmstadt were used as reference compounds for the aquatic snail, B. alexandrina and pure methiocarb (4-methylthio-3.5-dimethylphenyl Nmethyl- carbamate) was used as a reference standard for the land snail, T. pisana.

Stock solutions of the tested chemicals were prepared in dimethyl sulfoxide (DMSO) to enhance the solubility and this solvent was found to cause little distress to molluscan pests (Young and Wilkins, 1989). The same solvent was then used to achieve the desired tested concentrations. The final DMSO concentration in the experiments never exceeded 0.5% (v/v), and an equal amount was added to the control.

Experimental design

For the aquatic snail

The recommended World Health Organization bioassay procedure (WHO, 1965), was used for the evaluation of some naturally occurring compounds on B. alexandrina. The tests were performed in 150 ml glass beakers with ten adult healthy snails per assay. Each chemical was mixed with Tween 20, to ensure complete solubility of the chemical in water. The mixture was added to the glass beaker filled up to 100 ml of the dechlorinated water to give the desired concentration (w/v) up to 400 μ g/ml for each chemical or chemical + PB at the ratio of 1:2, respectively, were used. It was observed that there was no mortality up to 400 ug/ml of PB or Tween 20 alone. Four glass beakers were set up for each concentration of each chemical. The snails were exposed for 24 h to different concentrations of compounds or their mixture with PB and then transferred to dechlorinated water for another 48 h. Percentage kill were recorded after 72 h depending on the lack of snail reaction to irritation of the foot with a needle as a criteria for mortality.

For the land snail

The molluscicidal toxicity of the tested compounds against T. pisana snails was performed according to the method published by Hussien et al. (1994). Preliminary experiments were carried out to establish the effective range of the tested compounds. Six different concentrations, ranged from 2.5 to 25 g/liter for each chemical alone or chemical + PB at the ratio of 1:2, respectively, were prepared. It was observed that there was no mortality up to 750 µg/snail of PB alone. Four 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

micropipette containing 5 μ l after treatment. Dead animals were detected 48 hr after treatment by loss of response to touch by a thin stainless steel needle according to the WHO procedure (1965).

Synergistic effect

The joint action effect was carried out by mixing the tested compounds with PB in 1:2 ratio. Synergistic ratio was calculated according to Hewlett (1960).

Statistical analysis

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

The LC₅₀ values of some naturally occurring compounds against the fresh water snail, *B. alexandrina* along with the slope regression lines and the synergistic ratios (SR's) are shown in Table I. Thymol, *trans*-anethole, pulegone, cinnamyl aldehyde and eugenol have been shown to be the most potent components against *B. alexandrina* snails with LC₅₀ values of 13.92, 28.16, 49.93, 60.41 and 76.91 µg/ml, respectively. Anisole was found to be moderately active with LC₅₀ value of 185.48 µg/ml, while α -terpineol and phenethyl propionate exhibited low activity, displaying LC₅₀ values of 262.23 and 296.27 µg/ml, respectively. Benzyl alcohol and cinnamyl alcohol were inactive at concentrations up to 400 µg/ml.

The results of synergism which indicated as synergistic ratio (SR), showed that PB did not affect the toxicity against the snails up to 400 µg/ml. However, PB synergized all of the tested chemicals against *B. alexandrina* snails. The SR values varied from 2.22 to > 15.44 fold depending on the chemical. Mixing the chemical with the PB greatly improved the molluscicidal activity compared to the chemical alone. Cinnamyl alcohol and cinnamyl aldehyde were the most synergistic followed by benzyl alcohol and α -terpineol.

The LC₅₀ value of cinnamyl alcohol or benzyl alcohol alone was >400 μ g/ml, but when mixed in

1:2 ratio with PB, the LC₅₀ values highly decreased to 25.91 and 79.77 μ g/ml leading to SR's values of >15.44 and >5.02, respectively. In other words, the molluscicidal activity of cinnamyl alcohol was increased more than 15.44 times by the synergist, PB. However, the toxicity of anisole and thymol were slightly synergized with SR values of 2.22 and 2.35, respectively (Table I).

In case of *T. pisana* snails, thymol ($LD_{50} = 120.61 \mu g/snail$) was found to be the most effective chemical followed by eugenol ($LD_{50} = 125.82 \mu g/snail$) and pulegone ($LD_{50} = 361.79 \mu g/snail$), while cinnamyl alcohol, cinnamyl aldehyde and *trans*-anethole showed low activity, displaying LD_{50} values of 549.52, 582.73 and 668.97 $\mu g/snail$, respectively. α -terpineol and benzyl alcohol occupied an intermediate status; they have LD_{50} values of 417.52 and 447.71 $\mu g/snail$, respectively. The rest of the screening compounds were less effective (Table II).

The results also showed that PB synergized all of the tested chemicals except anisole against *T. pisana* with SR values ranged from 1.30 to > 1.24, depending on the chemical. Mixing the chemical with PB improved the molluscicidal activity compared to the chemical alone. Phenethyl propionate, benzyl alcohol, thymol and cinnamyl alcohol were the most synergized compounds against *T. pisana* snails.

DISCUSSION

In the present study, we tested the molluscicidal activity of ten naturally occurring compounds against B. alexandrina and T. pisana snails. Among these, thymol showed the greatest molluscicidal activity against the two tested snails. This result confirm the earlier findings reported that thymol is known to be bio-toxic against the aquatic, B. glabrata snails (Marston and Hostettmann, 1985), B. alexandrina and Helix aspersa snails (El-Zemity et al., 2001a,b), Bulinus truncates (Lahlou and Berrada, 2001) and B. alexandrina, Bulinus truncates and Lymnneae natalensis (El-Din, 2006). Encouragingly, thymol, trans-anethole, pulegone, cinnamyl aldehyde and eugenol showed significant molluscicidal activity against B. alexandrina snails with LC₅₀'s recorded (13.92-76.91 µg/ml) falling

Material	LC ₅₀ (µg/ml) after 72 hr	95% Confidence limits	Synergistic ratio ^a	Slope ± SE
				•
Benzyl alcohol ^b	>400			
Benzyl alcohol + PB	79.77	62.79 - 101.42	>5.02	1.02 ± 0.01
A – Terpineol	262.23	248.36 - 276.86		4.48 ± 0.11
A – Terpineol + PB	59.80	47.62 - 75.06	4.38	1.08 ± 0.01
Cinnamyl alcohol	>400			
Cinnamyl alcohol +PB	25.91	20.87 - 32.09	>15.44	1.43 ± 0.02
Thymol	13.92	13.47 – 14.39		7.99 ± 0.27
Thymol + PB	5.92	4.68 - 7.42	2.35	1.06 ± 0.05
Eugenol	76.91	71.24 - 83.04		4.17 ± 0.17
Eugenol + PB	24.20	19.49 - 30.12	3.18	1.10 ± 0.08
Anisole	185.48	149.86 - 229.87		1.45 ± 0.02
Anisole + PB	83.45	67.76 - 102.83	2.22	1.00 ± 0.01
trans-anethole	28.16	26.89 - 29.49		5.52 ± 0.15
trans-anethole +PB	8.15	6.49 – 9.56	3.45	1.75 ± 0.01
Phenethyl propionate	296.27	279.45 - 314.10		4.40 ± 0.12
Phenethyl propionate + PB	88.56	76.73 - 102.23	3.34	1.92 ± 0.02
Pulegone	49.93	42.29 - 58.89		2.05 ± 0.02
Pulegone + PB	13.08	10.63 - 16.08	3.82	1.11 ± 0.07
Cinnamyl aldehyde	60.41	57.88 - 63.04		6.96 ± 0.27
Cinnamyl aldehyde + PB	10.20	8.87 - 11.71	5.92	2.05 ± 0.02
Cupper sulphate ^c	2.90	2.32 - 3.63		2.30 ± 0.40
Niclosamide ^c	0.39	0.25 - 0.59		5.12 ± 0.03

well below the threshold of 100 μ g/ml, set for a

potential natural molluscicides by WHO (1965). In

 Table I. Toxic effect of some naturally occurring compounds alone and in combination with piperonyl butoxide (PB) 1:2 against *Biomphalaria alexandrina* snails.

^a Synergistic ratio (LC₅₀ of chemical / LC₅₀ of chemical + PB).

^b The chemicals which did show less than 50% mortality at the highest concentration have been presented as $LC_{50} > 400 \ \mu g / ml$. ^c Niclosamide and copper sulfate were used as standard molluscicides.

addition, the molluscicidal activity of thymol and eugenol against *T. pisana* snails were almost equivalent to that of the standard, methiocab. Accordingly, these chemicals can be classified as qualified molluscicide candidates.

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

The molluscicidal mechanism of these naturally occurring compounds may have a multiplicity of effects. Either the snail draws in the shell after ejection of the haemolymph, or the snail inflates and extends out of the shell by rupture of osmotic equilibrium which is under neurohormonal control (McCullough and Mott, 1983). Another possibility is that these components disrupt the cell membrane of the snail and change its permeability (Appleton, 1985).

It is evident from the obtained results that the use of PB to improve the efficacy of tested compounds resulted in high molluscicidal activity. Both cinnamyl alcohol and benzyl alcohol were strongly synergized by PB against B. alexandrina snails. The molluscicidal effect of these compounds with PB against the snails was still less active than the standard molluscicides (niclosamide and copper sulfate). However, the mixture of thymol, eugenol or benzyl alcohol with PB notably increased the molluscicidal activity over the standard molluscicide, methiocarb in killing T. pisana snails. PB usually exerts its synergistic action with synthetic pesticides and/or natural products by inhibiting the mixed function oxidase activity which detoxifies xenobiotics (Metcalf, 1967; Matsumura, 1985). Besides, it may increase the penetration of the pesticide resulting in a high titer at the active site synergistic action of the PB with the naturally

of action (Singh et al., 1998). In the present study,

Toxic effect of some naturally occurring compounds alone and in combination with piperonyl butoxide (PB) 1:2 Table II.against Theba pisana snails after 48 h

Material	LD ₅₀ μg/snail	95% Confidence limits	Synergistic ratio ^a	Slope ± SE
Benzyl alcohol	447.71	418.13 - 479.37		4.39 ± 0.14
Benzyl alcohol + PB	83.93	72.97 - 96.53	5.33	1.97 ± 0.04
α – Terpineol	417.52	398.12 - 437.85		8.08 ± 0.42
α – Terpineol + PB	157.49	135.51 - 183.12	2.65	1.74 ± 0.03
Cinnamyl alcohol	549.52	507.22 - 595.38		3.83 ± 0.14
Cinnamyl alcohol +PB	160.14	127.94 - 200.75	3.43	1.39 ± 0.03
Thymol	120.61	92.94 - 156.35		2.44 ± 0.09
Thymol + PB	34.96	27.16 - 44.88	3.45	1.69 ± 0.04
Eugenol	125.82	116.71 – 135.65		4.04 ± 0.07
Eugenol + PB	50.13	37.51 - 66.81	2.51	1.14 ± 0.03
Anisole ^b	>750			
Anisole + PB	>750			
trans-anethole	668.97	567.03 - 789.53		2.15 ± 0.08
<i>trans</i> -anethole + PB	514.72	436.91 - 606.54	1.30	1.73 ± 0.06
Phenethyl propionate	>750			
Phenethyl propionate + PB	602.14	532.57 - 680.91	>1.24	2.64 ± 0.09
Pulegone	361.79	323.19 - 404.98		2.43 ± 0.07
Pulegone + PB	256.49	224.65 - 292.79	1.41	2.50 ± 0.07
Cinnamyl aldehyde	582.73	526.89 - 644.54		3.14 ± 0.11
Cinnamyl aldehyde + PB	230.24	184.25 - 287.49	2.53	1.58 ± 0.06
Methiocarb ^c	107.34	87.83 - 124.35		2.43 ± 0.06

Synergistic ratio (LC_{50} of chemical / LC_{50} of chemical + PB).

^b The chemicals which did show less than 50% mortality at the highest concentration have been presented as $LD_{50} > 750 \mu g / snail$. ^c Methiocarb was used as a standard molluscicide.

occurring compounds may be due to the inhibition of microsomal oxidases which reduces the detoxification of the chemical components thus extending the effective dose at the active site. Singh et al. (1998) found that the molluscicidal activity of azadirachtin against Lymnaea acuminate snails was increased 3 to 11 times using PB at exposure period from 24 to 96 hours. Moreover, El-Zemity et al. (2001a,b) showed that β -citronellol and carvacrol were the most synergized by PB against B. alexandrina and H. aspersa snails.

In general, it could be concluded that the obtained results of these naturally occurring compounds were very promising particularly those of thymol, which exhibited high molluscicidal activity against the two tested snails. Moreover, mixing the chemical with PB led to an increase in the molluscicidal activity compared to the chemical alone. The molluscicidal activity of thymol, eugenol

and benzyl alcohol were more effective than the standard molluscicide, methiocarb, when mixed with PB against T. pisana snails. Accordingly, these chemicals could be developed or used as building block for synthesizing a new potent and safe molluscicide against the tested snails.

Our results indicate positive potential for these chemicals of plant origin in the snail control. Such compound is promising as a lead towards maximizing the biological activity as pesticides for future potential efficient alternatives that are expected to be much safer than the synthetic conventional compounds now in use.

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