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Molluscicidal potency of some naturally occurring compounds and their blends against schistosomiasis snail vector, *Biomphalaria alexandrina.*

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ABSTRACT

The present study describes the molluscicidal potency of some naturally occurring compounds and their blends against *Biomphalaria alexandrina* snail, a vector of *schistosoma mansoni* in Egypt. The role of the synerigst, piperonyl butoxide in improving the efficacy of these chemicals was also investigated. The results revealed that thymol exhibited marked potency with LC_{50} value of 13.92 ppm, followed by anethole and pulegone with LC_{50} values of 28.16 and 49.93 ppm, respectively. The toxic effect of these chemicals or their blends against the snails was less active than the standard molluscicides. The synergist, piperonyl butoxide enhanced the toxicity of all of the tested chemicals.

Keywords: Molluscicidal activity, natural products, plant essential oils components, *Biomphalaria alexandrina,* fresh water snails.

INTRODUCTION

The aquatic snails are of great concern in medical and veterinary vector control due to their main role as intermediate hosts for the trematodes causing schistosomiasis and fascioliasis in humans and domestic animals (Godan,1983). Schistosomiasis (Bilharziasis) is a human disease caused by parasitic blood flukes of the genus *Schistosoma*. These flukes are endemic in more than 70 tropical and subtropical countries and infect more than 250 million people worldwide (Anonymous, 1985 and Marston and Hostettmann, 1985).

Synthetic molluscicides are still considered the most effective measures for the control of schistosomiasis and considerable success have been achieved by the use of these chemicals such as copper sulphate, trifenmorph, niclosamide and sodium pentachlorophenate for the elimination of the snail intermediate host ,which is responsible for their transmission. However, these synthetic compounds may lead to problems of toxicity to non-target organisms in addition to deleterious long-term effects to the environment, especially the aquatic biota (El-Mofty *et al.*, 1982; Sabry, 1983 and Awad, 1985).

Plant products and extracts are currently receiving greater attention for their molluscicidal activity which may be useful for the control of snail populations and hence control transmission of schistosomiasis. Their use is simple, inexpensive and environmentally acceptable as an alternative for effective control of the aquatic snails (Lemma, 1970; Adewunmi and Sofowora, 1980 and El-Sawy *et al.*, 1981). The naturally occurring molluscicides have shown promising results for the control of fresh water snails (Kady *et al.*, 1986; Speiser *et al.*, 1992; Ekabo *et al.*, 1996 and Singh *et al.*, 1998). Therefore, the

present work is devoted to evaluate the molluscicidal activity of some naturally occurring compounds and their blends against *Biomphalaria alexandrina* snails, which is the main vector of human bilharziasis in northern Egypt, in order to find out a new natural molluscicide. The role of the synergist, piperonyl butoxide, in improving the efficacy of these chemicals was also investigated under laboratory conditions.

MATERIALS AND METHODS

Fresh water snails: The intermediate hosts for schistosomiasis (Bilharziasis) of human disease, Biomphalaria alexandrina snails (8 mm ± 2 mm diameter) were collected from fresh water ponds at Kafr El-Dwar, Behera Governorate during May, 2000, and were maintained in glass aquaria containing 5 liters of dechlorinated water supplied with vegetation and dry lettuce leaves as a feed. The aquaria were oxygenated for one hour daily, one week before testing.

Test chemicals: The following ten commercially available essential plant oils components and two of their blends were used in this study are supplied by Ecosmart company:- Benzyl alcohol (99%), cinnamyl alcohol (98%), cinnamyl aldehyde (99%), α-terpineol (98%), thymol (98%), eugenol (99%), anethole (99%), anisole (99%), pulegone (98%), phenylethylpropionate (98%), 3-blend (eugenol + α-terpineol + cinnamyl aldehyde) and 5-blend (eugenol + α-terpineol + cinnamyl aldehyde + thymole + anisole). The chemical structures of these chemicals are shown in Fig. 1.

Laboratory testing: The recommended World Health Organization (WHO) bioassay procedure (Anonymous,

1965), was used for the evaluation of some naturally occurringcompounds on snail-intermediate host of schistosoma, *B. alexandrina.* The tests were performed in 100 ml glass beakers with 10 snails per replicate. The tested chemicals were mixed with Tween 20 in 2:1 ratio. The mixture was added to the glass beaker which is then filled up to 100 ml of the dechlorinated water to give the desired concentration (w/v).

Different concentrations up to 400 µg/ ml for each chemical or chemical + piperonyl butoxide at the ratio of 1 : 2, respectively, were used. It was observed that there was no mortality up to 400 ug/ ml of piperonyl butoxide or Tween 20 alone. Three glass beakers were set up for each concentration of each chemical. Control assays with Tween 20 were carried out with appropriate dilutions in dechlorinated water without tested chemical. Niclosamide and / or copper sulphate was used as a reference. The snails were exposed for 24 h to different concentrations of compounds or their mixture with piperonyl butoxide at the ratio of 1 : 2 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.

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

RESULTS AND DISCUSSION

The LC_{50} values of some naturally occurring compounds and their blends against the fresh water snail, *B. alexandrina* along with the slope regression lines and the synerigstic ratios (SR's) are shown in Table 1.

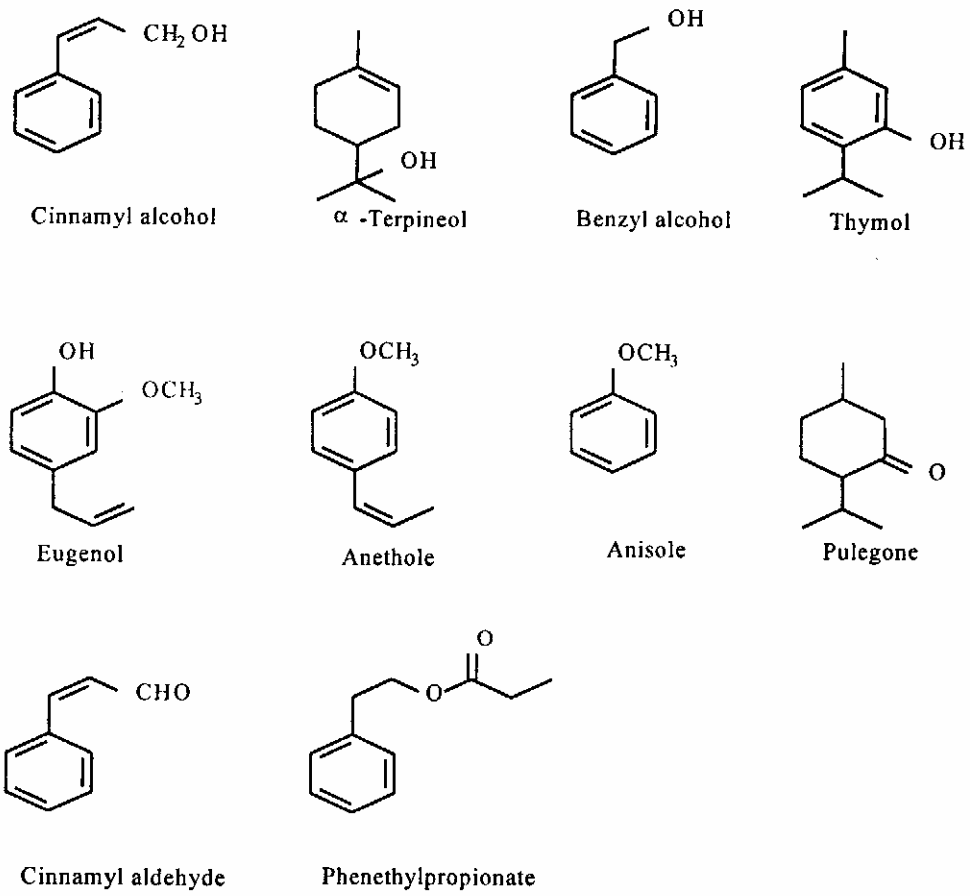

Fig 1. Chemical structures of the tested components

Thymol was shown to be the most potent among all the tested compounds with LC_{50} value of 13.92 ppm. This result coincides with the literature (Marston and Hostettmann, 1985), concerning the molluscicidal activity of thymol against the aquatic fresh snail, *B. glabrata*. In addition, the molluscicidal activity of anethole, pulegone and cinnamyl aldehyde was shown

to be moderate and respond positively to be synergized when mixed with piperonyl butoxide.

Variation as well as modification of the chemical structure proved to have a significant impact on the screened molluscicidal potency against the aquatic snails, *B. alexandrina*. The structure of thymol proved to be most favorable among the compared active ingredients (Fig.1). Its slight modification to the structure of pulegone did not favor the molluscicidal potency. Similarly, the para substitution with a propylene side chain in anisole seems to enhance the molluscicidal potency as shown in anethole which is para propenyl anethole. Besides, cinnamyl aldehyde proved to be more potent than the corresponding cinnamyl alcohol.

Mixing some of these components together was a successful approach as in 3 blend (eugenol, 76.91 ppm + α-terpineol, 262.23 ppm + cinnamyl aldehyde, 60.41 ppm) lead to an increase in the molluscicidal activity, whereas, the LC_{50} of 3-blend components was 44.13 ppm. Contrary, 5-blend components (eugenol, 76.91 ppm + α-terpineol, 262.23 ppm + cinnamyl aldehyde, 60.41 ppm + thymol, 13.92 ppm + anisole, 185.48 ppm) reduced the molluscicidal activity and the LC_{50} turned to be over 400 ppm.

Piperonyl butoxide usually exerts its synergistic action with synthetic pesticides and/ or natural products by inhibiting the mixed function oxidase activity which detoxifies 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. In the present study, synergistic action of the piperonyl butoxide with the naturally occurring components 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.

Table 1: Toxic effect of some natural occurring compounds and their blends alone and in combination with piperonyl butoxide (PB) 1: 2 against *Biomphlaria alexandrina* snails.

Material (s)	LC_{50} (µg / ml) after 72 hr	Synergistic Ratio [b]	slope & variance
Benzyl alcohol [c]	> 400		
Benzyl alcohol + PB	79.77 (62.79-101.42)[a]	>5.02	1.02 ± 0.011
α-Terpineol	262.23 (248.36-276.86)		4.48 ± 0.109
α-Terpineol + PB	59.80 (47.62-75.06)	4.38	1.08 ± 0.011
Cinnamyl alcohol [c]	> 400		
Cinnamyl alcohol + PB	25.91 (20.87-32.09)	>15.44	1.43 ± 0.015
Thymol	13.92 (13.47-14.39)		7.99 ± 0.276
Thymol + PB	5.92 (4.68-7.42)	2.35	1.06 ± 0.053
Eugenol	76.91 (71.24-83.04)		4.17 ± 0.174
Eugenol + PB	24.20 (19.49-30.12)	3.18	1.10 ± 0.080
Anisole	185.48 (149.86-229.87)		1.45 ± 0.021
Anisole + PB	83.45 (67.76-102.83)	2.22	1.00 ± 0.012
T-Anethole	28.16 (26.89-29.49)		5.52 ± 0.148
T-Anethole + PB	8.15 (6.94-9.56)	3.45	1.75 ± 0.014
Phenethyl propionate	296.27 (279.45-314.10)		4.40 ± 0.124
Phenethyl propionate + PB	88.56 (76.73-102.23)	3.34	1.92 ± 0.019
Pulegone	49.93 (42.29-58.89)		2.05 ± 0.025
Pulegone + PB	13.08 (10.63-16.08)	3.82	1.11 ± 0.069
Cinnamyl aldehyde	60.41 (57.88-63.04)		6.96 ± 0.271
Cinnamyl aldehyde + PB	10.20 (8.87-11.71)	5.92	2.05 ± 0.020
3 blend	44.13 (41.88-46.51)		6.49 ± 0.261
3 blend + PB	20.40 (17.10-24.35)	2.16	1.35 ± 0.01
5 blend [c]	> 400		
5 blend + PB	50.87 (42.37-61.03)	>7.86	1.43 ± 0.013
Cupper sulfate [d]	2.9 (2.32-3.63)		2.30 ± 0.040
Niclosamide[d]	0.39 (0.25-0.59)		5.12 ± 0.026

[a] 95 % confidence limits in parenthesis.

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

[c] The chemicals which did show less than 50% mortality at the highest concentration have been presented as LC_{50} > 400 µg / ml.

[d] Niclosamide and cupper sulfate were used as standard molluscicides

Synergism expressed as synergistic ratio (SR) was measured according to the equation of El-Sebae *et al.*, 1964 and Hewlett, 1969. The results showed that piperonyl butoxide synergized all of the tested chemicals against *B. alexandrina* snails. However, lower SR values may suggest that a mixed-function oxidase detoxification is not the specific site. The synergistic ratio (SR) varied from 2.16 to >15.44 fold depending on the chemical and its piperonyl butoxide ratio. Mixing the chemical(s) or their blends with the synergist piperonyl butoxide greatly improved the molluscicidal activity compared to the chemical or the blend alone. Cinnamyl alcohol, cinnamyl aldehyde and 5-blend were the most synergistic followed by benzyl alcohol and α-terpineol.

LC_{50} values of cinnamyl alcohol, 5-blend and benzyl alcohol were > 400 ppm, but when mixed in 1:2 ratio with piperonyl butoxide, the LC_{50} values highly decreased to 25.91, 50.87 and 79.77, ppm leading to SR's values of > 15.44, >7.86 and >5.02, respectively. In other word, the molluscicidal activity of cinnamyl alcohol was increased more than 15.44 times by the synergist, piperonyl butoxide. However, the toxicity of anisole and thymol were slightly synergized with SR values of 2.22 and 2.35, respectively.

Generally, it could be concluded that, four of the tested components caused considerable toxicity against *B. alexandrina* snails with LC_{50}'s lower than 50 ppm, i.e, thymol, anethole and pulegone and 3-blend. However, The molluscicidal effect of these components and their blends alone or with piperonyl butoxide against the snails was still less active than the standard molluscicides (niclosamide and copper sulphate). Thymol was the most potent either alone or with the synergist, pipronyl butoxide. 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. Niclosamide, being reported to be genotoxic and possible carcinogen to humans, is one of the first priority to be replaced by the newly successful natural products.

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الملخص العربى

الكفاءة الإبادية لبعض المركبات الطبيعية و مخاليطها ضد قوقع البيموفلاريا أليكذندرينا العائل الوسيط للبلهارسيا

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تصف هذة الدراسة الكفاءة الابادية لبعض المركبات الطبيعية ومخاليطها ضد قوقع البيموفلاريا أليكذندرينا العائل الوسيط للبلهارسيا فى مصر . وقد تم أيضاً دراسة دور المنشط "ببرونيل بيوتوكسيد" فى تحسين كفاءة هذة المكونات. أوضحت النتائج أن الثيمول قد أظهر فاعلية واضحة بقيمة LC_{50} تساوى ١٣,٩٢ جزء فى المليون تلاه فى ذلك مركب الأنيتول ثم البلجون بقيم LC_{50} تساوى ٢٨,١٦ ، ٤٩,٩٣ جزء فى المليون على التوالى، وكانت سمية هذه المكونات أومخاليطها منفردة ضد القوقع أقل من المركب الموصى به ، وقد زود المنشط ببرونيل بيوتوكسيد السمية لكل المركبات المختبرة.