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Evaluation of the Pesticide Emamectin and Methanol Extract of Wheat Bran against *Biomphalaria Alexandrina* Snails, Their Hemocytes and Their Infection with *Schistosoma Mansoni*

***Hanan S. Mossalem*[1*] *--- Gehan L. ElEnain*[2]**

[1]Department of Environmental Research and Medical Malacology, Theodor Bilharz Research Institute (TBRI), Giza, Egypt
[2]Department of Parasitology, TBRI, Giza, Egypt; Natural Sciences, University College, Abu Dhabi University, UAE

Abstract

The present study was carried out to evaluate the molluscicidal activity of the pesticide Emamectin (5% aqueous solution) and the methanol extract from the wheat bran (MEWB) against *B alexandrina* and their infection with *Schistosoma mansoni* was studied. The LC_{90} and LC_{50} values for Emamectin were 50.4 and 22.3 ppm, respectively. Infection of snails with *S. mansoni* under the effect of the tested agents was evaluated via four experimental groups, each of 50 snails For three consecutive days, one group of snails was exposed to 9.08 ppm aqueous Emamectin solution, another group received 100 ppm methanol extract of wheat bran (MEWB), a third group was administered by a combination of 9.08 ppm Emamectin and 100ppm MEWB, The fourth group was control maintained under similar experimental conditions. After three days, all the experimental groups were infected with *S. mansoni*miracidia and observed till shedding of cercariae. The physiological and histological changes were assessed before and after the infection.The changes in hemocytes of infected snails after administration with LC25 of Emamectin or wheat bran was significantly (20% and 30%, respectively) suppressed compared to 75% for the control snails. However, the snails treated with joint of Emamectin and MEWB were the least infected snails (10%). On the other hand, the biochemical test results showed a remarkable reduction of GOT ($p<0.01$) GPT ($p<0.05$) and total protein (0.05) in the heamolyph extracted from the snails treated with an aqueous Emamectin solution. Yet, the levels of GOT were significantly increased in the groups administered with the MEWB alone ($p<0.01$) or in combination with the Emamectin ($p<0.001$). As for the total protein levels, there were slightly declining ($p<0.05$) in the group exposed to the aqueous Emamectin and to the contrary, theses levels were significantly increased ($P<0.05$) in the snails of the two other experimental groups when compared to the controls. Moreover, the haemocytes cells showed a differentiation which varied in number when detected under the microscope. Administration of the aqueous Emamectin solution resulted in a significant increase ($p<0.05$) of the amaebocytes and a considerable decrease ($p<0.05$) in the number of granulocytes and hyalinocytes compared to the control snails. On the contrary, a remarkable surge ($P<0.05$) of granulocytes and decrease of the amaebocytes was detected in the haemolyph of snails treated with MEWB.

Keywords: Biomphalaria alexandrina, Emamectin, Wheat bran, Molluscicide, Antioxidant, Schistosoma mansoni.

Contents

** Corresponding Author*

1. Introduction

Schistosomiasis is the second major parasitic disease in the world after malaria [1]. It poses not only a health problem, but economic crisis as well. Relatively 200 million people are infected with *Schistosoma* and around 800 millions are at risk of infection [2].

Fresh water snails are the intermediate hosts that transmit the human schistosomiasis [3]. The intestinal Schistosomiasis is caused by *Scistosomamansoni* which is transmitted by the intermediate host *Biomophalaria alexandrina*, a fresh water snail found in Egypt [4].

It is well-known that control of the freshwater snails might play a key role in the transmission of schistosomiasis. Snails are approved to be the most vulnerable link in the schistosome life cycle and consequently, targeting their populations is an important approach to prevent the transmission of shistosomes. Still comprehensive information about snails is required [5] to assist in developing a harmless molluscicide.

Application of molluscicides (synthetic or of plant origin) can confer a rapid and efficient technique in tumbling the snail populations [6]. Attempts to reduce or eliminate populations of freshwater snails in Africa have been concentrated on the intermediate hosts of schistosomes with less attitude to use the chemicals for the purpose of reducing transmission of schistosomiasis [4]. The chemical molluscicides have not achieved the expected outcomes [7] and the synthetic ones were approved to be costly in relation to the restricted budgets [7] available for the control of communicable diseases in many countries [8]. The development of molluscicides from plant origin is currently of interest in measures for snail control because they are affordable, safe and useful in local community self-help projects [9]. There are some plants with antioxidant activity such as rice bran which play an important role in reducing the pollution of aquatic media and were proved to be safe for all manner of objects and the surrounding environment [10].

It possesses not only detect the molluscicidal activity of the aqueous Emamectin solution to assist in developing a candidate molluscicude, alone and combined with methanol extract from the wheat bran, as antioxidant agent, against *B. alexandrina*.

2. Materials and Methods

2.1. Snails

Snails used in this study were adult *Biomphalariaalexandrina* (8-10mm) collected from the River Nile and irrigation scheme near Cairo and brought to the laboratory, washed with dechlorinated water and examined for larval trematodes. Unhealthy and infected snails were excluded, the healthy snails were maintained in plastic aquaria under standard conditions ($25^{o}C \pm 1^{o}C$, PH = 7.2) for three weeks before being used in molluscicidal tests. Snails were daily fed boiled lettuce leaves and Blue green algae.

2.2. Miracidia

Schistosomamansonimiracidia were obtained from Schistosome Biological Supply Center (SBSC), TBRI, Egypt.

2.3. Emamectin (Vetoken)

Emamectin (Vetoken) is non-systemic insecticide which penetrates leaf tissues by translaminar movement. Paralyses the Lepidoptera, which stop feeding within hours of ingestion, and die 2-4 days.Uses For control of Lepidoptera on vegetables, brassicas, cotton and pine trees. The purchase company of Emamectin is Shanghai Agrochina, International, Trade, CO. LTD CHIN,The structural formula of Emamectin is (4″R) -4″-deoxy-4″-(methylamino) avermectin B1 and Mode of Action: Chloride channel activator. Acts by stimulating the release of g-aminobutyric acid, an inhibitory neurotransmitter, thus causing paralysis

2.3.1. Extraction of Methanol Extract of Wheat Bran

The wheat bran was dried in shade, then in an oven at $50^{o}C$. Ten grams powder was extracted with 250 ml 95% methanol at room temperature for one week. Methanol was evaporated under vacuum and the residues were used for the bioassay tests.

2.4. The Molluscicidal Tests Using Aqueous Emamectin and Methanol Extract of Wheat Bran

The potential molluscicidal activities of Emamectin and methanol extract of wheat bran were tested against adult B. alexandrina. Different concentrations in ppm were prepared on the basis of weight/volume. For each concentration three glass containers each containing one liter dechlorinated water to which the test material was added directly on the water surface and stirred properly then 10 snails were introduced. The glass containers were covered by porous plastic sheets and maintained for 24 hours of exposure at normal laboratory conditions ($25^{o}C \pm 1^{o}C$). After that, the snails were washed thoroughly with dechlorinated water, transferred to jars containing fresh dechlorinated water for another 24 hours for recovery. Three replicates of control snails (10snails/l) were prepared in dechlorinated water. Dead snails were distinguished and counted. Further experiments were carried out such that Laboratory bred *B. alexandrina* snails (8-10 mm) were deployed into four groups, each of 50 snails. They were continuously treated for 3 days with the tested agents one group Emamectin solution was treated with the LC50 of Emamectin, another group was treated with MEWB, the third was exposed to a combination of 9.08ppm Emamectin and 100ppm WEMB, whereas the fourth group was maintained at the same conditions in dechlorinated water as a control group. Dead snails were removed daily from all experimental groups. Snails were considered dead if they probed and remained motionless or if the shell looked discolored. After 3 days of treatment, the survived snails at all groups were subjected to to infection with *S. mansoni*miracidia and kept till shedding of cercaria. The extent of the survival of the experimental snails were estimated by relating them to their counterparts of the control snails. Determination of total protein, Haemocytes number, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transminase (GPT) in snails' hemolymph.

2.5. Heamatocyte Count

Haemolymph samples were collected according to Michelson [11] by removing a small portion of the shell and inserting a capillary tube into the heart. The haemolymph pooled from 10 snails were collected in a vial tube (1.5ml) and kept in an ice-box.

2.5.1. Total Protein, Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT)

Colorimetric estimation of total protein content in haemolymph of *B. alexandrina* snails was estimated according to the principle of Biuret-tartrate method [12]. that of GOT and GPT was detected as stated by Reitman and Frankel [13].

3. Statistical Analysis

The means of the different groups were compared globally using the student's t- test Sokal and Rohlf [14]. The Molluscicidal activity of the tested chemical was calculated by Probit analysis, SPSS Computer Program version19, Window7, MW Office 2007.

4. Results

4.1. Molluscicidal Activity of Aqueous Emamectin and Methanol Extract of Wheat Bran

The effect of various concentrations of the aqueous Emamectin *(5*%) and MEWB on adult *B. alexandrina* snails after 24hr exposure was evaluated (Table 1 and Fig. 1). All MEWB concentrations up to 100ppm were harmless to the snails. The LC_{25}, LC_{50} and LC_{90} obtained with the aqueous Emamectin solution against *B. alexandrina* snails were 9.08 ppm, 22.3ppm and 50.4 ppm, respectively. Surprisingly, administration of joint 100ppm MEWB and aqueous Emamectin solution changed the activity of the Emamectin against *B. alexanderia* snails to be 22.916ppm at LC25, 31.8 ppm at LC50 and 48.816 ppm in LC90, as depicted in table 2.

4.2. Moullscicidal Efficacy on the B. Alexandrina Infectivity withS. Mansoni

The percent *S. mansoni* infection to the experimental snails was remarkably lower ($p<0.001$) than that detected in the infected negative control snails. The lowest infection (10%) was determined by the group of snails administered by a joint of aqueous Emamectin and MEWB.

4.3. The Effect of Emamectin and MEWB on B. Alexanderina Physiologic and Histologic Activity

The enzyme activity measured by the levels of GOT, GPT and total protein showed variable responses to the administered substances. In the 9.08ppm Emamectin treated snails, there was a significant decline in the levels of the three types of protein, whereas administration of MEWB alone or in combination with the aqueous Emamectin solution to snails increased the levels of GOT, GPT and total proteins in the haemolymph, considerably (Table 2). Administration of the Emamectin resulted in a significant increase ($p<0.05$) of the amaebocytes and a considerable decrease ($p<0.05$) in the number of granulocytes and hyalinocytes compared to the control snails. On the contrary, a remarkable surge ($P<0.05$) of granulocytes and decrease of the amaebocytes was detected in the haemolyph of snails treated with MEWB (Table 3 & Fig. 1).

5. Discussion

The aqueous Emamectin (5%) is one of the most chemical pesticides used in cotton agriculture and causes pollution in the aquatic environment. The molluscicidal activity of aqueous Emamectin (5%) alone and joint with the methanol extract from wheat bran was evaluated against *B. alexandrina* snails. The lethal concentrations of this molluscicide obtained against *B.alexandrina* snails LC_{90} and LC_{50} values were 50.4ppm and 22.3ppm.The current data showed a severe decrease (20% and 10%) in the infection rate of snails infected with *S.mansoni* after being continuously exposed to the sublethal dose LC_{25} of Emamectin (5%) alone and in combination with the MEWB, respectively. This may be explained by the deteriorations of physiological parameters of snails making them incongruous for the parasite development [15]. Mahmoud, et al. [16] found that exposure of *B. alexandrina* and *B. glabrata* snails to sublethal concentration of *Cryptostegiagrandiflora* 3 days pre-miracidial exposure led to a significant reduction in the infection rate with *S. mansoni* by 55.47% and 58.9%, respectively. In another study [17] ,administration of LC_{10} of *Daturastramonium* and *Sesbaniasesban* during *S. mansoni*miracidial exposure reduced the infection rate of *B. alexandrina* snails by 41.7% and 52.2%, respectively compared to the control group. All the recorded results in the present study concerning total protein levels, GOT and GPT activities are mostly in agreement with with similar research work conducted by many authors [18-21].

The present investigation showed that haemocytes in the experimental snails were significant different in number when compared to the control ones. Three types of haemocytes were detected unevenly tribute in the haemolymph of *B. alexandrina* snails. They ranged from Granulocytes 60%, Amoebocytes 25% to Hyalinocytes 15% in the lymph of the control snails, which is supported by the results obtained in different studies [15, 22-26]. Administration of Emamectin (5%) reduces the number of Granulocytes and, Hyalinocytes (40% and 10%, respectivel) but increased the number of Amoebocytes by 50%. This observed surge in the number and size of Amoebocytes might be due to their main role in encountering any invaders/foe that attack the snails' tissue and being activated by engulfing such administered harmful pesticide. Previous similar observations were detected when snails were subjected to the molluscicideArtemether for 3 days and obvious abnormalities were detected in haemocytes morphology [10] In the present study, exposure to the wheat bran alone or in combination with the Emamectin has increased the number of haematocytes which might be due to the highly effective role of all extracts of wheat bran in inhibiting the Linoleic acid peroxidation as demonstrated by Laokuldilok, et al. [27]. It has been approved that rice bran has antioxidant

activity due to the high content of phytochemicals Norhaizan, et al. [28] which may indicate that the wheat bran also have antioxidants that improve the performance of the Emamectin and reduce its harmful pesticidal activity.
This work vigorously urge more researches on all types of bran that could be anti-pollutant agents and friendly environment substances.

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Table-1. Mortality rate of Vetoken in comination with methanol extract of wheat bran against Biomophalaria alexandrina snails

Concentration (ppm)	Total no. of snails	Total no. of dead snails
45	48	**40**
40	48	**35**
35	48	**30**
30	48	**22**
25	48	**11**
15	48	**8**
10	48	**1**

Table-2. Molluscicidal activity of Vetoken (5%) alone and joint with Wheat bran against B. alexandrina

PROBIT	Aqueous Vetoken (5%)		Vetoken (5%) combined with 100 ppm MEWB	
	95% Confidence Limits for Concentration			
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
LC_{90}	50.4ppm		48.816ppm	
	39.480	79.538	45.275	53.865
LC_{50}	22.3ppm		31.847	
	16.748	31.370	29.804	33.977
LC_{25}	9.08ppm		22.916	
	-5.032	15.834	19.937	25.235

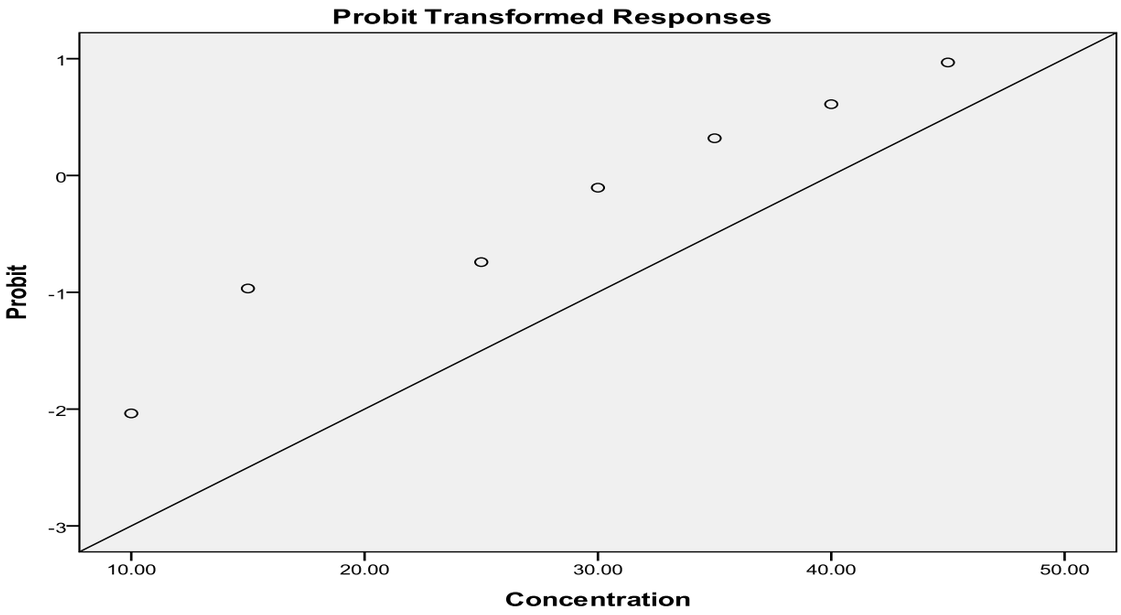

Fig-1. Dose/Probit regression line of joint Vetoken/MEWB on Biomophalaria alexandrina

Table-3. The average percentage of *S. mansoni* infection to *B. alexandrina* in the experimental groups

Concentrations	% infection	GOT (g/dl)	GPT (g/dl)	Total protein (g/dl)
	Mean ± SD			
9.08ppm Vetoken	20%***	0.318667±0.006351**	0.348667±0.005508*	5.44±0.1*
100ppm WEMB	30%***	0.415667±0.005508**	0.392333±0.005508*	6.776667±0.01527*
9.08ppm Vetoken + 100ppm WEMB	10%***	0.542667±0.009018***	0.415667±0.005508*	5.95±0.01*
Control	75%	0.350667±0.000577	0.353±0.001	5.748± 0.001

***high significant at p<0.001; ** moderate significant at <0.01; *slightly Significant at p<0.05

Table-4. The haematocytes countbefore and after three-consecutive days' exposure to aqueous vetoken and MEWB

Concentration	Granulocyte	Amoebocyte	Hyalinocyte
Control	60%	25%	15%
Vetoken	40%*	50%*	10%*
MEWB	70%*	15%*	15%

*significant at P<0.05

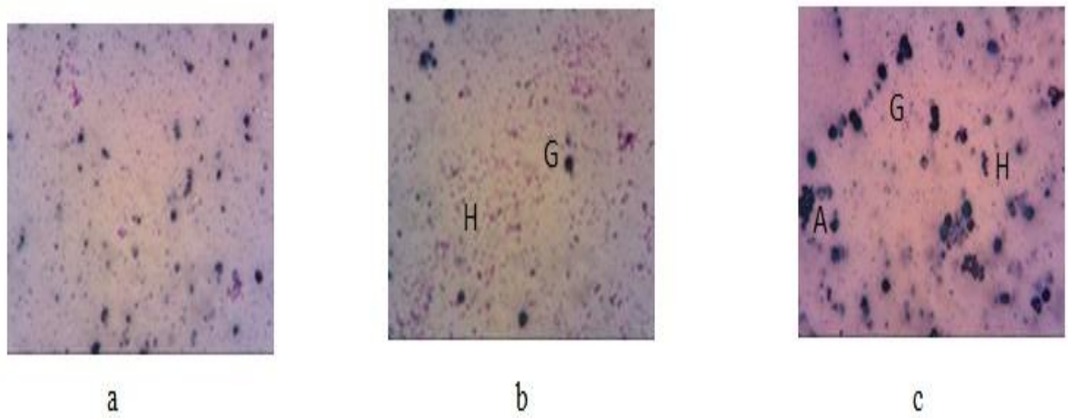

Fig-2. Haemocytes under light microscope showing G granulocyte, A amoebocyte, and H hyalinocyte a. Control; b. LC_{25}Vetoken-treated snails showed decreasing in the number of granulocytes; c. Haemocytes from 100ppm MEWB-treated snails showing an increase in the number and size of granulocytes. A is denoted for amaebocytes, G stands for granulocytes and H refers to hylinocytes (Light microscope, 200X)