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EFFECT OF DIFFERENT SYNTHETIC PESTICIDES FOR THE FRESHWATER SNAIL, *Lymnaea luteola* THE INTERMEDIATE HOST FOR *Fasiola hepatica*

Al-Megrin, Wafa A. I.
Department of Zoology, Girls Collage of Education, P.O. Box. 25701, Riyadh 11476, Saudi Arabia

ABSTRACT

The toxicity of three synthetic compounds namely (Diazinon, Phorate and Carbaryl) against eggs, immature, young mature and adult stages of *L. luteola* was studied. A comparison between the effects of the three compounds against eggs of 0-24 h old of *L. luteola* revealed that the three compounds gave low ovicidal effect on the base of LC_{50} and LC_{90} values. While, Carbaryl gave more effective than Phorate then Diazinon came in the last category against eggs and immature stages of *L. luteola* according to the LC_{50} also, Phorate gave more effect than Carbaryl then Diazinon came in the last category against young and mature stages of *L. luteola* according the LC_{50} vapues. The slope values indicated that the insect population had the same homogeneity in their response to both Phorate and Carbaryl, while it had less homogeneity in case of Diazinon for the tested stages of *L. luteola*.

INTRODUCTION

The freshwater snail, *Lymnaea luteola* is infested with the miracidia of *Fasiola hepatica* (common liver fluke), the parasite that causes faschiolasis in animals other than sheep, It is also found in cattle and humans. The parasite resides in the bile ducts inside the liver and it is a common parasite of sheep and cattle. Infection occurs when encysted metacercariae are ingested on raw vegetation accidentally. The metacercariae move through the intestinal wall and peritoneal cavity to the liver, where adults mature in the biliary ducts of the liver. Eggs of this parasite are passed through the bile ducts into the intestine and finally they passed in the feces. Symptoms of this condition include abdominal pain, an enlarged liver, fever, vomiting and diarrhea. In the chronic phase of the infection, symptoms result from the intermittent blockage of the bile duct and inflammation. In some cases, ectopic infections can occur in the intestinal wall, lungs or subcutaneous tissues (Rivero and Marcial 1989) and (Hastanesi *et al.,* 1999). *Fasciola hepatica* is widespread and about 250 million sheep and 350 million cattle are at faschiolasis risk worldwide. Thus in many countries about a quarter of the sheep and cattle population is exposed to the infection causing severe economic loss (approximately US$2 billion worldwide) in domestic livestock; economic damage is due to mortality, liver condemnation, secondary infections, reduced milk and/or meat production, abortions and fertility reduction Sukumaran *et al.* (2004).

The main purpose of this study is to study the relative toxicity of different insecticides against different stages of the freshwater snail *Lymnaea luteola*, i.e., eggs, immature, young mature, and adults infested with miracidia of *Fasciola hepatica*.

MATERIALS AND METHODS

1. Relative toxicity of different pesticides against the freshwater snail *Lymnaea luteola*:

The freshwater snails *L. luteola* were taken from a laboratory culture maintained in enamel bowls filled with dechlorinated water at room temperature 28±2ºC and relative humidity more than 75%. They were fed with spinach and the water was changed twice a week. The egg strips containing 0-24 h old eggs were used to study the ovicidal effect of molluscicides and the snails of different sizes i.e., immature (3-6 mm), young mature (9-12 mm), and adults (more than 12-mm) were used for the toxicity studies (Sukumaran *et al.,* 2004).

The toxicity of Diazinon & Phorate (organophosphorous compounds) and Carbaryl (carbamate compound) was screened as described by WHO (1965). In the case of ovicidal action, the total number of eggs in each egg strip was counted using dissection microscope before exposure to 200 ml of the tested solutions and kept in separate Petri dishes. They were exposed for 24 h and then transferred to normal water for further 24 h. Disintegration of embryos or absence of movement of the embryos was considered for calculating the per cent mortality of eggs. The toxicity of molluscicides against adult snails was carried out with ten *L. luteola* snails of each stage. They were exposed to water in enamel bowls containing different concentrations of the molluscicides for 24 h. After exposure, they were transferred to normal water for further 24 h and later the percent mortality of snails was calculated. All the experiments were replicated six times.

2. The following materials were tested:

a. Diazinon: is an organophosphorous compound is formulation as emulsifibale concentrate, Novertes Co., containing 60% a.i. was applied with different ppm values/liter of water.
b. Phorate: is an organophosphorous compound is formulation as emulsifibale concentrate, Cyanamid Co., containing 50% a.i. was applied with different ppm values/liter of water.
c. Carbaryl: is a carbamate compound is formulation as emulsifibale concentrate, Cyanamid Co., containing 50% a.i. was applied with different ppm values/liter of water.

The least susceptible young mature stage of *L. luteola* snails was used for this study. Ten snails in each of two groups were exposed to tap water containing LC_{90} concentration of Diazinon, Phorate, and Carbaryl, respectively. Soon after exposure, they were continuously observed at an interval of every 30 min for mortality. Extrusion of the whole head-foot region permanently outside the shell or lack of any movement of the body when touched the needle with tip was considered to determine mortality of the snail. The duration required for the mortality of all treated snails was recorded. Dead snails were removed immediately from the enamel bowl. The experiment was replicated six times. The mean duration required for the

death of the snail was calculated and the data were statistically analyzed and subjected to regression curve using sigma plot.

3. Statistical analysis:

The mortality percentages were calculated and corrected for natural mortalities by Abbott's formula (1925).

The corrected percent mortalities were statistically analyzed according to Finney (1971) and plotted on probit analysis paper. The tested compounds were compared for their efficiency on the snails according to their LC_{50}.

RESULTS AND DISCUSSION

1. Relative toxicity of different pesticides against the freshwater snail *Lymnaea luteola*:

1.1. Egg stages:

Data presented in Table (1) and graphically illustrated as toxicity lines in Fig. (1) show the potency of three compounds namely (Diazinon , Phorate and Carbaryl) compared with control against eggs, immature, young mature, and adult stages of *L. luteola*.

Tabulated data indicate that the potency of the tested compounds was varied tremendously due to the nature of tested compounds, the used concentration and the tested stage. As a general trend, data proved that at any of the tested compound the higher the concentration, the higher was the rate of mortality and vice versa.

In comparison between effects of the three compounds against eggs of 0-24 h old of *L. luteola*, the three compounds gave a low effect, in which the LC_{50} values were 2338.629, 5030.687 and 5646.395 ppm for Diazinon, Phorate and Carbaryl, respectively**.**

According to the slope values data in Table (1) show similar homogeneity in response of eggs to phorate and carbaryp, while less homogeneity was recorded to Diazinon, in which the slope values are 0.602 , 0.706 for Phorate and Carbaryl and Diazinon, respectively.

In animals, the principle metabolites of Diazinon (organophosphorous compound) are diethyl triophosphate and diethyl phosphate (Pardue, 1970). Also, phorate (organophosphorous compound) is metabolically oxidized to the sulfoxide and sulfone and their phosphorothioate analogues, followed by hydrolysis to dithio-, thio- and orthophosphoric acids (Suett. 1975). On the other hand, carbaryl (carbamate compound) does not accumulate into body tissues of animals, but is rapidly metabolites to non-toxic substances, particularly 1-napthol. This, together with the glucuronic acid conjugate, is eliminated predominantly in the urine and faeces (Cool and Jankowski, 1990).

Low ovicidal action of Diazinon, Phorate and Carbaryl against *L. luteola* may be due to protective covering of capsular jelly like material that covers and protects the freshly laid eggs from the external environment. A similar observation was also reported by Parashar *et al.* (1990 & 1995) for

nicotinanilide against *L. auricularia* (LC_{90} value against 0 to 1 day and 4 to 5 days old eggs was 1.03 mg/l and 0.57 mg/l respectively) and the eggs of *Indoplanorbis exustus* (the LC_{90} value was 0.087 mg/l). The values of LC_{50} of 4'-chloronicotinanilide against 1 to 4 days old eggs of *Biomphalaria glabrata* was reported as 10 ppm (Dunlop *et al.*, 1980, Duncan & Brown, 1983).

Table (1): LC_{50} values of the three molluscicides (Diazinon, Phorate and Carbaryl) against eggs of *L. luteola*.

Stages	Compound	LC_{50} ppm	Slope
Eggs	**Diazinon**	2338.629	1.24
	Phorate	5030.687	0.602
	Carbaryl	5646.395	0.706

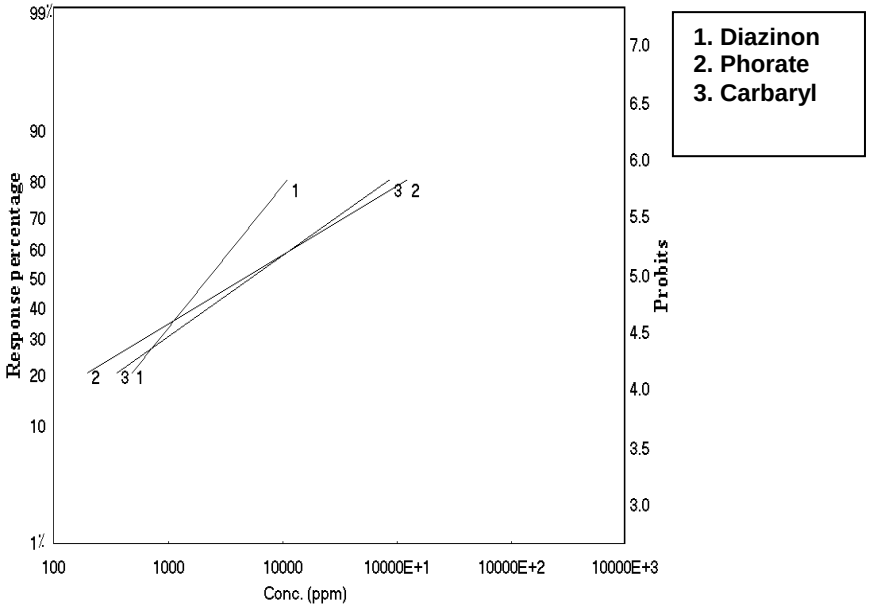

Fig. (1): Regression lines of the tested compounds (Diazinon, Phorate and Carbaryl) against eggs of *L. luteola*.

1.2. Immature stages:

Data presented in Table (2) and graphically illustrated as toxicity regression lines in Fig. (2) show the potency of three pesticides namely (Diazinon , Phorate and Carbaryl) compared with control against immature stages of *L. luteola*.

Data revealed that Carbaryl exhibited the highest toxic action followed by Phorate, while Diazinon was the least in toxicity values of LC_{50} were 69.44, 73.148 and 318.663 ppm, respectively.

Data in Table (2) show that the slope of immature stages of *L. luteola* in Diazinon, Phorate and Carbaryl are medium (1.785, 0.946 and 0.85) this meaning the insect population is homogeneity in response and not resistant to compounds.

Table (2): LC values of Diazinon, Phorate and Carbaryl against immature stages of *L. luteola*.

Stages	Compound	LC_{50}	Slope
Immature	Diazinon	318.663	1.785
	Phorate	73.148	0.946
	Carbaryl	69.414	0.85

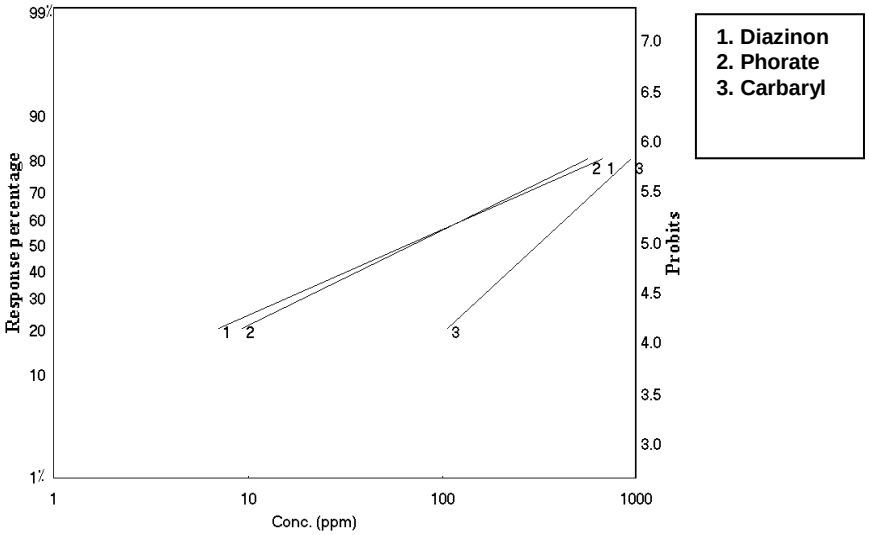

Fig. (2): Regression lines of the tested compounds (Diazinon, Phorate and Carbaryl) against immature stages of *L. luteola*.

1.3. Young mature stages:

Data presented in Table (3) and graphically illustrated as toxicity lines in Fig. (3) show the potency of tested compounds namely (Diazinon, Phorate and Carbaryl) compared with the control against young mature stages of *L. luteola*.

It is clear that, Phorate was the most potent one followed by Carbaryl and Diazinon. LC50 values were 134.508, 180.765 and 444.876 ppm, respectively.

As shown in Table (3), the slope values of immature stages of *L. luteola* in Diazinon, Phorate and Carbaryl are medium (1.866, 1.013 and 1.028) this meaning the insect population is homogeneity in response and not resistant to compounds.

Table (3): LC50 values of Diazinon , Phorate and Carbaryl against young mature stages of *L. luteola*.

Stages	Compound	LC_{50}ppm	Slope
Young immature	Diazinon	444.876	1.866
	Phorate	134.508	1.013
	Carbaryl	180.765	1.028

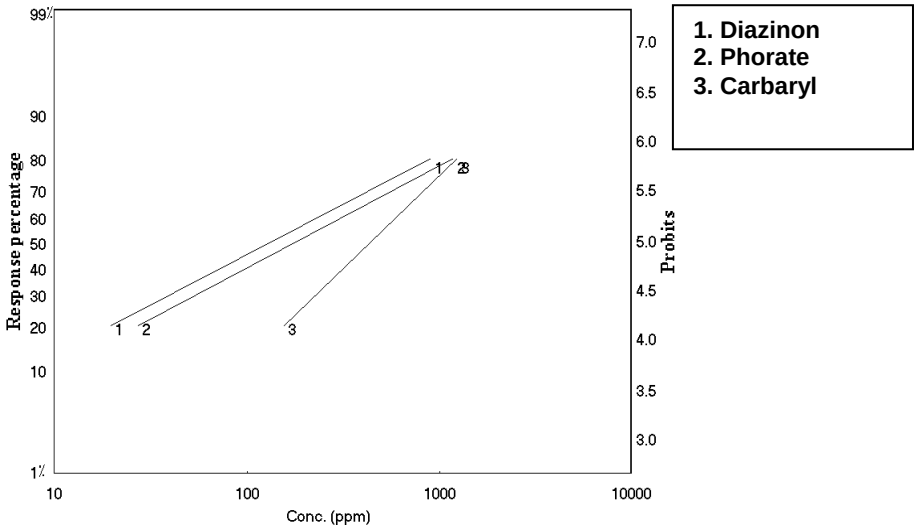

Fig. (3): Regression lines of the tested compounds (Diazinon, Phorate and Carbaryl) against young mature stages of *L. luteola*.

1.4. Adult stages:

Data presented in Table (4) and graphically illustrated as toxicity lines in Fig. (4) show the potency of the three compounds (Diazinon , Phorate and Carbaryl) compared with the control against adult stages of *L. luteola*.

Data tabulated in Table (4) revealed that Phorate the organophosphorous insecticide appear to be the most effective one followed by Carbaryl, while Diazinon showed the lowest order of activity LC_{50} values were 286.0330, 370.824 and 646.761 ppm, respectively.

Similarly, the slope values of Diazinon, Phorate and carbaryllin case of adult stages of ***L. luteola*** showed the same trend of homogeneity in response to the tested pesticides as mentioned above in case of immature and young mature stages.

The previous results are in agreement with those obtained by (White House 1964, Andrews *et al.* 1983), they recorded that the rapid action of niclosamide in killing snails is due to its toxic effect on the respiratory function of the snails by acting as uncoupler of oxidative phosphorylation at the mitochondrial level. They added that highest mortality of the *L. luteola* snails was observed during the period between 6 and 8 h after exposure. While, Singh and Agarwal (2006) studied the effects of carbaryl and phorate on the

survival of young snails, the fecundity and hatchability of the snail *Lymnaea acuminate*. It was observed that the fecundity of *Lymnaea acuminata* was reduced only at concentration of 11.00 mg/l for Carbaryl and Phorate, whereas a time- and dose dependent reduction in the hatchability of exposed eggs and the survival of young snails was observed.

According to the obtained results in the present study it was concluded that the tested compounds were more toxic to the immature stage than young mature and adult stages. While, they were less toxic to the egg stage. Parashar *et al.* (1990) reported the toxicity of nicotinanilide against *I. exustus* snails and indicated that young adult stage is the least susceptible.

Table (4): LC50 values of the three compounds (Diazinon , Phorate and Carbaryl) against adult stages of *L. luteola*.

Stages	Compound	LC_{50ppm}	Slope
Adult	**Diazinon**	646.761	1.642
	Phorate	286.033	1.0
	Carbaryl	370.824	1.06

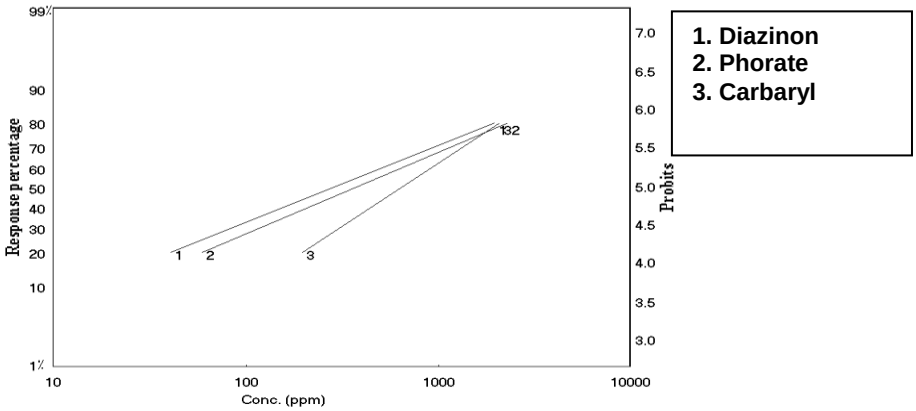

Fig. (4): Regression lines of the tested compounds (Diazinon, Phorate and Carbaryl) against adult stages of *L. luteola*.

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دراسة تأثير ثلاث مبيدات مصنعة علي قوقع *Lymnaea luteola* العائل الوسيط للفاشيولا *Fasiola hepatica*

وفا عبد الله إبراهيم المجرين

قسم الحيوان – كلية البنات – صندوق بريد 25701- الرياض 11476 – المملكة العربية السعودية

تم دراسة تأثير ثلاث مبيدات مصنعة (ديازينون- فورات- كرباريل) مقارنة بتجربة المقارنة ضد بيض، الأعمار غير الكاملة، الأعمار اليافعة، الأعمار الكاملة للقوقع *Lymnaea luteola*.

بمقارنة تأثير الثلاث مبيدات ضد البيض وجد أن الثلاث مبيدات تأثيرها منخفض علي البيض وذلك علي أساس قيم الجرعة السامة النصفية. في حين أعطي مركب الكرباريل نسبة خفض في التعداد أعلي من الفورات وجاء الديازينون أخيرا ضد الأعمار غير الكاملة علي أساس قيم الجرعة السامة النصفية ، كذلك أعطي مركب الفورات نسبة خفض في التعداد أعلي من الكارباريل بينما جاء الديازينون أخيرا ضد الأعمار الشابه علي أساس قيم الجرعة السامة النصفية كما أعطي مركب الفورات نسبة خفض في التعداد أعلي من الكارباريل كما جاء الديازينون أخيرا ضد الأعمار الكاملة علي أساس قيم الجرعة السامة النصفية.

وبمقارنة ميل خطوط السمية للمركبات الثلاث وجد أن إستجابة الأفراد المختبرة في نفس المستوي بالنسبة لمركبي الفورات والكرباريل وكانت أقل تجانسا في الإستجابة بالنسبة لمركب الديازينون.