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STUDY OF THE EFFECT OF SOME CARBAMATE AND ORGANOPHOSPHORUS PESTICIDES AGAINST SNAILS, *Biomphalaria alexandrina* AND *Bulinus truncatus* AND THEIR CHEMICAL STABILITY IN WATER

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ABSTRACT

Laboratory trials for the controlling two snails species namely *Biomphlaria alexandrina* and *Bulinus truncatus* were carried out. Three carbamate compounds (Trimethacarb· Thiodicarb and Methiocarb) and three organophosphorus compounds (Trichlorofon; Phormothion and Phorate) were tested for their molluscicidal activities at intervals with time up to 10 days. On basis of LD_{50} values after 24 h. or 48 h.exposure, the toxicity order for the carbamates compounds were trimethacarb > thiodicarb > methiocarb and that of organophosphorus compounds was trichlorofon > formothion > phorate. *Biomphalaria alexandrina* was found to be more sensitive to all the pesticides tested than *Bulinus truncates*, snails.

Also the chemical stability of the previous pesticides in water was determined using HPLC method for all pesticides tested. This was done by allowing the expected LC_{90} values for 24-hours exposure to stand for varying periods before testing them against snails. The stability of the previous pesticides were ranged as follow: trimethacarb > thiodicarb > methiocarb > formothion > phorate > trichlorofon, that with HPLC method and with toxicity test against *Biomphalaria alexandrina*, snails.

INTRODUCTION

In many countries of world, fresh water reservoirs are foci for snail pests, the intermediate host of Schistosomiasis and Fasciolasis , which cause immense damage to both livestock and water submerged crops. Of these Biomphalaria spp. and Bulinus spp. are intermediate host of Schistosoma spp. and Lymnaea acuminate is an intermediate host of Fasciola hepatica and Fasciola gigantica, the infection of which is endemic in these livestock of this countries. In other parts of the world, however, carbamate and organophosphorus compounds have been successfully used for controlling snails. (Hammer, 1962; Barry, 1969; Brar and Simwat, 1973; Garthwait and Thomas, 1969; Davidson, 1962; Getzin and Cole, 1964; Judge, 1969; Jurberg *et.al.*, 1995 and Sturrock, 1995) In view of the urgent for the elimination these snails, the present work studied the toxicity of various pesticide groups in order to develop specific candidate molluscicides for controlling pest snails. Many Authers studied the pharmacology of carbamate and organophosphorus compounds (trichlorfon, formothion and phorate) on the isolated heart, radular protractor muscle and rectum of Pila gloposa and found that these pesticides inhibited cholinesterase in these organs to varying degrees.The efficiency of a molluscicide must certainly be related to its chemical stability, but just how stable a compound need be for maximum efficiency has not been determined (Singh and Agarwal, 1978a&1978b; Singh and Agarwal 1979; Singh, 1995 and Caupland, 1996). Its possible that

individual differences in stability may prove desirable, because of other characteristics of compounds, or perhaps because of the types of snail habitat in which they must be used, their methods of application, the domestic needs for the treated water, or because of concern for ecological balances. In order to select a specific candidate molluscicides, laboratory trials on this study were carried out on three carbamate compounds (trimethacarb; thiodicarb and methiocarb) and three organophosphorus compounds (trichlorofon; phormothion; phorate) in order to determine such concentration of these, that kill 50% of the snails when exposed to pesticides for varying time period, also to determine the chemical stability in water by allowing the approximate LC90 values for 24-hour exposure to stand for varying periods before testing them against snails and the concentrations were determined after 10 days using High Performance Liquid Chromatograpyy (HPLC).

MATERIALS AND METHODS

Analytical methods

High Performance Liquid Chromatography (HPLC)

The molluscicidal concentration in the aqueous solutions were usually measured from direct samples with HPLC.Only at very low concentration levels , Solid Phase Extraction with C-18 modified silicagel was used as enrichment method. Quantification was performed by automatic integration of peak areas and calibration with external standards (Luhing *et.al.*, 1979; Muir and Grift, 1980 and Churchill and Ku, 1980)

HPLC: Pumpe: Modell 590 Fa. Water, Detedctor: UV/VIS- Diodenarray detector HP 1040 A with DPU multichannels Integrator (Fa Hewlett Packard). The chromatographic conditions for the molluscicides are listed below:
for carbamate compounds: Column 250x4 mm, Hypersil APS um, acetonitril / water 60:40, 1.2 ml/min. UV-detector 235 nm.for organophosphorus compounds: Column 250x4 mm, Hypersil ODS 5 um, Precolumn 20x4 mm, Nucleosil- C18, 30 um, Methomol/water 80:20, 1.2 ml/min., UV-detector 235 and 225.

Test animals:

Biomphalaria alexandrina (Eherenberg) and *Bolinus truncates* (Audotin), the tow common snails, which present the intermediate host of Schistosomiasis (Bilharisiose desease) in Egypt. These snails were collected from various parts (irrigation canals) located in North Eltahrir, Behera Governorate, Egypt, that had not been treated with molluscicides. Both snail species were held for 2 months prior to testing under laboratory condition in glass aquaria containing 15 liters of dechlorinated tap water by aeration, that had a total hardness of about 100 ppm (as $CaC0_3$), a pH of about 7.5 and at a temperature of 24-25 °C), normal lighting (fluorescent), and fed on fresh lettuce leaves daily. The water in the aquaria was changed twice weakly and the snails were acclimatized under these laboratory conditions for a period of at least two months before being used in the experimental tests (Hopf and Muller, 1962).

Pesticides used:

Carbamate compounds:

Trimethacarb: 2,3,5 (or 3,4,5)- trimethylphenyl methylcarbamate (Rohne Poulene Agrochemie.

Thiodicarb: Dimethyl N,N'- [thiobis [(methylimino) carbonyloxy]] bis [ethanimidothioate], (Rohne Poulene Agrochemie).

Methiocarb: 3,5 dimethyl-4-(methylthio) phenyl methylcarbamate, (Rohne Poulene Agrochemie).

Organophosphorus comopunds

Trichlorofon: 2,2,2- trichloro-1-hydroxyethyl phosphate, (Bayer A.G.).
Formothion:O,O-dimethyl-S-(N-methyl-N-formoylcarbamoyl-methyl) dithiophosphate, introduced by sandos Co. Ltd., Swissland.
Phorate: O,O-diethyl-S-(ethylthio)methyl phosphrothioate, Union Carbide Co.

Testing procedures

1- Effect of the tested pesticides against *Biomphalaria alexandrina* and *Bulinus truncates*, snails.

Experimental snails (2-3 mm and 3-4 mm in diameter for *Biomphalaria alexandrina* and *Bulinus truncates*, respectively) were kept separately in all glass aquaria containing 3:1 of dechlorinated water, each aquaria contained 10 experimental animals. Four aquarius (4 replicates of 10 snails) were set up for each concentration of each pesticide tested, equal number of animals were kept in a similar manner without the chemical (as control). Both species of animals were exposed to different concentrations of each pesticide for a period of 10 days. A total number of 200 of each snails were thus, exposed to each pesticide. The aquaria were covered with cloth netting secured with a rubber band in order to prevent the snails from escaping and were kept aerated through the duration of experiment, evaporated water during the experimental period was replaced Dead snails were removed as soon as possible in order to prevent the decomposition of the body in the aquaria, which was observed to cause rapid death in the remaining populations. Concentration of pesticides are given as final concentration of active ingredient in water.

Snail mortalities were recorded daily during an observation period of 10 days. The LC_{50} values (mg/l) of each pesticide for 24, 48, 72, 96, 168 and 240 hours were calculated by the method standardized by the American Public Health Association and others (1975).

2- Determination of tested pesticides stability by using *Biomphalaria alexandrina*, snails.

The chemical stability of pesticides tested was defined as the length of time its pesticidal activity remained unchanged when dissolved in water at a concentration close to the LC_{90} required for 24-hour exposure. The tests were performed under normal laboratory lighting (fluorescent) , in dechlorinated tap water by aeration, that had a total hardness of about 100 ppm (as $CaCO_3$), a pH of about 7.5 and at a temperature of 24-25 °C. Dechlorinated tap water

was used in preparing the final solutions, which were allowed to stand for 2, 4, 8, 16, or 32 days before snails, were added. Water that evaporated was replaced during the experimental period.The snails were exposed for 24 hours and the recovery period was sufficiently long for snails to return to normal activity or to die, the period was usually 24 hours, but for some pesticides was up to 72 hours. Mortalities did not occur among control snails, that were handled similarly but without chemical.

RESULTS AND DISCUSSION

The toxicodynamic properties of the carbamate compounds (trimethacarb thiodicarb and methiocarb) and organophosphorus compounds (trichlorofon; formothion and phorate) in order of their toxicity at six different time intervals on the snails tested have been summarized in Tables 1 and 2. It is obvious from the tables that within the range of doses tests LD_{50} values of all the six compounds could not be calculated after 24 h. exposure to the pesticides. Based on the data on LD_{50} values from 48 h. to 240 h. exposure time the toxicity of the carbamate compounds against both snails species were trimethacarb > thiodicarb > methiocarb, while those of the organophosphorus compounds were trichlorofon > formothion > phorate. It can be seen that even after 48 h. of exposure, failed to kill more than 50% of the snails within the tested dose range. Another fact which became obvious during these studies was that the LD_{50} values were time independent when the exposure time was increased from 24 h. to 240 h. (Tables 1 and 2). Of the two animals tested , it was found that *Biomphalaria alexandrina* was more sensitive to these pesticides tested as compared to *Bulinus truncates*, snails.

Table (1): Toxicity of pesticides tested against *Biomphalaria alexandrina*, snails at time intervals

Pesticide doses (mg/l)	LD_{50} (mg/l) of active ingredient at time intervals					
	24 h.	48 h.	72 h.	96 h.	168 h.	240 h.
Carbamate compounds Trimethacarb	3.32	2.65	2.5	1.75	1.02	0.96
Thiodicarb	-*	13.8	8.5	4.15	0.42	0.35
Methiocarb	30.0	24.5	17.5	15.5	11.25	10.5
Organophosphrus Trichlorofon	-*	2.08	1.5	0.38	0.28	0.06
Formothion	28.75	23.5	21.0	17.5	6.25	5.2
Phorate	-*	-*	27.25	23.5	15.75	5.10

*LD_{50} values could not obtained within the range of tested concentration

During these experiments it was found that except of trimethacarb, pesticide, all pesticides tested caused paralysis to tested snails within 24 h. of exposure to the different concentrations even though the animal remained alive for varying length of time. There was no mortality in the control group. These data on the relative toxicity of three carbamate compounds demonstrate that trimethacarb was the most effective, while methiocarb was

the least effective against the snails tested. It has been reported by Metcalf (1971) and Matsumura (1976) that carbamate compounds having N-methyl and N,N'-dimethyl group in their molecule possess a high degree of anti-AChE property. In the fact that trimethacarb possesses both trimethyl as will as n-methyl group in its molecule supports the above hypothesis thiodicarb has dimethyl group, whereas methiocarb has methyl and dimethyl group. Also methiocarb contains an Sulpher atom which is supposed to reduce the anti-AChE property of anti-AChE pesticides (Metcalf, 1971). Reports from earlier Workers show that the toxicity of carbamate compounds on other species of mollusks is also somewhat similar (Ishak *et.al.*, 1972; Chiang, 1977 and El-Emam and Ebeid, 1989).

Table (2): Toxicity of pesticides tested against *Bulinus truncatus*, snails at time intervals.

Pesticide doses (mg/l)	LD_{50} (mg/l) of active ingredient at time intervals					
	24 h.	48 h.	72 h.	96 h.	168 h.	240 h.
Carbamate compounds						
Trimethacarb	4.0	2.75	2.62	2.5	2.0	1.82
Thiodicarb	59.5	45.0	45.0	37.5	27.25	22.25
Methiocarb	-*	-*	202.0	165.0	88.5	80.0
Organophosphrus Compounds						
Trichlorofon	-*	28.5	25.9	9.38	3.0	2.2
Formothion	-*	75.0	52.0	41.0	24.5	10.5
Phorate	-*	-*	-*	43.8	31.2	18.4

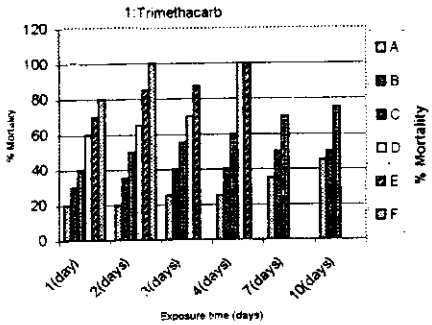
*LD_{50} values could not obtained within the range of tested concentration

The presented results in this study supports the previous studies of (Metcalf, 1971; Hartley, 1975; Jonson, 1977 and Singh, 1995). Trichlorofon which is a phosphate analogue was found to be highly toxic to *Biomphalaria alexandrina* and *Bulinus truncates*, whereas phorate being a phosphorothioate was the least effective amongst the three tested organophosphorus compounds. Formothion a lower LD_{50} as a compared to the latter against both species of snails. (Metcalf, 1971; Hartley, 1975: Jonson, 1977 and Singh, 1995)

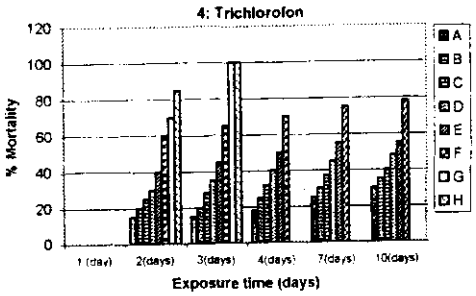
On the other hand the depicted results in table 1 shows that even though the LD_{50} values of trichlorofon were lower as compared to formothion yet the latter could kill more than 50% of the animals only after 24 h. of exposure time. Since it has been shown that the organophosphorus compounds tahe a longer time to act, the acute toxicity caused by formothion may be because of the presence of carbamoyl moiety in this compound (Singh and Agarwal. 1978b; Singh and Agarwal, 1979). In vew of the above findings it is suggested that low dosed of trimethacarb or trichlorofon be fried for longer periods, e.g. 10 days for the controi of these snails.

In conclusion the results depicted in Figures. (1) and (2), it was clearly that *Biomphalaria alexandrina* and *Bulinus truncatus* were sensitive to all concentrations tested with some variations in the exposure times to the different pesticides.

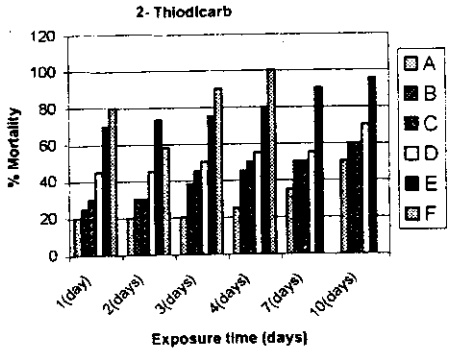
Fig 1: Toxicity test of some pesticides against *Biomphalaria alexandrina* at time intervals



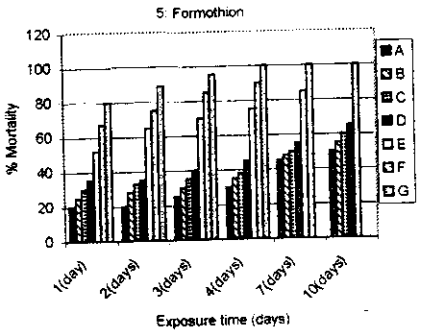
A=0.62 ppm B=1.25 ppm C=2.5 ppm
D=3.75 ppm E=5 ppm F=7.2 ppm



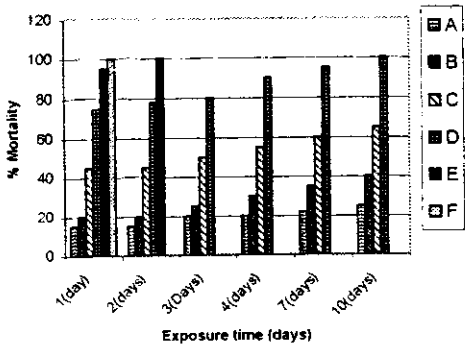
A=0.03 ppm B=0.05 ppm C=0.25 ppm
D=0.5 ppm E=1.5 ppm F=2.5 ppm
G=3.75 ppm H=4.5 ppm



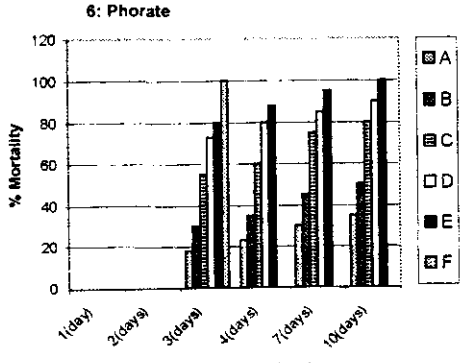
A=0.1 ppm B=0.5 ppm C=2 ppm
D=5 ppm E=15 ppm F=17 ppm



A=1.25 ppm B=2.5 ppm C=6.25 ppm D=12.5 ppm
E=37.5 ppm F=45 ppm G=60 ppm

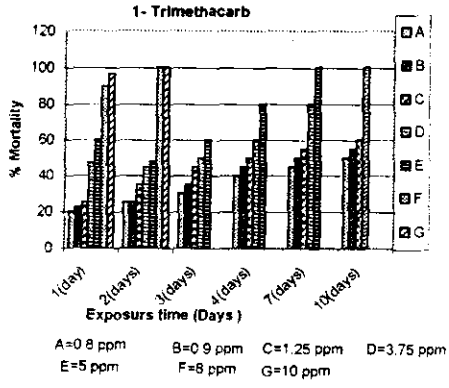


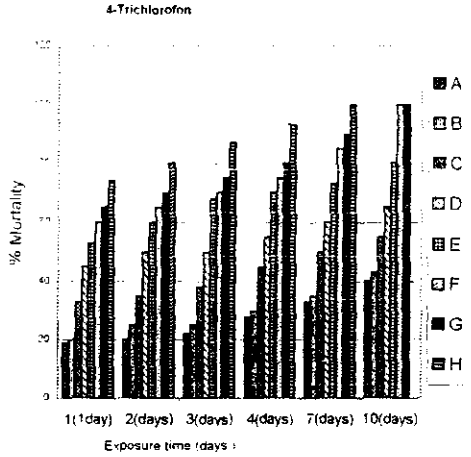
A=3 ppm B=6 ppm C=30 ppm
D=60 ppm E=90 ppm F=120 ppm

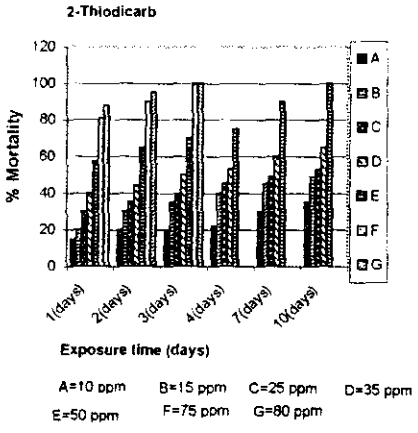


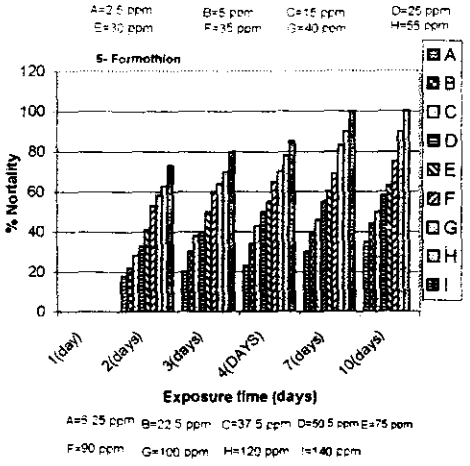
A=11.25 ppm B=15 ppm C=30 ppm
D=40 ppm E=45 ppm F=50 ppm

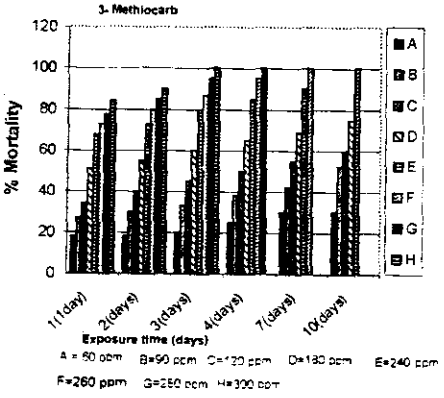
Fig. (2): Toxicity test of some pesticides against *Bulinus truncatus* at time intervals

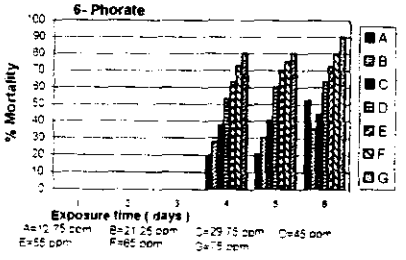












Also by this methods of treatment it was shown that small concentrations of pesticides can be used sucessefully to control these snails and to reduce the environmental pollutions. Also the speed of toxicant actions against snails explain that carbamates insecticides, trimethacarb; thiodicarb and methiocarb can be used as molluscicides, while the organophosphorus insecticides, trichlorofon; formothion and phorate showed less effective against these snails species.

The stability of the previous pesticides were determined using the HPLC methods. The spected LC90 values Against *Biomphalaria alexandrina* were used to determine the stability of pesticides tested. Results in table (3) showed that these pesticides were not stable in water up to 10 days. The concentrations were reduced to approximately 1/10 of the initial deposite concentrations for all pesticides tested.

Table (3): Detected concentrations of some pesticide residues in snails water aquarious, 10 days after treatment at the levels of LC_{90} by using HPLC methods

Pesticides	Initial deposite concentrations (ppm)	Concentrations (ppm) after 10 days
Trimethacarb	8.20	0.80
Thiodicarb	19.60	0.25
Methiocarb	85.00	9.25
Trichlorofon	9.25	0.04
Phormothion	68.75	405
Phorate	48.00	4.9

Also, the effectiveness of those concentrations were tested against, *Biomphalaria alexandrina*, snails. Results illustrated in table (4) showed that all pesticides were effective with 100 % mortalities of the experiment, then it was reduced to less than 30 % mortalities after 10 days of solutions preparations, which agree with the previous obtained results which determined using HPLC methods and explain, the figured results in table (1) for the effectiveness of these pesticides for a long time period of exposure.

Table (4): Chemical stability of some pesticides using the, LC_{90} concentrations (after 24-hour of exposures) to stand for varying periods before exposing snails, *Biomphalaria alexandrina*

Insecticides	Expected LC_{90} (ppm)	% Mortality after standing for (days)*	
		1 (day)	10 (days)
Trimethacarb	8.20	100	20
Thiodicarb	19.60	100	22.5
Methiocarb	85.0	95	50
Trichlorofon	9.20	100	18
Phormothion	68.75	100	28
Phrate	48.00	100	0.0

* 4 Replicates of 10 snails).

** Expected LC_{90} concentrations was calculated after 24 hours at the beginning of the experimental period.

The efficiency was probably related to the protection from sunlight afforded by the darkcoloured water, since in other endemic areas, where the water was clear, the basic stability of trimethacarb was not apparent methiocarb, which average stability and moderate vulnerability to physico-chemical factors, has proven very effective in both standing and free-flowing water, the two moluscicides that showed the lowest basis stability in the presence study (phorate and trichlorofon) have given commendable results in field trials (El-Gindy and Mohahamed, 1978; Calvin, 1981 Sullivan *et.al.*, 1994 and Emara, 1994).

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دراسة تأثير بعض مبيدات الآفات الكرباماتية والفوسفورية العضوية على قواقع البيومفلاريا ألكساندرينا *Biomphalaria alexandrina* والبولينوس ترنكاتس *Bolinus truncatus* ومدى ثبات هذه المبيدات فى الماء

محمد محمد فتحى النجار
قسم المبيدات – كلية الزراعة بكفر الشيخ – جامعة طنطا – مصر

فى هذا البحث تمت الدراسة المعملية لمكافحة نوعين من القواقع المائية واللذان يلعبان العائل الوسيط لاتمام دورة حياة ديدان البلهرسيا وهما قواقع البيومفلاريا ألكساندرينا *Biomphalaria alexandrina* والبولينوس ترنكاتس *Bolinus truncatus* واستخدم لهذا الغرض ستة من مبيدات الآفات الكرباماتية والفوسفورية العضوية وهى مبيدات (تراى ميثاكارب – ثيودای كارب – الميثيوكارب – تراى كلوروفون – فورموثيون – والفوريت).

ولقد تمت دراسة تأثير هذه المبيدات على القواقع المختبرة على فترات زمنية مختلفة حتى عشرة أيام وعلى أساس التركيزات المميتة لـ ٥٠ % من القواقع والتى حسبت بعد ٢٤ أو ٤٨ ساعة من المعاملة ، ومن النتائج المتحصل عليها يمكن ترتيب المبيدات الكرباماتية حسب قوتها الابادية الأكبر فالأصغر كالآتى: تراى ميثاكارب – ثيودای كارب – الميثيوكارب وبالنسبة للمركبات الفوسفورية العضوية فكان ترتيبها حسب قوتها الابادية الأكبر فالأصغر: تراى كلوروفون – فورموثيون – والفوريت.

ولقد أظهرت النتائج أن كلا النوعين من القواقع المختبرة كانت حساسة لجميع المبيدات المختبرة ولكن كانت قواقع *Biomphalaria alexandrina* أكبر حساسية لهذه المبيدات.

فى الخطوة الثانية تم دراسة مدى ثبات المبيدات المختبرة سالفة الذكر عاليه فى الماء باستخدام طرق التقدير بواسطة الكروماتوجرافى السائل عالى الكفاءة (HPLC) واسنتج من النتائج المتحصل عليها أن جميع المركبات المختبرة كانت غير ثابتة كيميائيا فى الماء حتى عشرة أيام وانخفضت تركيزاتها الى ١/١٠ من التركيزات التى بدئ بها (Initial time).

ومن ناحية أخرى أختبرت هذه التركيزات على قواقع *Biomphalaria alexandrina* وظهر من النتائج أن جميع المبيدات المختبرة فعالة وأعطت نسبة موت ١٠٠% لهذه القواقع بالتركيز المبدئى (Initial time) وبعد عشرة أيام انخفضت نسبة الموت لأقل من ٣٠% للمحلول المحضر والمطابق للتركيزات التى قدرت بواسطة (HPLC) ولكنها ظلت فعالة ضد القواقع حتى العشرة أيام (على المدى الطويل نسبيا) مدة التجربة.