

COMPARATIVE EFFICACY OF METHOMYL AND DIAZINON ON DIFFERENT AGES OF THE LAND SNAIL *Eobania vermiculata*

El-Deeb, H. I.**; E. A. Eweis *; M. A. Kandil *; Fatma K. Khidr** and Soha A. Mobarak**.

* Econ. Entomol., and Pesticides Dept., Fac., Agric. Cairo Univ.

** Harmful Animal Dept. Plant Protection Institute Research.

ABSTRACT

The comparative efficacy of methomyl 20% SL and diazinon 60% EC insecticides used as baits or contact poison were studied against different ages of *Eobania vermiculata*. The LC₅₀ and LC₉₀ values of each compound were determined for the different ages (one, three, six, 9-12 months and over 24 months) of *Eobania vermiculata*.

Serial concentrations of each compound were tested against land snail *E. vermiculata* using baiting and contact thin layer film (TLF) techniques. Mortality percentages were recorded after 24, 48 and 72 h., respectively. Methomyl was more toxic for *E. vermiculata* than diazinon, when used as poison bait at all different ages. Regarding the contact method, diazinon was more toxic to *E. vermiculata* at the three-month and (over 24 months) ages. Methomyl was more repellency to different ages of *E. vermiculata* than diazinon. The biochemical response of different ages (one, three, six, 9-12 month and over 24 months) of *E. vermiculata* treated with LC₅₀ of methomyl and diazinon compounds was also evaluated. Acetyl cholinesterase response of the three and six month's individuals differed with the rest ages to both compounds. The response of LDH and AST was obviously affected by the age of tested individuals and also application time. The effect of methomyl and diazinon on total protein and total lipids in the different ages fluctuated intervals of periods after treatment in different ages. Generally, it could be concluded that the response and susceptibility of tested snail to methomyl and diazinon differed according to different parameters i.e. chemical structure of pesticides, method of application, snail ages and time after treatment.

Keywords: Methomyl, Diazinon, Toxicity lines, *E. vermiculata*, Repellent (R₅₀), AchE, LDH, AST, Total protein, and Total lipid.

INTRODUCTION

Land mollusca are economic pests attacking several types of plants. Snails are considered key pests in many countries especially Egypt and European Union but mainly in France and Italy (Elmsile, 1989). Harmful snails consume a significant amount of plant and that highly affects the exportation and importation. This is the case for molluscicides, which are predominantly formulated into baits thus act as a feeding poisons. Chemical baits are suitable for mollusca control, in contrast to carbamate molluscicides, which are known to act as nerve toxins by inhibition of acetyl cholinesterase (Wilkinson, 1976; Young and Wilkins, 1989). On the other hand, control of snail individuals and their eggs whatever the method used is not simple (Nehmedo Abd-El-Karim 2000).

The present work outlines the following:

- 1- The susceptibility of different ages of the land snail *E. vermiculata* to methomyl and diazinon tested as poison bait or contact (Thin film technique).
- 2- Studies the repellency effect of the two tested compounds on different ages and determine the R_{50} values of each compound.
- 3- Studies the impact of both compounds on some biochemical parameters of the different ages of *E. vermiculata* i.e. acetylcholinesterase (AChE), lactic acid dehydrogenase (LDH), aspartate amino transferase (AST), total protein and total lipid.

MATERIALS AND METHODS

Chemical Insecticides used: Methomyl

Newmyl (20% SL) a carbamate compound obtained from KZ pesticides company, Egypt.

Diazinon (60% EC) an organophosphorus compound was obtained from Syngenta Company, Cairo, Egypt.

Tested Animals: Snail species of *E. vermiculata* were collected from untreated clover fields at Giza Governorate.

Health individuals were kept in separate glass terraria (70 ×40 ×35 cm) where snails in each terrarium were fed three times per week with potato slices, carrot slices, lettuce leaves and 50% of bean (Godan, 1983).

Toxicity test: Methomyl and diazinon were tested against the land snail *E. vermiculata* using baiting and contact techniques.

Mortality was calculated after 72 h and corrected according to Abbott's formula (1925).

The medium lethal concentration values were estimated and toxicity lines were drawn according to (Finney 1971). Thin layer film technique was used as a method of application according to Ascher and Mirian (1981). Some pesticides used at high concentrations cause a repellent effect against snails. Their molluscicidal effect is lost because the snails avoid them (Godan 1983).

Laboratory trails were conducted to determine R_{50} values of the two tested compounds according to Weil (1952). Ten snail individuals were put of each age (1, 3, 6, 9-12 and over 24 months) in a homogenizer for 3 minutes with 10 ml of phosphate buffer pH=7 at 1-4 °C and centrifuged (3500 r.p.m) for 10 min. The procedure Bergmeyer (1963) was followed. Acetylcholinesterase (AChE) was determined according to Ellman *et al.* (1961), while aspartate amino transferase (AST) was assayed by the method of Schmidt and Schmidt (1963). The method Caboud and Wroblewski (1958) assayed lactic acid dehydrogenase (LDH). Total protein was assayed by the method of Gornall *et al.* (1949) while total lipid was assayed by the method of Zollner and Kirsch (1962).

Statistical analysis was done using the student "T" test according to Snedecor and Cochran (1967) and Hill (1971).

RESULTS AND DISCUSSION

The data shown in Table (1) present the comparative effect of methomyl and diazinon used as bait or contact on different ages of *E. vermiculata*. LC₅₀ and LC₉₀ of methomyl used as bait were 241.6 and 1006.5 ppm one-month while LC₅₀ and LC₉₀ of diazinon bait 780.1 and 1238.5 for *E. vermiculata*. In addition, the same trend was induced with snail individuals of three and six month old treated with methomyl and diazinon (441.8, 772.9, 136.9 and 617.0) and (526.1, 1484.5, 3662.1 and 4227.2) for diazinon bait and contact against *E. vermiculata*, respectively. In case of adults and old adults data in Table (1) clearly showed that LC₅₀ and LC₉₀ of methomyl averaged 217.8, 719.4 for snail 9-12 months old. These values increased to 261.3 and 1183.6 for individuals over 24 months, respectively. Concerning diazinon bait, LC₅₀ and LC₉₀ were 2501.3, 6611.2 for (9-12 months) and 739.4, 4239.8 (over 24 months) when they were tested against older individuals, respectively. Methomyl was more toxic than diazinon when used as bait in all ages of *E. vermiculata*. Also, these findings clearly that age variation may give rise to differences in susceptibility to acute intoxication by different toxicants and there is no simple rule for relating age to toxic response. Result in Table (1) showed that LC₅₀ and LC₉₀ of methomyl and diazinon as contact gave the values (0.03 and 0.12 mg/cm²) and (0.47 and 1.09 mg/cm²) at one month old individuals, respectively. At three and six month old LC₅₀ and LC₉₀ values increased to (0.29, 0.76, 0.31 and 0.59 mg/cm²) and (0.29, 0.97, 1.81 and 2.45), respectively. Values of LC₅₀ and LC₉₀ for adult and old adult in contact treatments averaged (0.39, 4.61, 0.87 and 5.1 mg/cm²), respectively.

Table (1) Comparative LC₅₀ and LC₉₀ values for different ages of *Eobania vermiculata* treated with methomyl and diazinon by two techniques.

Molluscicides Ages	Methomyl				Diazinon			
	Bait (ppm)		Thin film (mg/cm ²)		Bait (ppm)		Thin film (mg/cm ²)	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
One month	241.6	1006.5	0.03	0.12	780.1	1238.5	0.47	1.09
Three month	441.8	772.9	0.29	0.76	526.1	1484.5	0.29	0.97
Six month	136.9	617.4	0.31	0.59	3662.1	4227.2	1.81	2.45
Adult (9-12 month)	217.8	719.4	0.39	0.53	2501.3	6611.2	2.48	4.61
Old (over 24month)	261.3	1183.6	0.27	1.24	739.4	4239.8	0.87	5.1

These differences in the susceptibility level may be due to the physiological status of the tested snail, which may change from one age to another. These finding agree with these obtained by Godan (1983) found that very young and newly hatched individuals were extremely susceptible from

the second month. Sensitivity to molluscicide diminished, so that three month old juveniles have a relatively higher degree of resistance. The present results are agree with Dauterman (1971) who found that the sensitivity to intoxication diminished by further increase in age and these differences could be attributed to the development of metabolizing enzyme as an age dependent factor rather than to differential cholinesterase pattern. Data in Table (2) summarize the efficacy of methomyl when it was used as bait or contact by using thin film technique. Results showed that mortality percentages increased proportion by increasing the compound concentration or exposure periods. the bait concentrations 100, 200, 300, 400, 500 ppm gave 0, 22.5, 40, 22.5 and 32.5% mortality after 24h post treatment, respectively. While the same concentrations caused 27.5, 32.5, 47.5, 80.0 and 100% mortality after 72h from the administration. The tested compounds were used as contact poisons it reached 7.5, 35.0, 50.0 and 62.5% after 24h at 0.0125, 0.025, 0.05, 0.1mg/cm², respectively. The same concentration induced 25.0, 50.0, 72.5 and 97.5% mortality after 72h, respectively.

Also, the bait concentration 300, 400, 500, 600 and 700 ppm induced 22.5, 40.0 50.0 77.5 and 90.0% mortality. At six month old individuals, both application gave 15.0, 50.0, 70.0, 75.0 and 97.55% in three month old snails, respectively mortality, when methomyl was used as bait at 50.0 100.0, 300.0, 450.0 and 600.0 ppm, respectively. When the toxicant was applied at 0.2, 0.3, 0.4, 0.5 and 0.6 mg/cm² it gave 25.0, 37.5, 72.5, 72.5, 85.0 and 100% mortality after 72h post treatment, respectively. In case of adult age, methomyl bait at level 600ppm achieved 100% mortality while the contact treatment 0.6 mg/cm² caused 97.5% mortality. On the other hand, in the old age all bait concentrations (100, 200, 300,400and 600ppm) did not gave any mortality after 24h and 48h reaching 25.0, 10.0, 12.5, 12.5 and 27.5. The same concentrations induced 25.0, 35.0, 50.0, 67.5 and 77.5%, respectively.

The results illustrated in Table (3) also *E. vermiculata* was susceptible to methomyl as contact for the most ages where 0.1, 0.6, 0.6, 0.6, and 0.6 mg/cm² concentrations achieved (97.5 and 97.5), (100 and 100), (100and 97.5), (97.5 and 75.0) and (100 and 82.5%) mortality post the some periods at the same ages consecutively. summarized the response of snail individuals of one month old to different concentrations of diazinon used as bait or contact. Data indicated at diazinon was toxic as contact than bait whereas it caused 100% mortality after 72h post treatment when it was used as contact at 0.9 mg/cm², while it caused only 50.0% and 75.0% after 24h and 48h post treatment, respectively.

Also, 0.75mg/cm² concentration caused 40.0, 70.0 and 85.0% after 24h, 48h and 72h, respectively. When it was used as bait 600.0, 750.0, 900.0, 1050and 1200.0ppm gave 0.0, 25.0, 32.5, 35.0 and 20.0% mortality after 24h post treatment, respectively. In addition, the same concentrations gave mortality percentages 30.0, 30.0 47.5, 75.0 and 45.0% after 48h. The same pattern was noticed after 72h where mortality percentages were of 30.0, 37.5, 62.5, 75.0 and 95.0%, respectively.

At three month old differed according to the application method. Bait concentration 1200ppm achieved 50.0, 85.0 and 85% mortality after 24h, 48h

and 72h from the administration, respectively. The contact treatment using 0.9 ppm caused 50.0, 100.0 and 100.0% after the three periods, respectively.

The effect of diazinon at 0.2, 0.3, 0.6 and 0.9 mg/cm² as contact whereas it achieved 0.0, 20.0, 47.5 and 50.0% after 24h 25.0, 52.5, 77.5 and 100% after 48h 35.0, 52.5, 77.5 and 100% after 72h, respectively. Regarding the contact method diazinon was toxic at the three month and old ages, on the other hand, complete kills occurred at one month age with 0.9 mg/cm².

Data in Table (3) revealed the response of six month age as bait or contact. Regarding the poison bait 4500 ppm gave 25.0, 75.0 and 97.5% mortality post 24, 48 and 72h of treatment. In addition, 3300ppm caused 0.0, 0.0 and 25.0% mortality after the same period. Mortality percentage with contact treatment whereas it was 5.0, 15.0, 22.5 and 25.0% after 24h with 1.5, 1.8, 2.1 and 2.4 mg/cm². After 48h, the same concentrations induced 27.5, 30.0, 40.0 and 50.0% mortality. Also, the previous concentrations occurred 27.5, 35.0, 77.5 and 90.0% after 72h post treatment. Effect of diazinon when used as bait or contact on the adult age. The lowest bait concentration 1800ppm achieved 25.0, 32.5 and 32.5% mortality after 24h, 48h and 72h while the highest bait concentrations 5400 ppm gave 50.0, 75.0 and 100.0% after the three tested periods. Concerning the contact treatment the compound caused 10.0, 25.0 and 35.0% mortality post 24, 48 and 72h at 1.9 mg/cm² concentration. The highest contact concentration 5.1 mg/cm² occurred 50.0, 90.0 and 97.5 mortality. The response of old adult individuals (over 24 month age). The compound when used as contact at 0.3, 0.6, 0.9, 1.2 and 1.8 mg/cm² gave 25.0, 32.0, 50.0, 62.5 and 100.0% mortality post 24, 48 and 72h from the treatment. These results are in agreement with those obtained by Brus and Hermen, (1971) and Godan, (1983).

Many chemical molluscicides are repellent at high concentrations but are attractive at very low concentrations, between these two extremes the response to them is indifferent (Godan, 1958, 1959).

Results in Tables (4, 5 and 6) showed the repellent effect of methomyl, diazinon and R₅₀ values (50% of tested animals consumed less than half offered treated food) of methomyl was low 16.8 time at one month, 1.2 time for three month age, 1.9 time to six month 3.1 time for adult and 1.9 time for the old age animals. The repellent effect increased with increasing whereas R₅₀ value of methomyl raised to 5.2 times for the three month age, 6.1 times for six months age, 6.3 times for adults and 5.3 times for old age than one month age. Finally it is cleared methomyl was repellency in all different ages. Concerning the R₅₀ value of diazinon compound with 4.7, 2.5, 1.2 and 1.5 times for one, three, six month age and adult animals, respectively but it reduced 0.87 time in case of the old aged individuals. R₅₀ values of diazinon the same pattern where it enhanced to 1.7, 4.1 times for six months age and adults than one month age while it reduced 0.98 times for three month age and 0.81 times with old aged animals. The repellent effect increased with increasing age where R₅₀ value enhanced 1.9, 6.9, 12.6 and 4.3 times for the three, six months, adult age and old animals than one month age respectively.

Table (4) Comparative repellent effect of methomyl on different ages of *Eobania vermiculata* land snails.

Repellency %									
One month		Three month		Six month		Adult (9-12 month)		Old (over 24month)	
Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%
50.0	36.8	400.0	42.7	400.0	46.6	400.0	40.0	400.0	46.4
100.0	48.3	800.0	68.8	600.0	68.0	800.0	63.6	600.0	68.7
200.0	76.2	1200.0	71.8	800.0	79.0	1200.0	84.7	800.0	77.3
400.0	97.7	1600.0	77.2	1000.0	95.0	1500.0	93.2	1000.0	94.3

Table (5) Comparative repellent effect of diazinon on different ages of *Eobania vermiculata* land snails.

Repellency %									
One month		Three month		Six month		Adult (9-12 month)		Old (over 24month)	
Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%
1200.0	28.4	1200.0	30.5	2700.0	40.9	5700.0	37.4	1200.0	25.0
1800.0	61.4	1800.0	57.0	3000.0	78.3	6900.0	59.6	1500.0	48.2
2000.0	88.2	2400.0	89.8	3300.0	91.8	8220.0	73.5	1800.0	62.4
3000.0	94.8	3000.0	94.4	3600.0	95.0	9852.0	98.0	2100.0	92.6

Table (6) Comparative effect of methomyl and diazinon on different ages of *Eobania vermiculata*.

R ₅₀ (ppm)									
One month		Three month		Six month		(9-12) month		Over24month	
M*	D*	M*	D*	M*	D*	M*	D*	M*	D*
83.7	1570.2	434.8	1549.9	508.4	2739.5	522.8	6399.6	439.0	1274.2

*M= Methomyl

D= Diazinon

The previous results indicate that repellency effect was high at one month age and old aged snails (over 24 month) also it was higher for the old animal than one month age while it decreased with the rest ages i.e. three, six month and adult age. The mortality rate provides indirect evidence for the altered behavior of gastropods in response to high concentrations of molluscicides, mortality is less at high concentrations of the molluscicide than at lower was explained by Crowell (1967).

Results in Table (7) indicate the effect of LC₅₀ of methomyl on the activity of cholinesterase in the different ages. Data showed the treatment with methomyl significantly decreased the enzyme activity to 673.2, 893.5 and 1090.9 nmol per min/mg protein after 24, 48 and 72h treatment with difference percentages -48.2, -31.3 and -16.1% respectively as compared with control for one month age. At the three month age the difference percentages were 15.2, -42.9 and -49% post 24, 48 and 72h variation as significant decreased -49.0%. Large variation was observed at six month age

whereas the difference percentages of the enzyme activity were 177.0, 152.0 and 383.2% post 24, 48 and 72h of treatment.

Regarding the adult animals (9-12) fixed decreased occurred 99.7, -99.7, 99.9% at the three tested periods. Concerning the aged old animals the different percentages increased to 9.9% at 24h and decreased to 1.5% at 48h while reduced to -57%. Our results are agreement with (Marti and Ronald 1998 and El-Deeb *et al.*, 1999).

The response of LDH enzyme to methomyl it fluctuated with increase and decrease to each age. The enzyme level enhanced to 1010.5%, while in case of old age level decreased to -73.8%. Our results are agreement with Gluchova (1985). On the other hand, the activity of AST increased to 244.0% at one month age while it reduced to -9.9% for old aged animals. Our results are agreement with Tilkian *et al.*, (1983). The level of total protein increased to 29.3% while it reduced to -44.0%. In addition, this ratio reached to 85.2% at adult age individuals post 72h after treatment. Our results are in harmony with that obtained by El-Essely (2002). The effect of methomyl on total lipid in different ages fluctuated intervals of periods after treatment.

Data in Table (8) explain the effect of LC₅₀ of diazinon on the activity of cholinesterase in the different ages. Concerning the diazinon treatment the enzyme activity raised to 62.0% for the three month age post 72h of treatment. On the other hand, at six month age, the increase of the enzyme activity reached to 1466.8% our results are agreement with Westalke *et al* (1981). Diazinon treatment raised LDH level to 489.0% at one month while it reduced to -52.0% at the old age. Our results are agreement with Ray *et al.* (1988).

The activity of AST enzyme fluctuated with increase and decrease, it enhanced to 244.0% for one month age post 72h from treatment while it reduced to -9.9% for old age. Our results are agreement with Amer *et al.* (1994-b). The effect of diazinon on total protein similar after 72h of treatment at the most ages except the six months and adult ages, but it reduced at the adult age. Our results are agreement with Wilson (1986).

The effect of diazinon on total lipid in different ages fluctuated intervals of periods after treatment. Generally it could be concluded that the response and susceptibility of snail to diazinon differed according i.e. method of application, age of snail and chemical structure.

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مقارنة كفاءة مبيد الميثوميل والديازينون على الأعمار المختلفة لقوقع ايوبانيا فيرميكولاتا.

حسن إبراهيم الديب² - عصام الدين عبد الرؤوف عويس¹ - محمد عبد الهادي قنديل¹ - فاطمة كامل خضر² - سها عبدالله مبارك².

- 1- قسم الحشرات الاقتصادية والمبيدات- كلية الزراعة- جامعة القاهرة.
- 2- قسم بحوث الحيوانات الضارة - معهد بحوث وقاية النباتات- مركز البحوث الزراعية.

تهدف الدراسة إلى مقارنة كفاءة تأثير مبيد الميثوميل والديازينون على الأعمار المختلفة لقوقع ايوبانيا فيرميكولاتا من خلال طريقتي معاملة هي الملامسة والطعوم السامة. تم تقدير خطوط السمية وتقييم قيم LC_{50} , LC_{90} لكل مبيد على حدة للأعمار المختلفة وأختبر تركيزات مختلفة لكل مبيد على حدة وسجلت نسب الموت بعد 24، 48، 72 ساعة من المعاملة.

أوضحت النتائج أن مبيد الميثوميل أكثر كفاءة وتأثير طارد للأعمار المختلفة للقوقع وله تأثير واضح للميثوميل والديازينون على الأعمار الصغيرة ثم الكبيرة للقوقع. أتضح أن الميثوميل أكثر تأثير على نشاط الأنزيمات الكولين استيريز واللاكتيك ديهيدروجينيز والانتقال والبروتين والليبيدات الكلى بالمقارنة بمبيد الديازينون.

Table (2) Comparative toxicity of methomyl 20%SL used as bait or thin film on different ages of *Eobania vermiculata*

Age	One month				Three month				Six month				Adult (9-12 month)				Old (over 24 month)			
	Conc.*	Mortality%			Conc.*	Mortality%			Conc.*	Mortality%			Conc.*	Mortality%			Conc.*	Mortality%		
		24h	48h	72h		24h	48h	72h		24h	48h	72h		24h	48h	72h		24h	48h	72h
Treatment																				
Bait	100.0	0.0	27.5	27.5	300.0	0.0	0.0	22.5	50.0	5.0	10.0	15.0	100.0	0.0	0.0	22.0	100.0	0.0	25.0	25.0
	200.0	22.5	27.5	32.5	400.0	15.0	20.0	40.0	100.0	42.5	50.0	50.0	250.0	25.0	50.0	50.0	200.0	0.0	10.0	35.0
	300.0	40.0	47.5	47.5	500.0	20.0	20.0	50.0	300.0	10.0	35.0	70.0	400.0	45.0	77.5	77.5	300.0	0.0	12.5	50.0
	400.0	22.5	32.5	80.0	600.0	5.0	75.0	77.5	450.0	10.0	47.5	75.0	600.0	25.0	50.0	100.0	400.0	0.0	12.5	67.5
	500.0	32.5	72.5	100.0	700.0	40.0	90.0	90.0	600.0	22.5	97.5	97.5	-	-	-	-	600.0	0.0	27.5	77.5
Thin Film	0.0125	7.5	17.5	25.0	0.2	25.0	25.0	32.5	0.2	0.0	0.0	25.0	0.34	0.0	27.5	27.5	0.1	22.5	22.5	22.5
	0.025	35.0	50.0	50.0	0.3	37.5	47.5	47.5	0.3	0.0	32.5	37.5	0.4	25.0	47.5	47.5	0.2	25.0	35.0	35.0
	0.05	50.0	72.5	72.5	0.5	52.5	77.5	77.5	0.4	27.5	72.5	72.5	0.5	50.0	75.0	82.5	0.3	25.0	50.0	50.0
	0.1	62.5	75.0	97.5	0.6	97.5	100.0	100.0	0.5	45.0	70.0	85.0	0.6	75.0	82.5	97.5	0.4	52.5	67.5	67.5
	-	-	-	-	-	-	-	-	0.6	45.0	75.0	100.0	-	-	-	-	0.6	75.0	82.5	100.0

* Methomyl concentrations represented by ppm in case of bait and mg a.i./cm² of thin film technique.

Table (3) Comparative toxic effect of diazinon 60% EC used as bait or thin film on different ages of *Eobania vermiculata*

Age	Treatment	One month				Three month				Six month				Adult (9-12 month)				Old (over 24 month)			
		Conc.*	Mortality%			Conc.*	Mortality%			Conc.*	Mortality%			Conc.*	Mortality%			Conc.*	Mortality%		
			24h	48h	72h		24h	48h	72h		24h	48h	72h		24h	48h	72h		24h	48h	72h
Bait		600.0	0.0	30.0	30.0	300.0	0.0	0.0	25.0	3300.0	0.0	0.0	25.0	1800.0	25.0	32.5	32.5	300.0	0.0	0.0	27.5
		750.0	25.0	30.0	37.5	600.0	0.0	27.5	55.0	3600.0	15.0	30.0	30.0	3000.0	35.0	52.5	60.0	600.0	12.5	42.5	42.5
		900.0	32.5	47.5	62.5	900.0	25.0	50.0	75.0	3900.0	25.0	50.0	75.0	4200.0	50.0	70.0	75.0	900.0	25.0	50.0	50.0
		1050.0	35.0	75.0	75.0	1200.0	50.0	85.0	85.0	4500.0	25.0	75.0	97.5	5400.0	50.0	75.0	100.0	1800.0	25.0	47.5	77.5
		1200.0	20.0	45.0	95.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thin Film		0.3	12.5	27.5	27.5	0.2	0.0	25.0	35.0	1.5	5.0	27.5	27.5	1.9	10.0	25.0	35.0	0.3	15.0	25.0	25.0
		0.45	25.0	42.5	42.5	0.3	20.0	52.5	52.5	1.8	15.0	30.0	35.0	2.7	25.0	50.0	50.0	0.6	25.0	30.0	32.5
		0.6	12.5	22.5	52.5	0.6	47.5	77.5	77.5	2.1	22.5	40.0	77.5	3.9	42.5	77.5	77.5	0.9	27.5	50.0	50.0
		0.75	40.0	70.0	85.0	0.9	50.0	100.0	100.0	2.4	25.0	50.0	90.0	5.1	50.0	90.0	97.5	1.2	40.0	62.5	62.5
		0.9	50.0	75.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-	1.8	50.0	75.0	100.0

* Diazinon concentrations was represented by ppm in case of bait and mg a.i./cm² of thin film technique.

Table (7) Effect of methomyl at LC₅₀ on total protein, acetyl cholinesterase (AChE), lactic acid dehydrogenase (LDH), aspartate amino transferase (AST) activities and total lipid in different ages of *Eobania vermiculata*.

Parameters	Periods	Ages				
		One month	Three month	Six month	Adult (9-12 month)	Old (over24 month)
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Total Protein (g/100ml)	Control	0.96±0.01	1.27±0.08	1.5±0.48	0.69±0.11	0.99±0.13
	24h	0.81±0.12	1.26±0.02	1.69±0.21	1.11±0.08	0.97±0.08
	48h	0.87±0.12	1.25±0.11	1.6±0.47	0.90±0.13	1.75±0.12
	72h	0.72±0.01	1.24±0.06	1.61±0.32	1.29±0.31	0.65±0.07
AChE (nmol/min/mg)	Control	1300.0±0.002	859.8±0.001	50.0±0.0001	135409.0±1.2	2190.0±0.004
	24h	673.2±0.002**	990.5±0.002	138.5±0.001	386.5±0.002	2407.4±0.004
	48h	893.5±0.001*	499.2±0.001*	126.4±0.0003**	286.0±0.0006	2223.5±0.01*
	72h	1090.9±0.001*	440.3±0.0001**	241.6±0.0006**	120.8±0.002	940.7±0.002*
LDH (U/L)	Control	113.2±25.2	64.5±17.1	364.7±145.9	109.7±19.7	350.0±5.8
	24h	3290.5±26.5**	280.7±79.4*	122.7±35.9	868.2±420.8	214.6±79.8
	48h	2033.2±1914.6	91.8±37.1*	179.0±56.2	442±177.5	222.6±22.4**
	72h	1257.0±920.8	537.3±96.3**	98.4±4.3	235.6±69.9	91.9±24.9**
AST (U/L)	Control	17.0±7.2	15.7±5.9	15.3±3.9	52.3±10.9	20.3±10.5
	24h	42.0±17.1	15.0±1.0	11.0±2.7	17.3±2.9	8.0±1.0
	48h	42.0±17.4	13.7±6.7	9.0±2.0	27.7±6.7	10.0±1.73
	72h	37.6±12.4	34.6±4.1	13.0±3.5	34.6±4.1	18.0±6.6
Total Lipid (gm/100ml)	Control	0.28±0.04	1.61±0.64	1.21±1.29	0.7±0.16	0.55±0.23
	24h	0.39±0.13	1.72±0.57	0.29±0.06	1.71±0.11	0.25±0.05
	48h	0.47±0.25	1.84±0.21	0.56±0.13	1.45±0.22	0.02±0.15
	72h	0.42±0.11	1.86±0.11	0.48±0.09	1.28±0.2	0.39±0.13

* Significant.

** High significant.

Table (8) Effect of diazinon at LC₅₀ on total protein, acetyl cholinesterase (AChE), lactic acid dehydrogenase (LDH), aspartate amino transferase (AST) activities and total lipid in different ages of *Eobania vermiculata*.

Parameters	Periods	Ages				
		One month	Three month	Six month	Adult (9-12 month)	Old (over 24 month)
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Total Protein (g/100ml)	Control	0.96±0.08	1.27±0.01	1.25±0.48	0.69±0.11	0.99±0.13
	24h	0.28±0.06**	0.42±0.14**	1.40±0.34	0.57±0.06	1.32±0.22
	48h	0.66±0.23	0.6±0.04**	1.17±0.53	0.44±0.14	1.76±0.35
	72h	0.6±0.21	0.83±0.1*	0.69±0.47	0.56±0.10	1.98±0.26**
AChE (nmol/min/mg)	Control	1300±0.002	859.8±0.001	50.0±0.0001	135409±1.2	2190.0±0.004
	24h	3343.0±0.001	1300.0±0.001**	423.4±0.001**	1089.0±0.002	530.0±0.001
	48h	1660.0±0.002	1560.0±0.001	334.2±0.0003*	707.5±0.0003	164.4±0.0001*
	72h	1170±0.001**	1394.0±0.001	783.4±0.003*	1532.0±0.002	315.5±0.001*
LDH (U/L)	Control	113.2±25.2	64.5±17.1	364.7±145.9	109.7±19.6	350.2±5.8
	24h	357.8±168.0	463.0±256.0	51.6±19.9*	91.9±30.8	321.1±71.2
	48h	348.0±72.7**	479.0±170.7*	503.5±268.7*	217.8±46.2*	246.9±137.8
	72h	666.7±180.0**	432.5±107.9**	43.6±26.8	143.6±11.3	167.8±28.7
AST (U/L)	Control	17.0±7.2	15.7±5.9	15.3±3.93	52.3±10.9	20.3±10.5
	24h	36.0±2.9*	33.7±7.12	13.0±1.73	14.0±1.0**	9.0±1.0
	48h	51.0±4.0**	38.7±4.3**	14.0±1.0	21.7±2.7*	10.0±1.73
	72h	58.6±10.1**	43.3±6.34**	9.0±1.0	21.0±5.0*	18.3±2.9
Total Lipid (gm/100ml)	Control	0.28±0.04	1.61±0.64	1.21±0.11	0.7±0.16	0.55±0.23
	24h	0.81±0.43	1.82±0.47	0.75±0.33	0.89±0.16	0.75±0.49
	48h	2.36±0.57**	1.29±0.37	0.61±0.19*	0.83±0.4	0.56±0.14
	72h	2.15±0.28**	0.97±0.19	0.39±0.11**	0.77±0.14	0.99±0.24

* Significant.

** High significant.