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TOXICITY AND BIOCHEMICAL STUDIES OF METHOMYL AND DIAZINON ON DIFFERENT AGES OF THE LAND GASTROPOD SPECIES *Monacha obstucta*

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ABSTRACT

The toxicity and biochemical of the two insecticides methomyl 20% SL and diazinon 60% EC used as bait or contact poisons were studied against different ages of *Monacha obstructa*. The LC_{50} and LC_{90} values of each compound were determined for the different ages of *M. obstructa*.

Different concentrations of each compound were tested against *M. obstructa* using baiting and contact thin layer film (TLF). Mortality percentages were recorded 24, 48 and 72 hrs., after treatment. Methomyl was toxic used as poison contact for all tested ages, while methomyl was moderately toxic when it was used as poison bait on all different ages (1, 3, 6, 9-12 and over 24 months). Methomyl and diazinon were toxic in high concentrations as contact than bait gave highly mortality percentages in adult and (over 24 months) than (one month, three month and six month) after 72h from treatment. Regarding the contact method, diazinon was toxic to the three-month and old individuals. The repellent effect of the two insecticides methomyl and diazinon against (one month, three month, six months, 9-12 month adult and over 24 months old) different ages of *M. obstructa* were studied and medium repellent different ages while vice versa occurred in case of diazinon, as it was repellency at the same ages. The biochemical response of the different ages of *M. obstructa* treated with LC_{50} of methomyl and diazinon compounds, whereas AchE response of the three and six months ages differed with the all ages to both compounds. The response of LDH and AST variation to each age of *M. obstructa*. On the other hand, effect of two compounds on total protein and total lipid similar in most ages except six month and old ages reduced after 72 h, treatment.

Keywords: Toxicity lines, Methomyl, Diazinon, *Monacha obstructa*, Repellent (R_{50}), AchE, LDH, AST, Total protein, and Total lipid.

INTRODUCTION

The land snails have become a serious pest during the last few years attacking different types of crops in the different governorates in Egypt.

The injured plants quickly repair the damaged parts and the yield of crops seems unaffected, but at least the quality a reduced, so that the vegetable crops get poorer marketing rating and reduced in value (El-Okda, 1980). The snails attack cereals, orchards, ornamental, fruits, vegetables and forestis.

As most of land snails are herbivorous of snails attack root crops such as potatoes. These pests can be controlled by several different ways: by chemical means molluscicides on the other hand, control of snail individuals and their eggs method used is not simple (Nehmedo, Abd-El-Karim 2000).

The molluscicides must be inhaled or absorbed over the body surface to reach the site of action. Presumably, the poison bait is ingested where the

toxic fraction of material is adsorbed on contact (Judge and kuhr, 1972). The molluscicideal activity of methomyl against the land snail *Monacha obstructa* was used as contact test, whereas the LD_{50} value after 72h, of treatment 11.9µg/snail (Hussein *et al.,* 1999). Effect of diazinon against snail gave 45% mortality after 2 hours, which the repellent effect of carbamate compounds at high concentration and attractiveness at low concentrations (Godan, 1965).

The present work aims to study the biochemical effect of both compounds on some biochemical parameters of different ages *M. obstructa* i.e. acetyl cholinesterase, lactic acid dehydrogenase, aspartate amino transferase, total protein and total lipid and the extent of alteration in biochemical responses caused by treatment with low and high doses of insecticide. The mode of action insecticides explained gradually increased with an increase in different treatment (Singh and Agarwal, 1978).

MATERIALS AND METHODS

Chemical: Insecticides used methomyl

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

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

Tested Animal: Snails of *Monacha obstructa* were collected from clover field at Zagazig district (Sharkia Governorate). Health individuals were kept in separate glass terraria (70 ×40 ×35cm) 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 *Monacha obstructa* using baiting and contact techniques. Mortality were 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). A thin layer film technique was used as method of application according to Ascher and Mirian (1981). Some pesticides used with high concentrations repel 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 individual of each age (1, 3, 6, 9-12 and over 24 months) were put in 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 to Bergmeyer (1963). 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) was used to assay 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 results in Table (1) presented the response of different ages to methomyl and diazinon insecticides, LC_{50} and LC_{90} methomyl values to different ages. Tolerance to methomyl as contact poison in all ages except the one and three month age individuals, which seemed less sensitive. The LC_{50} and LC_{90} values clear that *M. obstructa* was susceptible to both application methods of diazinon (bait or contact) at the most ages except for the adult and old animals. *Monacha obstructa* whereas LC_{50} was 1.18 while LC_{90} where it was 3.84.

Table (1): LC_{50} and LC_{90} values for different ages of *Monacha obstructa*.

Molluscicides	Methomyl				Diazinon			
	Bait (ppm)		Thin film (mg/cm^2)		Bait (ppm)		Thin film (mg/cm^2)	
Ages	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
One month	244.9	1087.4	0.03	0.07	626.8	1170.3	0.39	0.87
Three month	491.2	1045.7	0.19	0.41	511.4	2043.0	0.25	0.77
Six month	267.4	1699.6	0.39	0.67	3520.7	4180.4	1.77	2.44
Adult (9-12 month)	271.9	1031.6	0.49	0.78	2780.2	6749.9	2.48	3.69
Old (over 24month)	561.0	4818.0	0.30	1.65	1816.7	4358.7	1.18	3.84

The data in Table (2) indicated the effect of methomyl insecticides when administered as bait or contact by using technique against *Monacha obstructa* (one, three, six, adult and old). It is clear that one-month age was more susceptible age to methomyl than other ages. The contact effect was caused the last concentration 0.1 mg/cm^2 it gave 97.5% mortality. Methomyl was more efficient as contact poison than poison baits at three-month age these results in agreement with those obtained by (Okka *et al.*, 1996) who reported that effect of methiocarb was tested when used as bait or spray was 80.0% after 3 days of exposure it gave 90% mortality after six days of application when used as bait. At the six month age of *M. obstructa* susceptible to both application methods.

Data revealed that methomyl bait or contact on the adult age was toxic in three tested periods (24-48 and 72 h). The effect of methomyl bait or contact was effective in over 24-month age, the susceptibility level differed from time to another after treatment. Crowell, (1967), observed similar results. Many chemical molluscicides are repellent at high concentrations bait are attractive at very low concentrations, between these two extremes the response to them is different (Godan, 1958 and 1959).

Data (3) revealed that R_{50} values (50% of tested animals consumed less than half offered treated food) effect of methomyl on different ages to *M. obstructa* was lower at one month 8 times, 1-2 time for three month age, 1.9 time to six months, 3.1 time for adults and 1.9 time for old aged animals (over 24 month). R_{50} value of methomyl raised to 5.2 times for the three months age, 6.1 times for six months age, 6.3 times for adults and 5.3 times for the old age than one-month 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.

Table (3) Repellent effect of methomyl on different ages of *Monacha obstructa* land snails.

Repellency %									
One month		Three month		Six month		Adult (9-12 month)		Old (over 24month)	
Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%
50.0	26.7	400.0	31.4	400.0	17.3	400.0	29.3	400.0	41.9
100.0	41.5	800.0	69.2	600.0	35.6	800.0	43.0	600.0	60.4
200.0	49.0	1200.0	100.0	800.0	51.9	1200.0	65.2	800.0	87.9
400.0	70.4	1600.0	100.0	1000.0	57.3	1500.0	97.8	1000.0	90.9

Data in table (4) presents the results toxic effect of diazinon against different ages of *M. obstructa*, results illustrated diazinon was toxic as contact than bait it caused 100.0% mortality after 72h. *Monacha obstructa* exhibited high susceptibility for diazinon. The sensitivity level to diazinon at three-month age differed according to the application method, it clear response of six-month age. Our results are also in accordance with El-Bahi *et al.* (1992) who reported that reached mortality 100.0% after treatment diazinon against terrestrial. There is no large different between poison bait and contact diazinon, the highest 37.5, 75.0 and 100.0% mortality. The response of old adult (over 24-month age) diazinon was toxic in the two cases of treatment bait or contact. (Godan, 1983) in agreement with obtains our results.

In case of diazinon the repellent effect in Table (5) increased with increasing age of snail where R_{50} value enhanced 1.9, 6.9, 12.6 and 4.3 times for the three, six month, adult age and old animals than one month age respectively.

Finally in case of diazinon as it was repellency for *M. obstructa* at the different ages. The previous results indicate that repellency effect was high at one month age and old aged animals than one month age while it decreased with the rest ages i.e. three , six month and adult age.

Table (5) Repellent effect of diazinon on different ages of *Monacha obstructa* land snails.

Repellency %									
One month		Three month		Six month		Adult (9-12 month)		Old (over 24month)	
Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%
300.0	33.9	480.0	44.6	2340.0	44.3	4260.0	41.3	1200.0	45.4
360.0	57.2	576.0	62.3	2400.0	78.5	4320.0	47.9	1500.0	67.3
420.0	79.2	690.0	78.5	2460.0	84.0	4380.0	89.0	1800.0	82.5
540.0	98.2	830.0	95.0	2520.0	91.9	4440.0	95.5	2100.0	98.6

Data tabulated in Table (6) indicated the effect of LC_{50} of methomyl and diazinon on total protein, activity of acetyl cholinesterase (AchE), lactic acid dehydrogenase (LDH), aspartate amino transferase (AST) and total lipid in the different ages of *Monacha obstructa* after different periods. Plasma protein serves as source for rapid replacement of tissue proteins during tissue depletions, as buffers in acid-base balance and as transporters for constituents of the blood such as lipid, vitamins, hormones, iron, copper and certain enzymes (Wilson, 1986). Data revealed it reduced to -55.5, -5.95 and -6.75 at one-month age after 24, 48 and 27h respectively. Similar results was obtained at three month where protein decreased to the effect of diazinon results showed total protein level decreased significantly in the all tested periods at one, three month. At six-month age in methomyl, the different % raised to 35.4, 116.0, 28.5, 54.9, 29.3, and 67.3% during 24, 48 and 72h while in diazinon it raised to 55.8, 52.0 and 93.0% after the same periods. For adult and old individuals total protein levels decrease was observed in methomyl, while in diazinon it decreased significantly to -99.0, -28.3 and 44.0% after the same tested periods. Our results are in agreement with that obtained by El-Wakil and Radwan (1991).

Results in Table (6,7) showed the treatment with methomyl and diazinon occurred decrease the acetyl cholinesterase (AchE) activity.

AchE activity decrease to -34.5, -78.0 and -64.3% after 24h, 48h and 72h, respectively for one month age *M. obstructa* treatment of methomyl while the enzyme activity decreased significantly to -65.0 and -43.0% after 24 and 72h, respectively while increased to -25.0% post 47h from the treatment of diazinon. At the three month age the difference percentage were 28.6, 38.0 and 102.6% post 24, 48 and 72h treatment of methomyl, respectively in case of treatment diazinon the enzyme activity increased significantly to 136.0% after 24h and 72h, respectively while it increased to -25.0% post 48h from the treatment. Large variation was observed at six month age individuals whereas the difference percentages of enzyme activity were -58.0%, -67.0and -52.2% post 24, 48 and 72h of treatment methomyl regarding enzyme activity increased in three tested periods it reached 288.4, 1466.8 and 26.0% of treatment diazinon. Regarding the adult animals (9-12 month) it raised 257.4 after 24h and reduced to -36.0% post 48 and 72 h concerning old animals' acetyl cholinesterase activity increased to 312.6, 49.5 and 77.5% at three tested periods of treatment methomyl. Effect of diazinon on the enzyme it decreased to -44.0, -64.0 and 62.0% in the three tested periods respectively. These results are agreement with Marti and Ronald (1988).

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Data in Table (6,7) revealed the effect of LC_{50} of methomyl and diazinon on lactic acid dehydrogenase (LDH) in the different ages. Results it reached 48 and 72h in treatment methomyl in one-month age. While in three-month LDH level increased with increasing periods it reached to 24.5% after 24h and decreased to 12.2 post 48h, then increased to 22.5% after 72h. In contrast, diazinon these ratios decreased to -82.5, -49.4, 44.4, -20.7 and -79.3 and 61.0% with the three tested periods.

Regarding six-month age the difference percentages of LDH level it increased to 12.4, 30.9 and 36.8 after three tested periods treatment methomyl, while it enhanced to 250.0, 153.1 and 303.5% during three tested of diazinon. Concerning treatment of methomyl the adult age it decreased to -27.3, -62.6 and -33.3% for three to 206.5, 314.0 and 117.6% for treatment. In contrast treatment diazinon, it decreased -27.0, -52.0 and 41.3% after 24, 48 and 72h. Our results agreement with (Glulchova, 1985 and Ray et al., 1988).

The effect of LC_{50} of methomyl and diazinon on AST during the different ages in Table (6,7). Data pointed out that at one month the enzyme increased to 94.5, 121.2 and 32.0% after three-tested period's treatment methomyl, similar result in treatment diazinon. The enzyme level increased to 11.5, 12.5, 96.15, 25.0, 69.2, and 50.0% in three and six month after 24, 48 and 72h from treatment methomyl while in diazinon it decreased to 57.7, 11.5 and 11.5% in three month but six month enhanced. Regarding adult age of (AST) activity reduced to -26.7, -33.7 and -13.3% but in old aged the enzyme level increased to 128.8, 141.3 and 203.8% after treatment methomyl. Adult and old ages it reached to 62.5, 193.0 and 141.3% post 48h it increased to 91.3 and 108.8% after 72h from treatment with diazinon.

The results agreement with Tilkion *et al*. (1983).Lipids play extremely important roles in the normal function of a cell, not only to lipids nerve as highly reduced storage forms of energy but they also play an intimate role in the structure of cell membrane and organelles found in the cell (Willson, 1986).

Table (6,7) indicates the effect of methomyl and diazinon the obtained data revealed the difference % of total lipid level in comparison with control for one month in methomyl it decreased to -71.0, -27.0and -38.0 similar in diazinon decreased to -21.0, -84.9 and -60.6% after 24, 48 and 72h, respectively. The results were similar in three month age for methomyl and diazinon, it reduced to -64.6, -30.7 and -48.4% also it decreased in diazinon to -53.9, -27.5 and -7.5 after three tested periods. Significant decline occurred in case of six-month age in methomyl and diazinon 23, 5.0, 61.4, 40, 2, -55.6, -15.5 and -7.8%, respectively after 24, 48 and 72h periods. For adult stage reduced to -55.8, -41.9 and -48.8% in methomyl but its level periods of administration of diazinon, respectively. The old aged animals the level of total lipid decreased to -76.8, -43.5 and -53.2% after methomyl treatment, while in diazinon these values reduced to -66.0, -62.8 and -24.7% after 24, 48 and 72h periods. Our results are agreed with Godan (1983) who observed that three are different susceptibility levels at different times in the life cycle of snails. Also, he mentioned that the period of snail sensitivity, which is important for control program of snails.

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دراسات السمية والبيوكيماوية لمبيدى الميثوميل والديازينون على الأعمار المختلفة لقوقع موناكا أوبستركتا

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يهدف البحث لدراسة تأثير مبيدى الميثوميل والديازينون على الأعمار المختلفة لقوقع موناكا أوبستركتا من خلال المعاملة عن طريق الملامسة والطعوم السامة.
أجريت تقدير قيم LC_{90}, LC_{50} لكل مبيد على حدة للأعمار المختلفة. استخدم تركيزات مختلفة لكل مبيد على حدة وسجلت نسب الموت بعد 24 ،48 ، 72 ساعة من المعاملة.
أتضح أن مبيد الميثوميل له تأثير سام عند استخدامه كطعم سام أو بالملامسة لمعظم الأعمار المختلفة للقوقع وأن مبيد الديازينون له تأثير سام واضح عن طريق الملامسة للأفراد عمر 3 شهور والأفراد البالغة.
أوضحت النتائج أن التأثير الطارد للمركبين المختبرين واضح على الأعمار المختلفة للقوقع
أتضح أن الميثوميل أكثر تأثير طارد للأعمار المختلفة للقوقع عن الديازينون. أكدت النتائج أن مبيدى الميثوميل والديازينون لهما تأثير على النشاط الانزيمى من انخفاض وارتفاع لنشاط الإنزيمات وكذلك لتقدير البروتين والليبيدات الكلى تبعا للأعمار المختلفة للقوقع وظهر واضحا بعد 72 ساعة من المعاملة أوضحت النتائج أن اختلاف التركيب الكيماوى للمبيدين المختبرين وكذلك طرق المعاملة وفترة التعرض للأعمار المختلفة للقوقع لها تأثير واضح على النشاط الأنزيمى.

Table (2): Toxic effect of methomyl 20% SL used as bait or thin film on different ages *Monacha obstructa.*

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	100.0	25.0	25.0	25.0	300.0	0.0	0.0	25.0	50.0	0.0	0.0	0.0	100.0	0.0	0.0	15.0	100.0	0.0	0.0	0.0
	200.0	30.0	30.0	40.0	400.0	10.0	15.0	30.0	100.0	0.0	0.0	25.0	250.0	12.5	27.5	52.5	200.0	0.0	10.0	25.0
	300.0	25	52.5	52.5	500.0	25.0	50.0	50.0	300.0	0.0	25.0	50.0	400.0	27.5	40.0	60.0	300.0	10.0	25.0	37.5
	400.0	30.0	65.0	65.0	600.0	30.0	42.5	60.0	450.0	12.5	30.0	60.0	600.0	27.5	37.5	77.5	400.0	12.5	35.0	42.5
	500.0	35.0	77.5	77.5	700.0	35.0	60.0	77.5	600.0	25.0	50.0	75.0	-	-	-	-	600.0	15.0	50.0	50.0
Thin Film	0.0125	0.0	0.0	25.0	0.2	10.0	15.0	15.0	0.2	0.0	0.0	0.0	0.34	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	0.025	0.0	25.0	50.0	0.3	37.5	52.5	52.5	0.3	0.0	0.0	20.0	0.4	0.0	25.0	25.0	0.2	0.0	0.0	20.0
	0.05	25.0	45.0	77.5	0.5	67.5	77.5	77.5	0.4	20	50.0	50.0	0.5	25.0	50.0	50.0	0.3	25. 0	27.5	52.5
	0.01	37.5	70.0	97.5	0.6	55.0	80.0	100.0	0.5	25.0	65.0	75.0	0.6	25.0	65.0	75.0	0.4	50.0	75.0	75.0
	-	-	-	-	-	-	-	-	0.6	25.0	50.0	97.5	-	-	-	-	0.6	70.0	75.0	82.0

*** Methomyl concentrations represented by ppm in case of bait and mg a.i./cm^2 in case of thin film technique.**

Tale (4): Toxic effect of diazinon 60% EC used as bait or thin film o different ages *Monacha obstructa.*

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	15.0	50.0	50.0	300.0	0.0	0.0	0.0	3300.0	20.0	27.5	27.5	1800.0	22.5	22.5	22.5	300.0	0.0	0.0	12.5
	750.0	35.0	60.0	62.5	600.0	25.0	25.0	25.0	3600.0	25.0	50.0	50.0	3000.0	40.0	52.5	52.5	600.0	20.0	22.5	22.5
	900.0	12.5	62.5	75.0	900.0	25.0	50.0	60.0	3900.0	50.0	77.5	77.5	4200.0	50.0	72.5	77.5	900.0	30.0	32.5	32.5
	1050.0	67.5	80.0	97.5	1200	50.0	100.0	100.0	4500.0	60.0	80.0	100.0	5400.0	47.5	75.0	100.0	1800.0	47.5	50.0	50.0
Thin Film	0.3	0.0	10.0	35.0	0.2	20.0	30.0	32.5	1.5	20.0	25.0	25.0	1.9	10.0	12.5	25.0	0.3	0.0	0.0	10.0
	0.45	10.0	27.5	52.5	0.3	20.0	27.5	50.0	1.8	25.0	52.2	52.5	2.7	27.5	35.0	50.0	0.6	5.0	15.5	22.5
	0.6	25.0	77.5	77.5	0.6	62.5	75.0	75.0	2.1	35.0	42.5	75.0	3.9	27.5	50.0	97.5	0.9	25.0	25.0	30.0
	0.75	75.0	100.0	100.0	0.9	97.5	97.5	97.5	2.4	50.0	75.0	100.0	5.1	37.5	75.0	100.0	1.2	47.5	47.5	47.5
	0.9	100.0	100.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-	1.8	50.0	72.5	75.0

***Diazinon concentrations represented by ppm in case of bait and mg a.i. / cm^2 in case of thin film technique.**

Table (6) Effect of methomyl at LC_{50} on total protein, acetyl cholinesterase (AchE), lactic acid dehydrogenase (LDH), aspartate amino transferase (AST) activities and total lipid in different ages of *Monacha obstructa.*

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	1.13 ± 0.04	1.67 ± 0.08	0.37 ± 0.24	0.67 ±0.12	1.36 ±0.09
	24h	0.50 ± 0.021*	1.38 ± 0.16	0.81 ± 0.23	0.31 ± 0.24*	0.045 ±0.18**
	48h	1.06 ± 0.10	0.76 ± 0.33*	1.73 ± 0.33*	0.47 ± 0.06	1.06 ± 0.08*
	72h	1.05 ± 0.09	1.03 ± 0.01**	0.63 ±0.34	0.24 ± 0.08**	0.51 ±0.08**
AchE (nmol/min/mg)	Control	1664.0 ± 0.002	747.3 ± 0.002	1668.4 ± 0.001	927.2 ± 0.001	592.0 ± 0.005
	24h	1089.8±0.002**	960.8 ± 0.002	250.0 ± 0.003	3313.7 ± 0.002	2443.0 ±0.005
	48h	368.6±0.001**	1031.7 ± 0.004	553.3 ± 0.003	598.0 ± 0.013	885.0 ± 0.004
	72h	594.8±0.001**	1514.5 ± 0.01	797.4 ± 0.001	579.0 ± 0.013	1051.2 ± 0.002
LDH (U/L)	Control	203.3 ± 89.5	382.4 ± 193.1	162.7 ± 11.6	106.5 ± 44.4	101.6 ± 8.4
	24h	286.6 ± 88.3	288.8 ± 5.8	182.7 ± 7.8	77.4 ± 31.1	311.4 ± 18.6*
	48h	169.3 ± 75.5	335.6 ± 219.8	213.0 ± 39.2	39.9 ± 77.2	421.0 ± 97.9**
	72h	71.09 ± 52.7	467.9 ± 202.7	222.6 ± 82.4	71.0 ± 8.5	221.1 ± 70.2
AST (U/L)	Control	16.3 ± 3.76	26.0 ± 5.0	8.0 ± 1.0	15.0 ± 2.7	8.0 ± 1.0
	24h	29.0 ± 6.0	29.0 ± 1.16	9.0 ± 1.0	11.0 ± 2.7	18.3 ± 2.9*
	48h	31.7 ± 4.67*	51.0 ± 8.1*	10.0 ± 1.73	11.0 ± 2.0	19.3 ± 2.0**
	72h	21.5 ± 3.2	44.0 ± 1.73**	12.0 ± 2.7	13.0 ± 3.47	24.3 ± 3.53**
Total Lipid (gm/100ml)	Control	1.50 ± 0.88	1.39 ± 0.47	1.02 ± 0.36	1.29 ± 0.8	1.99 ± 0.76
	24h	0.43 ± 0.11	0.94 ± 0.24	1.26 ± 0.85	0.57 ± 0.24	0.46 ± 0.28
	48h	1.09 ± 0.9	0.97 ± 0.37	1.64 ± 0.08	0.75 ± 0.09	1.13 ± 0.55
	72h	0.93 ± 0.03	0.72 ± 0.23	1.43 ± 0.77	0.28 ± 0.2	0.94 ± 0.17

*** Significant.** **** High significant.**

Table (7) Effect of diazinon at LC_{50} on total protein, acetyl cholinesterase (AchE), lactic acid dehydrogenase (LDH), aspartate amino transferase (AST) activities and total lipid in different ages of *Monacha obstructa*

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	1.13 ± 0.04	1.67 ± 0.08	0.37 ± 0.24	0.67 ±0.12	1.36 ±0.09
	24h	0.80 ± 0.16	0.93 ± 0.15**	0.58 ± 0.42	0.55 ± 0.12	0.01 ± 0.09*
	48h	0.49 ± 0.08**	1.09 ± 0.09**	0.57 ± 0.37	0.68 ± 0.09	0.97 ± 0.13*
	72h	0.75 ± 0.08**	1.05 ± 0.05**	0.72 ± 0.26	0.66 ± 0.06	0.76 ± 0.05**
AchE (nmol/min/mg)	Control	1664.0 ± 0.002	747.3 ± 0.002	1668.4 ± 0.001	927.2 ± 0.001	592.0 ± 0.005
	24h	583.5 ± 0.001	1761.3 ±0.001	3425±0.008	936.0±0.002	6240.0±0.001
	48h	2074.0±0.001**	716±0.004*	5346±0.01**	333.6±0.001	400.8±0.002
	72h	942.3±0.001**	1040±0.001	1730.0±0.007*	355.0±0.001	339.0±0.001
LDH (U/L)	Control	203.3 ± 89.5	382.4 ± 193.1	162.7 ± 11.6	106.5 ± 44.4	101.6 ± 8.4
	24h	35.5 ± 14.08	193.6 ± 86.8	216.6 ± 53.2	372.7 ± 151.9	37.7 ±8.8**
	48h	112.9 ±36.4	440.5 ± 467.9	64.5 ± 11.7**	269.5 ± 85.9	74.2±6.5*
	72h	41.9 ± 17.9	182.3 ±467.9	62.0 ± 20.6**	429.7±124.4*	59.7±4.3**
AST (U/L)	Control	16.3 ± 3.76	26.0 ± 5.0	8.0 ± 1.0	15.0 ± 2.7	8.0 ± 1.0
	24h	10.0 ± 7.3	41.0 ± 10.4	17.3 ± 2.9*	29.0±6.4*	17.7 ± 9.2
	48h	11.0 ±4.0	29.0 ±1.16	13.0 ± 1.73*	44.0 ± 1.73**	19.3 ± 2.0**
	72h	9.0 ± 1.0	29.0 ± 64	15.3 ± 4.6	29.6± 3.7**	16.7 ± 5.24
Total Lipid (gm/100ml)	Control	1.50 ± 0.88	1.39 ± 0.47	1.02 ± 0.36	1.29 ± 0.8	1.99 ± 0.76
	24h	1.18 ± 0.38	0.64 ± 0.19	0.74 ± 0.32	0.83 ±0.62	1.5 ± 0.5
	48h	0.23 ±0.04	1.29 ± 0.49	0.45 ± 0.18	1.15 ± 0.34	0.68 ± 0.23
	72h	0.59 ±0.35	1.18 ± 0.57	0.94 ±0.71	0.48 ±0.13	1.51 ± 0.38

*** Significant.** **** High significant**