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Effect of Carbamates Pesticide on Instar I-II larvae and Adult Artemia urmiana

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ABSTRACT

The purpose of this assessment was to evaluate potential direct effects of acute and chronic toxicity of Maneb (Dithane-M22) and Carbaryl (Sevin) in Artemia urmiana. A study was conducted to determine the concentration incorporated in the contamination treatment of instar I-II larvae and Adult Artemia urmiana for 24 hr. and 48 hr. Nine-hundred Artemia urmiana were used in a 2 factorial experiments (time and concentration). Mortality was recorded during the different times as well as variable concentration. Acute intoxication of Maneb and Carbaryl were able to be induced at >33 ppm concentration, Carbaryl was more lethal than Maneb. Histopathological observations made from hematoxylin-eosin stained sections of Maneb and Carbaryl treated Artemia showed significant lesions which were found to be more extensive in Carbaryl treated Artemia than in those Maneb treated Artemia. Results of this study showed the potential of Carbaryl pesticide to cause serious damage of the nervous system of the Adult Artemia urmiana. On the other hand, Maneb pesticides was primarily highly toxic on instar I-II larvae.

INTRODUCTION

Iran has many natural saline lakes. One of the biggest and most important is the Urmia Lake located in the Northwestern part of the country [1]. (Crustacea, Anostraca), also known as brine shrimp, are typical inhabitants of extreme saline biotopes [2]. Artemia populations are found in about 500 natural salt lakes scattered throughout the tropical, subtropical and temperate climatic zones, along coastlines as well as inland [3]. Artemia urmiana is reported to exist only in Lake Urmia. [4-6]. Pesticides are an integral part of agriculture. Our environment also is favorable for the development and presence of beneficial organisms that positively affect our agricultural production and enhance our wildlife and plant communities. A side effect of usage of some

pesticides results in unfortunate consequences to our non target organisms. The study of synergistic interactions between natural and anthropogenic stressors across multiple species is an important study area in ecology as it will contribute to our understanding and ability to project how natural communities will change with increasing human impact. Among the anthropogenic stressors, pesticides are increasingly used worldwide [7] while presenting also a major threat to many natural ecosystems [8]. Carbaryl is registered for use as an insecticide on over 400 sites,

The use of these chemical compounds as prophylaxis against plant diseases is well documented including agriculture, professional turf management and ornamental production, and residential settings (U.S. Environmental Protection Agency (U.S. EPA). 1998). Carbamate pesticides Carbamate pesticides are used as substitutes for organophosphates. Various studies have been conducted on the activity of Maneb and Carbaryl in birds [9] fish [10], rat [11], and Bees [12], *Artemia salina* [13], but none has been conducted on pathology of carbamate in brine shrimp, *Artemia urmiana*. Histopathological investigations have proved to be a rapid and sensitive tool to detect direct effects of chemical compounds within target organs in laboratory experiments, however the histopathological effects of mane and carbaryl on *Artemia* have not been documented yet. For these reasons, present study was conducted to determine the histopathological effects of mane and carbaryl in different organs of *Artemia urmiana*.

MATERIALS AND METHODS

Selection of the Stage of the Larvae. A total of nine-hundred *Artemia urmiana* of both sexes, were used. One gram of disinfected *Artemia urmiana* cysts from the Lake Urmia in 1 liter of 45 millimicron-filtered autoclaved seawater 80% were incubated in Erlenmayer at pH 8.5 and 26°C in 2000 lux light and strong aeration. In order to obtain a population consisting of first instar larvae only, the hatched larvae appeared 14 hr. after the start of the incubation. Instar I larvae that sank to the bottom of the funnel were collected. One half of the populations was incubated at 26°C in an Erlenmayer was gently aerated seawater, after 24 hr. the larvae had molted into the instar II or even instar III stage. The larvae were then collected to carry out bioassays on *Artemia urmiana* larvae which were in the instar II stage. Similar procedure were used culture the Adult *Artemia* 1.67 gram of disinfected *Artemia urmiana* cysts in 2 liters of 45 millimicron filtered seawater 80% were incubated in an Erlenmayer at 28°C in 2000 lux light and strong aeration. Regular checks of the morphological stage of development showed that after several days of molting, 14 days after the larvae become Adult.

The live green-microalgae, *Dunaliella teriolecta*, encapsulated yeast, and soybean meal powder were used at 0.10 mg dry wt. food/*Artemia*/ml. seawater to feed to the *Artemia urmiana* from the onset of the feeding.

Preparation of the tests.

All experiments were carried out in triplicate with 10 larvae per glass beaker (diameter: 7cm; height: 8cm). The glasses were then filled with 10 ml. of the respective concentrations of toxicant in seawater. The amount of the poison needed contained Carbaryl (0.177 gram per liter ASW) and Maneb (0.125 gram per liter ASW). The purity percentage varies between 85 percent for carbaryl and 80 percent for Maneb. Ten-fold dilution of each sample was prepared using the calculated toxin dose. Then they were closed and placed in darkness in an incubation chamber (temperature of 26 ± 1°C) for the respective test periods. The ten treatment groups used for Carbaryl and ten treatment groups for Maneb were as follows: Group I: 100 ppm Carbaryl, Group II: 33 ppm Carbaryl, Group III: 10 ppm Carbaryl, Group IV: 3.30 ppm Carbaryl, Group V: 1.00 ppm Carbaryl, Group VI: 0.33 ppm Carbaryl, Group VII: 0.100 Carbaryl, Group VIII: 0.033

Carbaryl, Group IX: 0.10 ppm Carbaryl and Group X: non-treated group :Group I: 100 ppm Maneb, Group II: 33 ppm Maneb, Group III: 10 ppm Maneb, Group IV: 3.30 ppm Maneb, Group V: 1.00 ppm Maneb Group VI: 0.33 ppm Maneb, Group VII: 0.100 ppm Maneb Group VIII: 0.33 ppm Maneb, Group IX: 0.10 ppm Maneb and Group X: non-treated group. A triplicate experiment from each group was carried out for each concentration Maneb and Carbaryl pesticides. The contamination of Maneb and Carbaryl pesticides. The contamination treatment on instar I-II larvae and adult *Artemia urmiana* were continuously observed for 24 hr. and 48 hr. The percent mortality of the instar I II larvae, Adult *Artemia urmiana* exposed to the pesticides were recorded at 24 hr. and 48 hr. of exposure.

Histopathology of the instar I larvae II larvae, and Adult *Artemia urmiana*

The number of dead larvae and Adult *Artemia urmiana* were checked in each beaker. The larvae and Adult *Artemia* were considered dead when no moving of the appendage was observed within a few seconds. Representative samples from instar I-II larvae and Adult *Artemia urmiana* groups were fixed in 10 % buffered formalin, dehydrated in paraffin and sectioned with a microtome knife at 5 millimicron, stained with hematoxylin-eosin and examined microscopically to differentiate the nature of the lesions. Statistical Analysis: The mean and standards deviation of the different dose response based on mortality rate (24 h and 48 h.) were taken. Significance of data was evaluated using the Duncan, s Multiple test at $P < 0.05$.

RESULTS

Two experiments were conducted, in the first experiment after hatching of *Artemia urmiana* cysts into instars I-II larvae; light was used to attract the photo tactic larvae, so they could be collected separately into a pure sample (the first larvae appeared 14 hr after the start of the incubation, after 24 hr. the nauplii had molted into the instars II). The different concentration of Maneb and Carbaryl pesticides were prepared from each dilution. Experimental instar I-II larvae were contaminated with the different concentration. The mean dose response based mortality rate at 24 h and 48 h on instars I-II and Adult brine shrimp were computed

Maneb at 33 ppm concentration, the mortality rate of instar I larvae were discernable after 24 h. At 48 h. there was an increasing percent mortality, reaching 100% at 100 ppm concentration. While in the control treatment no mortality rate was recorded with instar I larvae at 33 ppm, 100 ppm concentration. On instar II larvae the mortality rate was manifested as early as 24 hr. At 33 ppm concentration, 100% mortality was seen on exposure after 48 h at 100 ppm concentration. While the control treatment no mortality rate was recorded with instar I larvae at 33 ppm, 100 ppm concentration. On instar II larvae, the mortality rate was manifested as early as 24 hr. at 33 ppm concentration, 100% mortality was seen on exposure after 48 hr. at 100 ppm concentration. While the control treatment no mortality rate after 24 h and 48 h on instars II larvae at 33 ppm and 10 ppm concentration. The concentration of 100 ppm caused 100% mortality among Adult *Artemia urmiana* after 24 h. While the control treatment no mortality after 24 h was recorded with Adult *Artemia urmiana* at 100 ppm concentration. With Carbaryl pesticides, the mortality rate on instar I larvae at 10 ppm concentration was detected, While the dose level containing 33 ppm-100 ppm concentration were capable of conferring 100% mortality rate 24 h after. At 48 h a slight increase in mortality rate were noted in dose levels ranging from 1 ppm, 3.3 ppm, 10 ppm, concentration treatment. However, a significant increase of 100% mortality rate observed at 33 ppm-100 ppm concentration.

Instar II larvae, results showed that a minimal increase of mortality rate at 0.33 ppm, 1 ppm, 3.3 ppm, 10 ppm, and a relative rise up to 100% mortality rate at 0.33 ppm-100 ppm concentration

24 hr. after. Forty-eight hour after from dose levels 0.1 ppm, 0.33 ppm, 1 ppm, and 3.3 ppm exhibited slight increased in mortality rate. However, an increase of 100% mortality rate at 10 ppm, 33 ppm-100 ppm was observed. Similar to instar I-II larvae, Adult *Artemia urmiana* showed a significant increase reaching up to 100% mortality at 100 ppm concentration within 24 h-48 hr. While in the control treatment no mortality rate was recorded with instar I-II and adult *Artemia urmiana* at 10 ppm, 33 ppm, and 100 ppm concentration. As shown in Table I, the results of the mean dose response based on mortality showed that with Maneb and Carbaryl at 33 ppm concentration were capable of conferring 100% mortality rate to instar I-II larvae while 100 ppm concentration conferred 100% mortality to Adult *Artemia urmiana*. The above results indicate that the dose response based on mortality rate will demonstrate the acute and chronic toxicity of Maneb and carbaryl pesticides on instar I-II larvae and Adult *Artemia urmiana*. The difference in the dose response based on mortality of the experimental instar I-II larvae and Adult *Artemia urmiana* is highlighted. Histopathology of the larvae and Adult *Artemia*. Figure 1. shows at 24 hours, histopathology of the instar I-II larvae (brine shrimp) treated with Maneb pesticides at 10 ppm concentration revealed the presence of erythrocytes and fibrin-like secretions between the muscle fibers. Figure 2 shows the intestinal histology of the instar I-II larvae treated with Carbaryl at 10 ppm concentration showed cell infiltration in the gastric intestinal lumen and increased cellularity of the lamina propria and the mucosa.

Figure 3 and 4. shows the ovaries of the instar I-II larvae treated with Maneb pesticides at 10 ppm concentration showed degenerative changes, nurse cells were either absent or necrotic. Zenkers necrosis in the skeletal muscles was also observed. Figure 5. shows the histology of the protocerebrum from the 14 day old *Artemia urmiana* treated with Carbaryl pesticides at 10 ppm concentration on revealed denuded globuli cells and secretory cells formation of vacuoles within the nervous tissue, severe necrosis and, degeneration in the cone cells and the retina of the compound eyes. No work comparing the effects of carbamate pesticides on instar I-II larvae and Adult *Artemia urmiana* has been done before. The results proved that toxic effect of Maneb pesticides was more profound in the instar I-II larvae while Carbaryl pesticides was generally more lethal than Maneb pesticides in the instar I-II larvae and adult *Artemia urmiana*.

Table 1. Effects of carbaryl in *Artemia Urmiana* at the beginning of phase II (Mean±SEM)

time	Concentration(p.p.m)									
	100	33	10	3.3	1	0.33	0.1	0.033	0.01	0
0.5h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12h	1±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
24h	10±0	10±0	1.75±0.29	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2d	10±0	10±0	9.25±0.29	3.75±0.29	0.75±0.29	0±0	0±0	0±0	0±0	0±0
3d	10±0	10±0	10±0	8±0.47	1.75±0.29	1±0	0.75±0.29	0±0	0±0	0±0
4d	10±0	10±0	10±0	10±0	3.25±0.29	2.25±0.29	1.75±0.29	1.25±0.29	0±0	0.25±0
5d	10±0	10±0	10±0	10±0	3.75±0.55	3.00±0.47	2±0	1.5±0.58	0.5±0.33	0.25±0
6h	10±0	10±0	10±0	10±0	4.25±0.29	3.25±0.55	2.25±0.29	1.75±0.55	0.75±0.29	0.25±0
7h	10±0	10±0	10±0	10±0	4.25±0.29	3.25±0.55	2.25±0.29	1.75±0.55	0.75±0.29	0.25±0

Table 2. Effects of carbaryl in Artemia Urmiana at the beginning of phase2(Mean±SEM)

time	Concentration(p.p.m)									
	100	33	10	3.3	1	0.33	0.1	0.033	0.01	0
0.5h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6h	1.75±0.29	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12h	6.25±0.29	0.75±0.29	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
24h	10±0	10±0	1.75±0.29	0.75±0.29	0.5±0.33	0±0	0±0	0±0	0±0	0±0
2d	10±0	10±0	10±0	4.75±0.29	2.25±0.29	1.25±0.29	0.50±0.33	0±0	0±0	0±0
3d	10±0	10±0	10±0	10±0	5.75±0.29	2.75±0.29	1.75±0.29	0.75±0.29	0±0	0±0
4d	10±0	10±0	10±0	10±0	8.5±0.58	3.25±0.29	2.5±0.33	1.5±0.33	0.50±0.33	0±0
5d	10±0	10±0	10±0	10±0	9.75±0.29	3.5±0.58	2.75±0.29	2±0.47	1.25±0.29	0±0
6h	10±0	10±0	10±0	10±0	10±0	4±0.47	3.25±0.29	2.50±0.33	1.50±0.33	0±0
7h	10±0	10±0	10±0	10±0	10±0	4±0.47	3.25±0.29	2.50±0.33	1.50±0.33	0±0

Table 3. Effects of carbaryl in adult Artemia Urmiana (Mean±SEM)

time	Concentration(p.p.m)									
	100	33	10	3.3	1	0.33	0.1	0.033	0.01	0
0.5h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6h	5±0	3.5±0.33	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12h	6.25±0.29	4.25±0.29	1.75±0.29	1±0	0±0	0±0	0±0	0±0	0±0	0±0
24h	10±0	5.75±0.29	4±0.47	3±0.47	1±0.47	0.75±0.29	0±0	0±0	0±0	0±0
2d	10±0	8±0	6.75±0.55	5.25±0.73	2.5±0.33	2±0.47	0.5±0.33	0.25±0.29	0±0	0±0
3d	10±0	10±0	10±0	7±0.82	3.25±0.55	2.5±0.75	1.5±0.33	1±0	0.25±0.29	0±0
4d	10±0	10±0	10±0	10±0	4.25±0.73	3.5±0.33	2.25±0.55	1.5±0.33	1.25±0.29	0±0
5d	10±0	10±0	10±0	10±0	5.25±0.29	4.25±0.29	2.75±0.29	2.25±0.29	1.75±0.55	0±0
6h	10±0	10±0	10±0	10±0	5.75±0.29	5±0.47	3.5±0.33	2.75±0.55	2.25±0.29	0±0
7h	10±0	10±0	10±0	10±0	5.75±0.29	5±0.47	3.5±0.33	2.75±0.55	2.25±0.29	0±0

Table 4. Effects of Maneb in Artemia Urmiana at the beginning of phase I1(Mean±SEM)

time	Concentration(p.p.m)									
	100	33	10	3.3	1	0.33	0.1	0.033	0.01	0
0.5h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2h	1.5±0.33	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6h	6±0.67	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12h	10±0	0.75±0.29	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
24h	10±0	8±0.47	1±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2d	10±0	10±0	7±0.47	2.5±0.33	1.5±0.33	0.75±0.29	0.5±0.33	0±0	0±0	0±0
3d	10±0	10±0	9.75±0.29	4.5±0.33	2.75±0.29	2±0	1.5±0.33	0.75±0.29	0±0	0±0
4d	10±0	10±0	10±0	5.25±0.29	3.50±0.58	2.75±0.29	2±0.47	1.75±0.29	0.5±0.33	0±0
5d	10±0	10±0	10±0	5.75±0.29	4±0.47	3.25±0.29	2.5±0.33	2.25±0.29	1±0	0.25±0.29
6h	10±0	10±0	10±0	6.25±0.29	4.25±0.29	3.75±0.29	2.75±0.55	2.25±0.29	1.25±0.29	0.25±0.29
7h	10±0	10±0	10±0	6.25±0.29	4.25±0.29	3.75±0.29	2.75±0.55	2.25±0.29	1.25±0.29	0.25±0.29

Table 5. Effects of Maneb in Artemia Urmiana at the beginning of phase I2 (Mean±SEM)

time	Concentration(p.p.m)									
	100	33	10	3.3	1	0.33	0.1	0.033	0.01	0
0.5h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2h	1.5±0.33	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6h	6±0.67	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12h	10±0	0.75±0.29	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
24h	10±0	8±0.47	1±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2d	10±0	10±0	7±0.47	2.5±0.33	1.5±0.33	0.75±0.29	0.5±0.33	0±0	0±0	0±0
3d	10±0	10±0	9.75±0.29	4.5±0.33	2.75±0.29	2±0	1.5±0.33	0.75±0.29	0±0	0±0
4d	10±0	10±0	10±0	5.25±0.29	3.50±0.58	2.75±0.29	2±0.47	1.75±0.29	0.5±0.33	0±0
5d	10±0	10±0	10±0	5.75±0.29	4±0.47	3.25±0.29	2.5±0.33	2.25±0.29	1±0	0.25±0.29
6h	10±0	10±0	10±0	6.25±0.29	4.25±0.29	3.75±0.29	2.75±0.55	2.25±0.29	1.25±0.29	0.25±0.29
7h	10±0	10±0	10±0	6.25±0.29	4.25±0.29	3.75±0.29	2.75±0.55	2.25±0.29	1.25±0.29	0.25±0.29

Table 6. Effects of Maneb in adult Artemia Urmiana (Mean±SEM)

time	Concentration(p.p.m)									
	100	33	10	3.3	1	0.33	0.1	0.033	0.01	0
0.5h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6h	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12h	10±0	2±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
24h	10±0	6±0	0.25±0.29	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2d	10±0	8.25±0.29	1.75±0.29	0.75±0.55	0.5±0.33	0.5±0.58	0±0	0±0	0±0	0±0
3d	10±0	9.25±0.55	4.25±0.29	2.75±0.99	1.75±0.73	1.25±0.29	0.75±0.55	0.5±0.58	0±0	0±0
4d	10±0	10±0	6±0	4.75±0.87	3.5±0.33	2.25±0.55	1.75±0.29	1.5±0.33	1±0.47	0±0
5d	10±0	10±0	7.25±0.29	6.25±0.73	4.5±0.33	3±0.47	2.25±0.29	1.75±0.29	1.25±0.55	0±0
6h	10±0	10±0	8.5±0.33	7.25±0.55	5.25±0.29	3.75±0.55	2.75±0.29	2.25±0.29	1.75±0.29	0±0
7h	10±0	10±0	8.5±0.33	7.25±0.55	5.25±0.29	3.75±0.55	2.75±0.29	2.25±0.29	1.75±0.29	0±0

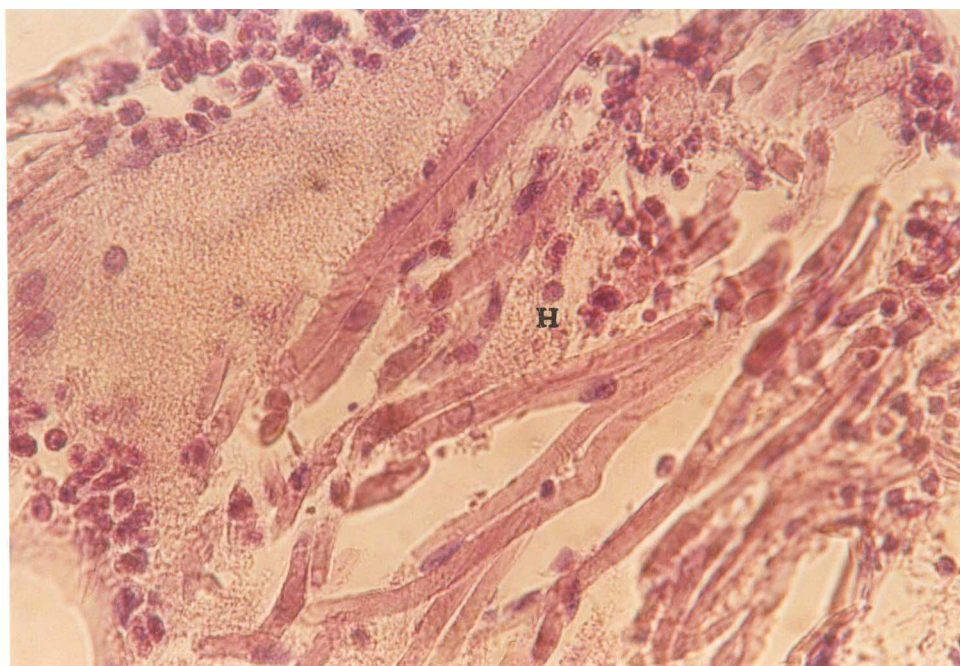


Figure. 1. RBC and fibrin like exudates between muscles. H-10 ppm concentration of Maneb (H&E*400)

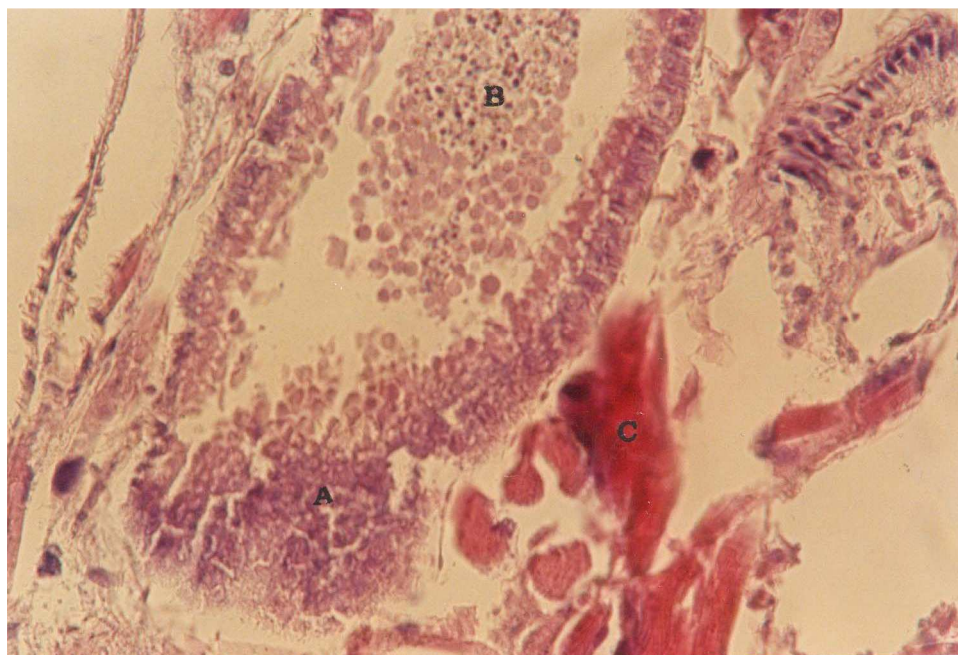


Figure 2. A hyperplasia in epithelial cells of gastric tract. B-infiltration of inflammation cells in gastric tract. c- Hyaline Degeneration and necrosis in muscles. - 1ppm concentration of carbaril (H&E*250)

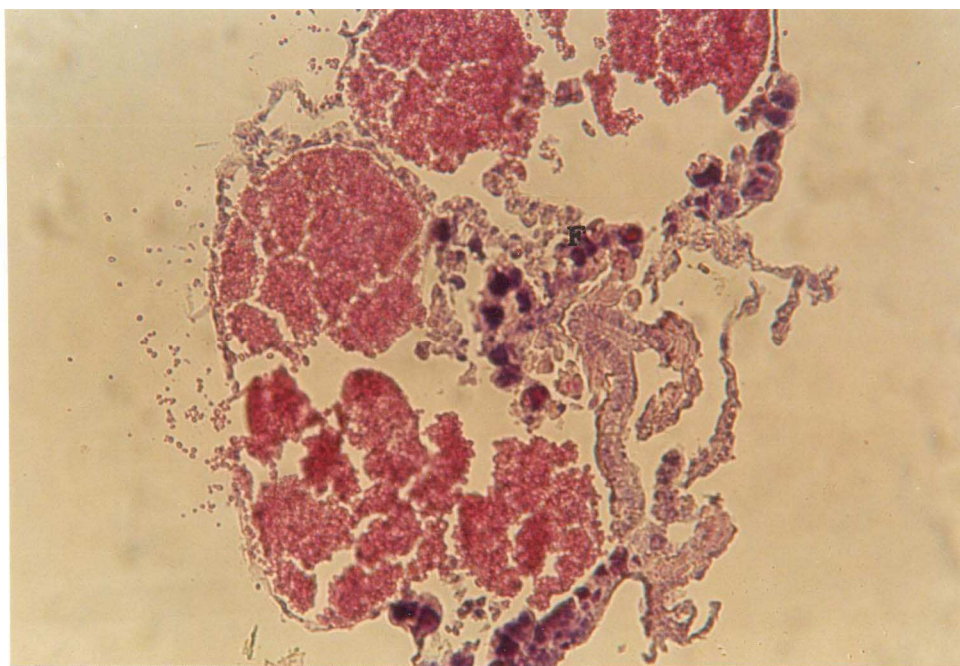


Figure3.Necrosis in ovarian Nurse Cells. F- 10ppm concentration of Maneb(H&E*250)

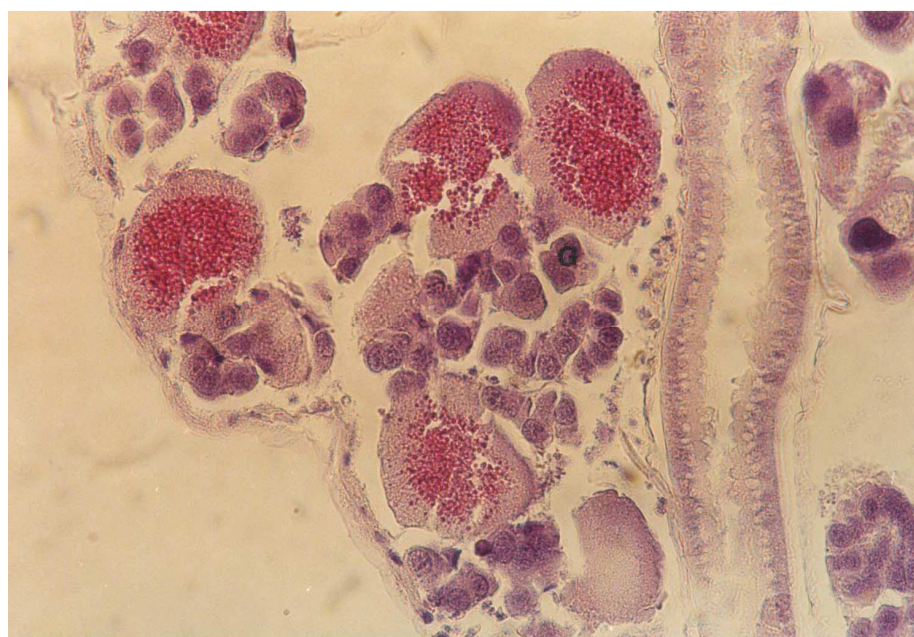


Figure4.Necrosis in ovarian Nurse Cells. G- 10ppm concentration of Maneb(H&E*250)



Figure 5. Vacuol formation in neurotic masses. I-decrease in globular and exudate cells. J- Fluid gathering and edema between neurotic masses and norolema. K- 1ppm concentration of Carbaril (H&E*250)

DISCUSSION

Acute toxicity data are abundant for carbamate insecticides with aquatic organisms [10,14] and our findings were confirmed by previous studies [4]. Another study by Vanhaecke, et al. [5] confirmed that Adult *Artemia* are very well suited for long term chronic bioassays. However, little information is available from histopathology conducted on *Artemia urmiana*. The exposure of aquatic organisms to sublethal concentration of pesticides in their environment may result in various biochemical, physiological, and histological alterations in vital tissues [15]. In the present study, histological lesions were observed in digestive, nervous, reproductive and musculoskeletal system exposed to carbaryl and maneb. Maneb exposed *Artemia* had less severe lesions in their organs than that of carbaryl exposed *Artemia*. In the Correlation of carbaryl and maneb concentrations and histopathological changes were weak in *Artemia urmiana* exposed to sublethal concentrations of these insecticides. The toxicity of the anticholinesterase (organophosphorous and carbamate) insecticides are due primarily to the inhibition or inactivation of acetylcholinesterase (AChE). The consequence of this enzyme inhibition is the accumulation of acetylcholine (ACh) at effector sites. The protection against acute poisoning offered by atropine and other cholinergic blocking agents supports this mechanism [13]. Additionally, induced reversal of cholinesterase inhibition by chemical compounds, such as oxime, derivatives, results in alleviation of symptoms of organophosphorous poisoning. Generally, the most effective treatment for organophosphorous intoxication is a combination of pharmacologic (atropine) and biochemical antidotes (oximes) [16].

Previous studies with *Artemia* larvae have shown that sensitivity to chemicals differed with age. Usually, older brine shrimp are more sensitive than younger ones. With *A. salina* larvae 72-h appears to be one of the more sensitive ages [17]. There is no information about the pathological study of the affect of pesticides on *Artemia*. In the present study, the significantly greater sensitivity of the different organs of *Artemia* at 72-h of age was observed for carbaryl, These results suggest that this age is a better indicator of carbamate insecticide potency than either the

24- or 48-h age organisms. The influence of age of *A. urmiana* on the toxicity of different chemicals may be related to the fact that larval development is rapid. Other authors have studied the relative sensitivity to several contaminants of different stages of the life cycle in aquatic organisms. Sleet and Brendel [18] reported that with cadmium and mercury LC50s decreased as *A. salina* nauplii aged and developed; whereas with azide LC50s did not vary. Puente et al. [19] showed that *Artemia* nauplii were very susceptible to low concentrations of chlorine dioxide, but the adults were slightly more resistant. Pickering et al.[20] showed that the sensitivity of 1-, 4-, and 7-day old fathead minnow, *Pimephales promelas* larvae to carbaryl was similar

CONCLUSION

There is an increasing concern about the adverse effect of the toxic action of some local agricultural pesticides such as Maneb (Dithane-M22) and Carbaryl (Sevin) in to the ecosystems of the lake Urmia, toxicological tests on the instar I-II and adult *Artemia urmiana* was undertaken not only to prevent economic losses but also to know the right concentration as a reference in brine shrimp *Artemia urmiana*. In summary, the acute toxicity of Maneb (Dithane-M22) and Carbaryl (Sevin) was influenced by age of test organisms. There was an increase in toxicity of the two carbamates following longer development of *A. urmiana*. Results of this study showed the potential of Carbaryl pesticide to cause serious damage of the nervous system of the Adult *Artemia urmiana*. On the other hand, Maneb pesticides was primarily highly toxic on instar I-II larvae.

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