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Effects of Chemical Herbicides and Insecticides on Mortality and Feeding of Silver Carp (*Hypophthalmichthys molitrix*)

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Abstract: Fingerlings of Silver Carp (*Hypophthalmichthys molitrix*) were used in an ecotoxicological investigation. The fish were exposed to (0.3-10 mg/l) Machete, (3-5 mg/l) Saturn, (1.5-13 mg/l) Diazinon and (1-10 mg/l) Malathion to measure the toxicity effect of these chemicals on mortality, feeding and ingestion rate. Methods for investigation on mortality were according to TRC (1984) and feeding behavior, according to Gould Equation (1951). Probit analysis was used for evaluation of obtained data. LC_{10} of these toxic compounds were 0.17-0.23, 2.85-3.73, 0.6-0.95, 0.87-1.34 mg/l for Machete, Saturn, Diazinon and Malathion respectively. Experiments showed that Machete and Diazinon in comparison with Saturn and Malathion were highly toxic for fingerlings of silver carp. At first fish showed to be disturbed, then moved rapidly and exhausted. A secretion of excess mucus was also noticed and eye-ball became expanded. There was also blanching of the skin. The fish surfaced sometimes on their side and became unbalanced which resulted in death. Abnormal behavior of fish became greater with the increase of pesticides concentration. In these exposures pH was decreased, in contrast with the amount of water hardness. LC_{10}, LC_{50} and LC_{90} from 24 to 96 hours were mostly decreased. Feeding experiments showed that 8 hours exposure below the LC_{50} values reduced 50 percent of feeding and ingestion rates.

KEYWORDS: Herbicide, Insecticide, Silver carp, Feeding, Mortality

Introduction

Fish culture in ponds can contribute to protein supply considering the recent demograhic population growth trend, but untreated agriculture wastes and its run-off from rice field and other agriculture activities in inland waters and rivers irrigation canal cause sometimes fish mortality and a decline in fish production. Therefore, it seems necessary to develoep a code of conduct in use for agricultural

chemicals and establishing of an enforcement law to control the application for chemicals in farms.

Recently, fish has been the most extensively used test organism in fershwater aquatic toxicology. Marty (1986) in his book "Toxicity of pesticides to fish" reviewed fundamentaly the effects of chemical toxicity on fish communities. Albaster (1969), Magne *et al.* (1994), Holden (1973), Johnson and Fimley (1980) studied the effects of pesticides and herbicides on fish and aquatic invertebrates.

Fish mortality have occurred in rivers, streams and fish ponds due to toxic chemical compounds used in agriculture. For example in Guilan province which is located at the north of Iran, recently aquaculture of Chinese carps have been developed widely. In this region, application of herbicides and pesticides in rice fields (230,000 ha) is regarded as a necessity of production. The rice field's water usually drain in rivers and streams, and may sometimes influence the fish culture water supply in the area. Aquatic toxicological study is regarded as a new research method for environmental investigations in the region. This study was carried out to examine the effects of herbicides and pecticides on silver carp fingerlings.

Materials and Methods

The pesticides such as Malathion, Diazinon and herbicides like Machete and Saturn were used for ecotoxicological studies, because these highly toxic chemicals are mostly applied in rice fields in the region. Silver carp fingerlings with 3-5 gr weight were brought from hacthery to the Guilan Fisheries Research Centre (GFRC) and kept in nursery fiber glass tanks. Tanks were filled with tap water and left for 24 hours for de-chlorination, then the young fish were placed in tank. After seven days, when the young fish accustomed, the tests initiated. Each replicate aquarium were filled with 20 liters of tap water and five fingerlings fish were released in each aquarium.

Toxicant in various concentrations were dissolved in distill water. Prior to definitive toxicity tests, range finding test were preformed to establish the range concentrations to be used. During the tests, physical and chemical parameters of water such as temperature, dissolved oxygen, pH, hardness and EC (electric conductivity) were measured daily. The aquaria were also aerated with aquaria pump. At the end of the day fish mortality and symptoms of death which were imposed by toxicants were recorded. This toxicity test was conducted according to the method described by TRC (1984). LC_{50} values of 96 hours were calculated

with a computer program using Probit analysis (Finney , 1971). LC_{10} , LC_{50} and LC_{90} were calculated for 24, 48, 72 and 96 hours. Machete (0.3-1 mg/l), Saturn (3-5 mg/l), Diazinon (1.5-13 mg/l) and Malathion (1-10 mg/l) were used to measure the toxicity of these chemicals on mortality, feeding and ingestion rate of silver carp fingerlings. In the nursery tanks fish were supplied with live food (algae) but during the test feeding was stopped.

Four LC_{50} concentrations were used for 3-5 gr silver carp fingerlings. The fish were exposed to 0.11, 0.23, 0.35 and 0.47 mg/l Machete and 0.72, 1.43, 2.12, 2.87 mg/l Diazinon with three replicates in each treatment. The algae *Anabaena flos aquae* were given to the fish based on 2% of the body weight. All aquaria were covered with a black curtain to hamper the algae growth. Duration of the tests were 8 hours. The number of algae in aquarium counted by an inverted microscope, both before the test and after its accomplishment. Filtration rate is defined as volume swept clear per unit of time. Ingestion rate is a straight forward unit referring to the biomass of number of cells consumed by a fish in an interval of time. For the calculation of average filtration rate (F: µl/g/h) and ingestion rate (I: cell/g/h), the equations used were as follows (Gauld , 1951):

$F = v/n. (Ln\ c0 - Ln\ ct)/t - A$

$A = (Ln\ c0 - Ln\ ct)/t$

$I = F.\sqrt{c0 . ct}$

where c0 and ct are initial and final algae concentration (cell/l), "t" is time (duration of experiment in hours), and "n" is the number of fish (ind) in volume (l), "A" is correction factor for changes in the control with final concentration of ct after time "t". The expression $\sqrt{c0.ct}$ expresses the geometric mean of algae concentration during time "t".The obtained data were evaluated by Probit analysis. LC_{10} , LC_{50} and LC_{90} were calculated and chosen for the feeding study, filtration and ingestion rates were used as the measures of feeding behavior.

Results

The effects of Machete, Saturn, Diazion and Malathion on mortality rate of silver carp fingerlings have been studied in 4 days toxicity tests with three replicates. Abnormal behavior of fish was increased with higher concentration of toxic chemicals. Mortality occured on fourth day of the test. Therefore the results of this study could emphasize that the process before 4 days, do not produce reasonable results. The experiments also showed that Machete and Diazinon in

comparsion with Saturn and Malathion were more toxic for silver carp fingerlings. As soon as the fish were exposed to the toxicant, rapid movement of fish was observed, but later they were exhausted. Mortality was occurred in high concentration of the toxic materials. The first mortality happened between 3 - 9 hours of the tests. Excess mucus secretion was also noticed and eye-balls became expanded. There was also blanching of the skin. The fish were going to the surface, sometimes on their side, and became unbalanced which resulted in death. Variations in water temperature may increas or decrease the effect of toxicants on the mortality of fish, but during the experiments temperature remained stable on 25 $\pm$ 2°C. Disolved oxygen was 80-100%, and pH reduced significantly from 7.57 to 6.1. It is likely that the reduction of pH might have an effect on fish mortality. Further studies would clarify the pH status on toxicants effect on the fish. In contrast with the decrease of pH, water hardness increased from 326 mg/l to 658 mg/l.

Obtained results concerning the total allowed concentrations is summarized in table 1 and fig. 1-4. In feeding experiments, the influence of Diazinon and Machete on filteration and ingestion rates are provided in table 2. Filteration rates for the two chemicals were 160.9 and 180.27 ml/gr/hr respectively.

Ingestion rate were 4×10^4 and 466×10^4 cell/gr/h respectively (Fig. 5-8). Feeding experiments showed that levels below the LC_{50} values are enough in 8 hours exposure to reduce filtration and ingestion rates to 50 percent.

Table 1: The impact of chemicals (mg/l) on silver carp fingerlings

Chemicals	LC_{10} mg/l				LC_{50} mg/l				LC_{90} mg/l			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Machete	0.28	0.19	0.18	0.24	0.63	0.49	0.41	0.37	1.51	1.29	0.92	0.60
Saturn	2.92	3.73	2.84	2.85	4.29	3.95	3.47	3.59	6.28	5.7	4.22	4.23
Diazinon	0.95	0.74	0.60	0.60	4.03	3.26	2.30	1.90	17.1	14.3	8.84	4/05
Malathion	1.32	1.02	0.87	0.87	6.74	5.64	3.71	2.90	34.1	31.0	15.7	6.25

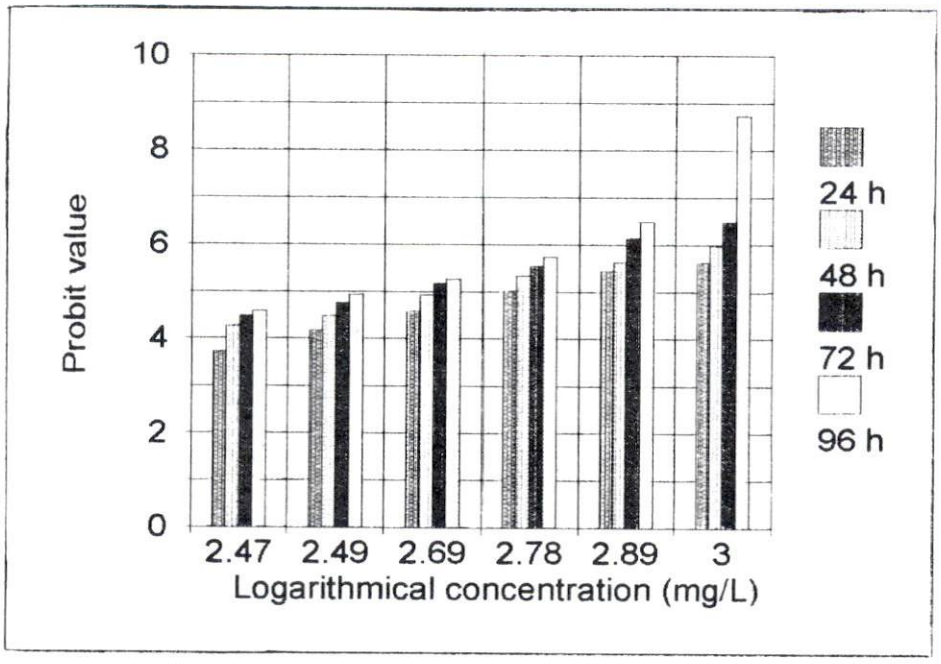

Fig.1: The effect of Machete on mortality of silver carp fingerlings

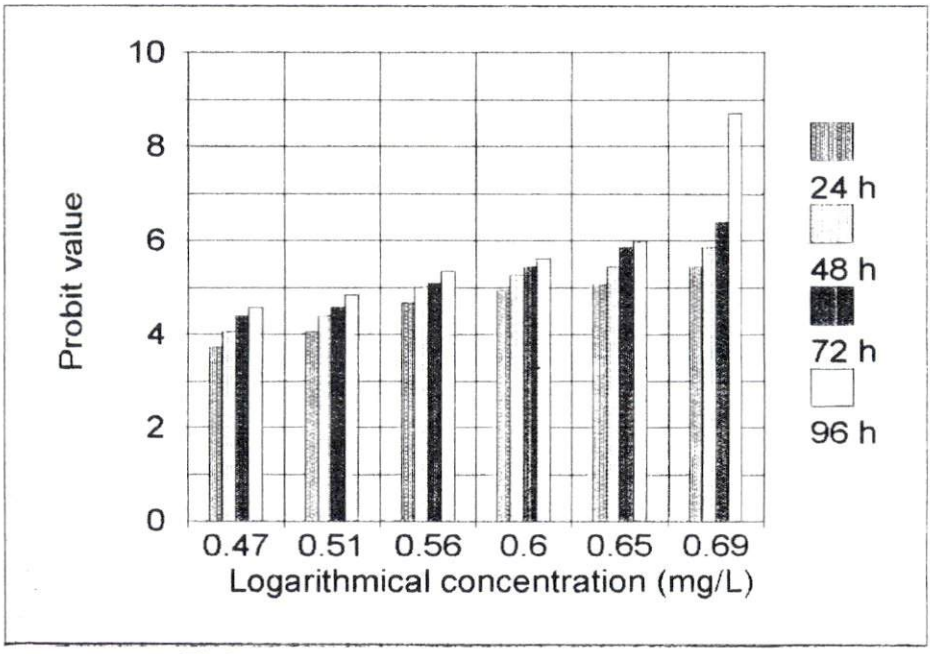

Fig.2: The effect of Saturn on mortality of silver carp fingerlings

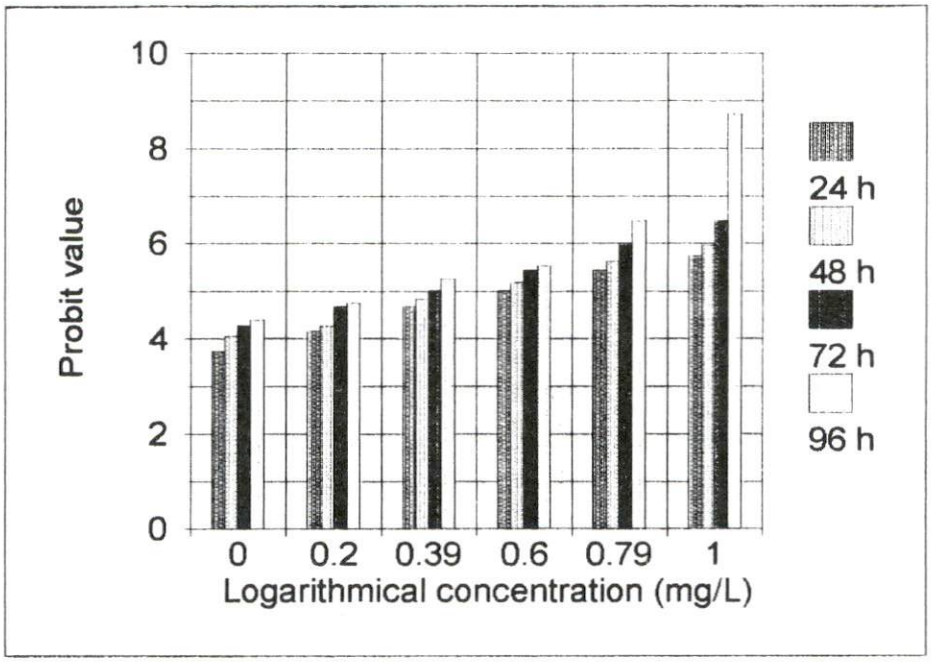

Fig.3: The effect of Diazinon on mortality of silver carp fingerlings

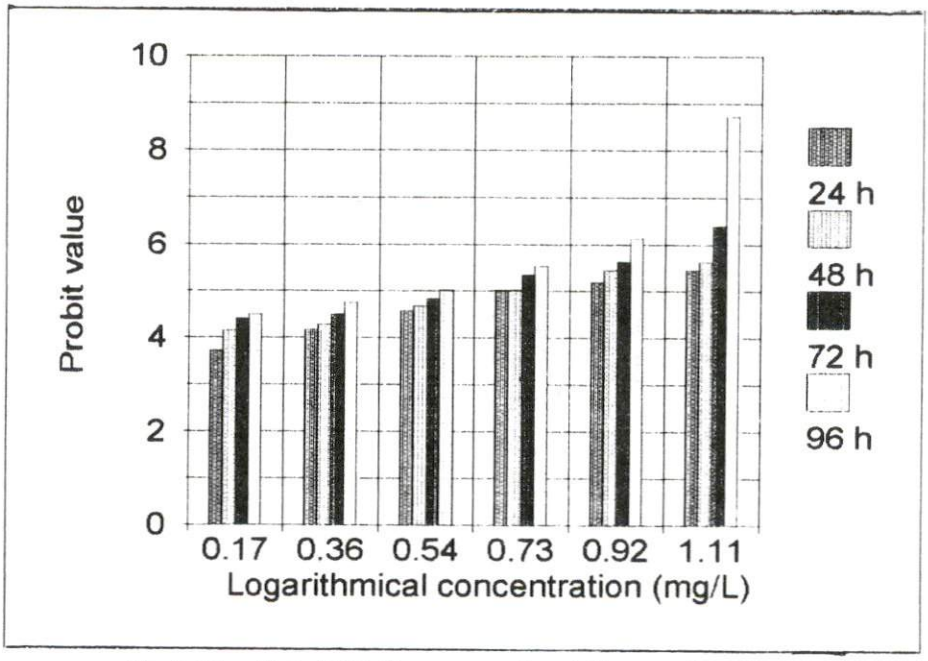

Fig.4: The effect of Malathion on mortality of silver carp fingerlings

Table 2: Examining the effect of Diazinon and Machete on the nutrition condition of silver carp fingerlings

Treatments	Chemical Concentration mg/l	Filtration rate µl/gr/h	Ingestion rate cell/gr/h
Machete	0.00	160922	40071959
	0.12	105113	27579239
	0.24	58560	15291762
	0.36	69211	18861912
	1.44	35625	9659817
Diazinon	0.00	180374	26696104
	0.72	126436	32746253
	1.44	79130	31285347
	2.15	50757	13932617
	2.87	42043	11626656

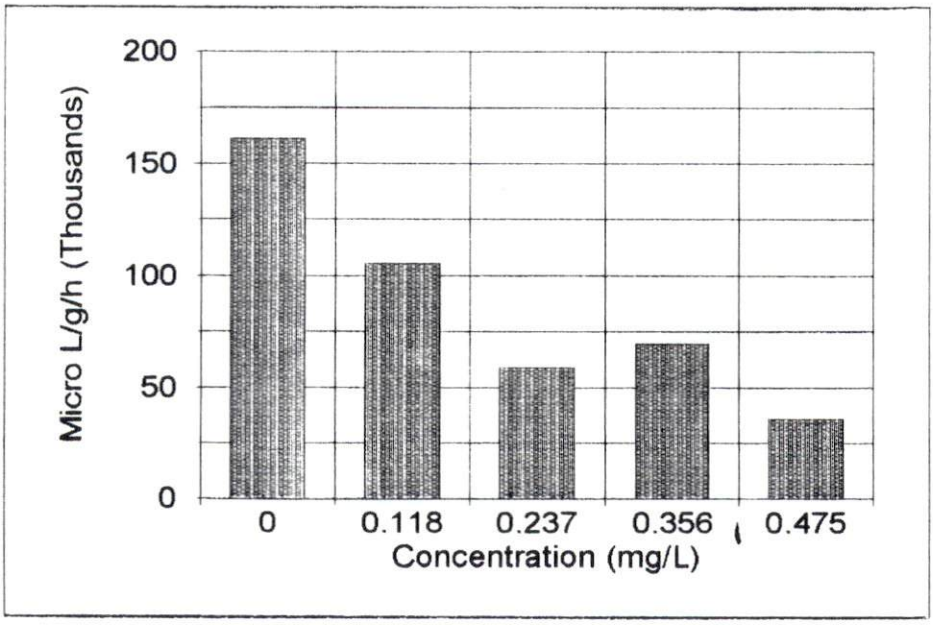

Fig.5: The effect of Machete on filtration rate of silver carp fingerlings

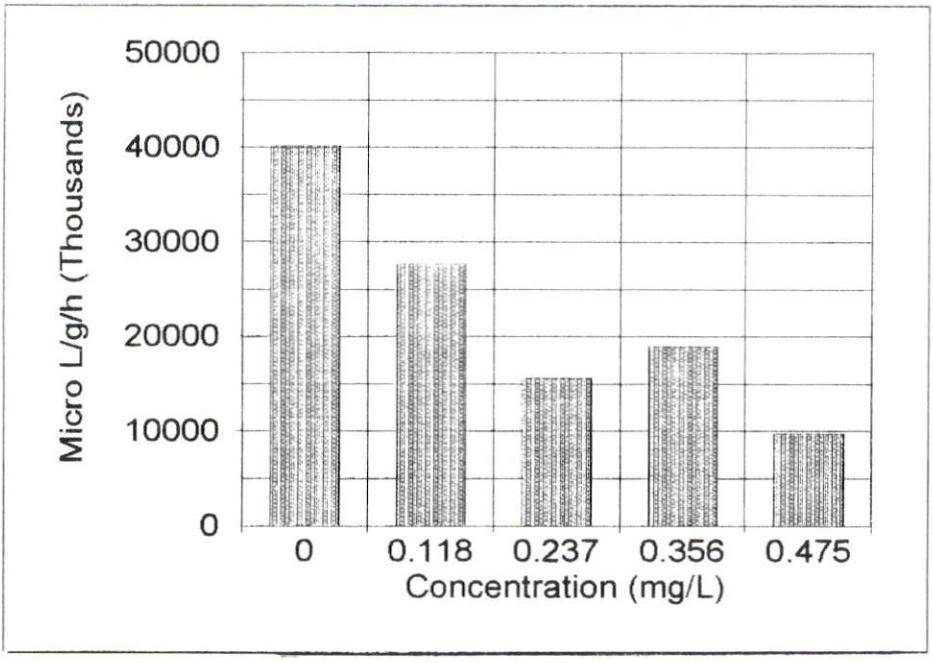

Fig.6: The effect of Machete on ingestion rate of silver carp fingerlings

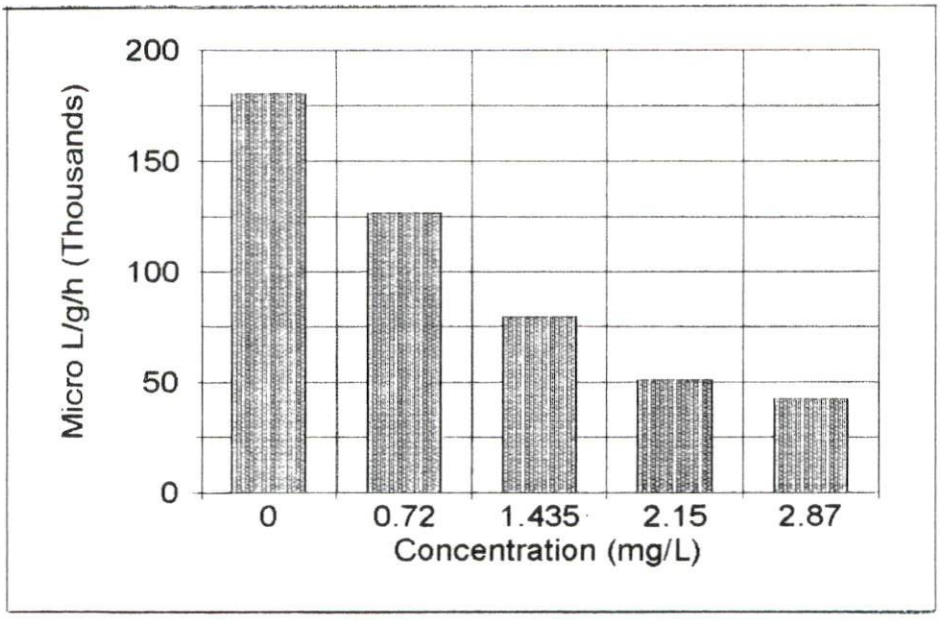

Fig.7: The effect of Diazinon on filtration rate of silver carp fingerlings

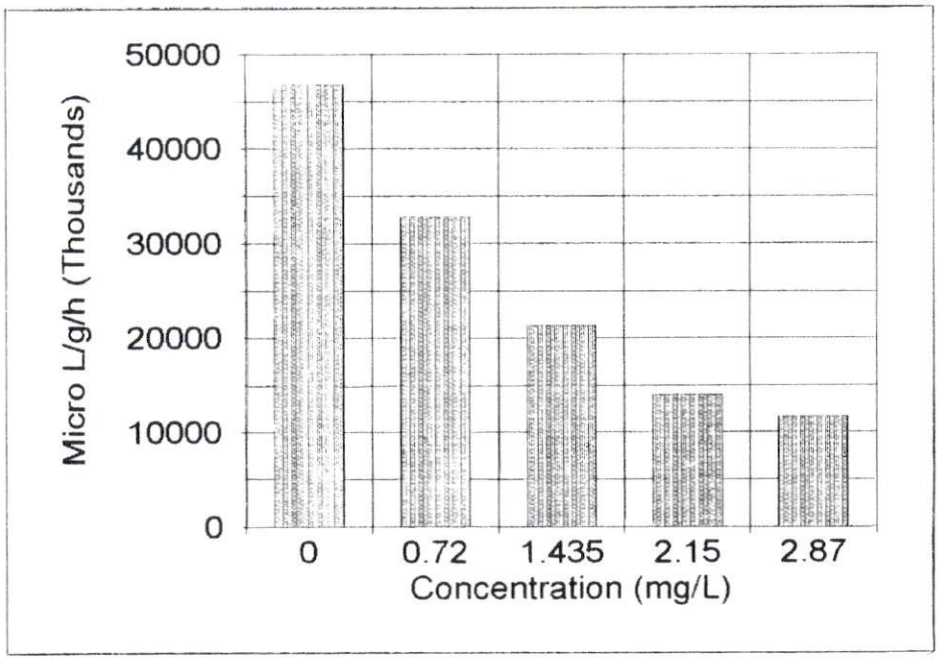

Fig.8: The effect of Diazinon on ingestion rate of silver carp fingerlings

Discussion

Considering the content of Wasserschadstoff-katalog (1973) and Pesticide Dictionary (1994), the present bioassay tests procedures are designed in a standared method

Holocombe *et al.*, (1982) and Lingaraja *et al.*, (1979) who recorded behavior of fish in toxicity test, expressed similar symptoms as was observed in the present experiments. Secretion of exess mucus, eye-balls expansion, blanching of the skin, unbalanced swimming and eventual death in acute toxicity condition, reported by Thamse and Gacutan (1994) and Rabia and Sharma (1990). The water colour in aquarium was quite milky. These could be caused by mucus residues and the effects of chemical decomposition process (Francisco *et al.*, 1994).

Regarding to filtration and ingestion rates in the tests, Spilttler (1981) reported a mean filtration rate of 241 ± 139 ml/gr/h and Antalfi and Tolg (1971) reported 128 ml/gr/h for silver carp. It is distinguished that exposing four concentrations (1/4, 1/2, 2/3 LC_{50}) of Diazinon and Machete cause a decrease in filteration and ingestion rates. In invertebrates, It is unknown which biochemical and

physiological process are affected by pesticide toxication, but the main effect of these compounds may be on the nervous system (Ware , 1983).

At first rapid movement of fish and later exhaustion and resting on the bottom of the aquaria indicated presence of high concentration of toxicants. This resulted also in a decrease of filtration and ingestion rates and finally death of the fish.

The reduction in pH might be caused by decomposition of chemical materials in aquarium. In an extreme case in the experiments pH was decreased to 5 and hardness increased from 76.8 to 710.4 mg/l. LC_{10} , LC_{50} and LC_{90} from 24 to 96 hours were mostly decreased. This means that the tests below 96 hours do not produce adequate data to assess the toxicity.

Total allowed concentration of chemical materials for fish, as a result of mortality and feeding test, were higher than those of algae and zooplankton. This means that in such concentrations the growth of algae do not stop but in contrast the death of zooplankton increase. Therefore chemical materials with such concentrations leaking to the aquatic ecosystems could contribute to plankton blooms.

The agriculture research centres with the aim of promotion of applied that research in the reduction of toxic chemical used in agriculture activities could mitigate hazardous chemical impact and substitute elements more environmentaly friend.

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