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AQUATIC BIOASSAY OF CERTAIN PESTICIDES USING DIFFERENT AQUATIC ORGANISMS

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ABSTRACT: Seven aquatic organisms namely; mosquitoes (three larval instars), two daphnid species, snails (two ages), aquarium fishes (three species), frogs (three instars) and tilapia fish (one age) were used as biomarkers to detect water contamination with pesticides. Three pesticides, belong to different groups of chemicals, were used, *viz.* the carbamate insecticide methomyl (Lannate SP 90%), the organophosphorus fungicide edifenophos (Hinosan EC 50%), and the acetanilide herbicide butachlor (Machete EC 60%).

The obtained results show that there was a wide range in sensitivity to the bioactivity of the three tested pesticides. Species, stage, instar, and age specificity took place in this respect. The two daphnid species water flea *(Daphnia magna and Moina australiensi)* revealed the highest sensitivity to the insecticide methomyl and the OP fungicide edifenophos (LC_{50} values ranged between 0.001 – 0.34 ppm). On the other hand, the aquarium fish species (molly fish *Poecilia sphenops,* sword fish *Poecilia latipinna and* gupy fish *Xinophorous hallen)* and *M.australiehsi* exhibited the highest sensitivity to the herbicide butachlor (LC_{50} values ranged between 0.24 – 0.91 ppm).

Key words: Hinosan, machete, lannate, fishes, mosquito larvae, water snails, frogs, water flea.

INTRODUCTION

In the last four decades the industrial pollutants and human activities increased many environmental problems arising from the release of toxic contaminates in the aquatic environments (APHA, 1985).

Toxicity tests represent an important methodological approach

to the identification, characterization and assessment of chemicals. Aquatic organisms have been used extensively as biomarkers for water pollutants.

The major aim of aquatic bioassay is the investigation of minor deleterious effect, caused by ware pollutants, against the aquatic beneficial organisms. The advantages of this system include simplicity, economic of space and time, tolerance to uncontaminated water from a wide variety of sources, capability of testing small samples of water in order to reduce problems of transportation, and capability of detecting toxicants at levels at or below the LC_{50} for common species of beneficial aquatic organisms (Strickman, 1985).

The present work aimed to detect the sensitivity of seven aquatic organisms, belong to five classes, namely mosquitoes, daphnids, frogs, water snails, and fishes to three pesticides (the insecticide methomyl, the herbicide butachlor and the fungicide edifenophos) belong to three chemical groups. These pesticides are widely used as pest control agents.

MATERIALS AND METHODS

1. Pesticides

The carbamate insecticide methomyl (Lannate SP 90%) [S.methyl, N-(methylcarbamoyloxy) thioacetimidate], the acetanilide herbicide butachlor (Machete 60% EC) [N-butoxymethyl -2-chloro–2, 6-diethyl acetanilide], and the organ ophosphorus fungicide edifenophos (Hinosan 50% EC) [0-ethyl S, S-diphenylphosphorodithiate] were used.

2. Test organisms

Seven organisms were used *viz*

A. Invertebrate organisms

2.1. The first, second, and fourth instars larvae of *Culex pipiens*

2.2. The neonate and adult stages of *Daphnia magna*

2.3. The adult stage of *Moina australiensi*

2.4. The juvenile and adult stages of the molluscan species (snails) *Biomphloria alexandrina*

B. Vertebrate organisms

2.5. Seven-day old fingers of aquarium fishes molly fish, *Poecilia sphenops*, sword fish, *Poecilia latipinna* and gupy fish, *Xinophorous hallen*.

2.6. Four-dayold fingers of the Nile fish *Oeochromis (Tilapia) niloticus*

2.7. The first, third and fiftieth larval instars of the Egyptian frog *Bufo regularis*

The preceding organisms were either laboratory colonies (mosquitoes, daphnids, snails, aquarium and tilapia fishes) reared under laboratory conditions for more than one year away from any contamination with pesticides or field population obtained from streams (frogs).

3. Bioassay

3.1. Acute toxicity

A series of dilution, at least six concentrations (mg a.i./L), were made using aged tap water. The stock solution of each pesticide was prepared daily. One hundred ml from each dilution was placed in a convenient container (250-glass beaker). Twenty five individuals of each test organism were transferred to each jar. With fishes and frog instars 10 individuals were used. At least 100 individuals were used with each concentration. The individuals were obtained either from the laboratory stock culture or stream. A parallel experiment was carried out using water only and was considered as a control. For test species the jars were kept at room temperature 25±2°C for different holding periods until mortality percentages were established. Observation during the different holding periods showed that 24h were quite enough for the three tested pesticides to manifest their toxicities. Data were analyzed by a a probit computer program (EAP probit analysis program) and by plotting mortality data on a probit graph paper.

RESULTS AND DISCUSSION

The toxicity of the acetanilide herbicide butachlor, the organophosphorus fungicide edifenophos and the carbamate insecticide methomyl to seven aquatic organisms *viz.*, mosquito larvae, 4 fish species, two species of daphnids, snails and frogs is compited in Tables 1, 2 and 3.

Toxicity of methomyl (Lannate SP 90%)

It is obvious that *D.magna* exhibited the highest sensitivity to methomyl at both levels of toxcicity. Both stages, i.e., the juvenile and adults behave similar, the LC_{50} for each stage was 0.001 ppm. Although *M.australiensi* and *D.magna* are categorized under the

Table 1: Sensitivity of some aquatic organisms to the insecticide methomyl (Lannate SP 90%)

Test organisms		LC_{50} (90% Conf. Lim.)*	LC_{90}	Slope
Mosquito	**1st instar larvae**	0.45 (2.21 – 2.68)	6.58 (5.60 – 8.21)	2.98
	2nd instar larvae	2.39 (2.13 – 2.66)	7.67 (6.26 – 10.27)	5.53
	4th instar larvae	2.88 (2.7 – 3.06)	5.9 (5.14 – 7.23)	4.08
Water flea	**Juvenile**	0.001 (0.01 – 0.001)	0.016 (0.010 – 0.028	1.12
	Adult	(0.02 0.001 (0.000 – 0.005	0.020 (0.004 – 0.060)	0.96
Monia	**Adult**	0.34 (0.014 – 0.078	0.7 (0.022 – 5.19)	0.97
Nile fish	**4-day – old**	1.25 (0.094 – 1.074	4.33 (3.11-5.55)	2.17
Aquarium fish	**Molly fish**	0.59 (0.58 – 0.61)	0.84 (0.78 – 093)	8.46
	Sword tail	0.45 (0.44 – 0.46)	0.62 (0.58 – 0.68)	9.14
	Gupy fish	0.47 (0.44 – 0.49	0.88 (0.79 – 1.05)	4.59
Frogs	**3rd instar lavae**	50.80 (49.23 – 52.28)	72.88 (69.27-77.97)	8.18
	50th instar larvae	3.02 (2.93 – 3.10)	4.24 (4.01-4.57	8.67

* All values are in mg/L (ppm).

Table 2: Sensitivity of some aquatic organisms to the fungicide edifenophos (Hinosan EC 50%)

Test organisms		LC_{50} (90% Conf. Lim.)*	LC_{90}	Slope
Mosquito	**1st instar larvae**	0.81 (0.66-0.99)	11.63 (7.22 – 23.26)	1.12
	2nd instar larvae	2.21 (1.82 – 2.79)	17.1 (10.85 – 33.38)	1.44
	4th instar larvae	0.41 (0.29 – 0.54)	10.45 (6.02 –25.00)	0.91
Water flea	**Juvenile**	0.003 (0.001-0.004)	0.56 (0.21 – 2.25)	0.54
	Adult	0.061 (0.0046-0.119)	0.98 (0.13 – 1.45)	0.72
Monia	**Adult**	0.028 (0.021 – 0.038)	0.83 (0.475 – 1.742)	0.87
Snails	**7 – day – old**	6.72 (6.42 – 7.03)	10.96 (9.98 – 12.65)	6.03
	30 – day - old	7.91 (7.62-8.21)	11.76 (10.90 – 13.17)	7.44
Aquarium fish	**Molly fish**	0.96 (0.82 – 1.15	5.92 (4.19 – 9.63)	1.62
	Swordtail	2.03 (1.63 – 2.79)	12.34 (7.23 – 30.04)	1.64
	Gupy fish	1.19 (1.05 – 1.39	4.12 (3.19 – 5.89	2.39
Nile fish	**4- day – old**	1.85 (1.13 – 2.31)	2.56 (2.11 – 7.54)	3.76
Frogs	**3rd instar lavae**	2.45 (2.28 – 2.62)	4.75 (4.17 – 5.12)	4.72
	50th instar larvae	4.61 (4.56 – 4.67	5.25 (5.12 – 5.44)	22.94

* All values are in mg/L (ppm).

Table 3: Sensitivity of some aquatic organisms to the herbicide butachlor (Machete EC 60%)

Test organisms		LC_{50} (90% Conf. Lim.)*	LC_{90}	Slope
Mosquito	**1st instar larvae**	10.07 (9.42 – 10.72)	21.9 (19.75 – 25.06)	3.79
	2nd instar larvae	13.35 (12.43 – 14.36)	30.4 (26.33 – 37.26)	3.58
	4th instar larvae	11.31 (10.63 – 12.00)	23.7 (21.34 – 27.20)	3.98
Water flea	**Juvenile**	1.98 (1.43 – 2.52)	7.71 (5.12 – 16.93)	2.13
	Adult	3.12 (2.92 – 3.33)	5.77 (5.17 – 8.36)	4.81
Monia	**Adult**	0.266 (0.24 – 0.28)	0.687 (0.601 – 0.817)	3.11
Snails	**7 – day – old**	1.73 (1.29 – 2.28)	2.77 (2.15 – 8.61)	6.82
	30 – day - old	3.86 (3.71 – 4.03)	5.73 (5.31 – 6.39	7.5
Aquarium fish	**Molly fish**	0.24 (0.19 – 0.29)	1.47 (1.11 – 2.25)	1.6
	Swordtail	0.91 (0.80 – 10.10)	5.85 (5.18 – 7.14)	1.67
	Gupy fish	0.34 (0.25 – 0.45)	7.68 (4.40 – 18.98	0.95
Nile fish	**4- day – old**	0.7 (0.33 – 0.095)	9.18 (6.22 – 18.26)	1.25
Frogs	**1st instar lavae**	0.25 (0.19 – 0.30)	1.92 (1.35 – 32.34)	1.45
	3rd instar larvae	0.39 (0.28 – 0.48)	1.4 (0.79 – 2.02)	2.03
	50th instar larvae	7.18 (6.98 – 7.39)	9.56 (9.11 – 10.20)	1.25

* All values are in mg/L (ppm).

term daphnids, the former species tolerated much higher amounts of methomyl (340 times). The sensitivity of the three species of aquarium fishes was almost similar to that of Moina; the LC_{50} ranged between 0.45 – 0.59 ppm. It is obvious that the Nile fish *O.niloticus* tolerated higher levels of methomyl (2 times) compared with the three aquarium fish species. The three larval instars of mosquito tolerated much higher concentrations compared with *D.magna* (2390 – 2890 times). There was no great differences between the three instars in this respect. Frogs revealed the highest tolerance to methomyl; LC_{50} values ranged between 3.02 to 50.80 ppm. It is not expected to find that the younger instar larvae (3^{rd} instar) was much more tolerant (16.8 times) than the older one (the 50^{th} instar). At both levels of toxicity the sensitivity of the preceding six organisms, could be descending arranged as follow: *D.magna* > *Moina SP* > aquarium fishes > Nile fish > mosquito larvae > frogs larvae.

Toxicity of edifenophos (Hinosan EC50%)

The picture with the organophosphorus fungicide edifenophos was, however, similar to that with the carbamate insecticide methomyl (Table 2). Data show that all the tested species revealed either slight tolerance or higher susceptibility depending on species and the tested instar or age of the used species.

It is obvious that, snails (7- and 30 – days – old) were the most tolerant organism; tolerance levels, compared with juvenile daphnids (the most susceptible species) ranged between 2240 – 2636 times. In contrary with methomyl, the wide range in the susceptibility of the two frog larval instars to methomyl disapeared with edifenophos; the LC_{50} values ranged between 3.02 – 50.8 and 2.45 – 4.61 ppm with methomyl and edifenophos, respectively. Also, moina adults which revealed high levels of tolerance to methomyl (340 times), compared with *D.magna* juveniles, showed, however, relative susceptibility to the action of edifenophos (93.3 times). The susceptibility of the other two organisms mosquito and fish as well as the tested ages of *D.magna* were, however, more or less similar in edifenophos treatment.

Toxicity of the acetanilide butachlor (Machete EC60%)

Data presented in Table 3 show that the seven used organisms did

behave differently to the action of the third tested pesticide butachlor compared with the two preceding pesticides. The three aquarium fish species, which occupied on intermediate position between the test organisms, as regard their sensitivity to methomyl and edifenophos were the superior organisms in this respect. The same phenomenon took place with frog larval instars which were the most tolerant species to the preceding two pesticides. *D.magna* which revealed the highest sensitivity to methomyl and edifenophos tolerated much higher levels of butachlor in comparison with the other five organisms. It is obvious that mosquito larvae were the least sensitive organisms.

In general, data presented in the three Tables show that species specificity and to less extent stage and instar specificities were obvious. The LC_{50} values ranged between 0.001-50.80, 0.003-4.61, and 0.24-7.18 ppm for methomyl, edifenophos and butachlor; respectively. The two crustacian invertebrates of daphnids, however, revealed the highest sensitivity to the used insecticide and fungicide, whereas aquarium fishes were the most sensitive to the herbicide butachlor. Slope values indicated the extremely heterogeneous nature of the used populations.

As mentioned before, the two daphnid species revealed the highest sensitivity to methomyl and edifenophos. Several investigators (e.g., Burton, 1991; ASTMD, 1993, Sanna, and Matti, 1995 and Sakai, 2001) referred to the same conclusion.

The relative susceptibility of the different larval instars of mosquito *C.pipiens*_to pesticides were studied by Molta *et al.* (1988), Kawakamiy (1989), Unat *et al.* (1994), Berchi (2000) and Mostafa and Allam (2001). Different susceptibility was found between the four instars.

The acute toxicity of some carbamates, organophosphates and herbicides, to fishes, frogs, daphnids and fresh water snail, was studied by several investigators (e.g., Wan *et al.*, 1990; De-Croux *et al.*, 1999; Arora *et al.*, 1986; Vismara *et al.*, 2000 and 2001; Sampath *et al.*, 2002 and Daniel *et al.*, 2003). The authors showed that there was a wide range in the susceptipility of the used organisms to the action of the three groups of chemicals.

In conclusion, further screening trials are needed to

establish categories of aquatic organisms had high sensitivity against specific group of chemical pollutants using earlier toxic symptoms with the aim to detect levels below that mentioned in this work.

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التقييم الحيوى المائى لعدة مبيدات آفات باستخدام كائنات مائية مختلفة

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