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ACUTE TOXICITY OF MALATHION, DICHLORVOS AND TEMEPHOS IN CLIMBING PERCH (*Anabas testudineus*)

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ABSTRACT

Malathion, dichlorvos and temephos are used globally to control a wide range of invertebrate pests especially in Malaysia. These pesticides usually enter aquatic environment by direct application or through overspray, runoff and watersheds. However, applications of pesticides may cause adverse impacts to many non-target organisms such as fish. The objective for this study was to determine the 96 hours lethal concentration (LC_{50}) of each pesticide in climbing perch, *Anabas testudineus*. A total of 130 *A. testudineus* was subjected to 13 aquariums. Fish were exposed to different concentrations of each pesticide for 96 hours. Fish were observed daily and dead fish were removed immediately. The 96h LC_{50} value for malathion, dichlorvos and temephos was determined as 0.25 mg/L, 2.35 mg/L and 25.0 mg/L respectively. The results obtained were based on the probit analysis method as described by Finney 1952. From the values obtained, malathion, dichlorvos and temephos can be classified as highly toxic pesticides since it can kill 50 percent of the population even in lower concentration. Thus, the information in this study can be used as a guide to help environmental management to assure the effective use of these pesticides and to prevent indiscriminate use of pesticides.

Key words: *Anabas testudineus,* malathion, dichlorvos, temephos, acute toxicity

INTRODUCTION

Widespread use of pesticide is now a worldwide phenomenon (Omitoyin *et al.*, 2006). Pesticides become readily available in the food chain and subsequent bioaccumulation in both aquatic and terrestrial flora and fauna, with possible unquantifiable disastrous consequences on the ecosystem (Odiete, 1999).

Pesticide is defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (UNEP, 2005). Pesticides are toxic and were designed to repel or kill unwanted organisms and when used for their different purposes they may be brought to water bodies killing or influencing the lives of aquatic organisms (El-Sayed *et al.*, 2007).

Therefore, due to indiscriminate use of pesticides, contamination levels in aquatic environments have greatly increased in recent years as a consequence of intense human activities, which in some areas have resulted in a substantial impact (van de Oost *et al.*, 2003). Due to the residual effects of pesticides, important organs like kidney, liver, gills, stomach, brain, muscle and genital organs are damaged in fish exposed to pesticide (Odiete, 1999).

It has been estimated that only about one percent of applied pesticides land on the target and that the rest contaminates the environment (Lawsom *et al.,* 2011). Organophosphates are the most widely used class of pesticides. Exposure of aquatic ecosystems to these pesticides is difficult to assess because of their short persistence in water column due to low solubility and rapid degradation. However monitoring of these insecticides is important, because they are highly toxic to aquatic organisms. Moreover, organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Even though, organophosphates degrade faster than the organochlorides, they have greater acute toxicity, which may cause poisoning risks to people who may be exposed to large amounts (Halappa & David, 2009).

Malathion is a pesticide that can cause serious metabolic disturbances to non-target organisms. It is an organophosphate pesticide that has been

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widely used as an alternative to carbamate pesticide (Svoboda *et al.*, 2001). While dichlorvos is an organophosphate compound used as an agricultural insecticide on crops, animals and stored products (EPA, 2007). At sub-lethal concentration, dichlorvos inhibited oxygen consumption by gill, brain and muscle tissues. Moreover, it also inhibits AChE activity (Rath & Misra, 1981). Next, temephos is a larvicide which is non-systemic organophosphates. The mode of action by inhibiting AChE then causes accumulation of acetylcholine (Ach) in neuromuscular synapses. Temephos is not persistent, thus it easily breaks down in water in a few days (Auib *et al*., 2002).

Fishes represent the largest and most diverse group of vertebrates. They are excellent experimental models for toxicological research. Fishes are important component of the food chain. So any effect of toxicant may have adverse influence on the nutritive value of fish and on human being through their consumption (Gupta & Srivastava, 2006).

Anabas testudineus, the climbing perch is a species of fish in the family of Anabantidae. It inhabits fresh waters mostly in rivers, canals, lakes, ponds, swamps and paddy fields. It can live in water with low in oxygen, polluted water and also water with rotting vegetation where the fish rises to surface and gulps for air. This is a very hardy fish, due to the presence of accessory respiratory organ and is of considerable fisheries interest. This species improves its marketability because it is important as a food fish due to its ability to survive out of water for extended periods of time (Pal & Chaudhry, 2010).

MATERIALS AND METHODS

Chemicals and raw materials

All the chemicals used were of analytical grade and purchased from Sigma-Aldrich Co (St Louis, Missouri) and Merck (Darmstadt, Germany). *A. testudineus* fish was supplied by Alam Akuarium, Shah Alam Malaysia.

Experimental fish species and acclimatization

Total of 130 *A. testudineus* with average size of 10-12 cm body length and average of 55-65 g weight were selected from unpolluted water body without any injury. The fishes were brought to laboratory and stocked in 13 different aquarium of size 45 cm x 30 cm x 30 cm. Each aquarium consisted of 10 fishes with 20 ml of dechlorinated tap water. The fishes were acclimatized in laboratory conditions with temperature of 27 to 29°C and pH of 7.4 to 7.8 for 15 days. Commercial fish food was given once a day at the beginning of 48 hours after acclimatization and stopped 48 hrs before the acute test. In addition, the water exchange was made at three day intervals with fresh test solutions in each experimental aquarium (EPA, 1996).

Experimental acute toxicity (LC_{50}) for 96 hours

The acute toxicity test (LC_{50}) at 96 hours was conducted. One control and 12 different concentrations of malathion, dichlorvos and temephos were prepared. The test concentrations used were 0.15 mg/L, 0.25 mg/L, 0.35 mg/L and 0.45 mg/L for malathion. Then, 0.5 mg/L, 1.5 mg/L, 2.5 mg/L and 3.5 mg/L for dichlorvos. Lastly for temephos, 5.0 mg/L, 15.0 mg/L, 25.0 mg/L and 35.0 mg/L test concentrations were used. The LC_{50} values for different pesticides were calculated for 96 hours of exposure time. Dead fishes were removed and mortality was recorded at 6, 24, 48, 72 and 96 hours. The average mortality in each concentration was taken to determine the LC_{50} by plotting a graph of probits mortality against log concentration of pesticide, according to the probit analysis method by Finney 1952 (Finney, 1952). The experiment was repeated for second replicate (EPA, 1996).

RESULTS AND DISCUSSION

Mortality of *A. testudineus* at different concentration of pesticides and LC_{50} determination for 96 hours exposure period

Based on the results obtained, the control group indicated that no mortality was recorded. Table 1 to Table 3 shows the mortality of *A. testudineus* at different concentration of different pesticides for 96 hours exposure period. While Fig. 1 shows the values of 96h LC_{50} based on the graphs of percentage in mortality against log concentration.

Based on Table 1 for malathion, for the test concentration of 0.25 mg/L, 50 percent population of the fish was dead. This result was supported by previous research which summarized the 96 hours LC_{50} of malathion was varied from the range of 0.091 mg/L to 22.09 mg/L for different fish species (Vittozi & De-Angelis, 1991). In addition, other studies in acute toxicity for malathion on fish *Clarius batrachus* found that the 96 hours LC_{50} value was also 0.25 mg/L (Yogesh *et al.*, 2009).

Next, Table 2 for dichlorvos shows that the value of 96h LC_{50} obtained was 2.35 mg/L. This finding also supported by previous study which highlighted that 96h LC_{50} of dichlorvos for fishes were in the range of 0.004 to 11.6 mg/L. Another study on the acute toxicity of dichlorvos on guppies (*Poecilia reticulata*) and carps (*Cyprinus carpio*) showed the 96h LC_{50} values were 1.84 mg/L and 2.51 mg/L respectively (Shepard, 1986).

Table 1. Mortality of *A. testudineus* at different concentration of malathion for 96 hours exposure period

Conc. of Malathion (mg/L)	Log Conc.	Fish Exposed	Fish Dead Replicates		% of Mortality	Probits of Mortality
			1	2		
0.15	-0.824	10	1	2	15%	3.06
0.25	-0.602	10	5	5	50%	5.00
0.35	-0.456	10	6	7	65%	5.30
0.45	-0.347	10	9	8	85%	6.04

Table 2. Mortality of *A. testudineus* at different concentration of dichlorvos for 96 hours exposure period

Conc. of Dichlorvos (mg/L)	Log Conc.	Fish Exposed	Fish Dead Replicates		% of Mortality	Probits of Mortality
			1	2		
0.5	-0.301	10	0	0	0%	0
1.5	0.176	10	2	2	20%	4.16
2.5	0.398	10	6	5	55%	5.13
3.5	0.544	10	9	8	85%	6.04

Table 3. Mortality of *A. testudineus* at different concentration of temephos for 96 hours exposure period

Conc. of Temephos (mg/L)	Log Conc.	Fish Exposed	Fish Dead Replicates		% of Mortality	Probits of Mortality
			1	2		
5.0	0.699	10	0	0	0%	0
15.0	1.176	10	2	1	15%	3.06
25.0	1.398	10	5	5	50%	5.00
35.0	1.544	10	8	8	80%	5.84

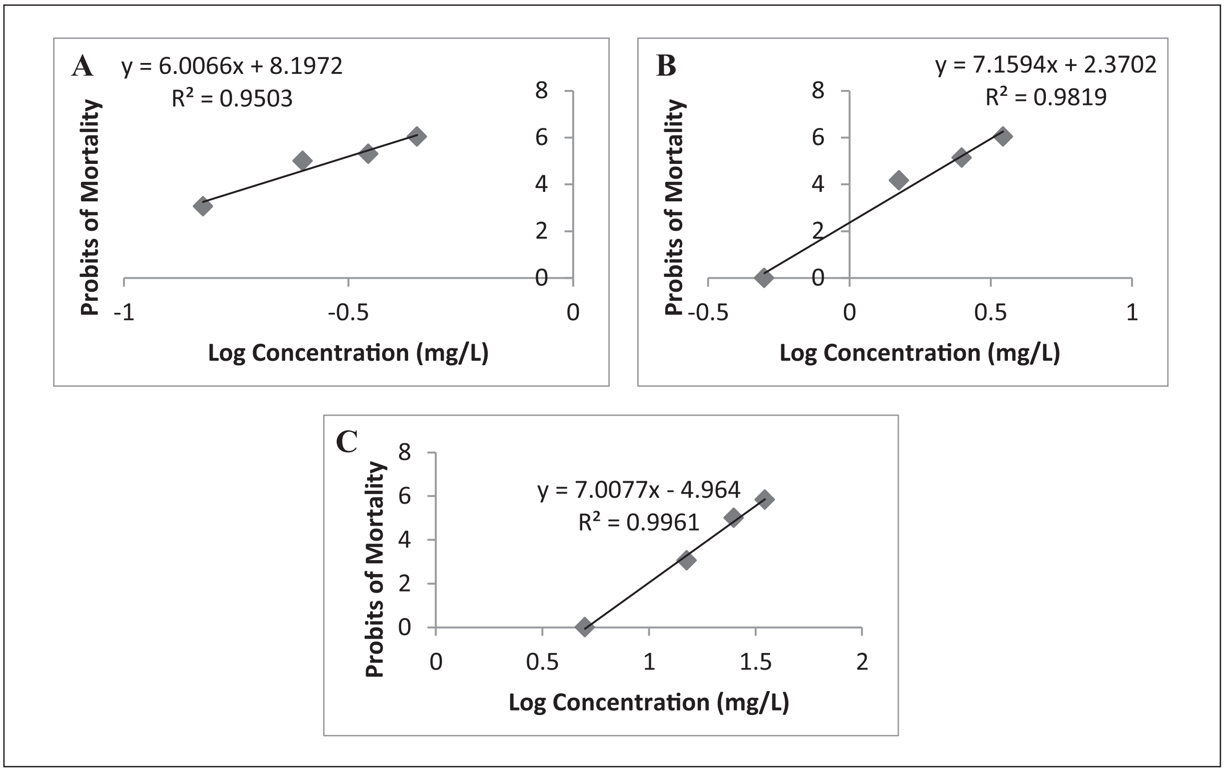

Fig. 1. Lethal concentration (LC_{50}) for 96 hours exposure of each pesticide in *A. testudineus* (**A**) Malathion (**B**) Dichlorvos (**C**) Temephos

Then, in Table 3, the value of 96h LC_{50} obtained for temephos was 25.0 mg/L. Based on previous finding, 96h LC_{50} for temephos in fishes were around 20.5 mg/L to 44.2 mg/L. Other research on *Tilapia melanopleura* with exposure of temephos showed a value of 30.2 mg/L for the 96h LC_{50} (Anadu, 1996).

During acclimatization, *A. testudineus* exhibited erratic movement and aggression when placed in the aquariums. Some fish attempted to jump out of the aquarium. The behavior continued for a few hours then their movement becomes normal and calm. Then, the acute toxicity test showed that all of the fish in control medium survived, while mortalities were observed in every treatment as shown in the Table 1. The result of mortality increased with the increasing concentration of the pesticides was supported by Omoregie *et al*. (2009) and Umar *et al*. (2010).

In addition, based on observation during acute toxicity study, the fishes showed different behavioural changes. There was gulping of air at water surface, increase in mucus secretion, hanging vertically in water, sudden quick movement and jumping, respiratory distress and state of calmness. Moreover, the fishes also showed restlessness and uncoordinated swimming pattern in treated aquariums and were found lying motionless at the bottom of the aquarium, which was subsequently followed by death. Mucus was observed on the gills of the dead fish in all treatments group. Previous study reported that accumulation of mucus on gills reduces respiratory activity due to inability of the gill surfaces to actively carry out gaseous exchange and thus lead to mortalities (Omoniyi *et al.*, 2002). Another research also reported respiratory distress may be due to decreased in the dissolved oxygen contents due to the introduction of pesticides into an aquatic system (Dede & Kaglo, 2001). These behavioural changes were supported by other studies that were exposed to organophosphate pesticide. Observation on *Labeo rohita* treated with malathion showed the same effects (Patil & David, 2008). Next, similar behavioural changes were observed in chlorpyrifos and diazinon treated fishes (Kopriicii, 2006). Other than behavioural changes, the treated fishes also showed external change of bleached body with lesions which can be concluded as indicative of damage caused by the pesticides. According to Fafioye *et al*. (2001), skin abnormalities such as lesions could expose the fish flesh to various other diseases.

Furthermore, the three pesticides used in this experiment were specifically identified to inhibit cholinesterase enzymes (Omoregie *et al.*, 2009). These pesticides could bind to acetylcholine receptors in the nervous system thus caused the excitation and behavioural changes which lead to the resultant of jumping and restlessness.

Therefore, the result of this study indicated that malathion, dichlorvos and temephos exert toxic effects on fish. Thus, the use of these pesticides should be properly and strictly controlled and regulated by appropriate legislation in order to prevent its bioaccumulation in the environment. Besides, based on all the results above and given the fact that, pesticides are not selective and affect non target organisms; it is not surprising that a chemical that acts on the pest with different systems will elicit similar effects in higher forms of life.

CONCLUSIONS

Thus, based on the abnormalities in behaviour and mortalities of *A. testudineus* as a result of exposure to pesticides under laboratory conditions, it is approved that malathion, dichlorvos and temephos were toxic and highly poisonous to *A. testudineus*. The finding was supported by previous studies. Therefore, this study can be used to help the environmental management agency to enhance public awareness in order to prevent indiscriminate use of pesticides and also to assure the effective use of these pesticides also for regulatory purposes.

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REFERENCES

Aiub, C.A.F., Coelho, E.C.A., Sodre, E., Pinto, L.F.R. and Felzenszwalb, I. 2002. Genotoxic evaluation of the organophosporus pesticide temephos. *Genetic Molecular Resources*, **1**: 159-166.

Ajani, E.K., Adesina, B.T. and Okuagu, C.N.F. 2006. Toxicity of lindane (Gamman Hexachloro Cyclohexane) to *Clarius garippinus*. *World Journal of Zoology,* **1**: 57-63.

Anadu, D.I. 1996. Acute toxicity of the insects' larvacide Abate® (Temephos) on the fish *Tilapia melanopleura* and the dragonfly larvae neurocordelia-virginiensis. *Journal of Environmental Science and Health* Omitoyin, B.O., Part B. Pesticides food contaminants and agricultural waste. **31**: 1363-1375.

Chuiko, G.M. 2000. Comparative study of acetylcholinesterase and butyrylcholinesterase in brain and serum of several freshwater fish: specific activities and in vitro inhibition by DDVP, an organophosphate pesticide. *Comp. Biochemical Physiology*, **127**: 233-242.

El-Sayed, Y.S., Saad, T.T. and El-Bahr, S.M. 2007. Acute intoxication of deltamethrin in monosex Nile Tilapia, *Oreochromis niloticus* with special reference to the clinical, bi-ochemical and hematological effects. *Environmental Toxicology and Pharmacology,* **24**: 212-217.

Fafioye, O.O., Adeogun, O.A., Olayinka, E.A. and Ayoade, A.A. 2001. *Nigerian Experimental Biol,* **5**(1): 61-68.

Finney, D.J. 1952. Cambridge University Press 333. Environmental Protection Agency (EPA). Ecological Effects Test Guideline. OPPTS 850.1075 Fish acute toxicity test, freshwater and marine, 1996; 96-118. Environmental Protection Agency (EPA). TEACH Chemical Summary, 2007; 1-13.

Gupta, P. and Srivastava, N. 2006. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus* (Bloch), *Journal Environmental Biology,* **27**: 211-215.

Halappa, R. and David, M. 2009.Behavioural responses of the freshwater fish, *Cyprinus carpio* (Linnaeus) following sublethal exposure to chloropyrifos, *Turk. J. Fish. Aquatic. Science,* **9**: 233-238.

Kopriicii, S.S., Kopriicii, K., Ural, M.S., Ispir, U. and Pala, M. 2006. Acute toxicity of an organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.), *Pesticide Biochemistry and Physiol*ogy, **86**: 99-105.

Lawson, E.O., Ndimele, P.E., Jimoh, A.A. and Whenu, O.O. 2011. Acute Toxicity of Lindane (Gamma Hexachloro Cyclohexane) to African Catfish (*Clarias gariepinus*, Burchell, 1822), *International Journal of Animal and Veterinary Advances,* **3**(2): 63-68.

Odiete, W.O. 1999. Environmental physiology of animals and oxygen consumption of *Channa puntatus* (Bloch) and Pollutions. Published by Diversified Resources Proc. Symp. *Environmental Biology*, 343-348.

Omoregie, E., Malachy, N.O., Ajino, L., Romanus, I. and Kazimierz. W. 2009. *Acta Ichthyological*, **39**: 103-110.

Omoniyi, I.T., Agbon, A.O. and Sodunke. S.A. 2002. *Applied Science Environmental. Management,* **6**(2): 37-41.

Pal, M. and Chaudhry, S. 2010. *Anabas testudineus*. The IUCN Red List of Threatened Species, 2010. Version 2014.3. Retrieved from www.iucnredlist.org.

Patil, V.K. and David, M. 2008. Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton), Turkish *Journal of Fisheries and Aquatic Sciences*, **8**: 233-237.

Rath, S. and Misra, B.N. 1981. Toxicological effects of dichlorvos (DDVP) on brain and liver AChE activity of *Tilapia mossambica*. *Toxicology*, **19**: 239-245.

Shepard, T.H. 1986. Catalog of Teratogenic Agents, Fifth Edition. Johns Hopkins University Press, Baltimore. MD. 5-10.

Svoboda, M.M., Luskova, V., Drastichova, J. and Zlabek, V. 2001. The effect of Diazinon on hematological indices of common carp (*Cyprinus carpio* L.). *Acta. Vet. Brno,***70**: 457-465.

Umar, M., Stephen, S.H., Abdullahi, M. and Garba, M. 2010. *Scientific Research and Essay,* **5**(1): 49-54.

United Nations Environment Programme (UNEP). 2005. Ridding the world of persistent organic pollutants: Aguide to the Stockholm convention on persistent organic pollutants. *United Nations Environment Programme*, 11-13.

Van der Oost, M., Beyer, R.J. and Vermeulan, N.P.E. 2003. Fish bioaccumulation and biomarkers in environment risk assessment a review. Environ. *Toxicology Pharmacology*, **13**: 57-149.

Vittozi, O.L. and De-Angelis, G. 1991. A critic review of comparative acute toxicity of data on fresh water fish. *Aqua. Toxicol,* **19**: 167-204.

Yogesh, H.W., Yashshri, A.G. and Prakash, P.A. 2009. Sublethal and chronic effects of carbaryl and malathion on *Clarins batrachus* (Linn.). *Journal Applied Science Environmental Management*, **13**(2): 23-26.