

Comparative Study on the Acute Toxicity of Synthetic Pesticides, Permethrin 25% and Monocrotophos 36%, and Neem-Based Pesticide, Neem Gold EC 0.03%, to Juvenile *Cyprinus carpio* Linn

Reza Davoodi¹ and Gholamreza Abdi^{2*}

¹ Fisheries and Aquaculture Department, Agricultural and Natural Resources College, Persian Gulf University, Boushehr, IRAN

² Persian Gulf Research and Studies Center, Persian Gulf University, Boushehr, IRAN

ABSTRACT

Fish and other organisms are affected by pesticides which pollute the natural water through agricultural runoff. The aim of this study was to determine the acute toxicity of botanical pesticide, neem gold, and two synthetic pesticides, permethrin and monocrotophos, which are extensively used in agricultural farms. The mortality rate of *Cyprinus carpio* was monitored under laboratory conditions for the periods of 96 hrs. The toxicity tests were done separately for each pesticide. Data obtained from the toxicity tests were evaluated using the Probit Analysis Statistical Method. The toxicity tests gave 96 hours LC50 values of 75.49, 72.26, 56.89 mg/l for neem gold, monocrotophos and permethrin respectively. The fish exhibited respiratory distress (such as gasping air), loss of balance and erratic swimming prior to mortality. It could be concluded that the application of neem gold bio-pesticides for the control of unwanted organisms in agricultural farms are much safer and more environmental friendly than synthetic pesticides.

Key Words: Azadirachtin, LC50, monocrotophos, neem gold, permethrin, pesticide, toxicity.

INTRODUCTION

The aquatic environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms (Kumar *et al.*, 2010). In the search for environmentally safe pesticides, much research has been done on the use of plants for the protection of crops in the field or in storage. Especially in tropical regions, the application of botanical material to protect a crop against insects is often traditional and centuries old (Van Huis *et al.*, 2004). Modern fish culture is classified in lucrative production, preservation of the environment and social development. Fish culture also depend mainly on the ecosystem they are inserted and which should remain even to attain the assurance of the activity (Valenti, 2000). Pesticides are used in agro-ecosystems, forests, and recreational areas such as golf courses, but it may enter aquatic systems such as streams, rivers, and lakes if used in adjacent areas or if an accidental spill occur. These pesticides are carried into aquatic ecosystem by surface runoff from sites of application and therefore the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides (Singh *et al.*, 2010). These pesticides are also found to be highly toxic not only to fish but also to other organisms which constitute food of the fish.

Among different classes of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment (Singh *et al.*, 2010). Synthetic pyrethroid insecticides are also extensively used in place of organochlorine, organophosphorus insecticides and carbamates to control pests. These insecticides are more likely to be toxic to fish and other aquatic organisms (Elliot 1977; Casida *et al.*, 1983; Smith and Stratton 1986; Moore and Waring 2001).

In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of pest control that minimize this damage, there has been extensive research on pest control by substances from plants (Wan *et al.*, 1996). One of the most promising natural compounds is azadirachtin, an active compound extracted from the neem tree (*Azadirachta indica*), whose antiviral, antibacterial and antifungal properties have been known for several years (Isman *et al.*, 1990; Harikrishnan *et al.*, 2003; ICAR, 1993). It is generally considered less harmful to the environment than other more commonly used pesticides (Mordue [Luntz] and Blackwell, 1993). Therefore, neem-based insecticides are being investigated as alternatives to synthetic insecticides for the control of agricultural insect pests. However, adverse effects of azadirachtin against beneficial organisms have been reported (Schmutterer and Holst, 1987; Beckage *et al.*, 1988; Hoelmer *et al.*, 1991; Price and Schuster, 1991).

Neem has also been used successfully in aquaculture systems to control fish predators (Dunkel and Ricilards, 1998). Martinez (2002) stated that aqueous extract of neem leaves and other neem-based products have been extensively used in fish-farms as alternative for the control of fish parasites and fish fry predators such as dragon-fly larvae. Although neem extract is considered of low toxicity towards non-target aquatic life, water

* Corresponding author: astoags@gmail.com

extracts of the bark of the neem plant caused respiratory problems in *Tilapia zilli* (Omorieg and Okpanachi, 1997), while long exposure to low concentrations of the crude extract of *A. indica* delayed the growth of this cichlid fish (Omorieg and Okpanachi, 1992). Also, the use of pesticides in aquaculture systems to control fish diseases, parasites and other pests not only leads to high levels of residues in the animals but also may interfere with the maintenance of their homeostasis and thus affect their performance (Barton and Iwama, 1991; Wendelaar Bonga, 1997).

Our research was conducted to determine the acute toxicity effects of two synthetic pesticides one commonly used in Iran, permethrin 25%, and one commonly used in India, monocrotophos 36%, and to compare them with the acute toxicity of a commercial neem product, Neem Gold, which have not been used in agricultural farms of Iran, on the survival of common carp under laboratory conditions.

MATERIALS AND METHODS

Juvenile of common carp (*Cyprinus carpio*) was brought from a fish farm in south of Iran and were acclimated in 1000 liter indoor tanks filled with well water for two weeks prior to the experiment. They were fed twice a day and feeding were ceased 24 hours before the start of the tests. During the acclimatization period constant aeration was applied to the tanks. Temperature ($20\pm 0.5^{\circ}\text{C}$) conductivity ($4.01\pm 0.02\ \mu\text{S}/\text{cm}$) and pH (8.02 ± 0.06) were monitored constantly during the experiments. In order to obtain information about the range of concentrations to be used in the main test, a series of range-finding tests were run on the pesticide.

Healthy fishes weighing of $5.02\pm 1.2\ \text{g}$ and length of $6.42\pm 0.43\ \text{cm}$ ($n=180$) were separated on the morning of the day of test and placed in 64L aquarium tanks contained 20L well water and 10 fishes per each aquarium. To determine the LC₅₀ of each pesticide, 15 aquariums were divided into 3 replications of each of 5 concentrations. The concentrations used include 25, 50, 67.5, 91 and 122.5 mg/l for Neem Gold (0.03% EC); 25, 39, 61, 96 and 150 mg/l for monocrotophos (36%) and 10, 18, 31.5, 56, 100 and 125 mg/l for permethrin (EC 25%). Three controls without the test substance were run simultaneously for each pesticide. Observations were made and recorded every 12 hours until the end of 96 hours. Test species were considered dead if they showed no movement at all. The experiments were carried out in semi-static systems with regular batch wise renewal of test solutions after 24 hours. The results of the experiments were analyzed by linear regression probit analysis (Finney, 1971) using SPSS computer program.

RESULTS AND DISCUSSION

The 96 hrs LC₅₀ values along with its 95% lower and upper confidential limits for each pesticide are presented in table 1. The greatest LC₅₀ was 75.49 mg/l for neem gold and the least was 56.90 mg/l for permethrin. The LC₅₀ value for monocrotophos was 72.26 mg/l which stands between other two pesticides and shows that permethrin is more toxic than monocrotophos too. Therefore, the most toxic pesticide was permethrin, monocrotophos and neem gold respectively.

After induction of pesticide, the intoxicated fish were aggregated at the corner of the aquarium resting at the bottom and frequently come to the surface followed by heavy breathing with stronger opercular movements and loss of equilibrium. Also the over secretion of mucus was observed on the fishes treated to pesticide. This has been observed in fishes treated with other pesticides also (Toor and Kaur, 1974; Kumar *et al.*, 1984). The same activity also reported in the gills of catfish treated with azadirachtin (Kumar *et al.*, 2010). In case of permethrin and monocrotophos, fishes appears excited within few minutes of exposure to higher concentrations however, they calm down and gathered at the corner of aquarium. There was no mortality recorded for neem gold and monocrotophos even in higher concentration in the first 24 hours of the test, but in case of permethrin it was different. However, it was shown that prolong exposure of *Clarias gariepinus* to monocrotophos has a significant reduction effect on weight gain, specific growth rate and protein efficiency ratio (Yaji and Auta, 2007).

To reduce the contamination in the environment it is suggested that the use of plant based pesticides should be encouraged (Schmutterer, 1990) because they disintegrated easily into constituent elements without leaving any indelible impression in different regions of environment (Khan and Ahmad, 2000). Plant base pesticides contain active principles with low half life period and their toxic effect on the environment are not too detrimental (Das and Mukharjee, 2003). The amount of active compounds in a given weight of neem varies widely with the part of the plant, its place of origin or even the individual tree (Luo *et al.*, 1999 and Winkler *et al.*, 2007). However the toxicity of two Nimbesidine and Neemgold on a fresh water loach *Lipidocephalichthys guntea* has been reported (Mondal *et al.*, 2007). Compared to other synthetic pesticides used in fish farming,

such as carbamates and organophosphates, neem based products are certainly less toxic to fish (Wan *et al.*, 1996). The neem leaf extract applications appeared to affect the abundance of the major crustacean zooplankton groups (Mamdouh Mousa *et al.*, 2008). Fish exposed to higher concentration of the plant extract exhibited respiratory distress, erratic swimming, off feed and nervous manifestations. Winkaler *et al.*, (2007) noticed that fish exposed to all neem extract concentrations exhibited damaged gill and kidney tissue.

From the present study, it could be concluded that permethrin and monocrotophos contamination is dangerous to aquatic ecosystems, and this fact should be taken into consideration when this insecticides are used in agriculture or in the control of mosquito populations. it can be also concluded that although neem based pesticides are considered as less toxic and environmental friendly, but precautions must be taken when it is used in fish inhabiting areas since the excess application can affect the life of organisms living near the farming area and cause the high mortality among them.

Table 1. The 96 hours lethal concentrations and 95% lower and upper confidential limit values of tested pesticides against common carp.

96 hours LC50	Dose	95% confidential limits			
		LCL 0.05	UCL 0.05	LCL 0.01	UCL 0.01
Neem Gold	75.49	70.46	80.44	68.71	82.88
Monocrotophos	72.26	64.24	81.44	61.66	84.98
Permethrin	56.90	47.71	70.11	45.09	76.31

REFERENCES

- Beckage N. E., Metcalf, J. S., Nielson, B. D. and D. J., Nesbit (1988). Disruptive effects of azadirachtin on development of *Cotesia congregata* in host tobacco hornworm larvae. Arch. Insect Biochem. Physiol. 9: 47-65.
- Barton B.A., and G.K., Iwama (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1: 3-26.
- Casida J.E., Gammon D.W., Glickman A.H. and L.J. Lawrence (1983). Mechanism of pyrethroid insecticides. Ann. Rev. Pharmacol. Toxicol. 23: 413-418.
- Das B.K. and S.C., Mukharjee (2003). Toxicity of cypermethrin in Labeo rohita fingerlings: Biochemical enzymatic and hematological consequences. Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 134: 109-121.
- Dunkel F.V. and D.C., Ricalards (1998). Effect of an azadirachtin formulation on six non target aquatic macroinvertebrates. Environ. Entomol. 27: 667-673.
- Elliot M (1977). Synthetic Pyrethroids. American Chemical Society, Washington pp.1-28.
- Finney D.J (1971). Probit analysis, 3rd Edition. Combrige Univ. Press, London.
- Harikrishnan R., Rani, M. N. and C., Balasundaram, (2003). Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture 221: 41-50.
- Hoelmer K. A., Osborne, L. S. and R. K., Yokomi (1991). Effects of neem extracts on beneficial insects in greenhouse culture, pp. 100-105. In J. C. Locke and R. H. Lawson [eds.], Proceedings. USDA Workshop. NeemÖpotential in pest management programs, 16-17 April 1990, USDA-ARS, ARS-86. USDA, Beltsville, MD.
- ICAR (1993). World Neem Conference Souvenir ICAR, Bangalore, India.
- Isman M. B., Koul, O., Luczyski, A. and J., Kaminski (1990). Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Agric. Food Chem., 38: 1406-1411.
- Khan M.F. and S.M., Ahmad (2000). Toxicity of crude neem leaf extract against housefly, *Musca domestica* L. adults as compared with DDVP, Dichlorvos. Turk. J. Zool., 24(4): 219-223.
- Kumar S. and S.C., Pant (1984). Organal damage caused by aldicarb to a freshwater teleost *Barbus conchonus* (Hamilton). Bull. Environ. Contam. Toxicol., 33:50.
- Kumar A., Prasad, M.R., Srivastava, K., Tripathi, S. and A.K., Srivastava (2010). Branchial Histopathological Study of catfish *Heteropneustes fossilis* following exposure to purified Neem Extract, Azadirachtin. World journal of zoology. 5(4): 239-243.
- Luo X., Ma, Y., Wu, S. and D., Wu (1999). Two novel azadirachtin derivates from *Azadirachta indica* J. Nat. Prod. 62, 1022-1024
- Mamdouh A. A. Mousa, Ahmed M. M. El-Ashram, and Hamed., Mona (2008). Effect of Neem Leaf Extract on Freshwater Fishes and Zooplankton Community. 8th International Symposium on Tilapia in Aquaculture 2008. 307-318
- Martinez S.O (2002). NIM — *Azadirachta indica*: natureza, usos múltiplos produção. Instituto Agronômico do Paraná (IAPAR), Londrina, PR.
- Mondal D., Barat, S. and M.K Mukhopadhyay, (2007). Toxicity of neem pesticides on a freshwater loach, *Lepidocephalichthys guntea* (Hamilton Buchanan) of Darjeeling district in west Bengal. J. Environ. Biol., 28(1): 119-122.
- Moore A. and C.P. Waring (2001). The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in atlantic salmon (*Salmo salar* L.). Aquatic Toxicol. 52: 1-12.
- Mordue [Luntz], A. J., and A Blackwell (1993). Azadirachtin: an update. J. Insect Physiol. 39: 903-924.
- Omeregie E. and M.A., Okpanachi (1992). Growth of *Tilapia zillii* exposed to sublethal concentrations of crude extracts of *Azadirachta indica*. Acta Hydrobiol., 34: 281-286.
- Omeregie E. And Okpanachi, M.A., (1997). Acute toxicity of water extracts of bark of the Neem plant, *Azadirachta indica* (Lodd) to the cichlid *Tilapia zillii* (Gervais). Acta Hydrobiol. 39, 47-51.
- Price J. F. and D.J., Schuster (1991). Effects of natural and synthetic insecticides on sweetpotato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) and its hymenopterous parasitoids. Fla. Entomol. 74: 60-68.

- Schmutterer H., and H., Holst (1987). On the effects of the enriched and formulated neem seed kernel extract AZTVR- K on *Apis mellifera* L. J. Appl. Entomol. 103: 208-213.
- Schmutterer H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entomol., 35: 271-297.
- Singh R.N., Pandey P.K., Singh N.N. and V.K., Dass (2010). Acute toxicity and behavioral responses of common carp *Cyprinus carpio* (Linn.) to an Organophosphate (Dimethoate). World Journal of Zoology. 5 (3): 183-188.
- Smith T.M. and G.M. Stratton (1986). Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Rev. 97: 93-119.
- Toor, H.S. and K., aur (1974). Toxicity of pesticides to the fish *Cyprino carpio communis*. Ind. J. Exp. Biol., 12: 334-336.
- Valenti W.C (2000). Aquicultura no Brasil. Bases para um desenvolvimento sustentavel. Brasilia: CNRq/ Ministerios da Ciencia e Tecnologia, 399p.
- Van Huis A., Dicke M., I., Rietjens (2004). Safety evaluation of neem (*Azadirachta indica*) derived pesticides. Journal of Ethnopharmacology. 94: 25-41.
- Wan M.T., Watts, R.G., Isman, M.B. and R., Strub (1996). Evaluation of the acute toxicity to juvenile pacific northwest salmon of azadirachtin, neem extract, and neem based products. Bull. Environ. Contam. Toxicol., 56: 432-439.
- Wendelaar Bonga, S.E (1997). The stress response in fish Physiol. Rev. 77: 591-625.
- Winkaler E.U., Santos, T.R.M., Machado-Neto, J. G. and C.B.R., Martinez (2007). Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus* Comparative Biochemistry and Physiology, Part C 145: 236-244.
- Yaji A.J. and J., Auta (2007). Sub-lethal effect of Monocrotophos on Growth and food Utilization of the Aferican Cat Fish *Clarias gariepinus* (Teugels). Journal of Fisheries International, 2(2):127-129.