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Acute toxicity of azadirachtin to a teleost, *Heteropneustes fossilis*

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ABSTRACT. The acute static renewal test of a botanical pesticide - azadirachtin for the freshwater catfish, *Heteropneustes fossilis* has been performed to determine the LC_{50} values at different exposure period. The LC50 values at various exposure periods are 173.06 mg L^{-1} for 24h; 80.69 mg L^{-1} for 48h; 58.57 mg L^{-1} for 72h and 52.35 mg L^{-1} for 96h. The upper confidence limits were 196.87, 86.91, 79.20 and 70.04 mg L^{-1} for 24, 48, 72 and 96 h and lower confidence limits were 154.01, 74.24, 37.33 and 33.83 mg L^{-1}, respectively. These results indicate that azadirachtin exposure to the fish caused toxic effects.

Keywords: Neem, azadirachtin, LC_{50}, *Heteropneustes fossilis*, fish.

Toxicidade aguda da azadiractin para um teleósteo, *Heteropneustes fossilis*

RESUMO. A renovação do ensaio estático agudo de um pesticida botânico - azadiractin para o peixe de água doce, *Heteropneustes fossilis* foi realizada para determinar os valores de LC_{50} em diferentes períodos de exposição. Os valores de LC_{50} em diferentes períodos de exposição são 173,06 mg L^{-1} por 24h; 80,69 mg L^{-1} por 48h; 58,57 mg L^{-1} por 72 h e 52,35 mg L^{-1} por 96h. Os limites de confiança superiores foram 196,87; 86,91; 79,20 e 70,04 mg L^{-1} para 24, 48, 72 e 96h os limites inferior e confiança foram 154,01; 74,24; 37,33 e 33,83 mg L^{-1}, respectivamente. Estes resultados indicam que a exposição do peixe à azadiractin causou efeitos tóxicos.

Palavras-chave: Neem, azadiractin, LC_{50}, *Heteropneustes fossilis*, peixes.

Introduction

Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which is caused by the use of pesticides by the human beings for their benefits – control of insect vectors of disease and increased yield of many crops. After entering into aquatic ecosystems, these pesticides have a negative effect on aquatic life. The effects of these pesticides may be lethal or sub-lethal. The young growing stages of fish are particularly more affected by these pesticides which may be disastrous for the future of the stock.

The use of persistent organochlorines and organophosphates has led to hazardous effects on environment and human beings which directed the attention towards the use of alternative chemicals. The alternative for the chemical pesticides came in the form of botanical pesticides which are plant-derived materials. The isolation and identification of pyrethroids from *Chrysanthemum cinerariifolium*, has revitalized the interests in plants which contain chemical compounds. However, simply because of compound is a natural product does not ensure that it is safe, therefore, toxicological and environmental properties of the compound must be considered before use.

The neem tree, *Azadirachta indica* is so far the most promising example of the plant currently being used. Azadirachtin is the principal active ingredient of neem tree (MORGAN, 2009). This is a naturally occurring substance related to an organic molecule class – tetranortriterpenoids (limonoids) and is now used for control of pests and other harmful animals (KREUTZWEISER, 1997; MONDAL et al., 2007; MORGAN, 2009; OKUMU et al., 2007; PUNZO; PARKER, 2005; SENTHIL NATHAN et al., 2008; SHAFEEK et al., 2004; SHANMUGASUNDARAM et al., 2008; SINGH et al., 2007; SU; MULLA, 1998; WINKALER et al., 2007). Azadirachtin is structurally similar to insect hormone ecdysones (which control metamorphosis) and has been suggested to be an ecdysone blocker. It is used in fish farms for the control of fish parasites and fish fry predators (WINKALER et al., 2007). In fish farms the neem insecticides has been applied at 50 g ha^{-1} and the expected environmental concentration (EEC) is 35 μg L^{-1} (KREUTZWEISER et al., 2004). In the present study, the acute static renewal test of a botanical pesticide -- azadirachtin for the freshwater

catfish, *Heteropneustes fossilis* has been performed to determine the LC_{50} values at different exposure period.

Material and methods

Adult freshwater catfish *Heteropneustes fossilis* (both sexes; body wt 27-32 g) were collected locally and acclimatized under laboratory conditions for 15 days in plastic pools and fed daily 2-3 times with wheat flour pellets and ground dried shrimps.

To determine the LC_{50} value of azadirachtin the four-day static renewal acute toxicity test (APHA/AWWA/WEF, 1998) was used. Five replicates of each containing 10 fish (kept in glass aquaria in 30L tap water) were exposed to each concentration (25, 50, 75, 100, 125, 150, 175, 200, 225, 250 mg L^{-1}) of azadirachtin (Ozoneem Aza containing Aza A 23.78% and Aza B 3.59%, Batch No. AZA-351, manufactured by Ozone Biotech, India). A control group with five replicates each of ten fish kept in 30L tap water was also run.

The media (the control and test solutions) in the aquaria were renewed daily (as azadirachtin breaks-down after exposure to light) (DUREJA; JOHNSON, 2000). The DT_{50} (time required for 50% disappearance of the initial concentration) for azadirachtin A has been reported as 31.5 day (25°C) and for azadirachtin B 42.3 day (25°C) (STARK; WALTER, 1995). The fish were not fed 24h before and during the experiment. Dead fish were removed immediately. The physico-chemical conditions of the tap water (this is measured as the toxicity of any chemical depends on the pH, hardness, temp. etc.) used in experiment are – temperature – 26.74 ± 2.11 C; pH – 7.26 ± 0.09; hardness – 167.97 ± 5.69 mg L^{-1} as $CaCO_3$; dissolved oxygen – 7.85 ± 0.36 mg L^{-1}; electrical conductivity – 307.16 ± 65.12 µmhos cm^{-1} and no free chlorine.

At different exposure periods (24, 48, 72 and 96h), the mortality of the fish was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC_{50} and 95% confidence interval.

Results and discussion

The per cent mortality of *H. fossilis* after exposure to various concentrations of azadirachtin for 24, 48, 72 and 96 h has been depicted in Figures 1, 2, 3 and 4. The LC_{50} values at various exposure periods are 173.06 mg L^{-1} for 24h; 80.69 mg L^{-1} for 48h; 58.57 mg L^{-1} for 72h and 52.35 mg L^{-1} for 96h. The LC_{50} values and their upper and lower confidence limits have been given in Table 1.

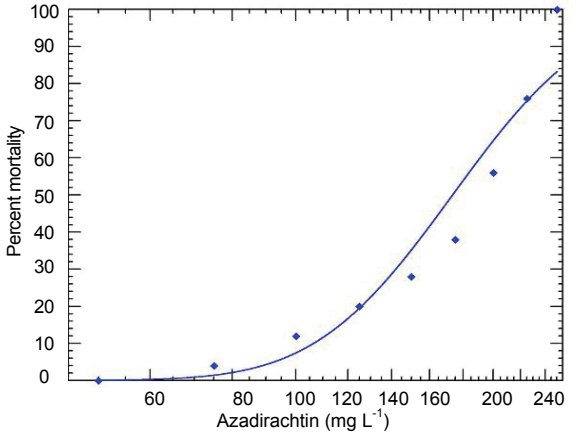

Figure 1. Percent mortality of the fish *Heteropneustes fossilis* after 24h exposure to different concentrations of azadirachtin (mg L^{-1}).

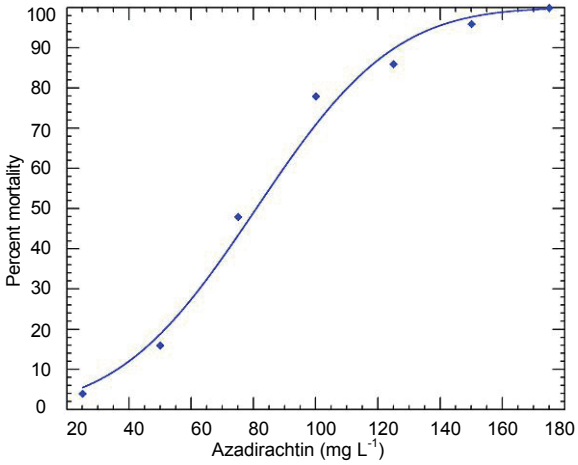

Figure 2. Percent mortality of the fish *Heteropneustes fossilis* after 48h exposure to different concentrations of azadirachtin (mg L^{-1}).

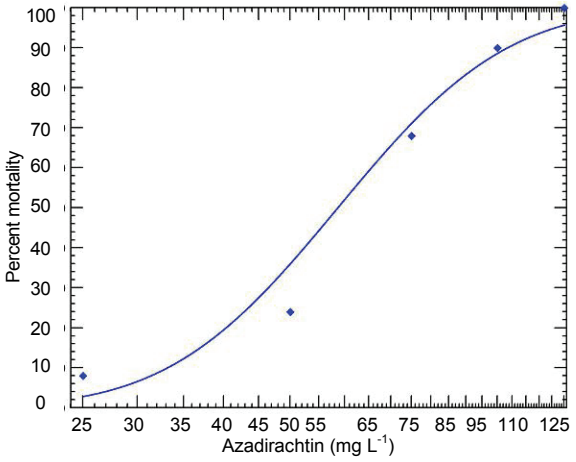

Figure 3. Percent mortality of the fish *Heteropneustes fossilis* after 72h exposure to different concentrations of azadirachtin (mg L^{-1}).

Mondal et al. (2007) have reported 96h LC_{50} value for fingerlings of a freshwater loach *Lepidocephalichthys guntea* as 0.135 mg L^{-1} for Nimbecidine and 0.525 mg L^{-1} for Neem Gold. They have found Nimbecidine

more toxic than Neem Gold. The 24h LC_{50} of neem leaf extract for juveniles of *Prochilodus lineatus* has been reported to be as 4.8 g L^{-1} (WINKALER et al., 2007). Osuala and Okwuosa (1993) have reported 96 h LC_{50} for freeze-dried aqueous extract of neem (stem bark) for the fish *Aphyosemon gairdneri* is 15.1 mg L^{-1}. For juvenile salmon the 96h LC_{50} of azadirachtin (AZA 49% purity) is greater than 4 mg L^{-1} (WAN et al., 1996). The toxicity of water-extract of mesocarp of neem (*Azadirachta indica*) fruit has been tested for hybrid, *Heteroclarias* and LC_{50} for 96h was found as 81.28 mg L^{-1} (AKINWANDE et al., 2007). Stalin et al. (2008) have found that azadirachtin (96h LC_{50} – 0.011 mg L^{-1}) is less toxic to fish *Poecilia reticulata* as compared to a pyrethroid, deltamethrin (96h LC_{50}– 0.0019 mg L^{-1}).

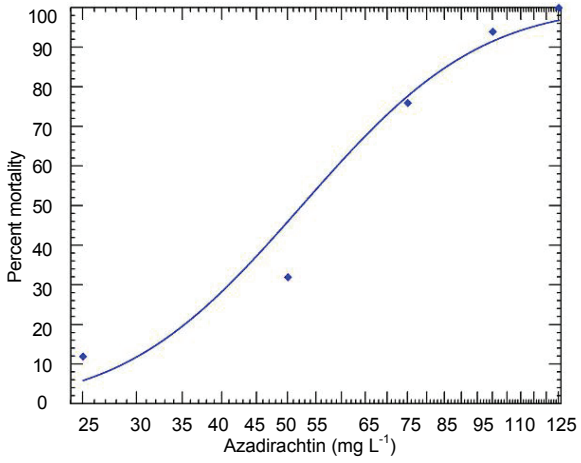

Figure 4. Percent mortality of the fish *Heteropneustes fossilis* after 96h exposure to different concentrations of azadirachtin (mg L^{-1}).

Table 1. LC_{50} value, and confidence limits for azadirachtin at different intervals for the fish *H. fossilis*.

	24h	48h	72h	96h
LC_{50} (mg L^{-1})	173.06	80.69	58.57	52.35
Upper Confidence Limit*	196.87	86.91	79.20	70.04
Lower Confidence Limit*	154.01	74.24	37.33	33.83

*The upper and lower confidence limits for LC_{50} values calculated at 0.05 level.

Conclusion

From the above facts it is clear that *Heteropneustes fossilis* is hardy fish when compared to other fish species (adult) regarding the toxicity of azadirachtin. Toxicity of azadirachtin to different fish species is difficult to compare as they are influenced by various factors such as light and water. Moreover, the toxicity also depends upon solvents and the emulsifiers used for formulating the materials (WAN et al., 1996) as well as on the species differences. However, from these reports it can be concluded that neem-based products may cause significant fish kills if large amounts reach the water reservoirs and it is safer to use these products as they have greater margin of safety to fishes as compared to other synthetic chemical pesticides.

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