ISSN 0976-5417 Cross. Res.: Dec. 2014 Vol. 5 No.2

Median Lethal Concentration (LC_{50}) of neem, *Azadirachta indica* leaf extract for the ornamental fish, *Poecilia latipinna*

Rosemin Shyli and *Mettilda, S. Dept. of Zoology, Holy Cross College (Autonomous), Nagercoil.
*Corresponding Author: Email Id: metti.silvester@yahoo.com

Abstract

The application of medicinal plants in the management of aquaculture is gaining momentum because they are safe, effective, widely available and inexpensive. A study was conducted to determine the median lethal concentration of neem, A. indica leaf extract for the ornamental fish, *P. latipinna* to standardize the appropriate lethal dose, sublethal dose and safe exposure period. Static acute toxicity (24 h, 48 h, 72 h, and 96 h) test was conducted to determine the LC_{50} value of neem leaf extract. The results indicated that the fish mortality was dose dependent. With the increase of concentration and exposure duration significant increase in mortality level was observed. The 24 h, 48 h, 72 h and 96 h median lethal concentration (LC_{50}) values of A. indica leaf extract for the fish, *P. latipinna* were found to be 1862.08 ppm, 1766.03 ppm, 1690.44 ppm and 1644.37 ppm respectively. The fishes exhibited marked behavioural changes followed by mortality when exposed to higher concentration. Thus, the present study reveals that A. indica leaf extract has impact on the exposed fishes. Hence the safety level should be assessed before using in aquaculture farms.

Key words: Aquaculture, *Azadirachta indica*, LC_{50}, Mortality, *Poecilia latipinna*, Toxicity

1. Introduction

In recent years, the use of medicinal plants in aquaculture as effective alternatives of synthetic antibiotics, pesticides and fertilizers has gained importance especially to combat problem both in fish and aquatic environment because they are highly toxic and adversely affect the quality of fish and their status [1]. There have been extensive studies on pest and diseases control by using biopesticides or plant extracts in aquaculture [2, 3]. The application of medicinal plants in the management of aquaculture is encouraged because they are safe, effective, widely available and inexpensive. Among the various plants used, neem, *Azadirachta indica* (*A. juss*) is found to be more promising. The leaves, barks, fruits and roots of the plant are highly appraised for their medicinal purposes. Neem leaf exhibits a wide range of pharmacological activities viz, anti-inflammatory, anti-hyperglycaemic, anti-ulcer, anti-malarial, anti-fungal, anti-bacterial, anti-viral, anti-oxidant, anti-mutagenic, anti-pyretic, anti-carcinogenic, insecticidal, biosorbent and immune modulatory [4, 5, 6]. The extract of *Azadirachta indica* has been successfully used in aquaculture system to control fish diseases, predators and parasites [7]. Application of biopesticides cause changes on the physiological activities of the fishes by influenzing the activities of several antioxidant enzymes [8] which alters heamatological and biochemical parameters [7].

Doses of *A. indica* that are not high enough to kill the fishes are associated with the changes in behaviour and reproduction of fish. These results indicate that neem extracts added to water may cause disturbance on fish and therefore it is important to determine the LC_{50} value to assess the safety level of the neem extract. Hence the present study was conducted to evaluate the toxic effects of aqueous leaf extract of *A. indica* on the fish, *P. latipinna* to establish the safe limits.

2. Materials and Methods

Experimental animal: Healthy fingerlings of *P. latipinna* measuring 3.0 ± 0.5 g body weight and 4.7 ± 0.5 cm body length were obtained from J. J. aquafarm and safely brought to the laboratory and acclimatized for 20 days in a large trough prior to the experiment. During the acclimatization period, fish were fed *adlibitum* with artificial feed.

Preparation of aqueous neem leaf extract: The leaves of *A. indica* were collected, dried and finely powdered. Aqueous extract of neem leaf was prepared by soaking the neem leaf powder (25 g) in one liter of water and kept at room temperature for 24 hours. The mixture was filtered and the

extract (25 g/ l) serves as the stock solution (25,000 ppm). This was used immediately in the experiment at different concentrations.

Determination of 24 h, 48 h, 72 h, and 96 h LC_{50} value of neem leaf extract: Static acute toxicity (24 h, 48 h, 72 h, and 96 h) test was conducted to determine the LC_{50} value of neem leaf extract. Feeding was withheld during the toxicity studies. Toxicity assay was carried out using different concentrations of neem leaf extract ranging from 1 to 10,000 ppm. 10 fishes were randomly selected and introduced in the experimental aquaria of different concentrations to observe the morality of fish to calculate the median lethal concentration (LC_{50}) using Finney's method [9]. Behavioural changes as well as the mortality of the fishes were recorded during the assay. The fishes were considered dead when they failed to show movements.

3. Results

For preliminary study different concentrations of neem leaf extract (i.e.) 1, 10, 100, 1000, 5000, 10000 ppm were prepared from the stock and used to find LC_{50} value for 24 h, 48 h, 72 h, and 96 h. When the fishes were exposed to wide range of concentrations like 1, 10, 100, 1000, 5000, 10,000 ppm it was observed that 100% morality occurred at and above 5000 ppm and no morality below 1000 ppm. When the fishes were exposed to concentrations like 1000, 2000, 3000, 4000, 5000 ppm 100% morality was observed at and above 3000 ppm and no morality was recorded at 1000 ppm. When the fishes were exposed to narrow range of concentrations like 1000, 1250, 1500, 1750, 2000, 2250, 2500 ppm no morality was observed below 1500 ppm and 100% morality was observed above 2250 ppm.

Since the preliminary study revealed that LC_{50} lies between 1250 ppm and 2250 ppm, seven different concentrations like 1375, 1500, 1625, 1750, 1875, 2000 and 2125 ppm were designed and the mortality percentage of the fishes were observed. Mortality results clearly indicate that the fish mortality was dose dependent. Exposure of fishes below 1375 ppm of aqueous leaf extract of *A. indica* showed no mortality up to 96 hours, except for some behavioural changes. Exposure of fishes at and above 2250 ppm showed 100% mortality (Table - 1). The 24 h, 48 h, 72 h and 96 h mortality was assessed and presented in the form of median lethal concentration (LC_{50}) (Table 2 to 5 and Figure 1 to 4). LC_{50} value for 24 h, 48 h, 72 h and 96 h of *A. indica* leaf extract on the fish *P. latipinna* was 1862.08 ppm, 1766.03 ppm, 1690.44 ppm and 1644.37 ppm respectively.

Changes in the behavioural activities of fish, *P. latipinna* were noted after the introduction of *A. indica* leaf extract. All the fishes immediately settled down at the bottom of the troughs. Erratic movements, body imbalance, surface floating, restlessness and loss of equilibrium were observed in the affected fishes. The opercular movement and mucous secretion increased with increasing neem extract concentration.

Table-1: *A. indica* leaves extract concentrations and fish mortalities at different exposure period

Neem extract Concentration (ppm)	Mortality %			
	24 h	48 h	72 h	96 h
1375	0	0	0	0
1500	0	0	0	10
1625	0	10	30	40
1750	20	40	60	80
1875	50	70	80	100
2000	80	100	-	-
2125	100	-	-	-

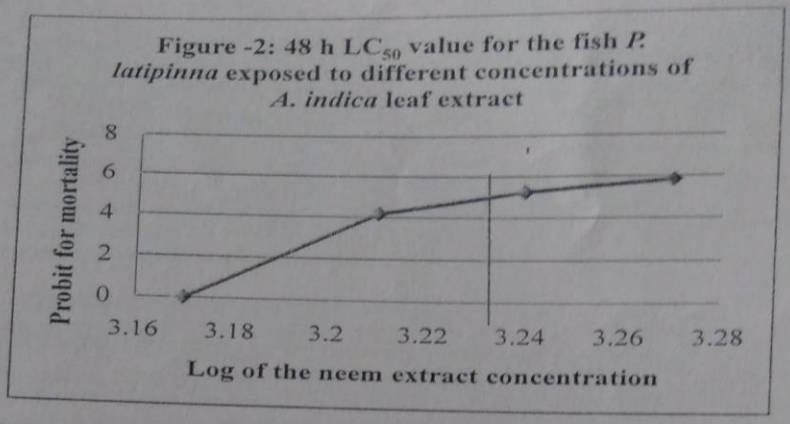

Figure -2: 48 h LC_{50} value for the fish *P. latipinna* exposed to different concentrations of *A. indica* leaf extract

Table-4: 72 h Calculated probit values

Neem extract Concentration (ppm)	Log of the Concentration	Mortality %	Probit for mortality
1500	3.17	0	0
1625	3.21	30	4.48
1750	3.24	60	5.25
1875	3.27	80	5.84
2000	3.30	100	7.37

Figure-3: 72 h LC_{50} value for the fish *P. latipinna* exposed to different concentrations of *A. indica* leaf extract

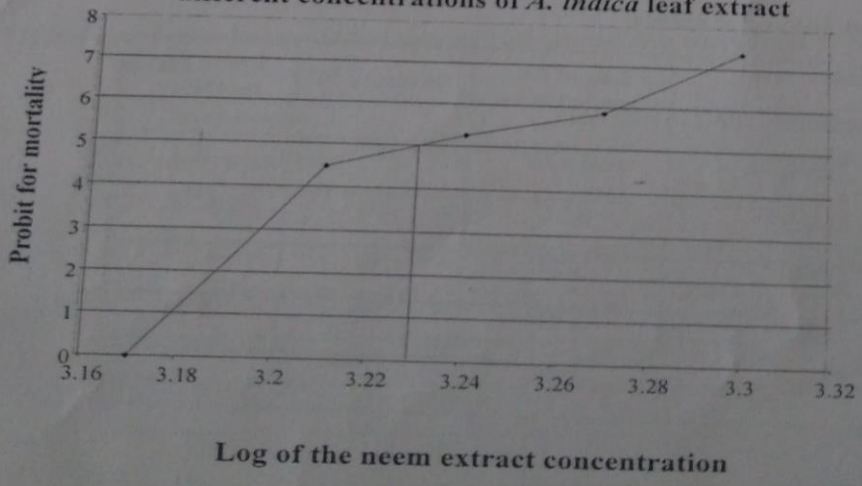

Table-5: 96 h Calculated probit values

Neem extract Concentration (ppm)	Log of the Concentration	Mortality %	Probit for mortality
1375	3.13	0	0
1500	3.17	10	3.72
1625	3.21	40	4.75
1750	3.24	80	5.84
1875	3.27	100	7.37

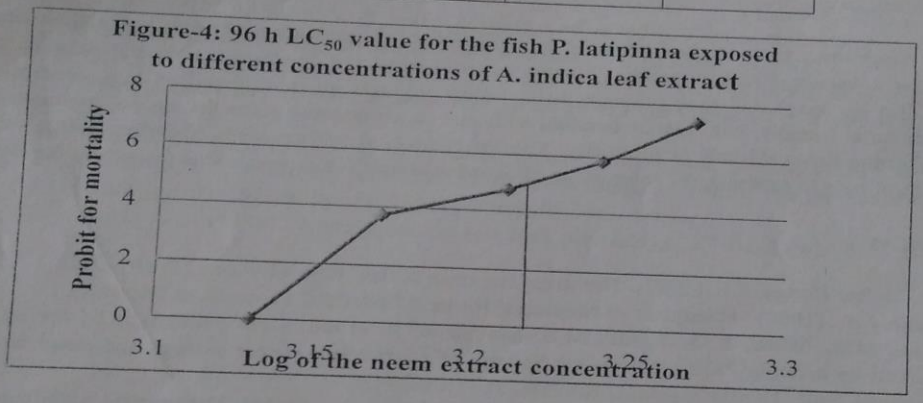

Figure-4: 96 h LC_{50} value for the fish P. latipinna exposed to different concentrations of A. indica leaf extract

4. Discussion

Comparison of the sensitivity of different fish species to neem are questionable, since the amount of active compounds in a given weight of neem varies widely with the part of the plant [10], its place of origin or even the individual tree [11]. Moreover, the toxicity also depends upon solvents and the emulsifiers used for formulating materials [3] as well as the species differences. The acute toxicity values of several neem products for different fish species have been reported earlier by Das *et al.* [12] and Hassanein *et al.* [13]. The variation in the LC_{50} values is due to its dependence upon various factors viz., sensitivity to the toxicant, its concentration and duration of exposure, type and size of the test animal and so on.

Behavioral toxicology is a tool for hazard assessment of water pollution. In the present investigation during acute treatment, behavioral responses such as erratic movements, increasing mucous secretion, body imbalance, surface floating, restlessness and loss of equilibrium were observed. Similar observations were also observed by Tiwari and Singh [14] and Mondal *et al.* [15] when exposed to plant extracts. The initial increase in opercular movement can be taken as the index of the stress felt by fish exposed to plant extract media. This may be a passive response to prevent excess entry of extract molecule present in the medium to minimize damage to gill

epithelium [16]. Control group fishes are free from such type of behavioral changes, so it was clear that only leaf moieties were responsible for the altered behavior and fish mortality.

Plant toxins even at low concentration are known to elicit mucous production on the body and gills of fish [17]. Increased mucous secretion in fish exposed to toxicants is a defense response by which the fish attempts to reduce entrance of the toxicant through the skin and gill surface [18]. The mucous forms a thin film on the delicate and sensitive gill tissue thus minimizing exchange of gases, particularly intake of oxygen [16]. The affected fishes were motionless possibly due to the loss of muscular contraction as a result of interference of the poison with normal functioning of nervous system and consequently the coordination of muscular activities [19]. The mortality rate was concentration dependent suggesting that the degree of exhaustion may have been raised by increasing concentration of the extract [20] and may be due to the entry of active moieties in the fish body [21]. Heath [22] observed that when death occurs in fish under toxicant action, it is usually due to the failure in gill function which may be the case with the exposed fishes.

From the present study it could be concluded that the application of neem leaf extract can be used in aquaculture as environment friendly material instead of deleterious synthetic chemicals. It can also be concluded that although neem based products are considered as less toxic and environmental friendly, but must be handled with care in aquaculture since the excess application can affect the fishes directly or indirectly. The safety level should be assessed before using; this might be of help to establish the safer usage of aqueous extracts of *A. indica* in aquaculture farms.

References

1. Wendelaar Bonga, S.E. (1997). The stress response in fish. Physiol. Rev., 77: 591-625.
2. Duke, J.A., (1987). Handbook of Medicinal Herbs, 5th ed. CRC Press. Boca Raton, FL.
3. Wan, M.T., Watts, R.G., Isman, M.B. and Strub, R. (1996). An evaluation of the acute toxicity to juvenile Pacific Northwest Salmon of azadirachtin, neem extract, and neem based products. Bull. Eviron. Contam. Toxicol., 56 (3): 432-439.
4. Okpanyi, S.N. and Ezeukwv. G.C. (1981). Anti-inflammatory and Anti-pyretic activities of Neem, *Azadirachta indica,* Planta Medica, 41: 34-39.
5. Subapriya, R. and Nagini, S. (2005). Medicinal properties of Neem leaves: a review. Curr. Med. Chem. Anticancer Agents, 5: 149-156.
6. Farah, M.A., Bushra Ateeq and Waseem Ahmad (2006). Antimutagenic effect of Neem leaves extract in freshwater fish, *Channa punctatus* evaluated by cytogenetic tests. Science of the Total Environment, 364 (1-3): 200-214.
7. Winkaler E.U., Santos, T.R.M., Machado-Neto, J.G. and Martinez, C.B.R. (2007). Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish, *Prochilodus lineatus*. Comp. Biochem. Physiol., 145C: 236-244.
8. Regoli, F. and Principato, G. (1995). Glutathione, glutathione-dependent and antioxidant enzymes in mussles, mytilus, galloprovincialis exposed to metals under field and laboratory conditions: Implications for the proper use of biochemical biomarkers. Aquatic Toxicol., 31 (2): 143-164.
9. Finney, D.J. (1971). Probit Analysis. 3rd Edition, Cambridge University Press, London.
10. Luo, X., Ma, Y., Wu, S. and Wu, D. (1999). Two novel azadirachtin derivates from *Azadirachta indica*. J. Nat. Prod., 62: 1022-1024.
11. NRC (National Research Council) (1992). Neem: A Tree for solving Global Problems. National Academy Press, Washington, D.C.

12. Das, B.K., Mukherjee, S.C. and Murjani, O. (2002). Acute toxicity of Neem (*Azadirachta indica*) in Indian Major Carps. J. Aquaculture in the Tropics, 17 (1): 23-33.
13. Hassanein, H.M.A, Okail, H.A. and Mohamed, N.K. (2007). Biochemical changes in proteins and DNA in *Ctenopharyngodon idella* due to environmental pollution with the biopesticide (Trilogy). 10 ICCA, Garyounis University, Benghazi, Libya, 18-21.
14. Tiwari, S. and Singh, A. (2004). Piscicidal and anti-acetylcholinesterase activity of *Euphorbia royleana* stem bark extracts against freshwater common predatory fish, *Channa punctatus*. Environ, Toxicol. Pharmacol., 18 (1): 47-53.
15. Mondal, D., Barat, S. and Mukhopadhyay, M.K. (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.
16. Sambasiva Rao, K.R.S (1999). Pesticide impact on fish metabolism. Discovery publishing House, New Delhi (India), 66-70.
17. Ayuba, V.O. and Ofojekwu, P.C. (2002). Acute toxicity of the root of Jimson's weed, *Datura innoxia* to the African catfish, *Clarias gariepinus* fingerlings. J. Aqua. Sci., 17 (2): 131-133.
18. Agbede, S.A., Adedeji, O.B. and Adeyemo, O.K. (2000). Tissues and organs involved in the non-specific defence mechanism in fish: a review. J. Fish. Tech., 2: 66-72.
19. Gbem, T.T., Balogun, F.A., Lawal, F.A. and Ann, P.A. (1990). Some aspects of the acute toxicity of tannery effluent to juvenile, *Clarias gariepinus* (Teugels). J. Tropical Bio- Science. 20 (1): 53-57.
20. Warren, C.E. (1977). Biology of pollution control. Philadelphia. Saunders Co., 434.
21. Goodman, L.S., Gillman, A.G., Rall, T.W. and Murad, F. (1985). The pharmacological basis of therapeutics. Macmillan Publishing Company, New York.
22. Heath, A.G. (1991). Water pollution and Fish physiology. 2nd Ed. Lewis Publ. Boca Raton, 359.