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Acute Toxicity Assessment of Imidacloprid 70 % WG to a Guppy, *Poecilia reticulata* (Peters)

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Abstract: An experimental routine static bioassay test has been performed to determined the acute toxicity of Imidacloprid 70 % WG to juveniles, males, females and mixed population of a freshwater fish, *Poecilia reticulata* (Peters) for 96 hrs, using water of hardness 560 ± 5 mg/l and pH 7.4 ± 0.3. The LC_{50} values and the 95% confidence limits for different concentrations and time intervals (24, 48, 72 and 96 hrs) were evaluated by Probit analysis statistical method. The 96 hrs LC_{50} values of Imidacloprid 70% WG were estimated as: 68.443, 90.056, 180.593 and 117.614 ppm respectively for juveniles, males, females and mixed population of *Poecilia reticulata*. However, the value of Presumable safe and Safe dischargeable concentrations of Imidacloprid 70 % WG were found to be ranged in between 21.948 ppm to 56.079 ppm and 1.048 ppm to 1.084 ppm respectively. The Juveniles of *Poecilia* were found to be extremely sensitive to this pesticide than males followed by females. The studied fishes also exhibits changes in behavioural pattern during the course of bioassay test, has been noticed.

Key words: Acute toxicity · Imidacloprid 70 % WG · *Poecilia reticulata* and LC_{50} value

INTRODUCTION

Imidacloprid is a neonicotinoid insecticide belongs to Chloronicotinyl nitroguanidine chemical family [1, 2]. Imidacloprid is used for controlling insects, termites and flea on domestic pets and to treat soil, seeds, crops [3]. Neonicotinoids now becomes the largest selling class of insecticide and seed treatments in the world [4]. Neonicotinoid insecticides are synthetic derivatives of nicotine, an alkaloid found in the leaves of many plants like tobacco [5- 7]. It is broken down in water by photolysis [8] but stable to hydrolysis in acidic or neutral conditions. However, hydrolysis increases with increasing alkaline pH and temperature [9]. It has been recommended that toxic product of imidacloprid and other neonicotinoids in water bodies make a correlative links to reduced aquatic insect populations [10] and several insectivorous farmland birds [11] in the Netherlands. Imidacloprid was found to be acutely toxic to adult fish at relatively high concentrations (over 80 ppm), whereas, juvenile fish being considered more susceptible to it. However, it was reported to be extremely toxic at low concentrations to some species of aquatic animals, including the freshwater crustacean, *Hyalella aztecais* and the estuary crustacean, *Mysidopsis bahia* [12].

Thus, looking to the importance of Imidacloprid from agricultural point of view, the attempt has been made to evaluate the LC_{50} values, the 95 percent confidence limits, presumable safe and safe dischargeable concentrations of this pesticide to juveniles, males, females and mixed population of an experimental freshwater fish, *Poecilia reticulata* (Peters, 1859).

MATERIALS AND METHODS

Experimental Fish: All the specimens of the experimental fish, *Poecilia reticulata* (juveniles, males and females) were collected from local sources, were acclimatized separately in plastic tank of 250 liters capacity for 10 days and fed rice bran and oil cake (1:1). Healthy juveniles, males and females of equal sizes 1.0 ± 0.2, 2.8 ± 0.2 and 3.9 ± 0.3 cm respectively were selected for the bioassay tests. For the preparation of stock solution for Imidacloprid 70 % WG, following formula was used:

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$N_1V_1 = N_2V_2$,

where,
N_1 = Concentration of available pesticide,
V_1 = Volume of available pesticide,
N_2 = Required concentration of pesticide to be prepared,
V_2 = Volume of solution required for application.

The series of different concentrations (in ppm) of selected pesticides were prepared by adding the common stock solution into the measured diluents water with the help of micro-pipette. The series of different concentrations of Imidacloprid 70 % WG used in the full scale static bioassay tests were based on the progressive bisection of intervals on a logarithmic scales [13]. The experimental routine static bioassay (up to 96 hrs) for Imidacloprid 70 % WG to the juveniles, males, females and mixed population of *Poecilia* were carried out in 1 liter capacity glass jar containing experimental water of hardness 560 ± 5 mg/l and pH 7.4 ± 0.3.

Preliminary or Screening Tests: The test range for Imidacloprid 70 % WG for the full scale bioassay was taken between the highest and lowest concentrations at which most of the test fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs.

Full Scale Bioassay Test: The toxicities of Imidacloprid 70 % WG were measured by testing various concentrations in the range known by preliminary exploratory test. The test containers of 1 litre glass jars filled with one litre toxicant solution were placed in three rows and each container was labelled with the details of the experiment such as concentration, replicate number, date and time of the experiment. The acclimatized juveniles, males and females of *Poecilia* were transferred to these jars after about 20 minutes of the preparation of test solutions. The bioassays for juveniles, males, females and mixed population of *Poecilia* were conducted for selected pesticides separately. Ten acclimatized tests specimens of fish were taken in each experimental glass jars, however proper controls were run simultaneously. The test solutions were renewed after each 24 hrs by fresh toxicant solutions and the experiments were continued for a period of 96 hrs. The number of test fishes died in each concentration of toxicant solution were observed carefully and noticed at the time intervals of 24, 48, 72 and 96 hrs. The dead fishes were removed from the test solution after knowing their exact mortality (with no body movements). The LC_{50} values and the 95 per cent confidence limits were estimated statistically at different concentration and time intervals (24, 48, 72 and 96 hrs) for selected pesticides by Probit Analysis methods [14]. Presumable safe and safe dischargeable concentrations of Imidacloprid 70 % WG for juveniles, males, females and mixed population of *Poecilia* were calculated by using the formula of Hart *et al*. [15]. Behavioural changes in the studied fishes (juveniles, males and females) were also observed carefully during the course of bioassay test.

RESULTS

The LC_{50} values were recorded for Imidacloprid 70 % WG to the juveniles, males, females and mixed population of *Poecilia* for the time intervals of 24, 48, 72 and 96 hrs have been summarized in table 1 and compared in figure 1. The 24, 48, 72 and 96 hrs LC_{50}'s for juveniles , males and females were observed as: 88.427, 80.405, 71.561 and 68.443 ppm; 125.788, 106.787, 98.210 and 90.056 ppm and 230.417, 207.493, 191.074 and 180.593 ppm respectively. Whereas, the 24, 48, 72 and 96 hrs LC_{50}'s of Imidacloprid 70 % WG for the mixed population of *Poecilia* were recorded as: 152.093, 132.626, 123.398 and 117.614 ppm respectively. The trend of sensitivity for the studied fishes to Imidacloprid 70 % WG was ordered as:

Females < Mixed population < Males < Juveniles (Table 1 and Fig. 1).

Presumable safe concentrations of Imidacloprid 70 % WG were obtained as 21.948, 27.218 and 56.079 and 34.718 ppm however, the safe dischargeable concentrations were recorded as 1.048, 1.084, 1.053 and 1.070 ppm respectively for juveniles, males, females and mixed population respectively (Table 2).

The changes in behavioural pattern in the studied fishes were noted as:

- Lack of coordination in juveniles, males and females at high concentration,
- Imbalanced position,
- Restless movement,
- Dullness with very less/no opercular movement,
- At the time of death, fish settled down on bottom with belly upside and deposition of whitish slimy substances on the bottom after death.

The upper and lower confidence limits for median lethal concentrations (LC_{50}'s) of the Imidacloprid 70 % WG and their significant ratios for juveniles, males, females and mixed population of *Poecilia reticulata* were estimated and depicted in Table 3.

Table 1: Median lethal concentrations (LC_{50}'s) of Imidacloprid 70 % WG (in ppm) for 24, 48, 72 and 96 hrs to juveniles, males, females and mixed population of *Poecilia reticulata*.

	LC_{50}'s of Imidacloprid (ppm)			
Duration (hrs)	Juveniles	Males	Females	Mixed population
24	88.427	125.788	230.417	152.093
48	80.405	106.787	207.493	132.626
72	71.561	98.210	191.074	123.398
96	68.443	90.056	180.593	117.614

Table 2: Safe or harmless and safe dischargeable concentrations (as ppm) of Imidacloprid 70% WG for juveniles, males, females and mixed population of *Poecilia reticulata*.

Concentrations (as ppm)	Juveniles	Males	Females	Mixed population
Safe or harmless	21.948	27.218	56.079	34.718
Safe dischargeable	1.048	1.084	1.053	1.070

Table 3: 95 per cent confidence limits for 24, 48, 72 & 96 hrs LC_{50}'s of Imidacloprid 70 % WG for the juveniles, males, females and mixed population of *Poecilia reticulata*.

	Juveniles			Males			Female			Mixed population		
Duration (hrs)	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R
24	77.892	122.252	1.569	108.756	234.273	2.154	208.490	264.445	1.268	135.264	198.563	1.467
48	72.555	91.546	1.261	92.462	132.230	1.430	181.531	236.619	1.303	116.337	155.023	1.332
72	63.442	79.094	1.246	81.457	115.485	1.417	167.929	210.599	1.254	108.310	137.965	1.273
96	61.185	74.625	1.219	67.167	103.646	1.543	140.882	206.556	1.466	53.949	147.461	2.733

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; R = Confidence Ratio (UCL/LCL)

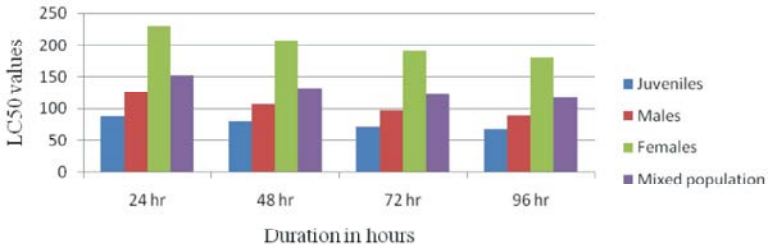

Fig. 1: Bar diagram showing Median lethal concentrations (LC_{50}'s in ppm) of Imidacloprid 70% WG for 24, 48, 72 and 96 hrs to juveniles, males, females and mixed population of *Poecilia reticulata*

DISCUSSION

Findings of present investigation illustrate that Imidacloprid 70% WG is acutely toxic to the Freshwater fish, *Poecilia reticulata* as their LC_{50} values were recorded in ppm. The 96 hrs LC_{50} values of Imidacloprid 70% WG were noticed as as: 68.443, 90.056, 180.593 and 117.614 ppm respectively for juveniles, males, females and mixed population of *Poecilia reticulata*.

According to EXTOXNET database, Oregon State University [16], 48 hr LC_{50}'s of Imidacloprid for *Daphnia magna* was recorded as 85 mg/l, whereas, 96 hr LC_{50} value of Imidacloprid for mysid shrimp and rainbow trout were estimated as 37 and 211 mg/l respectively. However, LC_{50} values of Imidacloprid for a 96 hrs exposure were recorded as 237 mg/l for golden orfe (*Leuciscus idus*) and 21 mg/l for rainbow trout *Oncorhyncus mykiss* [2]. Stoughton *et al*. [17] have assessed the toxicity of imidacloprid to the aquatic invertebrates, *Chironomus tentans* and *Hyalella azteca* for 96 hrs using both technical material (99.2 % pure) and Admire, a commercially available formulated product (240 g/l). Theses authors estimated the 96 hrs LC_{50} values of technical Imidacloprid and Admire as: 65.43 and 17.44 µg/l respectively for *H. azteca* and 5.75 and 5.40 µg/l respectively for *C. tentans*. Pestana *et al*. [18] have estimated the EC_{50} value of imidacloprid for *Daphnia magna* as 96.65 mg/l. However, this value declined to 90.68 mg/l upon adding the predators to the water as an additional stress. U.S. EPA [19] has reported the LC_{50} for Imidacloprid to the wide spread freshwater crustacean, *Hyalella azteca* as 55 ppb and is also classified as very highly toxic. Imidacloprid's LC50 for the

estuary crustacean, *Mysidopsis bahia* was reported as 37 ppb. Further, U.S. EPA [20] also noticed behavioural effects in animals, survived during the exposure *viz*; letharginess and loss of equilibrium. Further, it has been reported that mayflies (Ephemeroptera) and caddisflies (Trichoptera) becomes the most sensitive species with LC_{50} and EC_{50} values in the range of 0.1– 0.3 µg/l in both acute and chronic tests among aquatic fauna and immobility of mayflies and caddisflies were noticed in the range of 0.1 to 0.2 µg/l imidacloprid concentrations after a 96 hour exposure [21].

Whereas, Qadir *et al*. [22] noticed a decline in total Serum protein (mg/dl) in Imidacloprid treated fish, *Labeo rohita* for 2, 4 and 8 days at sub-lethal concentration (120 mg/l) and estimated these value as 2.85±0.90, 3.83±0.58 and 1.95± 0.07 mg/dl for control and 0.85±0.08, 2.85±0.68 and 1.65±0.21 mg/dl for treated fish respectively. Devashree *et al*. [23] observed the effect of Imidacloprid (pesticide) on the microbial population (i.e. fungi, actinomycetes and bacteria) of tea soil and rhizosphere. However, recent work on Imidacloprid for different plant and animal species have been carried out by various researchers *viz*; Abbas *et al*. [24] against whitefly, jassid and thrips on different cotton cultivars, Ibrahim *et al*. [25] in neonatal Rats, Iqbal *et al*. [26] in stored wheat grain at different temperatures. Findings of present investigation are also in agreement with the literature cited by previous authors in terms of most of the parameters has been estimated.

Since, aquatic invertebrates have a risk factor to become sensitive, due to persistence and transfer of neonicotinoids insecticides in the water bodies which directly affects the aquatic vertebrate, linked to it through food chain [27, 28]. Therefore, we recommended here to the user of Imidacloprid (a neonicotinoid insecticide), that they should be careful regarding environmentally safe concentrations to manage the health of aquatic ecosystem from aquaculture point of view and also to prevent the detoriation of natural resource .

REFERENCES

1. Wismer, T., 2004. Novel Insecticides. Clinical Veterinary Toxicology, Plumlee, K.H., (Ed.), Mosby, St. Louis, MO, pp: 184-185.
2. Tomlin, C.D.S., 2006. The Pesticide Manual, A World Compendium, 14th ed., British Crop Protection Council, Surry, England, pp: 598-599.
3. Meister, R.T., 2000.Farm Chemical Handbook 86. *Meister Publishing Company*, Willoughby, OH.
4. Jeschke, P., R. Nauen, M. Schindler and A. Elbert, 2010. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem., 59: 2897-2908.
5. Copping, L.G., 2001. The Biopesticide Manual, 2nd ed., British Crop Protection Council, Surrey, England, pp: 202-203.
6. Ware, G.W. and D.M. Whitacre, 2004. The Pesticide Book, Meister Pro Information Resources, Willoughby, OH, pp: 70-71.
7. Costa, L.G., 2008. Toxic Effects of Pesticides. Casarett and Doull's Toxicology: The Basic Science of Poisons, 7th ed.; Klaassen, C.D. (Ed.) McGraw Hill, New York, NY, 907: 922-930.
8. Liu, W., W. Zheng, Y. Ma and K. Liu, 2006. Sorption and Degradation of Imidacloprid in Soil and Water. Journal of Environmental Science and Health, 41: 623-634.
9. Zheng, W. and W. Liu, 1999. Kinetics and mechanism of the hydrolysis of imidacloprid. Pesticides Science, 55: 482-485.
10. Van Dijk, T.C., M.A. Van Staalduinen and J.P. Van der Sluijs, 2013. Macro-invertebrate decline in surface water polluted with imidacloprid. PLoS One 8, e62374.
11. Hallmann, C.A., RP.B. Foppen, C.A.M. Van Turnhout, H. De Kroon and E. Jongejans, 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature, 511: 341-343.
12. Pesticides News No. 62. , 2003. Imidacloprid retrieved from http://www.pan-uk.org/pestnews/Actives/imidaclo.htm on 2010-08-31.
13. American Public Health Association (APHA), 2005. American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Sandard methods for the examination of water and wastewater, 21st Edn, American Public Health Association, Washington D. C.
14. Finney, D.J., 1971. Probit Analysis, University Press, Cambridge, pp: 333.
15. Hart, W.B., P. Doudoroff and J. Greenbank, 1945. The evaluation of the toxicity of industrial wastes, chemical and other substances to freshwater fishes. Atlantic Refining Co. (Phill), pp: 317.
16. Extension Toxicology Network (EXTOXNET), 1998. Imidacloprid Pesticide Information Profile. [Online]. Available at http://extoxnet.orst.edu/pips/imidaclo.htm. (Verified 09 January 2006). Oregon State University, Corvallis, OR.

17. Stoughton, S.J., K. Liber, J. Culp and A. Cessna, 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions. Archives of Environmental Contamination and Toxicology, 54(4): 662-73.
18. Pestana, J.L., A.C. Alexander, J.M. Culp, D.J. Baird, A.J. Cessna and A.M. Soares, 2009. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. Environmental Pollution, 157(8-9): 2328-34.
19. U.S. Environmental Protection Agency, 1992a. Office of Prevention, Pesticides and Toxic Substances NTN 33893 (imidacloprid), data evaluation records. Memo from A.F. Maciorowski, *Ecological Effects Branch*, to D. Edwards, Registration Div. Washington, D.C., Dec.22.
20. U.S. Environmental Protection Agency, 1992b. Data evaluation record, NTN 33893. MRID No. 420553-19. Washington, D.C., Mar. 27.
21. Roessink, I., L.B. Merga, H.J. Zweers and P.J. Van Den Brink, 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. Environ. Toxicol.Chem., 32: 1096-1100.
22. Qadir, S., L. Abdul, A. Muhammad and I. Furhan, 2014. Effects of Imidacloprid on the hematological and serum biochemical profile of *Labeo rohita*. Pakistan J. Zool., 46(4): 1085-1090.
23. Devashree, Y., B.K. Dutta, S.B. Paul and S. Choudhury, 2014. Effect of Imidacloprid on the Soil and Rhizosphere Microflora of Tea Agro-Ecosystem. Global Journal of Biotechnology & Biochemistry, 9(2): 35-40.
24. Abbas, Q., M.J. Arif, M.D. Gogi, S.K. Abbas and H. Karar, 2012. Performance of Imidacloprid, Thiomethoxam, Acetamaprid and a Biocontrol Agent (*Chrysoperla carnea*) Against Whitefly, Jassid and Thrips on Different Cotton Cultivars. World Journal of Zoology, 7(2): 141-146.
25. Ibrahim, K.A., M.A. El-Desouky, H.M. Abou-Yousef, K.H. Gabrowny and A.S.M. El-Sayed, 2015. Imidacloprid and/or Esfenvalerate Induce Apoptosis and Disrupt Thyroid Hormones in Neonatal Rats. Global Journal of Biotechnology & Biochemistry, 10(3): 106-112.
26. Iqbal, S., S. Saied, R. Uddin, A. Bhutto, S. Nadir Ali, N. Sahar and M. Ahmed, 2014. Half-Life Study of Imidacloprid in Stored Wheat Grain at Different Temperatures. American-Eurasian J. Agric. & Environ. Sci., 14(2): 172-178.
27. Goulson, D., 2013. Review: an overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol., 50: 977-987.
28. Gibbons, D., C. Morrissey and P. Mineau, 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ. Sci. Pollut. Res., 22: 103-118.