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ORIGINAL ARTICLE

A Comparative Analysis of Acute Toxicity of Synthetic Pesticide Nuvan and Biopesticide Neem Oil On Freshwater Catfish *Clarias batrachus*

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ABSTRACT

An extensive challenge of agronomist is to augment food materials to fulfill the rapidly growing world population demands, without any adverse impact on the environment. Today, any edible thing in market is not without the use of any hazardous chemical or pesticide. In modern agricultural practices, the pest's management is normally accomplished by means of the enormous use of pesticides and other harmful agrochemicals, which leads to health and environmental hazards. Aquatic animals mostly fishes are more frequently exposed to such pollutants through the runoff from the agricultural fields and that may affect the humans and other animals through the food chain. Hence, biopesticides or natural pesticide can select as a better option for replacing the toxic chemicals and synthetic pesticides which enable to safer pest management and prominent for environmental security. Among the different biopesticides, neem oil is one of the beneficial biopesticide and minutest toxic to humans and other livings, so it is most optimistic for the control of various pests without adverse effects on the environment. In this work acute toxicity of Nuvan (synthetic pesticide) and Neem Oil (biopesticide) was separately accomplished on catfish Clarias batrachus. Experimental data collected from the toxicity study were assessed by using the Probit Analysis Statistical Method. The 96h LC_{50} value of Nuvan and Neem-Oil on test fish Clarias batrachus was found to be 0.274 ml/L and 0.848 ml/L respectively. Nuvan showed most toxic effect at low concentration compare to neem oil for Clarias batrachus. Biopesticides are completely biodegradable and target specific compare to highly non-degradable broad spectrum synthetic chemicals and other toxicants. Therefore, use of biopesticides is cost effective alternative to synthesized pesticides and more eco-friendly.

Key words –Nuvan, Neem-Oil, *Clarias batrachus*, Toxicity, 96h LC_{50}

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INTRODUCTION

Pesticides and other harmful chemicals nowadays are used in enormously of the accompanying substances available in the market, through which the food can be preserved for a maximum time. The agricultural farmers and aqua precisian apply toxic chemicals and pesticides in their agricultural fields for enhancing crop yield and for the control of pests and insect vectors of diseases. Although, such type of different pesticides is not always useful, it has also adverse effect on the environment. Residues of harmful pesticides can be noted in a great variety of our foods and beverages, including for instance fruit juices, water, packed foods, animal feeds, wine and other refreshments. Furthermore, washing and peeling of food materials cannot surely remove the residues from these materials. Different types of pesticides after being used in agricultural purposes ultimately notice their way into aquatic ecosystems including freshwater resources. They also contaminated rivers, ponds, and lakes by the runoff, after rain fall, spillage, drifts, industrial effluents, areal sprays and transport from soil treated with pesticide through agricultural fields and other resources and ground water through percolation and leaching becomes the reason of water pollution which is not safe for animal's health including humans and fishes. Not only that but also pesticides make a mark their toxic effects in different forms ranging from changes within a single cell, whole organism or even alterations in whole population [1]. Such type of negative

effects of pollutants become significant when they affect economically essential organisms or affect those living being which are consumed by economically essential human beings and other animals create stressful conditions either in the form of physiological, behavioral, biochemical, histo-pathological, damage to vital organs or even death of living beings of all types of ecosystem. Worldwide use of pesticides for agricultural purposes is now a worldwide concern.

Natural water conditions are necessary for proper fish culture. But present time several types of pesticides like organophosphate, carbamates, organochlorine and pyrethroids are used in agriculture and other purposes, which cause pollution in aquatic bodies. Nuvan is an organophosphorus pesticide and inhibits the activity of acetyl cholinesterase enzyme. The inhibitory impacts of organophosphorus pesticides are rely on its binding affinity to the enzyme active site and through its rate of phosphorylation connection to the age and behavior of any living. This compound attacking the nervous system and to kill the pests quickly and effectively and causing inactivation of acetyl cholinesterase enzyme, which is accountable for controlling the nerve impulse transmission. Nuvan or DDVP or Dichlorvos is registered to control insects and pests in agro-fields, commercial, industrial, institutional sites; in and around homes etc.

Because of serious setbacks originated from the use of chemical or synthetic pesticides on living systems and environment, the use of plant originated bio-pesticides or botanical pesticides is gaining momentum. The biopesticides are less persistent and biodegradable came into continuation and are used as alternative to synthetic organophosphorus pesticides recently in agricultural purposes. Biopesticides have phyto-chemicals which are manufactured naturally for defense against phytophagous and disease spreader pests, insects, and parasites. Day by day the plant-derived substances or natural pesticides are now being used mostly for the controlling of insect and pests [2, 3]. Biopesticide Neem oil is extracted basically from the seeds or leaves of the neem tree. The neem tree (*Azadirachta indica)* a medicinal plant is the source of neem oil biopesticide belonging Family- Meliaceae which contains approximately 35 biologically active compounds. Azadirachtin, main component of neem oil is the predominant insecticidal active substance that fulfils several of the criteria needed for a good pesticide. Azadirachtin is recently used for control of pests and other harmful insects [4, 5]. Azadirachtin is biodegradable which diminishes within 100 hours when exposed to light and water and exhibits very low toxicity to the mammals [6]. Neem oil is also used in aquatic ecosystem for the control of fish fry predators and fish parasites [7]. It kills harmful insects and pests but does not any harm to beneficial insects like honey bee, beetles etc. because harmful insects swallow the leaves of the plants and crops while beneficial insects do not eat the plant and crop leaves.

Fishes are most frequently sensitive to the aquatic environment or water contamination. However, most farmed fishes have much loaded than wild fishes from natural and man-made toxicant, e.g., pesticides, and other chemical pollutants. The toxicants from fishes can pose health issues to unsuspecting consumers particularly like pregnant and nursing women and other livings through the food chain. The adjustment and international failure for aquaculture trades are uncommonly compound, with different agencies managing aquaculture practices, including polluted control, water quality, feed supply, food safety and site selection [8]. Poisoning by synthetic pesticides to people, livestock, and wildlife have found when appropriate cares do not take. Pesticides practitioners must be highly careful to avoid health hazards. Biopesticides are expected to hold a major share of the market in future owing to their multiple advantages over their chemical counterparts [9]. This work is an endeavor to evaluate the toxic concentration of Nuvan and Neem Oil in fish *Clarias batrachus* to determine the safe dose for sustainable development of environment.

MATERIAL AND METHODS

Healthy and active adult *Clarias batrachus* which is affiliated with the class Actinopterygii, has been selected as an experimental test animal for this study. They were obtained from local fish market of Agra (U.P) in the month of December and nourished in laboratory conditions when room temperature ranged between 15^0C-25^0C to acclimatize the fish for two weeks prior to start work for laboratory environment. Test fishes weighed range 60-120 g and their length was within the range 20-25 cm. The test fish *Clarias batrachus* were kept in large and clean aquaria measuring 75cm x 37cm x 37cm. The experimental fishes were carefully noticed and treated with 2% $KMnO_4$ solution prior to stocking to get rid of any dermal infection. The fishes were provided commercial fish food materials daily at 2.5% of body weight which was given at morning hours. Throughout the course of work, tap water was used. At alternate day the water of aquarium was replaced and daily draws off faecal matter and uneaten feed was done. Different physiochemical properties of test water like hardness, temperature and pH were continuously noted. Neem oil and Nuvan (DDVP) purchased from local market at Agra. Nuvan (Dichlorvos 76% EC) insecticide

is manufactured by insecticides (India) limited and Neem oil is a product of Coromandel Agro Products and Oils Pvt. Ltd which is used in present observation. Acute toxicity study was carried out to decide the potency of synthetic pesticide nuvan and biopesticide neem oil for static but renewal type of bioassay.
The harmful concentration of synthetic pesticide nuvan and bopesticide neem oil recognize as the median lethal concentration (LC_{50}) at which 50% fish mortality take place. The test fishes *Clarias batrachus* were divided in to four different groups (A, B, C, and D) in four aquaria to determine the LC_{50} value of nuvan at first and then after neem oil similarly. Each glass aquarium filled with 20 L dechlorinated water and consists of ten experimental fishes in each group. Different concentration of nuvan (1, 5, 10 and 15 ml) and neem oil (10, 15, 20 and 25 ml) separately were given to the experimental fishes with 20 L of water in each aquarium and a control set was run with the experimental groups simultaneously. The survival numbers of *Clarias batrachus* fishes were noted for each concentration after 24hrs, 48hrs, 72hrs and 96 hrs. After 96 hours the mortality percentage was recorded. The control (unexposed), nuvan and Neem Oil exposed fishes were continuously observed during test periods. Dead fishes (if any) were removed from aquaria to avoid water fouling. The LC_{50} concentration for 96h was analyzed by probit analysis method of Finney, [10]. On the basis of two variables, log-dose and empirical probit, regression line was depicted on the simple graph paper to find out the expected probit which is necessary for LC_{50} determination.

RESULTS

The 96h LC_{50} value of synthetic pesticide Nuvan and biopesticide Neem Oil was found to be 0.274 ml/L and 0.848 ml/L respectively. The LC_{50} concentration of both nuvan and neem oil for 96h was calculated by probit analysis method of Finney, [10]. Table- (1) and (2) shows the mortality percentage of experimental fishes *Clarias batrachus* after exposure of Nuvan and Neem Oil respectively. Table (3) represents evaluation of nuvan and neem oil toxicity respectively to *Clarias batrachus* specifying fiducial limits and LC_{50}. Graphs (I and II) below show the plot of Finney's probits against log dose for assessing LC_{50} value of both nuvan pesticide and neem oil biopesticide.

Table 1 – Number of survived fishes and mortality percentage of *Clarias batrachus* with different doses of synthetic pesticide Nuvan

Group	Doses of Nuvan (ml/20L)	No. of fishes	Exposure time (hours)				No. of died fishes	No. of survived fishes	Mortality Percentage
			24 h	48 h	72 h	96 h			
A	1	10	1	0	0	0	1	9	10%
B	5	10	2	1	0	0	3	7	30%
C	10	10	2	2	1	1	6	4	60%
D	15	10	3	3	2	2	10	0	100%

Table 2- Number of survived fishes and mortality percentage of *Clarias batrachus* with different doses of biopesticide Neem oil-

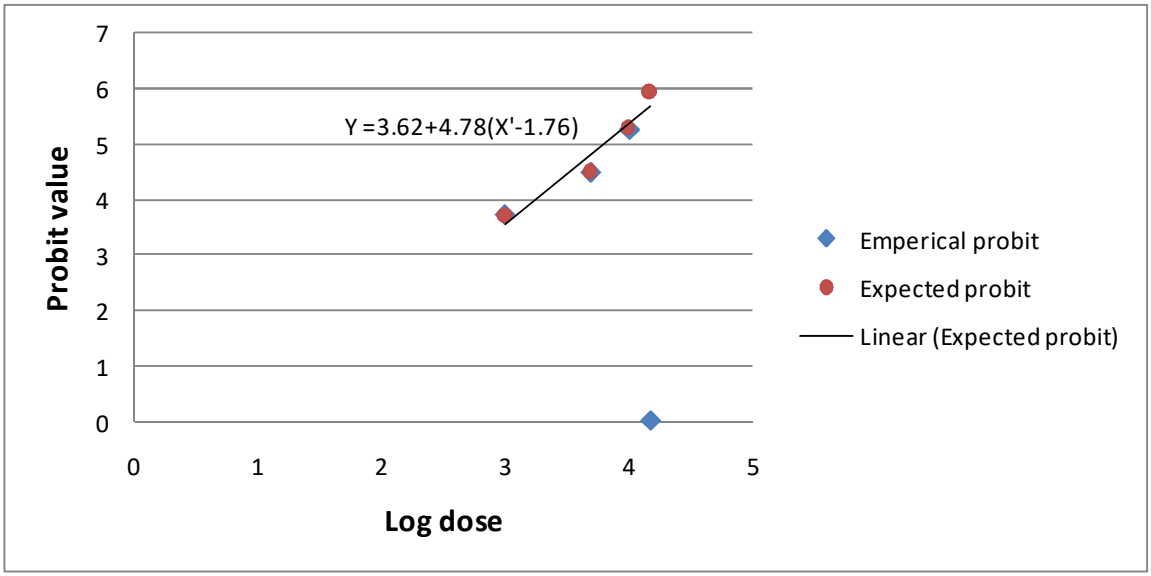
Group	Doses of Neem Oil (ml/20L)	No of fishes	Exposure time (hours)				No, of died fishes	No, of survived fishes	Mortality Percentage
			24 h	48 h	72 h	96 h			
A	10	10	-	-	1	-	1	9	10%
B	15	10	2	-	1	-	3	7	30%
C	20	10	2	2	1	1	6	4	60%
D	25	10	3	2	2	1	8	2	80%

Table 3 -Toxicity assessment of Nuvan and Neem oil to *Clarias batrachus* for determining fiducial limits and LC_{50}

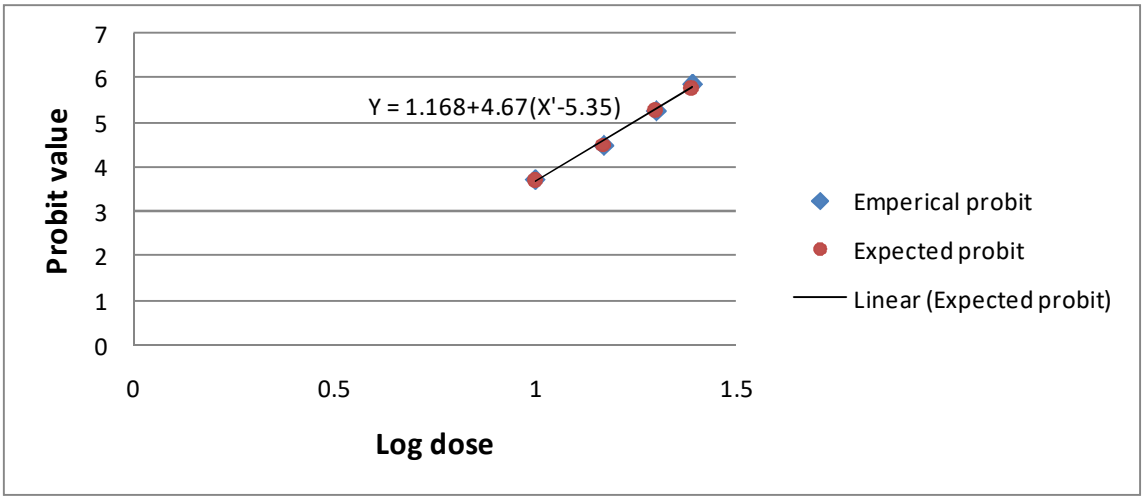
Test fish	Test exposure compound	Regression Equation	Variance	Fiducial limits	Mean of F. limits (M)	LC_{50}
Clarias batrachus	Nuvan (76%) (DDVP)	Y=3.62+4.78(X'-1.76)	0.017	3.76332(+) 3.69668(-)	3.73	5.4869 ml/20L (or) 0.274 ml/L
	Neem oil	Y=1.168+4.67(X'-5.35)	0.002	1.23292(+) 1.22508(-)	1.229	16.96 ml/20L (or) 0.848 ml/L

After exposure of both the pesticides, the experimental fishes *Clarias batrachus* showed many behavioral alterations. They were aggregated at one corner of the aquarium and showed irregular and erratic swimming movements and loss of equilibrium. The specimens showed hyper excited, became lethargic

slowly, restless, and excess mucus secreted all over their bodies. The experimental fish *Clarias batrachus* showed unusual behavior of trying to come out from the pesticide concentrations, indicated as an escaping phenomenon. They generally scrolled with gapping and lastly, sank to bottom with their slow opercula movements and died. The behavioral responses of experimental fishes were more prominent for toxic synthetic organophosphorus pesticide Nuvan as compared to biopesticide Neem oil. Hence, percentage of fish mortality found more at low concentration of nuvan while more concentration of neem oil showed less mortality.



Graph (I) - Finney's probits plot against log dose for calculating LC_{50} value of synthetic pesticide Nuvan to freshwater catfish *Clarias batrachus*.



Graph (II) - Finney's probits plot against log dose for calculating LC_{50} value of bio-pesticide Neem Oil to freshwater catfish *Clarias batrachus*.

DISCUSSION

Recently botanical pesticides or biopesticides are developed to replace toxic synthetic pesticides. The synthetic pesticides are effective and target specific but their impact on the environment of all organisms is mostly dangerous. Biopesticides contain active compounds with short half-life period and do not elevate the levels of harmful residues in environment, unlike other chemical pesticides [11]. Biopesticide neem oil containing azadirachtin is less toxic to test fishes *Clarias batrachus* compared to nuvan. The same concentration of both nuvan and neem oil have not similar impacts on test fishes because biopesticide is comparatively less effective compare to nuvan for the experimental catfishes *Clarias batrachus* which can be seen in the fishes behavioral changes and fish mortality percentage. Different

behavioral alterations due to various pesticides on *Clarias batrachus* have been studied by various workers [12, 13, 14]. In this study, the nuvan and neem oil can cause marked behavioral responses in fish and thus evaluated an effective sign of physiological stress in fish [15]. Hence it is also realized that specimen more reactive at very low concentration of organophosphorus pesticide nuvan compare to biopesticide neem oil.

An essential amount of pesticide to cause death is known as lethal concentration. Acute toxicity investigation for the model fish is first step to determining water quality and the studies disclose the harmful concentrations that cause the fish mortality even at less concentration of exposure [16]. The conventional LC_{50} assessment in aquatic toxicology is mostly used to analyze the potential risk of an unsafe chemical. In the present study it was found that 96h LC_{50} value of synthetic pesticide nuvan is 0.274 ml/L. But LC_{50} value of botanical pesticide or biopesticide neem oil is four time much higher (0.848 ml/L) than the nuvan. Thus higher value of neem oil is indicating the less harmful nature of the botanical pesticide [17]. Similarly, Israel *et al*. [18] observed 96 h LC_{50} of azadirachtin and deltamethrin for *Poecilia reticulate* and found that LC_{50} value of azadirachtin has greater than that of the chemical pesticide deltamethrin.

Rani and Gautam [19] recorded median lethal concentration of nuvan for freshwater fish *Channa punctatus* 0.27 ml/L at 96 h. The comparative hazardous effects of nuvan and chlorpyrifos analyzed by Ashade *et al*., [20] and noted LC_{50} value due to exposure of nuvan after 96 h as 0.184 ml/L of African catfish *Clarias gariepinus* while in *Channa punctatus* at 96 h LC_{50} value analyzed 0.024 ml/L by Kumar [21]; Kumar and Gautam, [22] and they also investigated biochemical alterations due to toxicity of nuvan in *Channa punctatus* (Bloch.). Gautam *et al*., [23] evaluated LC_{50} value at 96 h as 0.07 ml/l. and harmful impact of nuvan on biochemical indices of catfish *Clarias batrachus*. Worldwide research records found effect of pesticides on aquatic livings [24]. In the present work LC_{50} value calculated as 0.274 ml/L for catfish *Clarias batrachus* due to the exposure of nuvan which is greater than the value reported by many other workers like Verma *et al*., [25] noted LC_{50} 8.9 mg/L at 96 h in *Clarias batrachus*; in *Cirrhinus mrigala* for 96 h it was 9.1ppm [26]; Bhat *et al*., [27] recorded LC_{50} value as 42.66ppm in fish *Labeo rohita* after 96 h ; in *Heteropneustes fossilis* LC_{50} value after 96 h reported as 6.45 mg/L by Ahmad and Gautam, [28] treated with nuvan. LC_{50} value of carbaryl as 2.5 ml and parathion LC_{50} as 0.45 ml in freshwater catfish *Clarias batrachus* observed by Rather, [29] which is greater than the LC50 value of nuvan noted in present investigation.

Consequently, the effects of Neem oil on catfish *Clarias batrachus* were investigated and on other fishes studied by many other researchers. Hassanein *et al*., [30] evaluated LC_{50} value as 112 ppm of a biopesticide (Triology) at 96 h on the grass carp fish, *Ctenopharyngodon idella*. Cagauan *et al.,* [31] evaluated LC_{50} value of neem to Nile tilapia *Oreochromis niloticus L*. and mosquito fish *Gambusia affinis* Baird and Girard were 12.4 ml/L and 8.31 ml/L respectively and the corresponding LC_{50} values were 2.57 and 3.0 ml/L at 96 h. LC_{50} value of *Azadirechta indica* leaves extract as 785.4 mg/L which was 68 more-times greater than LC_{50} value of acetamiprid as 11.62 mg/L analyzed by Alam *et al.,* [32] in fish *Labeo rohita*. . The LC_{50} value for 96 h of azadirachtin for *Heteropneustes fossilis* is 52.35 mg/L were observed by Kumar *et al*., [33]. The lethal effects of neem oil to freshwater catfish *Clarias batrachus* expressed as 96 hours LC_{50} values found in the present experiment (0.848 ml/L) is manifold larger than the earlier findings [34, 35, 36]. Such differences in the lethality may be due to differences in used fish species, test methods and water quality, their age, sex and size [31].

Further, variation in median lethal concentration (LC_{50}) value for freshwater catfish *Clarias batrachus* in the present study may be associated to their strong nature and activeness to survive with unfavorable condition for being air-breathing catfish [37]. The susceptibility of various fish species to neem oil may be depending on its origin, parts or even individual neem tree and also related to differences in the amount of active compound azadiractin present in neem oil (Lue *et al.,* [38]. With the progress of exposure time toxicity element increases for nuvan and neem oil to *Clarias batrachus* in the present observation that is conformity to the conclusion noted by Dhara *et al*., [39] to *O. mossambicus*. Oniovosa *et al.* [40] studied that neem leaves extract administered by feed at sub lethal treatment was comfortably tolerated by the African catfish (*Clarias gariepinus*) with none or negligible adverse effects on organ histology, biochemical parameters and haematological indices. Because of greater LC_{50} value of Neem oil compare to nuvan, it has considered as less toxic towards non-targeted organisms including fish.

CONCLUSION

It is already known that the concept of profitable food production needs to be implemented in order to provide safe food items to the society. The lethal toxicity values of nuvan and neem oil of *Clarias batrachus* in the present investigation serve as basic information on its lethality which may be supportive

in formulating the dose of nuvan and neem oil in aquaculture management. To decline the chemical loads on the terrestrial and aquatic environment, it is recommended that use of biopesticides or plant based pesticides should be encouraged [41] which can prove to be helpful in creating an environment free from toxicants. Biopesticides disintegrate normally into components leaving without any ineradicable impression in different zone of environment [42].

Although neem oil is considered as low toxic and eco-friendly, but precautions must be taken when these are used in different purposes since the overdose can affect the life of the organisms. This work can also be helpful to compare the liability of different species of aquaculture and potency of synthetic pesticides and biopesticides using LC_{50} values so generate safe concentration for sustainability of environment. A low dose of a highly toxic pesticide (nuvan) may be more harmful than a large dose of a low toxic pesticide (neem oil). It is therefore imperative to explore sustainable, public health and environment friendly alternative for prevention of adverse health effects of many creatures including humans and fishes.

REFERENCES

1. Giari, L., Simoni E., Monera M., Dezfuli B.S., (2008). Histo-cytological responses of *Dicentrarchus labrax* (L.) following mercury exposure, Ecotox. Environ. Safe. 70: 400–410.
2. Sileshi, G.W, Nyeko P., Nkunika P.O.Y., Sekematte B.M., Akinnifesi F.K., Ajayi O.C. (2009). Integrating ethno-ecological and scientific knowledge of termites for sustainable termite management and human welfare in Africa. Ecology Society. 14: 48.
3. Dubey, N.K., Shukla R., Kumar A., Singh P., Prakash B., (2010). Prospects of botanical pesticides in sustainable agriculture. Curr. Sci. 98: 479–480.
4. Mondal, D., Barat S., Mukhopadhyay M.K., (2007). Toxicity of neem pesticides on a fresh water loach, *Lepidocephalichthys guntea* (Hamilton Buchanan) of Darjeeling district in West Bengal, J. Environ. Biol. 28:119–122.
5. Senthil Nathan S., Young Choi M., Yul Seo H., Hoon Paik C., Kalaivani K., Duk Kim J. (2008). Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stal), Ecotox. Environ. Safe. 70: 244–250.
6. Wikipedia. Available online (2018).
7. Winkaler, E.U., Santos J.G., Machado-Neto, C.B. (2007). Martinez, Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus*. Comp. Biochem. Physiol. C 145: 236–244.
8. David, W.C., Richard C., Steven J., Gaydos J.G and William WC, (2009). Aquaculture Environmental Toxicological, and Health tissues. Int. J. Hyg. Environ Health, 212: 369-377.
9. Kumar Jitendra, Ayyagari Ramlal, Dharmendra Mallick and Vachaspati Mishra (2021). Review- An overview of some biopesticides and their importance in plant protection for commercial acceptance. Plants. 10; 1185.
10. Finney, D.T., (1971). Probit analysis. Cambridge University Press: Cambridge. 333.
11. Menezes, ML, Dalbeto AC, Cruz C, Neto JMG. (2004). Determination of biopesticides Azadirachtin in samples of fish and in samples of water of fish ponds, using chromatography liquid of high performance. Salusvita 23: 401–414.
12. Narra, Madhusudan Reddy (2016). Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus*: A long-term monitoring. Chemosphere 144: 966-974.
13. Soni Rakesh, Sushant Kumar Verma (2018). Acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to herbicide pretilachlor. Heliyon 4: e01090.
14. Saha, S.; Chukwuka, A.V.; Mukherjee, D.; Patnaik, L.; Nayak, S.; Dhara, K.; Saha, N.C.; Faggio, C. (2021). Chronic effects of diazinon® exposures using integrated biomarker responses in freshwater walking catfish, *Clarias batrachus*. Appl. Sci. 11: 10902.
15. Åkerblom N. (2004). Agricultural pesticide toxicity to aquatic organisms- a literature reviews. Miljöanalaya, Department of Environmental Assessment, Swedish University of Agricultural Sciences, Uppasala, Rapport: 16.
16. Pandey, S., Kumar, R., Sharma, S., Nagpure, N.S., Srivastava, S.K., (2005). Acute toxicity bioassays of mercuric chloride and melathion on air breathing fish *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety.61: 114-120.
17. Ghimeray, A.K., Jin, C., Ghimire, B.K. And Cho, D.H., (2009). Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica*, a Jussgrown in foothills of Nepal. African J. Biol., 8: 3084-3091.
18. Israel, S.S., Kiruba, S., Sam, S. And Manohar, D. (2008). A comparative study on the toxicity of a synthetic pyrethroid, deltamethrin and a neem based pesticide, Azadirachtin to *Poecilia reticulate* Peters 1859 (Cyprinodonti formes: Poeciliidae). Turk. J. Fish. aquat. Sci., 8: 1-5.
19. Rani R. and Gautam R.K (2012). Assessement of nuvan toxicity to lipids in snake headed freshwater fish *Channa punctatus* Environment Conservation Journal 13 (3): 87-93.
20. Ashade, O.O., Ashiru A.W. and Obiri C.M., (2011). The comparative study of the toxic effects of 2-3 diclorovinyl dimethyl phosphate (DDVP) and chlorpyrifos on the behaviour and haematology of African-(*Clarias gariepinus*). Int. J. Sci. Soc. Yaba. 1 (1): 38-47.
21. Kumar S., (2014). Acute toxicity evaluation of Nuvan in liver of *Channa puncatus* (Bloch). Adv. Res. Agri. Veter. Sci., 1(1): 31-34.

22. Kumar, S. and Gautam R.K., (2014). Study of biochemical toxicity of nuvan in *Channa punctatus* (Bloch.). Adv. Res. Agri. Veter. Sci., 1(1): 31-34.
23. Gautam, R.K, Shakya S., Shamim I. and Khajuria V., (2014). Toxic effect of Nuvan (Organophosphate) on blood biochemistry of freshwater fish *Clarias batrachus*. Int. J. Interdiscip. Res. 1 (5): 1-7.
24. Ahalavat Shikha and Wajid Hasan (2018). Acute Toxicity and Behavioural Response in Fresh water Fish *Labeo rohita* Exposed to Floraguard and Neem-On Biopesticides. Int. J. Pure App. Biosci. SPI: 6 (3): 611-616.
25. Verma, S.R., Rani S., Tonk I.P. and Dalela R.C. (1983). Pesticide induced dysfunction in carbohydrate metabolism in three fresh water fishes. Environ Res. 32: 127-133.
26. Velmurugan, B., Selvanayagam M, Cengiz EI and Unlu E. (2009). Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos. Braz Arch Biol Technol; 52(5): 1291-1296.
27. Bhat, Bilal Ahmad, Imtiyaz Ahmad Bhat, Santosh Vishwakarma, Alok Verma and Geeta Saxena (2012). A comparative study on the toxicity of a synthetic pesticide, dichlorvos and a neem based pesticide, neem-on to *Labeo rohita* (Hamilton). Current World Environment; 7(1): 157-161.
28. Ahmad, S.I. and Gautam R.K., (2014). Effect of organophosphate pesticide, nuvan on serum biochemical parameters of fresh water catfish *Heteropneustes fossilis* (Bloch.). Int Res J Environ Sci.; 3(10): 1-6.
29. Rather, Ajaz Ahmad (2015). Biochemical responses induced by sub lethal concentrations of carbaryl and parathion on certain enzymes of freshwater catfish *Clarias batrachus* (Linn.). Int. Res. J. Biological Sci., 4(10): 52-56.
30. Hassanein, H.M.A., Okail H.A., 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.
31. Cagauan, A.G., Galaites M.C. and Fajardo L.J. (2004). Evaluation of botanical piscicides on Nile tilapia *Oreochromis niloticus* L. and Mosquito fish *Gambusia affinis* Baird and Garard. Proceedings on ISTA, 12-16 September, Manila, Phillipines: 179-187.
32. Alam, A., Amtul Bari Tabinda, Mahmood-ul-Hassan, and Abdullah Yasar (2014). Comparative Toxicity of Acetamiprid and *Azadirachta indica* Leave Extract on Biochemical Components of Blood of Labeo rohita Pakistan J. Zool. 46 (6): 1515-1520.
33. Kumar A, Prasad M., Mishra D., Srivastav S.K., Srivastava Ajai K., (2010). Acute toxicity of azadirachtin to a teleost, *Heteropneustes fossilis*. Acta. Sci. Biol. Sci.
34. Suzuki, N., Tabata M.J., Kambegawa A., Ajai K., Srivastav A., Shimada H., Takeda H., Kobayashi M., Wada S., Katsumata T., Hattori A., (2006). Tributylin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts, Life Sci. 78: 2533–2541.
35. Mousa, M.A.A., El-Ashram AMM and Hamed M. (2008). Effect of neem leaf extract on freshwater fishes and zooplankton community. Proceedings of the 8th International Symposium on *Tilapia* in Aquaculture, Central Laboratory for Aquaculture Research, October 12-14, Cairo, Egypt: 307–318.
36. Dhara, K., and Karmakar SR. (2016). Acute toxicity of Neem (*Azadirachta indica* A. Juss) leaf extracts to snake headed fish, *Channa gachua* (Ham.) with special reference to their Ethological responses and some Haematological Parameters. International Journal of life Sciences and Scientific Research; 2(5): 552-558.
37. Bhattacharya S. (1995). Possibilities and magnitude of heavy metal pollution in aquaculture. In: Nutrient Management in Aquaculture, Chattopadhyay GN (Eds), Institute of Agriculture, Palli Siksha Bhavana, Visva Bharati, Sriniketan, India: 90-92.
38. Lue X., Ma Y., Wu S. and Wu D. (1999). Two novel azadirachtin derivates from *Azadirachta indica*). Journal of Natural Products; 62: 1022-1024.
39. Dhara, K., Biswas S.J. and Roy Karmakar S. (2016). Study on the toxicity of neem (*Azadirachta indica* A. Juss) leaf extracts as phytopiscicide on three life stages of Mozambique tilapia (*Oreochromis mossambicus* Peters) with special reference to their ethological responses. Int J Exp Res Rev; 3: 7-13.
40. Oniovosa, U., Aina, O., Alarape, S., Babalola, O., Adeyemo, O., (2017). Effects of Neem leaves aqueous extract on organ histology, haematological parameters and biochemical indices in catfish. Alex. J. Vet. Sci. 54 (1), 17-24.
41. Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entomol., 35: 271-297.
42. Khan, M.F. and Ahmed, S.M. (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.