

Determination of Lethal Concentration (LC 50) of Channa Striatus to organophosphate Diazinon

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ABSTRACT

During the present research tenure, 10 percentile of 96 h LC50 of Diazinon concentrations were used against Channa striatus with an average length and weight of 12.5 ± 1.1 to 13.0 ± 1.2 cm and 32.7 ± 1.0 to 43.1 ± 2.3 g, respectively. The calculated LC50 for Diazinon concentrations (LC50 = 24.16 ppm) were tested in Channa striatus in different concentrations. The 50% mortality started in between 25 ppm (LC50) to 30 ppm (LC60), which reveals the calculated LC50 = 24.16 ppm. The mortality of 47.6% was recorded in 96 h LC50 (25 ppm) concentration with expected response of 23.2, residual of 1.8 and probability value of 50.00. ANOVA of the fish mortality and concentration of Diazinon revealed no significant differences ($P < 0.05$), the lower and higher 95% values were observed as -1.0 and 2.7 respectively.

1. Introduction

At the present time, the most widely used pesticides belong to the organophosphorus group. Overall, organophosphorus compounds account for 36% of total pesticides used globally (Kanekar *et al.*, 2004). Continuous and excessive use of organophosphorus compounds has led to the contamination of several ecosystems in different parts of the world (EPA, 1995; McConnell *et al.*, 1999; Cisar and Snyder, 2000; Tse *et al.*, 2004). Recent evidences indicate that fish, an extremely valuable resource, are quickly becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life.

The significant increase of chemical emissions in the water resources has lead to deleterious effects on aquatic organisms (Livingstone, 2001; Matsumoto *et al.*, 2006). Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important, because they are highly toxic to aquatic organisms.

The concern for our environment is mainly owing to the increasing chemical pollution when pesticides are transported into rivers, seas and other aquatic bodies. Evidence suggests that pesticides pose potential health hazards not only to livestock and wildlife, but also to fish, birds, mammals and even to human beings. The ecological impact of these pesticides in the aquatic system is evident in the ongoing research in large scale at global, but less in national and least in regional levels. Henderson and Pickering *et al.*, (1962), Jackson and Brungs (1966) have used fish for their monitoring studies. As the fishes constitute one of the important sources of protein, a thorough understanding of the effect of pesticides on fishes would be

useful for fish conservation and development of the fisheries (Holden, 1970).

There is vast literature on the toxicity of the organophosphate pesticides to different fish (Durairaj and Selvarajan, 1992; Fulton and key, 2001; Tilak and Marina, 2001; Rao *et al.*, 2005; Agrahari *et al.*, 2006; Manoj, 2014, 2015). The toxicity of different groups of organophosphorus insecticides to fish *Channa striatus* was found to be scanty, so the present study was under taken to evaluate the toxicity of Diazinon on the test fish species.

The present study has been undertaken to have a basic understanding of the acute toxicity of Diazinon 60 EC on the experimental fish *Channa striatus* species. Assessing the cumulative toxicity of the pesticide has therefore been an enduring challenge for environmental health research as well as ecotoxicology for the past several decades. So an attempt has been made to assess the acute toxicity of the above organophosphate pesticides. The importance of acute toxicity study is to prevent a massive kill of the fish by the contamination with the pesticide in nearby water bodies and the overall objective in taking up of such a work is to assess the damage done by the pesticide.

Depending on the toxicity study of the above pesticides to the test fish, one of the most toxic commercial and technical grade compound has been selected (Diazinon). The active ingredient in diazinon is phosphorothioic acid 0,0- diethyl 0- (6-methyl-2-(methylethyl)-4-pyrimidinyl) ester. Its molecular formula and molecular weight are $C_{12}H_{21}N_2O_3PS$. The technical grade is light amber to dark brown, and boils at 83 to 84°C. Diazinon is soluble in water to 40 mg/l, and dissolves readily in aliphatic and aromatic solvents, alcohols, and ketones. Diazinon may be stored on the shelf for at least 3 years with negligible degradation.

The pesticide Diazinon is also one among the commonly used chemicals on crops and the information gathered from literature are not adequate to understand the total impact of this organophosphate on freshwater fishes. A further and more detailed investigation of the effect of Diazinon on freshwater fishes would help to assess the damage caused and also to verify the non-toxic consumability of the fish, which would help to assess the health hazards, if any, in human population of the study area.

2. Materials and Methods

Freshwater murrel *Channa striatus* (SL 12.5 ± 1.22 cm, 38 ± 2.3 g) were collected from fresh water habitat and kept in large glass aquaria bearing tap water for 20 days in the laboratory for acclimatization in normal photoperiod and temperature and starved for 24 hours prior to experimentation. Fishes were fed daily with minced fish and chicken liver, and water was renewed every day. The test solution of Diazinon were prepared by using distilled water (having Dissolved Oxygen=6.0mg/l; pH=7.1 and room temperature $28 \pm 2^{\circ}$ C). 96-hour acute toxicity test were done by using various concentration of Diazinon. The data found were statistically evaluated by using Finney's probit analysis method.

3. Results

The evaluation of toxic impact of Organophosphate pesticide Diazinon on the fresh water fish *Channa striata* was selected for the toxicological study. No fish died during the period of acclimatization (20 days) before exposure to pesticide Diazinon. During the present research tenure, 10 percentile of 96 h LC₅₀ of Diazinon concentrations were used against *Channa striatus* with an average length and weight of 12.5 ± 1.1 to 13.0 ± 1.2 cm and 32.7 ± 1.0 to 43.1 ± 2.3 g, respectively. The Diazinon concentrations (LC₅₀ = 24.16 ppm) were tested in *Channa striatus* in different concentrations: Control (Normal water habitat); 5ppm (LC₁₀), 10 ppm (LC₂₀), 15 ppm (LC₃₀), 20 ppm (LC₄₀), 25 ppm (LC₅₀), 30 ppm (LC₆₀), 35 ppm (LC₇₀), 40 ppm (LC₈₀), 45 ppm (LC₉₀), 50 ppm (LC₉₉).

The mortality of *Channa striatus* fingerlings against various concentrations of Diazinon is presented in **table 1**. The 50% mortality started in between 25 ppm (LC₅₀) to 30 ppm (LC₆₀), which reveals the calculated LC₅₀ = 24.16 ppm. Probable, expected and residual mortality at LC₅₀ is presented in table 2 and graphically in **Figure 1**. **Figure 2** depicts the residual values of mortality plotted against the various concentrations of Diazinon. The mortality of 47.6% was recorded in 96 h LC₅₀ (25 ppm) concentration with expected response of 23.2, residual of 1.8 and probability value of 50.00. While as the mortality of 66.7% was recorded in 96 h LC₅₀ (30 ppm) concentration with expected response of 32.1, residual of -2.1 and probability value of 59.1. The mortality was lesser in the induction level of 5 ppm (LC₁₀) with mortality of 9.5%, expected response of 5.3, residual of -0.3 and probability value of 13.16. 100% mortality was observed in fishes exposed to 50 ppm (LC₉₉), with expected response of 47.7, residual value of 2.3 with probability of 95.5. The tabular presentation of the

analysis of variance is recorded in **table 3**. ANOVA of the fish mortality and concentration of Diazinon revealed no significant differences ($P < 0.05$), the lower and higher 95% values were observed as -1.0 and 2.7 respectively.

4. Discussion

Diazinon is an organophosphorus compound with an anticholinesterase mode of action. Diazinon degrades rapidly in the environment, with half-time persistence usually less than 14 days. But under conditions of low temperature, low moisture, high alkalinity, and lack of suitable microbial degraders, diazinon may remain biologically active in soils for 6 months or longer. Among sensitive aquatic organisms, LC-50 (96 h) values of 1.2 to 2.0 ug/l were derived for freshwater cladocerans, and 4.1 to 5.9 ug/l for marine shrimps; freshwater teleosts were comparatively resistant, with all LC-50 (96 h) values greater than 90 ug/l. Different authors have reported different lethal concentrations for different varieties of fish. According to EPA, diazinon is highly toxic (median lethal concentration, LC₅₀, less than 1 ppm) or very highly toxic (LC₅₀ less than 0.1 ppm) to about 60 percent of the fish species. These include bluegill sunfish; brook, cutthroat, lake, and rainbow trout; and striped mullet. Fish species that are sensitive to diazinon have enzymes that activate diazinon more quickly, enzymes that break it down more slowly, or nerves that are more affected by diazinon (Keizer, *et al.* 1995).

During the present study, the LC₅₀ for Diazinon EC60 was calculated to be 24.16 ppm. Svoboda *et al.* (2001) enumerated the LC₅₀ and LC₅ values of Basudin 600 EW for common carp juveniles in the respective time intervals as 26.7 mg.l⁻¹ of Basudin 600 EW preparation which is 16.0 mg.l⁻¹ of diazinon. Svoboda *et al.* (2002) enumerated the LC₅₀ after 96 h of action of the diazinon-based organophosphorous pesticide Basudin EW 600 at a concentration of 32.5 mg.l⁻¹. In yet another experiment, *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* were exposed to 5.62, 6.25, 6.87, 7.50, 8.12 and 8.75 ppm; 1.13, 2.26, 3.39, 4.52, 5.65 and 6.78 ppm; and 2.00, 2.50, 3.00, 3.50, 4.00 and 4.50 ppm of Diazinon 60 EC, respectively. The median lethal concentration (LC₅₀) values of Diazinon 60 EC on *A. testudineus*, *C. punctatus* and *B. gonionotus* were 6.55, 3.09 and 2.72 ppm for 96 hrs of exposure, as depicted by Rahman *et al.* (2002).

An experiment on the effect of Diazinon on Glutathine and Acetylcholinesterase in Tilapia (*Oreochromis niloticus*) by Nagwa *et al.* (2007) led to the toxicity results for LC₅₀ of Diazinon for 96 h as 2.8 mg L⁻¹ using 600 EC, while as LC₁₀ and LC_{33.5} were calculated and equaled 0.28 mg L⁻¹ and 1.89 mg L⁻¹. The low LC₅₀ is the result of the 600EC Diazinon active ingredient. Adedeji (2008, 2010) used African catfish (*Clarias gariepinus*) as test animal to assess the changes in blood profile due to Diazinon pesticide, containing 162 mg/l active ingredient. The authors

depicted the 96hr LC₅₀ value of Diazintol for the African catfish as 6.6 mg l⁻¹. In yet another experimental trial on Diazinon toxicity, the common carp (*Cyprinus carpio*) was exposed to diazinon to determine the lethal concentration (LC₅₀) values at different times and effects of sub-lethal concentrations on haematological and biochemical parameters. The LC₅₀ values at varying times were recorded as high as 18.57 mg/l (24 h), 14.87 mg/l (48 h), 12.29 mg/l (72 h) and 9.76 mg/l (96 h). The findings of the author lend complete support to our findings.

Khalid (2013) studied the effect of diazinon on haematological indices in common carp (*Cyprinus carpio* L.) in which fishes were exposed to six different concentrations of diazinon (0, 5, 10, 20, 40, 80 ppm) for 96 h. The authors reported LC₅₀-96h for diazinon (20 ppm), indicating diazinon as highly toxic to *C. carpio*. There was no recorded mortality in fish during the acclimation period before exposure and in the control group during acute toxicity tests. The authors reported 100% mortality at 80 ppm within the 96h after exposure, which completely supports our observations. Forouhar *et al.* (2013) worked on the effect of Diazinon and Deltamethrin to compare the acute toxicity in tench (*Tinca tinca*) larvae and fingerling. LC₅₀ of 24 h, 48-h, 72 h and 96 h were determined using a probit. Fish samples (21 fish in each test group) were exposed to different concentrations of diazinon and deltamethrin (diazinon: for fingerling between 1-20 ppm and larvae 0.25-2 ppm) for 96 h and mortality were recorded. The authors reported LC₅₀ 96 h of diazinon for fingerlings and larvae as 6.77 and 0.63, respectively.

Zebra Cichlid (*Cryptoheros nigrofasciatus*) samples were exposed to different concentrations of Diazinon (60%) (0, 2, 4, 8, 16 and 32 ppm) by Sadeghi and Hidayati (2014), for 96 h within the 100 L glass aquaria and cumulative mortality of Zebra Cichlid fish was

calculated in 24-h interval. The very low LC₅₀ was obtained for diazinon (5.06±0.37 ppm) by the author. The low LC₅₀ in their case may be associated with the sensitivity of the test fish to the insecticide. A semistatic experiment was conducted to find out the acute toxicity of diazinon as an aquatic pollutant on some hematological parameters of Indian carp (*Cirrhinus mrigala*) by Rauf and Arain (2013). The 1, 24, 48, 72, and 96 h LC₅₀ values of diazinon for Indian carp were estimated as 17.32 mg/L, 15.39 mg/L, 12.64 mg/L, 10.98 mg/L, and 8.15 mg/L respectively by the authors, which pertains to our results. Sub-lethal effects of Diazinon on haematological indices and blood biochemical parameters in Indian carp, *Cirrhinus mrigala* (Hamilton) were observed by Haider and Rauf (2014). The mean LC₅₀ values of diazinon recorded for Indian carp, *C. mrigala* at 1, 24, 48, 72 and 96 h clearly indicated a significant decrease in LC₅₀ values as exposure time to diazinon increased. 96 h LC₅₀ was found to be 8.15 mg/L of diazinon for *C. mrigala*. The authors reported that fish mortalities were not recorded during sub-lethal toxicity experiment at 10% and 20% of 96 h LC₅₀.

5. Conclusion

On the basis of above results and discussion, it can be concluded that the Diazinon may had a higher assimilation and toxicity in aquatic organisms. The pesticides discharged into aquatic environment caused chronic stress conditions that have harmful influence on aquatic life. The useful qualities of pesticides, they attract the farmer and health departments to use them against pest control. But these compounds are found to be highly toxic to aquatic biota and especially to fish, creating serious threat to the food webs in the aquatic ecosystems. So, there was a dreadful need to evaluate the hazardous potential of these pesticide residues in aquatic ecosystems.

Table 1: Half lethal concentration (LC₅₀) of Diazinon in *Channa striatus* (Bloch) (N = 21) exposed to various concentrations.

Conc. (ppm) (A)	Conc. (diff.) (B)	No. Alive (C)	No. Dead (D)	Mean Dead (E)	BXE
0.00	00	21	00	00	00
5.0	5.00	19	02	01	05
10.0	5.00	17	04	03	15
15.0	5.00	15	06	05	25
20.0	5.00	13	08	07	35
25.0	5.00	11	10	09	45
30.0	5.00	07	14	12	60
35.0	5.00	05	16	15	75
40.0	5.00	03	18	17	85
45.0	5.00	01	20	19	95
50.0	5.00	00	21	20.5	102.5
LC ₅₀ = 24.16 ppm					542.5

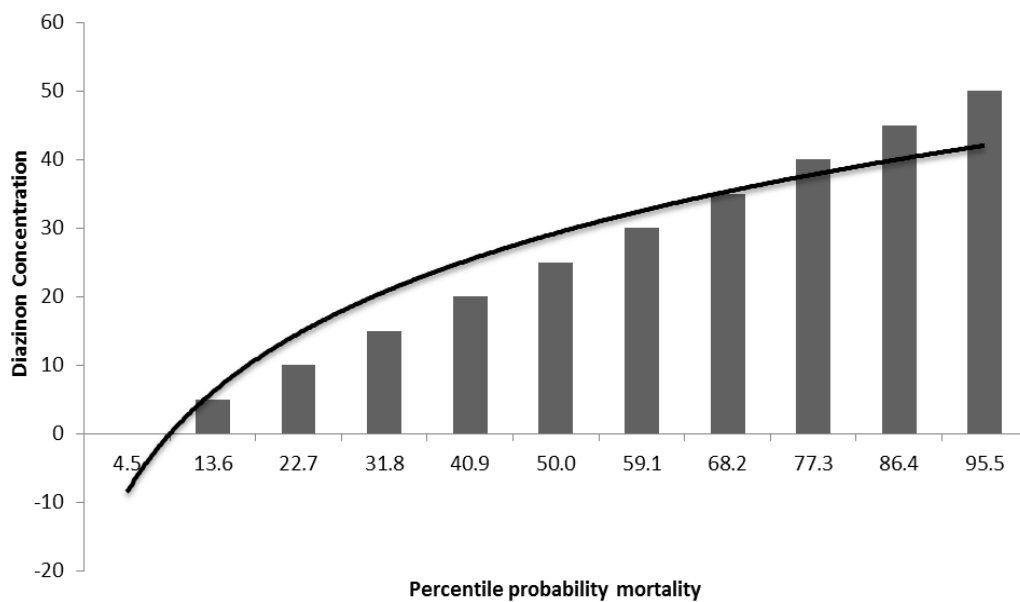
Table 2: Probable, expected and residual values of Diazinon impact on *Channa striatus* (Bloch).

Diazinon (ppm)	Log. Conc.	Mortality (%)	Expected Responses	Residual	Probability
Control	-	0.0	0.9	-0.9	4.5
5	0.7	9.5	5.3	-0.3	13.6
10	1.0	19.0	9.8	0.2	22.7

15	1.2	28.6	14.3	0.7	31.8
20	1.3	38.1	18.7	1.3	40.9
25	1.4	47.6	23.2	1.8	50.0
30	1.5	66.7	32.1	-2.1	59.1
35	1.5	76.2	36.5	-1.5	68.2
40	1.6	85.7	41.0	-1.0	77.3
45	1.7	95.2	45.5	-0.5	86.4
50	1.7	100.0	47.7	2.3	95.5

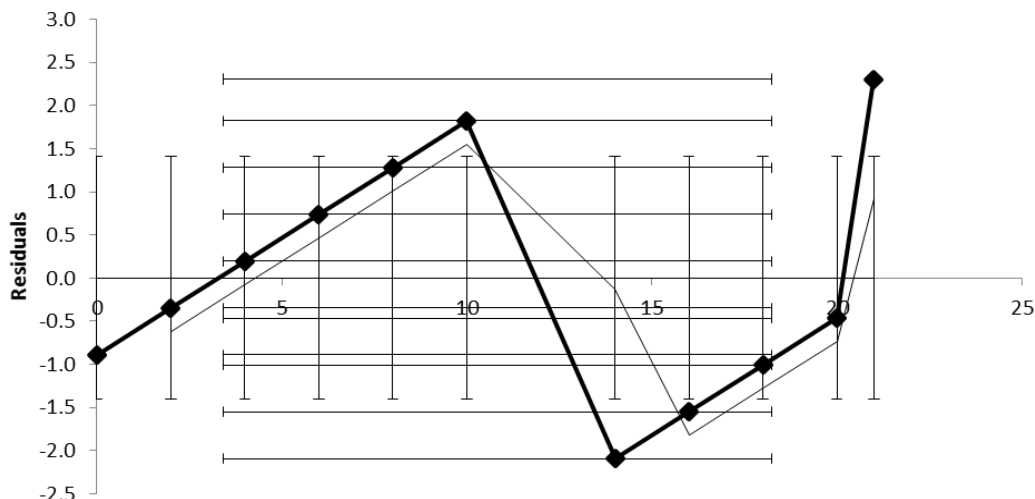
Table 3: ANOVA of mortality versus the concentration of Diazinon in *Channa striatus*.

	Df	SS	MS	F	Significance F	Lower 95%	Higher 95%
Regression	1.0	2730.2	2730.2	1241.9	0.0	-1.0	2.7
Residual	9.0	19.8	2.2				
Total	10.0	2750.0					



Graph 1: Percentile probable mortality of *Channa striatus* (Bloch) on exposure to various concentration of Diazinon.

X Variable 1 Residual Plot



Graph 2: Residual plot of Diazinon concentration based mortality in *Channa striatus* (Bloch)

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