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Research Article

ASSESSMENT OF ACUTE TOXICITY AND BEHAVIORAL CHANGES IN FRESHWATER FISH *CHANNA PUNCTATA* EXPOSED TO ORGANOPHOSPHATE PESTICIDE, ACEPHATE

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ABSTRACT

The present study assesses the acute toxicity and behavioural alterations due to Acephate, an organophosphate pesticide on *Channa punctata*. The most common acute toxicity test is acute lethality and LC_{50} is customary to represent the lethality of a test species in terms of mortality and time. The healthy juveniles of fish *C. punctata* were used for this study. The water quality parameters were in normal range, indicating good water quality for experimentation. The Median lethal concentration values of *C. punctata* for Acephate in the static test for 24, 48, 72, and 96 h were 1470, 1240, 1080 and 910 mg/l respectively. Zero percent mortality of the fish was observed at the concentration of the toxicant at and below 0.54, 0.48 0.24 and 0.30 mg/l for 1, 2, 3 and 4 days. A hundred percent mortality of fish was observed at the concentration of the toxicant at and above 2470, 2240, 2080 and 1810 mg/l for 1, 2, 3 and 4 days respectively. In the present study, irregular swimming, loss of balance, restlessness, excess secretion of mucous, surfacing activity and gradual decrease in the opercular movement were the common observations in the present study. There were no deaths and behavioral changes were also observed in the control group throughout the experiment.

Keywords: Keywords: Acephate, *Channa punctata*, LC_{50}, Mortality, Behavioral changes.

INTRODUCTION

Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue because of the extent of damage caused to the aquatic ecosystems and the disruption in the natural food chain by several agricultural practices such as insecticidal and herbicidal application. The increasing population has put a stress on resources, resulting in the excessive use of organophosphate pesticides and fertilizers to meet the demand. These substances ultimately pollute the aquatic environment and cause severe damage to the aquatic life especially the non-target species. Among the different groups of pesticides organophosphates are being used commonly as insecticides due to their facilitation properties like low mammalian toxicity, less persistence and rapid biodegradability in nature (Somaiah *et al*., 2015).

Acephate (O,S-Dimethyl acetylphosphoramidothioate) is an organophosphate pesticide used in agricultural practices, primarily to control a sap-feeding insect which includes various beetles, mites, grubs, and worms. This compound has been found to produce a lot of toxic effects in different fish species and is able to bring about changes in their metabolic pathways. Acephate is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops. Commercial names of Acephate are Asataf 75% SP; Tremor 75 SP etc. and its molecular formula is $C_4H_{10}NO_3PS$ (Akila *et al.*, 2015).

Acute toxicity of a pesticide refers to the chemical's ability to cause injury to an animal from a single exposure, generally of short duration. The acute toxicity test of pesticides to fish has been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to test organisms (Lakshmaiah *et al.*, 2014). The most common acute toxicity test is acute lethality and LC_{50} is customary to represent the lethality of a test species in terms of mortality and time. LC_{50} is the concentration of the chemical that results in the 50% death rate of the test organisms.

Water is one of the most essential needs for the survival of life on earth. Water covers 71% of the earth's surface and is vital for all forms of life. The aquatic

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environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms including fish. Pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. Heavy dependence modern agriculture on agrochemicals such as pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. Pesticides are carried into the aquatic ecosystems by surface runoff from sites of application and therefore the health of aquatic ecosystem is being adversely affected because they serve as an ultimate sink for these pesticides. These pesticides are also found to be highly toxic not only to fish but also to other organisms which constitute food of the fish. Among synthetic pesticides organophosphates are widely used in agriculture and in health and hygiene (Lakshmaiah *et al.*, 2014).

MATERIALS AND METHODS

Experimental Fish: The healthy juveniles of fish *C. punctata* with body length ranging 10 ± 0.9 cm and weighing 10 ± 0.8 g were obtained from local fish farm at Kuchipudi in Guntur district in Andhra Pradesh state, India. Prior to conducting experiment, the fish were acclimatized for a period of 10 to 15 days in dechlorinated tap water under laboratory conditions at room temperature (27 ± 3°C). The container was aerated with rich oxygen. Hygienic conditions were maintained by renewed water regularly and fish were daily fed with supplementary feed consisting of rice bran and fish pellets.

Water Quality Analysis: The physico-chemical parameters of water such as temperature, turbidity, pH, total hardness, total suspended solids, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), sulphates and Dissolved Oxygen were estimated during the course of the study according to the standard protocols (APHA, 1998).

Pesticide: The commercial grade formulations of Acephate 75% SP an organophosphate pesticide is used as a toxicant in the present experiment. Commercial names of Acephate are Asataf 75% SP; Tremor 75 SP etc. and its molecular formula is $C_4H_{10}NO_3PS$.

Preparation of Stock Solution: Stock solution of Acephate was prepared by dissolving 1 gram of pesticide in 100 ml of acetone and the required quantity of Acephate was drawn from the stock solution to maintain the suitable concentration of 1 mgl^{-1} in the container. The fish were separated into several groups and each containing 10 individuals. Pilot experiments were conducted to derive the LC_{50} determinations. These groups were exposed to different concentrations for acute toxicity estimation, ranging from 210 mg/L to 2470 mg/L for 24, 48, 72 and 96 hours. During the whole experiment a control group was maintained with acetone for comparison. The experimental design included three replicates. The fish were not fed on the day before the beginning of the experiment.

The concentration of the pesticide that caused 50% mortality at 96 h was taken as the LC_{50} value in the test organisms. Each day number of dead fish were counted and removed immediately from the test container. Percent mortality was carried out and the values were pooled up into probit scale. These values were determined and analysed by using Finney's Probit analysis method (Finney *et al.*, 1987).

Statistical Analysis: The data statistically were analyzed by SPSS statistical package.

RESULTS

Before the start of experiment, the water that will be used for maintenance of fish was analyzed for its quality. Thus, Table 1 gives the values of different water quality parameters which are in normal range indicating good water quality for experimentation.

Table 1. Estimation of physico-chemical parameters of water used for experimentation.

Water Parameter	Value
Temperature	27 ± 3ºC
Turbidity	7.8 silica units
pH value at 28ºC	7.42
Total Hardness as (CaCo3)	158 (mg/l)
Total suspended solids (TSS)	3.2 (mg/l)
Conductivity	175(mg/l)
BOD	7-10 ppm
Sulphates (SO4)	Trace amounts
Phosphates (PO4)	Trace amount
Dissolved Oxygen(DO)	6-7 mg/l

In the present study, the fishes (*C. punctata*) were subjected to various concentration of Acephate and their behavioural pattern was observed after exposure to the toxicant. Initially, the fishes were hitting against the container walls and started disturbing themselves. Irregular swimming, loss of balance, restlessness, excess secretion of mucous and surfacing activity with gradual decrease in opercular movement were the common observations in the present study. With the increased exposure, the gills changed from reddish to brownish colour and the body of the fish observed to be pale in colour.

Moreover the secretion of mucous has been increased before mortality of fish which shows that *C. punctata* has undergone pesticidal stress. During the experiment the treated fish body weight gradually decreased when compared with the control fish. Therefore, the present study could be taken as an indicator of aquatic pollution.

The percentage of mortality rate was observed at 24, 48, 72 and 96 h at different concentrations of the toxicant. Zero percent mortality of the fish was observed at the concentration of the toxicant at and below 210, 440, 280 and 210 mg/l for 1, 2, 3 and 4 days. Hundred percent mortality of the fish was observed at the concentration of the toxicant at and above 2470, 2240, 2080 and 1810 mg/l

for 1, 2, 3 and 4 days (Table 2 -5) respectively. There were no deaths and behavioral changes observed in the control group throughout the experiment. The theoretical spontaneous response in the control group was zero. The Median lethal concentration values of *C. punctata* for Acephate in static tests for 24, 48, 72, and 96 h were shown in Tables 2-5. Thus the average LC_{50} for 96 h is determined to be 910 mg/l, 95% upper and lower confidence limits were found to be 725-1220 mg/l, respectively. The mortality rate was gradually increased, as the concentration of the toxicant increased in the experiment. Table 6, shows the estimated values of the LC_{50} for 24, 48, 72 and 96 h as 1470, 1240, 1080 and 910 mg/l respectively.

Table 2. Effect of Acephate on survival of *Channa punctata* for 24 hours.

S. No.	Conc. of toxicant(mg/l)	No. of Exposed	No. of Dead	Per. of mortality	Probit Mortality
Control	---	10	0	0	---
1	670	10	0	0	---
2	870	10	0	0	---
3	1070	10	1	10	3.72
4	1270	10	3	30	4.48
5	**1470**	10	5	**50**	5.00
6	1670	10	6	60	5.25
7	1870	10	7	70	5.52
8	2070	10	8	80	5.84
9	2270	10	9	90	6.28
10	2470	10	10	100	8.09

Table 3. Effect of Acephate on survival of *Channa punctata* for 48 hours.

S. No.	Conc. of toxicant (mg/l)	No. of Exposed	No. of Dead	Percentage of mortality	Probit Mortality
Control	---	10	0	0	---
1	440	10	0	0	---
2	640	10	1	10	3.72
3	840	10	3	30	4.48
4	1040	10	4	40	4.75
5	**1240**	10	5	**50**	5.00
6	1440	10	6	60	5.25
7	1640	10	7	70	5.52
8	1840	10	8	80	5.84
9	2040	10	9	90	6.28
10	2240	10	10	100	8.09

Table 4. Effect of Acephate on survival of *Channa punctata* for 72 hours.

S. No.	Conc. of toxicant (mg/l)	No. of Exposed	No. of Dead	Percentage of mortality	Probit Mortality
Control	----	10	0	0	---
1	280	10	0	0	---
2	480	10	1	10	3.72
3	680	10	2	20	4.16
4	880	10	4	40	4.75
5	**1080**	10	5	**50**	5.00
6	1280	10	6	60	5.25
7	1480	10	7	70	5.52
8	1680	10	8	80	5.84
9	1880	10	9	90	6.28
10	2080	10	10	100	8.09

Table 5. Effect of Acephate on survival of *Channa punctata* for 96 hours.

S. No.	Conc. of toxicant (mg/L)	No. of Exposed	No. of Dead	Percentage of Mortality	Probit Mortality
Control	---	10	0	0	---
1	210	10	0	0	---
2	410	10	2	20	4.16
3	610	10	3	30	4.48
4	710	10	4	40	4.75
5	**910**	10	5	50	5.00
6	1110	10	6	60	5.25
7	1310	10	7	70	5.52
8	1510	10	8	80	5.84
9	1710	10	9	90	6.28
10	1810	10	10	100	8.09

Table 6. Estimated LC_{50} values and confidence limits of fish *Channa punctata.*

Time of Exposure	Toxicant Conc. (mg/l)**	95% Confidence Intervals (CI) UCL* - LCL*
24 h	1470	725-1220
48 h	1240	856-1489
72 h	1080	915-1189
96 h	910	870-990

** 24-96 h LC_{50} values, *UCL= Upper confidence limits, *LCL= Lower confidence limits.

DISCUSSION

Earlier studies revealed that the LC_{50} of a chemical for a species may vary under different environmental condition like time of exposure, size, and other impacts. Several reports were given for different LC_{50} values of various pesticides on fresh water fish (Dubey & Rani, 1990; Mathivanan *et al,* 2004; Ramasamy *et al.,* 2007; Sadhu *et al,* 1993; Santhakumar & Balaji, 2000; Sivakumar *et al.,* 2005).

The other impact of the pesticide could be observed by the behavioral changes like surfacing, erratic movement, increased mucous secretion, decreased opercular movement and loss of balance. Similar observations were made by (Sivakumar *et al.,* 2005) in *L. rohita* when exposed to endosulfan. The erratic swimming of the treated fish indicates that the loss of physiological equilibrium and the hyper-excitability of the fish invariably in the lethal and sublethal exposure of chemical may be due to the inhibition of cholinesterase (Singh *et al.,* 2012). Abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular coordination

Opercular movement has been decreased with increase in toxicant concentration and accumulation more fecal matter was observed in the container and similar results were observed by Bhat *et al.* (2012). The fast opercular movements might be due to accumulation of mucus over gills to the pesticide and similar observation was expressed by Prasanth *et al*. (2005). Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Behavioral changes of *L. rohita* have been studied for various chemicals by Marigoudar *et al*. (2009) and Pandey & Rizvi (2009). The treated fishes also showed fading of their body color before death, these changes can be considered as symptoms of stress on account of the toxicological nature of the environment. The behavioral changes showed by the fishes after Acephate intoxication are similar to those observed in other fishes exposed to organophosphate pesticides. Bayne & Scullard (1977) reported that altered movement of *Channa gachua* at different concentrations of dimethoate. The surfacing phenomenon of fish might be due to hydro toxic condition of the fish; these results are supported by Rao & Rao (2009) and Rathod *et al.* (2008). The decrease in body weight could be due to excessive expenditure of more energy on metabolism in fish growth and it was proportionate to the concentration of the pesticides. Similar results were reported by Fava *et al.* (2008) and Cook & Thomas (2005).

Acephate has been reported to be highly toxic to nontargeted organisms. In India, the effects of Acephate were studied on circulating leucocytes of *C. punctata*. The hemoglobin levels, as well as the number of small lymphocytes decreased while the number of large lymphocytes, thrombocytes and total leucocytes counts

increased in the fish. In an overall response against the pesticide, the fish showed restlessness, rapid body movement, convulsions, difficulty in respiration, excess mucous secretion, change in colour and loss of balance on exposure to pesticides. This pesticide has also been reported to cause disorders in carbohydrate and lipid metabolism in *Clarias batrachus*, a freshwater fish. It depleted the cholesterol level and elevated the amount of alkaline phosphatase and bilirubin in the blood serum (Mohanty *et al.,* 2011).

In the present investigation, Acephate caused 100% mortality of *C. punctata* at 1810 mg/l, 50% mortality at 910 mg/l during 96 h exposure and insecticidal toxicity influenced by factors like temperature, size, etc. (Kumaravel *et al.*, 2013) has reported monocrotophos caused 100% mortality of *L. rohita* at 0.0044 and 50% at 0.0036 ppm and also suggested that the lambda cyhalothrin caused mortality 100 % at 0.0029 and 50% at 0.0021 mg/l. Jang *et al.* (2002) determined the 96 h LC_{50} value of Kethrin to *L. rohita* as 21.68 ppm.

Mohanty *et al.* (2011) evaluated the tissue specific genotoxic effects of Acephate in *Labeo rohita* and to appraise the in vivo DNA repair ability of the fish. *L. rohita* (rohu) fingerlings were exposed to different concentrations (0.001, 0.002 and 0.01 ppm) of Acephate, an organophosphate pesticide; samplings were done at 24, 48, 72 and 96 h. The present study was carried out to evaluate the tissue specific genotoxic effects produced by Acephate on three different tissue systems and to assess DNA repair response in fish. Results of tissue specific DNA damage experiments showed low baseline damage in blood cells followed by gill and liver cells in control individuals whereas more DNA breaks were found in liver followed by gill and blood cells of treated individuals.

Sila *et al.* (2014) reported the acute toxicity of organophosphates and carbamates on *Catla catla* fingerlings. In his study he has reported the acute effects of commercial formulation of triazophos, profenofos, carbofuran and carbaryl. Pesticides were applied to fingerlings that had been grown under optimized standard conditions under a maintained static bioassay system. Probit analysis was used for the estimation of LC_{50} values, which were ascertained as 4.84, 0.19, 0.99 and 7.89 mg/l for triazophos, profenofos, carbofuran and carbaryl, respectively. 100% mortality of *Catla catla* was observed with a 2.8 mg/l dose of carbofuran at 96 hours with a significant difference. Acute toxic stress was noticed with subjects exhibiting behavioral intoxication, including suffocation, lying on the bottom, erratic swimming, lethargy and downward movements and gulping prior to mortality.

The present findings closely correlated with the acute toxic effects of an organophosphate insecticide Acephate on freshwater fish *Puntius sophore* reported by Gavit & Patil (2016). The results were recorded as 10% to 90% mortality during the experiment, no mortality was found in control fishes at 24, 48, 72 and 96 hours. The LC_{10} values of the pesticide at different concentration of Acephate were 1219 ppm, 980.2 ppm, 782.1 ppm and 622.3 ppm at 24, 48, 72 and 96 hours respectively, and the LC_{50} values found to be 1762 ppm, 1509 ppm, 1281 ppm and 1117 ppm at 24, 48, 72 and 96 hours respectively. The toxicity of the Acephate pesticide is very low to the fish *P. sophore* and it was considered to confer low toxicity to the fishes (Gavit & Patil, 2016).

Cambria & Hussain (2015) investigated the acute toxicity, behavioral response and biochemical parameters of blood of *Catla catla* to toxicity of an Organophosphate insecticide, Dimethoate were carried out in a static renewal system. The LC_{50} values of Dimethoate at various exposure times were 21.0 mg/l for about 84 h, 21.5 mg/l for 72 h, 22.0 mg/l for about 60 h, 22.5 mg/l for 48 h, 23.0 mg/l for 48 h, and 23.5 mg/l for 24 h. The *C. catla* showed behavioral alterations against Dimethoate intoxication viz, uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, drowning and change in body pigmentation, muscle fasciculation, moribund lethargy, refusal of feeding and respiratory distress. These symptoms became more apparent with increase in duration of exposure at all test concentration of Dimethoate. These results closely related to our present reports.

CONCLUSION

In conclusion, our study shows the Acephate is most toxic to the experimental fish and it has negatively acted on fish behaviour. From this study it could be concluded that the excess use of chemical pesticides not only results in the extermination of the target organisms but also of a large number of non-target organisms affected in such a way that their normal physiological mechanisms are hampered. The result revealed that death of the fishes thereby decreasing their population and bringing imbalance in ecosystem. Thus this study could be used as a tool for creating awareness among the local farmers so that the use of these deadly pesticides could be minimized. Thus the Acephate use is not safe for ecosystem and non-target organisms.

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