

## Impact of toxicity of Triazophos on their behavioural responses of a fresh water fish *Channa punctatus* (Bloch).

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### Abstract

Fishes serve as important bio-indicators for aquatic contamination to assess the changes caused by human activities effectively and reliable monitoring bio-system to recognize and predict hazardous effects of pollutants. As such exposure of pesticides induces physiological and behavioural alterations in the fishes. In present investigation, it was found that the quantity of Triazophos requires to constitute  $LC_{50}$  dose for 24 hrs and 48 hrs duration of treatment was 38.0 mg/l and 26.8 mg/l respectively and for the sub lethal it was 16.180 mg/l. Besides, fishes exhibited following behavioural changes: restlessness rapid superficial movement of the fins and increased rate of opercular movements, sign of loss of equilibrium, secretion of large quantity of mucus and in the dying fishes, feeble opercular movement and expanded bronchial chamber was observed. All these behavioural changes in fish was dose and duration dependent.

**Key Words:** LETHAL CONCENTRATION ( $LC_{50}$ ), TRIAZOPHOS, CHANNA PUNCTATUS, BEHAVIOURAL RESPONSE.

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Date of Submission: 26-10-2020

Date of Acceptance: 06-11-2020

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### I. Introduction

Pesticides are toxic substances, chemically they are designed to control, reduce or repel, mitigate and to kill a wide range of agricultural insect pests, plant pathogens, weeds, fungi or other organisms and microorganisms. In India's agricultural sector, the use of pesticides plays a significant role in modern agricultural practices including in the process of achieving higher agricultural yields, ensuring food security and in sustaining substantial food production. In the last few decades the importance of agricultural pesticides for protecting crops and livestock from pest infestations are undeniable. However, apart from their numerous beneficial effects, improper, unplanned and indiscriminate application of these chemical compounds has posed serious risks to human health and other life-forms. The problem of the pesticide toxicity and other related chemicals to non-target organisms become a major environmental issue around the world, as these harmful compounds persist and bioaccumulate in the ecosystem for longer duration and impose adverse effects on non-target organisms and natural ecosystems. In addition, these chemical pesticides ultimately find their way to natural water bodies through agricultural runoff and affect not only the target organism but also many other non-target organisms in different degrees (Sanchez-Bayo F, *et al.*, 2011). Among these non-target organisms, fish are commonly used as bio-indicators of aquatic pollution due to their sensitivity to the surrounding environment and considered as one of the most important specimens for toxicological studies. They can play a significant role in assessing potential risk associated with contamination in aquatic environments (Lakra and Nagpure, 2009). Alterations in the chemical composition of the natural aquatic environment usually affect behavioural and physiological systems of inhabitants, particularly fish (Radhaiah *et al.*, 1987; Khan and Law, 2005). One of the organophosphorus pesticide most commonly used in India is Triazophos, a non-systemic, broad spectrum used to control various types of pests. It is neurotoxic in nature and leads to accumulate in neurotransmitter acetylcholine in synapse resulting continuous flow of neuromuscular signals causing paralysis and death of the insect (Kamanyire and Karalliedde, 2004). The bioaccumulation of this toxicant in fish species affects the behavioural patterns by interfering with the nervous system and sensory receptors and consequently it can lead to disorders in the fish response to environmental stimuli. The evaluation of the toxic effect of Triazophos on fish physiology and behavioural changes has not been done thoroughly. Therefore, the aim of the present study was to evaluate the toxic effect of selected formula grade pesticide, namely Triazophos on the behavioural responses of the freshwater fish, *Channa punctatus* towards the toxic pollutants.

## II. Materials And Methods

**EXPERIMENTAL FISH:** The live freshwater fish *Channa punctatus* (Bloch), was selected for the present study. As the fish *C. punctatus* is well known, most edible, easily available in fresh water resources of India belongs to the family: Channidae and Order: Channiformes. *C. punctatus* inhabits muddy stagnant water and commonly found throughout the plains of India. The species are economically as well as medicinally important having great potential for aquaculture and fisheries.

**CHEMICAL:** The commercial grade pesticide **Triazophos** was used for the evaluation of the toxicity test on *Channa punctatus*.

**Triazophos(O,O-diethyl O-1-phenyl -1 H-1,2,4- triazol -3 -yl phosphorothioate )**, mostly marketed as 40% EC (Emulsifiable Concentration) with the trade name is Hostathion. It is a non-systemic insecticide with contact and stomach action on crops or stored pests with 39 mg/l at 20°C solubility in water. The chemical compound is susceptible to highly toxic and flammable substances. Triazophos is an organophosphate pesticide used as insecticide, acaricide and also possesses nematicidal properties against certain free-living nematodes that damage agricultural and horticultural crops and shows a broad-spectrum activity against a wide range of sucking insect pests, mites and some Lepidoptera. It is applied as a foliar spray on several crops like cotton, sugarcane, maize, rice, potatoes, vegetables and fruits etc.

Live fishes were collected from the fishing sites and also from the local fish market of Ara town situated in Bhojpur district. The fishes were brought to the laboratory in large polythene bags containing pond water. The care was taken to reduce hyperactivity and physical injuries to the fish. They were individually examined for any pathological symptoms and washed with 0.5% Potassium Permanganate (KMnO<sub>4</sub>) Solution for 2-3 minutes and then transferred to many large sized (90 x 60 x 45 cms<sup>3</sup>) aquaria. All the aquaria were carefully washed and cleaned with tap water before putting fishes in them for acclimatization. Healthy adult fishes having average body weight ranging from 45g to 60 g and length 21.5 cm to 25.3 cm were selected for the experiment and kept separately in glass aquaria filled with running tap water having a physico-chemical characteristics of: Temperature- 26 ± 28° C, pH - 7.2 ± 0.3, Dissolved Oxygen- 5.8 ± 0.4 mg/l, free CO<sub>2</sub> - 12.1 ± 0.8 mg/l and total alkalinity- 165 ± 2.5 for proper acclimatization to the laboratory condition for ten days. During this period, fish were fed with pieces of goat's liver and commercial fish diet twice daily ad-libitum. If mortality occurred, dead fishes were also removed immediately to prevent any contamination. The hygienic condition of aquaria was maintained by changing the water on every alternate day. In addition, the aquarium water was properly aerated to maintain the DO. (Dissolved Oxygen) condition throughout the experiment. The whole experiment was conducted for a period of 45 days under natural photoperiod and temperature. The water quality characteristics were assessed by using guidelines of APHA (1989).

**Determination of lethal and sub lethal concentration:** Well acclimatized stock fishes were divided into two groups: control and experimental ones. The control fishes were kept in separate aquarium containing normal tap water whereas experimental group of fishes were kept in eight separate aquariums containing different concentrations of Triazophos made by dissolving different quantity of pesticide in 10 liters of tap water. The toxicity tests of the pesticide Triazophos were conducted by employing the static bioassay method designed by Doudoroff *et al.*, (1951) and also recommended by APHA (1989). For the determination of LC<sub>50</sub> value of Triazophos, one set of experiments was conducted for 24 hrs and the second for 48 hrs respectively. In each concentration of the pesticide, 20 fishes from the stock were placed for the conduction of the experiment. Prior to the initiation of experiment, feeding was stopped for the maintenance of bioassay test. The experiments were monitored round the clock and the number of dead fishes during the experiment in each concentration of each set of experiment was recorded. The experiments were repeated twice to get average results and minimize any error. With the help of the records of dead fishes in different concentrations, the LC<sub>50</sub> for 24 hrs and 48 hrs of exposure for the pesticide Triazophos under consideration were determined graphically by Probit Analysis (Finney, 1971). After determining the LC<sub>50</sub> values the sub lethal concentration or safe concentration of the pesticide was determined as per the methods of Hart *et al.*, (1945). During the study, behavioural changes of the exposed fishes due to lethal toxicity of Triazophos were also observed regularly and compared with that of control fish for any marked variations.

## III. Results And Discussion

In the present study, the results of median lethal concentration or LC<sub>50</sub> values of Triazophos show that to constitute Lc<sub>50</sub> dose for 24 hrs and 48 hrs duration of treatment 38.0 mg/l and 26.8 mg/l respectively of pesticide is required. After determining the LC<sub>50</sub> dose, the sub lethal concentration of the pesticide was calculated as per the formula of Hart *et al.*, (1945). Accordingly. The amount of Triazophos required to constitute safe concentration was 16.180 mg/l. The evaluated results are summarized in table no 1, 2 & 3.

**Table 1:** Determination of mortality in *Channa punctatus* at different concentrations of Triazophos after 24 hrs of exposure to calculate LC<sub>50</sub> dose.

Sl. No.	Concentration of pesticide (in ml)	'Log' Concentration of pesticide	No. of fishes exposed	No. of fishes killed	Percentage of kill	Emperical probit
1	30	1.4771	20	2	10	3.72
2	50	1.6990	20	5	25	4.32
3	70	1.8451	20	7	35	4.61
4	90	1.9542	20	9	45	4.88
5	110	2.0414	20	12	60	5.25
6	130	2.1139	20	14	70	5.52
7	150	2.1761	20	17	85	6.05
8	170	2.2304	20	19	95	7.19

LC<sub>50</sub> dose = 95 ml or 38.00 mg/l.

**Table 2:** Determination of mortality in *Channa punctatus* at different concentration of Triazophos after 48 hrs exposure to calculate LC<sub>50</sub> dose.

Sl. No.	Concentration of pesticides (in ml)	'Log' Concentration of pesticide	No of fishes exposed	No of fishes killed	Percentage of kill	Emperical probit
1	20	1.3010	20	3	15	3.97
2	40	1.6021	20	6	30	4.48
3	60	1.7782	20	9	45	4.88
4	80	1.9031	20	12	60	5.25
5	100	2.000	20	15	75	5.67
6	120	2.0792	20	18	90	6.28
7	140	2.1461	20	19	95	7.19
8	160	2.2041	20	20	100	8.09

LC<sub>50</sub> dose =67 ml or 26.80 mg/l.

**Table 3:** Abstract of the quantity of Triazophos required to constitute the LC<sub>50</sub> for 24 hrs and 48 hrs of duration and the amount required to constitute sublethal dose of the fish *Channa punctatus*.

Sl. No.	Name of pesticide	LC <sub>50</sub> 24 hrs	LC <sub>50</sub> 48 hrs	SL dose
1	Triazophos (in mg/l)	38.0	26.8	16.18

### BEHAVIOURAL RESPONSE

The control fishes appeared very active with well-coordinated movement. However, the fishes, when exposed to pesticides exhibit very agonizing behaviour due to the poisoning effect. In present investigation, it was observed that when the fishes to different concentrations of Triazophos, marked deviation in its normal behaviour throughout the experiments was observed. The fishes due to the toxic effect of the pesticide become excited and restless immediately on exposure. The agnostic behaviour does not subside but rather gradually increases in proportion to the concentration and duration of exposure. The fishes were observed coming to the surface of the water in aquarium more frequently than does the control fishes and it was more pronounced in the fishes exposed to higher concentration of pesticides. After approximately about six hours of exposure, the restlessness in the fishes subsides and the fishes settled quietly on the bottom of the aquaria. But simultaneously, a rapid superficial movement of the fins and increased rate of opercular movements was noticed. The fishes start showing sign of loss of equilibrium and sensitivity approximately after 12 hours of exposure to the higher concentration of the pesticide. The movement of fins becomes slow and fishes observed lying on its side on the bottom of aquaria. A large quantity of mucus was observed on the body of the fishes and as such fishes appeared slimier. When dead fishes were examined, a thick slimy coat of mucus was found on the body of the dead fishes. In the dying fishes, feeble opercular movement and expanded bronchial chamber was observed. On the external examination of dead fishes red spots on the skin was noticed, suggesting subcutaneous hemorrhage and the gill epithelium and filaments was also found damaged.

It has been observed that fishes are directly affected by bioaccumulations of different pesticides in the aquatic medium (Rao and Pillala, 2001). A view also supported by (Srivastava *et al.*, 2016). Behavioural changes have also been observed due to bioaccumulation of xenobiotics in aquatic species cause major threat to aquatic life. Many fish species show uptake and accumulation of many contaminants such as pesticides,

polychlorinated biphenyls, polycyclic aromatic hydrocarbons and heavy metals (Pandey *et al.*, 2009). Pesticides induce different types of toxicity in fished which leads to changes in fish behaviour such as rendering fish sluggish and alter their swimming ability making them more susceptible to the toxicity of the aquatic pollutants. (Murthy *et al.*, 2013) were of the view that in general behaviour allows an organism to adjust to external and internal stimuli in order to adapt environmental variables and is a highly structural and predictable sequence of activities design to ensure maximal fitness and survival of the individual as such, fishes are able to uptake and retain different toxicants dissolve in water via active or passive processes (Satyavardhan, 2013; Rani and Kumaraguru, 2014). Therefore, behavioural modification during environmental stress is one of the most sensitive indicators of adjustment for survival in adverse condition (Byrne and O'Halloran, 2001).

Ullah *et al.*, (2014) have reported that even sub-lethal concentrations of pesticides in aquatic environments causes structural and functional changes in aquatic organisms mortality. Kalavathy *et al.*, (2001) observed erratic swimming, jerky movements and convulsions before death in *Sarotherodon mossambicus* due to Dimethoate poisoning and concluded that the behavioural changes shown by the fishes were due to asphyxiation resulting into gasping to death. Prasad *et al.*, (2002) have found that under environment toxicants stress, fishes show aggressive behaviour viz nudge and nip and also exhibits change in orientation and locomotors pattern. Malfunctioning of neuron- transmitters followed by hyper and hypo opercular activity, loss of equilibrium and excess secretion of mucus all over the body in *Labeo rohita* on exposure to SL concentration of Cypermethrin was observed by Marigoudar *et al.*, (2009). Whereas, Singh *et al.*, (2018) observed alterations in the behaviour of *Channa punctatus* of different degrees with varying concentrations of the pesticides as compared to control fish and were of the view that the effects of pesticides were found to be in dose and duration dependent manner and these effects may be due to AChE activity which is effective both at neural and neuromotor junctions present in muscles. Tilak and kumari (2009) also observed a decrease in oxygen consumption in *Ctenopharyngodon idella* when exposed to lethal concentration of Nuvan. A drastic variation in respiratory rate was observed by Ramesh and David (2011) in *Cyprinus carpio* when exposed to the SL concentration of Chlorpyrifos after fourteen days.

The behavioural changes shown by *C. punctatus* in the present study on exposure to the different concentrations of Triazophos were in conformity with the findings of the investigators cited above.

#### IV. Conclusions

In conclusion, the present study indicates that the toxic effect of the pesticide cause damage to gill epithelium and filaments and thereby disrupt normal functioning of the nervous and neuromuscular system by inhibiting AchE, the fishes try to get more oxygen by increasing their opercular movements. The scholar is of the view that on exposure to different concentrations of the Triazophos , the divergences in the behavioural responses may be due to neurotoxic characteristics of the pesticide.

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Minakshi Kumari. "Impact of toxicity of Triazophos on their behavioural responses of a fresh water fish *Channa punctatus* (Bloch). *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(10), (2020): pp 10-14.