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ACUTE TOXICITY OF AN ORGANOPHOSPHORUS PESTICIDE, CHLORPYRIFOS AND ITS EFFECT ON THE BEHAVIOR OF A FRESHWATER FISH *CHANNA PUNCTATUS*

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ABSTRACT

In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding. Contamination by pesticides in aquatic ecosystem is a serious problem and fishes are more frequently exposed to these pollutants and may taken in through gills, skin and contaminated foods. Chlorpyrifos is a widely used organophosphate pesticide, second largest selling in India and used for more than a decade to control pests on cotton, paddy field, pasture and vegetable crops. Aquatic contamination of pesticides causes acute and chronic poisoning in fish and other organisms directly or indirect via food chain. A bioassay experiment was carried out to determine the acute toxicity of chlorpyrisfos (Commando 20% EC) on freshwater Channa punctatus for different time interval viz; 24h, 48h, 72h and 96h. The LC50 were determined by the probit analysis method using Finney's table. The 96 h LC50 values was found 6.27µl/L. The present study was aimed to determine the acute toxicity of chlorpyrifos and its effect on the behavior of *Channa punctatus*. During experiment fish show erratic and jerky movement. It is observe that the fish show decrease opercular movement, increase surface air gulping and decrease resting time, respiratory trouble and excess secretion of mucous from all over the body and gills. The behavioral changes observed in chlorpyrifos induced *Channa punctatus* indicate that this pesticide is toxic and are highly sensitive to this chemical.

Keywords: Acute Toxicity, Behavior, Chlorpyrifos, Freshwater Fish, Lethal Concentration.

1. INTRODUCTION

According to UNEP (2005), pesticides are defined as substances used to prevent, destroy or repel pests. Their extensive global usage has led to widespread environmental contamination (Omitoyin et al., 2006), with studies estimating that less than 1% of applied pesticides affect the target organism (Rand and Petrocelli (1984); Lawson et al., 2011). Organophosphates are among the most widely used pesticides, favored for their high insecticidal activity, low mammalian toxicity and rapid biodegradability (Singh et al., 2009). Synthetic pesticides like chlorpyrifos remain dominant tools in pest control due to their immediate effectiveness and affordability. Chlorpyrifos (CPF), an organophosphate insecticide is widely utilized in global commercial agro-farming for pest control due to its broad-spectrum efficacy and low environmental persistence (Chernyak et al., 1996; Hasanuzzaman et al., 2018; Livingstone, 2001; Silva & Samayawardhena, 2002; Matsumoto et al., 2006). According to Joseph and Raj (2011), modern agricultural practices frequently result in agrochemical runoff that contaminates aquatic environments, thereby affecting the tolerance limits of aquatic fauna and flora. The aquatic toxicity of organophosphates is difficult to monitor due to their low solubility and rapid degradation in water, yet their toxicity to aquatic life remains high (Halappa & David, 2009). Bioassays are commonly employed to assess the toxicity of chemical substances and to determine sensitive organisms, helping to establish water quality criteria and effluent discharge standards (Finney, 1971). In India, it ranks as the second most commonly used synthetic pesticide (Stalin et al., 2019). While pesticides have greatly benefited agriculture and public health by enhancing crop yields and controlling disease vectors, their indiscriminate use has posed significant risks to non-target aquatic organisms. However, the application of insecticides in agricultural fields often leads to their entry into aquatic ecosystems through canals, rainfall, and surface runoff. Although pesticides contribute significantly to increased agricultural productivity and improved food quality especially in developing countries their infiltration into water bodies presents a significant threat to aquatic biodiversity (Abhilash & Singh, 2009). Exposure to pesticides can occur in humans and terrestrial animals through chronic exposure, ingestion of contaminated food and water, and domestic contact (Ullah et al., 2018). Despite their low persistence in aquatic environments, these substances can still be harmful to non-target species, particularly fish (Ali et al., 2009).

2. MATERIALS AND METHOD

Live fish *Channa punctatus* (mean length: 16.0±0.9 cm; mean weight: 26.0±1.2 g) were collected from local fish market and transported to the laboratory. Fish were disinfected using 0.05% $KMnO_4$ solution for 5 minutes and acclimatized in glass aquaria (with 20 liter water capacity) with aerated water, pH 7.4–7.5, DO 5.9–7.1 mg/L and temperature 27–28°C. were measured following APHA (1998) for 15 days. During a 15-day acclimatization period, fish were fed commercial pellets two times daily and the water was renewed daily to remove waste and dead fish. Feeding was stopped 24 hours before toxicity tests. The physico-chemical parameters of the test water used during the acute toxicity experiment were monitored daily over the 96-hour exposure period.. In the present study, exposure of *Channa punctatus* to varying concentrations of Hilban (chlorpyrifos 20% EC) resulted in dose-dependent mortality. A concentration of 25 µl/L led to 100% mortality within 96 hours. The calculated 96-hour LC50 was 6.27 µl/L. LC50 values were calculated using Finney's Probit Analysis (1971) with regression lines plotted between log concentrations and probit values. Sub-lethal concentrations were derived as 1/10th and 1/50th of 96-h LC50 values.

3. RESULTS AND DISCUSSION

Aquatic pesticide contamination causes both acute and chronic poisoning in fish (Heger et al., 1995; Velmurugan et al., 2007). The responses of fish to toxicants vary by species, concentration, exposure duration and water quality (Fisher, 1991; Richmonds & Dutta, 1992; Venkateswara, 2004). Behavioral bioassays are increasingly used as early-warning indicators in ecotoxicology (Drummond & Russom, 1990;) and have shown promise in unicellular organisms (Tadehl & Hader, 2001), insects (Martin, 2003; Venkateswara et al., 2004), and vertebrates like fish (Hansen et al., 1999; Rao et al., 2005). Behavioral endpoints such as swimming patterns, opercular movements, surface gulping, mucus secretion, reflex responses, and fin drooping were observed during the acute test period (Gupta & Dua, 2010; Nimila & Nandan, 2010; Srivastav and Chaturvedi 2012a, 2012b; Chaturvedi et al., 2012; Srivastav et al. 2017; Srivastav and Srivastava 2018; Srivastava and Srivastav, 2019; Srivastav et al., 2025). Behavioral alterations due to pesticide exposure in fish include erratic swimming, loss of equilibrium, and morphological deformities (Devi & Mishra, 2013; Srivastav and Chaturvedi 2012a). Numerous studies have documented the toxic effects of organophosphates in fish, including significant behavioral and morphological changes (Gul, 2005; Pandey et al., 2005; Reza & Gholamreza, 2012; Ishi & Patil, 2017; Bridi et al., 2017). At higher concentrations, fish became lethargic or motionless and exhibited erratic swimming patterns. Hyperactivity was more prominent at elevated concentrations and was likely due to inhibition of acetylcholinesterase, resulting in the accumulation of acetylcholine at synaptic junctions (Fulton & Key, 2001), leading to peripheral nervous system overstimulation, elevated metabolic activity, and increased oxygen demand (Rao, 1989). Additional behavioral manifestations included disrupted schooling, hyper-excitability, pectoral fin extension, frequent surfacing and air-gulping, avoidance behavior, escape attempts, fin drooping, vertical hanging, and progressive sluggishness leading to mortality. These observations support previous findings (Venkata et al., 2008) that abnormal swimming and loss of equilibrium are linked to neuro-muscular coordination deficits under toxicant-induced stress. Convulsions were particularly evident prior to death, with severity increasing at higher CPF concentrations. Initial exposure triggered avoidance behaviors such as rapid swimming, partial emersion from water, and attempts to jump out of aquaria. Abrupt, erratic swimming and vertical posture reflected physiological imbalance, likely due to acetylcholinesterase inhibition (Rao et al., 2005; Patil & David, 2008). Opercular movements, a key stress indicator, increased significantly with CPF concentration. Control fish showed 85–90 beats/min, whereas Hilban-exposed fish displayed rates up to 130 beats/min. Other behavioral and morphological abnormalities included pale body coloration, skin lesions, eye and fin deformities, excessive mucus secretion, and depigmentation. These changes were likely stress responses induced by the toxicant (Koprucu et al., 2006; Wasu et al., 2009; Ree & Paney, 1997; Pandey et al., 1990). Fish ultimately exhibited bottom-dwelling, motionlessness, and died with open mouths—symptoms similarly reported by previous studies (Koprucu et al., 2006; Patil & David, 2008; Susan et al., 2010). In the present study, *Channa punctatus* were exposed to a short-term concentration of 1 mg/L of chlorpyrifos, administered every 24 hours for four consecutive days. Both species exhibited a range of abnormal behavioral responses indicative of physiological stress. Observable behaviors included frequent surfacing to gulp air, bottom-dwelling, wall-following movements within the test jars, and attempts to escape by leaping out. These actions were accompanied by increased opercular activity, erratic and rapid swimming, and profuse mucus secretion from the gills and body surface. On the first day of exposure, a temporary decrease in opercular movement was noted. However, by the fourth day, the opercular rate had significantly increased, suggesting a delayed physiological response to the toxicant. Such behavioral changes reflect the acute and cumulative impacts of chlorpyrifos on the respiratory and neuromuscular systems of exposed fish. Quantitative observations included the recording of resting time and opercular movements (measured over one-minute intervals), as well as air-gulping frequency (measured over ten minutes), as per Gupta and Dua (2010). The exposed fish demonstrated frequent jumping, erratic swimming, and increased mucus production. These changes are indicative of respiratory distress and an attempt to mitigate exposure to the toxicant. Fish, as ectothermic (cold-blooded) vertebrates, are highly sensitive to environmental changes and aquatic pollutants. Exposure to toxicants such as chlorpyrifos can lead to disruptions in homeostasis, often manifesting as endocrine dysfunction and maladaptive behaviors. Industrial and municipal pollutants entering aquatic ecosystems have been shown to adversely affect fish survival, growth, and reproductive success. The degree of toxicity is influenced by factors such as body weight (Pickering, 1968), developmental stage and physiological condition of the organism. Behavioral alterations—such as hyperactivity, increased opercular movement, and disorganized swimming—are recognized as reliable biomarkers of sub-lethal toxicity. These responses serve as early indicators of water quality deterioration and are widely used in aquatic toxicity assessments (Halappa & David, 2009). Even at sub-lethal levels, pesticides can impair fish behavior, compromise fitness, and ultimately reduce population viability (Susan et al., 2010). Consequently, fish serve as valuable bioindicators in ecotoxicological studies evaluating the impact of pesticides, heavy metals, and other aquatic contaminants. Increased and erratic swimming behaviors observed in this study may be attributed to the breakdown of normal shoaling and locomotor control, commonly triggered by chemical-induced stress (Venkata et al., 2008). Oxygen deficiency caused by compromised gill function may further exacerbate this response, as evidenced by heightened opercular movements and air-gulping behavior (Susan et al., 2010). Similar findings have been reported in previous studies involving fish exposed to organophosphates, heavy metals, and agricultural runoff (Srivastava et al., 2010; Wasu et al., 2009). The act of air-gulping likely serves as a physiological strategy to reduce prolonged contact with the toxic medium (Katja et al., 2005). The behavioral symptoms reported here are consistent with those observed in other studies involving exposure of fish to a variety of pollutants including pesticides, metals, dyes, detergents, and fertilizers. These pollutants have been shown to elicit avoidance behaviors such as erratic swimming and jumping, as well as visible morphological stress indicators (Srivastava et al., 1995; Yadav et al., 2005; Chaturvedi et al., 2012; Srivastav & Chaturvedi, 2012). Notably, endocrine-disrupting chemicals (Srivastav et al., 2025), synthetic dyes (Srivastava et al., 1995), heavy metals, detergents (Yadav et al., 2005), and agricultural fertilizers have all been implicated in inducing similar maladaptive behaviors in freshwater fish. The entry of pesticides into water bodies through runoff significantly affects non-target organisms by damaging vital organs and altering behavioral and reproductive functions. Given the ecological significance and food value of fish, it is imperative to assess and monitor the environmental toxicity of pesticides using biological markers, histopathology, hematology, and behavioral studies. Currently synthetic pesticides are most suitable and dominant agent for eradication of insects/pests. These are considered quick and cheap means of controlling pests. In contrast the indiscriminate use of pesticides causes serious problems to the environment. Pesticides enter the aquatic environment through runoff water or may be applied directly, causing toxicity to non target organisms especially fishes (Nwani *et al.*, 2013; Tiwari andAnsari, 2014; Sunanda *et al.*, 2016). Exposure of toxicants causes fish poisoning which damage their vital organs, altering biochemical parameters and decline in reproductive ability. It has been reported that it inhibits acetylcholinesterase (AChE) activity in non- target animals especially fishes Black and Read, 2013). After exposure chlorpyrifos binds with the enzyme cholinesterase that prevents breakdown of acetylcholine at synapse. Restriction of AChE activity causes over stimulation of neuron tends to neurotoxicity (Jin *et al.*, 2015). Toxicity tests are the principal indicator to evaluate the effect of pesticides on aquatic fauna such as fishes. The effects of pesticides on fish population and other organisms depend on concentrations and exposure periods (Khare, 2015; Mishra and Verma, 2016). Toxicity is species- specific having different levels of responses to the same dose and time interval of a toxicant (Bridges and Semlitsch, 2000). Many chemical contaminants target physiological system and exert their effects on behavior. The investigation of morpho-behavioral markers are becoming the most potent and sensitive tool of ecotoxicology to evaluate the toxic effects (Cong *et al.*, 2009; Onyedineke *et al.*, 2010). Behavior is the cumulative manifestation of genetical, physiological and biochemical processes (Kane *et al.*, 2005; Dube and Hosetti, 2010). It allows an organism to adjust itself to external and internal stimuli in most challenging environment (Halappa and David, 2009). Some authors have been reported abnormal behavioral and morphological changes in fishes due to different pesticide exposure (Reza and Gholamreza, 2012; Ishi and Patil, 2017; Bridi *et al.*, 2017). Several researchers have also been reported the toxicity and behavioral alterations in fishes induced by organophosphate compounds (Mishra and Poddar, 2014; Misha and Verma, 2016; Majumder and Kaviraj, 2018).

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1. Control *Channa punctatus* showing settle on the bottom.

2. 1/10th of LC50 value for 1 day showing jerking movement for fish *Channa punctatu.*

3. 1/10th of LC50 value for 4 days showing jumping from the upper surface of water for fish *Channa punctatus.*

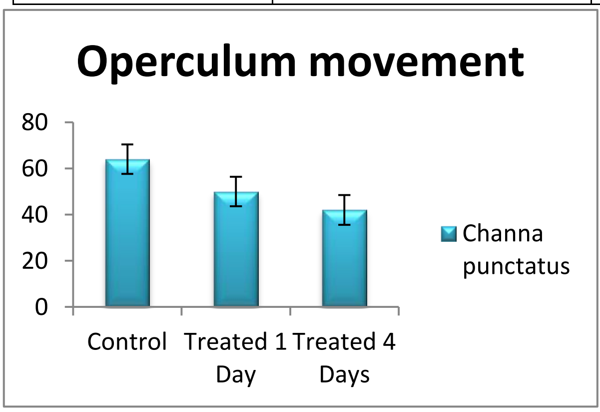
4. showing discoloration on the whole body of fish *Channa punctatus* for 1 day treatment.

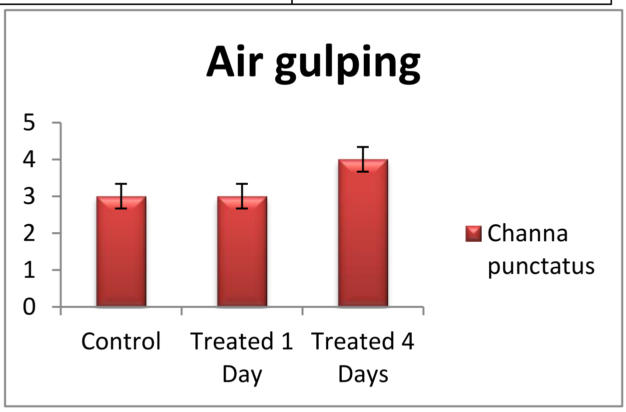
5. showing discoloration on mid dorsal to caudal region with lateral sides of fish *Channa punctatus* for 4 days treatment.

Parameter	Control	Treated 1 Day	Treated 4 Days
Mucous Secretion	-	+	++
Jumping	-	++	+++

(-) indicate no secretion/jumping; (+) indicate mild secretion/jumping; (++) indicate moderate secretion/jumping; (+++) indicate high jumping for fish *Channa punctatus*.

Fish	96hrs LC0 (µl/L)	96hrs LC50 (µl/L)	96hrs LC100 (µl/L)
Channa punctatus	0.01147625	6.2723459	308.460814





4. CONCLUSION

The *Channa punctatus* are valuable aquaculture species due to their air-breathing capability and ability to thrive in suboptimal environments. However, their exposure to organophosphate pesticides such as chlorpyrifos, increasingly introduced into aquatic ecosystems due to anthropogenic activities, poses serious threats. The study highlights dose- and time-dependent toxicity of CPF and its pronounced behavioral and morphological effects *Channa punctatus*, air-breathing freshwater species, are highly sensitive to chlorpyrifos, an organophosphorus insecticide widely used in agricultural practices. Exposure to chlorpyrifos, particularly at a concentration of 1 mg/L over short durations (24–96 hours), resulted in marked behavioral abnormalities, including erratic swimming, frequent jumping, excessive mucus secretion, reduced opercular movement, and intermittent air-gulping. These behavioral disturbances are indicative of underlying physiological stress and respiratory impairment, which ultimately contributed to increased mortality rates among exposed fish. The findings of this study establish a clear relationship between chlorpyrifos-induced toxicity and behavioral and physiological dysfunctions in fish, underscoring the ecological risks posed by indiscriminate pesticide use. This research serves as an important tool for raising awareness among farmers and agricultural stakeholders regarding the adverse effects of chlorpyrifos on aquatic life. It further emphasizes the necessity for regulated pesticide application and the implementation of safer, environmentally sustainable pest management practices. Moreover, the outcomes of this study support the need for continuous monitoring of pesticide residues and heavy metals in aquatic ecosystems to prevent long-term ecological and human health impacts. These results can inform policymakers and environmental authorities in developing guidelines and strategies to mitigate pesticide pollution, thereby safeguarding both biodiversity and public health.

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