Effects of Aquatic Vegetation and Water Turbidity on Chlorpyrifos-Induced Mortality of Nile Tilapia *Oreochromis niloticus*

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ABSTRACT

Chlorpyrifos is a common organophosphorus insecticide, and agricultural runoff containing this agrochemical pollutes adjacent water bodies. Static bioassays were carried out in the laboratory to evaluate if the presence of aquatic vegetation and water turbidity could reduce acute toxicity of the emulsified concentrate (EC) of chlorpyrifos (20% EC) to Nile tilapia *Oreochromis niloticus*. The 72 h-LC_{50} of chlorpyrifos alone on Nile tilapia was determined as 52 $\mu g \cdot L^{-1}$, whereas the LC_{50} value of chlorpyrifos was 80 $\mu g \cdot L^{-1}$ in the presence of the floating aquatic weed (AW) *Azolla pinnata* (150 $g \cdot 10\ L^{-1}$). The LC_{50} value of the formulation of chlorpyrifos with aquatic weed (CPF+AW) was about 1.45 to 1.7 times higher than that of chlorpyrifos (CPF) alone in clean water. The toxicity of chlorpyrifos decreased over time, but remained unchanged after 72 h in the presence of *A. pinnata* (150 $g \cdot 10\ L^{-1}$) in water. Chlorpyrifos toxicity to fish was also reduced when the turbidity of water was increased, both through the addition of organic compost and clay materials. In conclusion, the biological and physicochemical parameters of the receiving aqueous medium influence the toxicity of chlorpyrifos to fish.

Keywords: Aquatic Ecosystem, *Azolla*, LC_{50}, Nile tilapia, Organophosphate, Toxicity

INTRODUCTION

Chlorpyrifos is a broad-spectrum organophosphate used worldwide to control agricultural and structural pests. Agricultural runoff containing agrochemicals may contaminate adjacent freshwater ecosystems. Chlorpyrifos is potentially toxic to non-target aquatic organisms (Demetrio *et al*., 2014; David *et al*., 2018; Hatami *et al*., 2019). It inhibits acetylcholinesterase activity (Majumder and Kaviraj, 2019), triggers histological and cytological abnormalities (Velmurugan *et al*., 2020), and induces oxidative stress in fishes (Marigoudar *et al*., 2018; Majumder and Kaviraj, 2019). Chlorpyrifos residues have been detected in surface water (Chowdhury *et al*., 2012), sediments (Wang *et al*., 2016), and both farmed and wild fishes (Sun and Chen, 2008; Zahran *et al*., 2018). The US EPA classifies chlorpyrifos as moderately toxic (Li *et al*., 2015). An assessment by the US EPA and the FQPA identified a risk to human health and a 10X safety factor for children who inhale too much chlorpyrifos (Sud *et al*., 2020). In humans, chlorpyrifos can cause nerve disorders in cases of acute poisoning (Albers *et al*., 1999), while chronic exposure may lead to neurological, developmental and autoimmune disorders, and even retard mental development in children (Prasad *et al*., 2015).

Chlorpyrifos persists in the environment for a comparatively long time due to its low volatility and rate of degradation under aerobic conditions (John and Shaike, 2015). It is less soluble in water due to its non-polar nature (John and Shaike, 2015). Different adsorbents (soil, clay, organic matter, inorganic minerals, and sediments) influence the mobility and persistence of chlorpyrifos in soil and water (Gebremariam *et al*., 2012). Chlorpyrifos dissipation occurs more rapidly in aquatic ecosystems (Barron and Woodburn, 1995). Volatilization is

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Received 14 December 2021 / Accepted 17 May 2022

the main way of chlorpyrifos loss from water. The estimated volatility half-life of chlorpyrifos in pond water varies from 3.5 to 20 days, while photolysis half-life is 3-4 weeks (Kamrin, 1997). The toxicity of chlorpyrifos is enhanced when it gets degraded into chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinol (TCP) (John and Shaike, 2015). The toxicity of chlorpyrifos to aquatic organisms has been found to vary with ambient physicochemical conditions of the aquatic medium (Patra *et al*., 2015; Pawar *et al*., 2020). Chlorpyrifos was shown to become more toxic to fish with increasing water temperature, both in warm water (*Bidyanus bidyanus, Melanotaenia duboulayi, Hypseleotris klunzingerii*) and cold water (*Oncorhynchus mykiss*) species (Patra *et al*., 2015). On the other hand, no significant effect was found in the development of embryos or larvae of Zebra fish (*Danio rerio*) during the combined exposure to chlorpyrifos and different temperatures (Scheil and Köhler, 2009). Low salinity and temperature can cause higher chlorpyrifos toxicity in post-larval and juvenile shrimp (*Litopenaeus vannamei*) (Pawar *et al*., 2020). Salinity acclimation in juvenile *O. mykiss* may be affected due to chlorpyrifos exposure (Amiri *et al*., 2018). The toxicity of chlorpyrifos may change as the pH of the medium changes. *Salmo clarki* shows comparatively higher susceptibility to chlorpyrifos at pH 9.9 (96 h-LC_{50}: 5.4 ppb) than at pH 7.5 (96 h-LC_{50}: 5.4 ppb). Likewise, pH of the medium alters chlorpyrifos toxicity in *Salvelinus namaycush* (96 h-LC_{50}:140 ppb, 98 ppb and 205 ppb at pH 6.0, 7.5 and 9.0, respectively) (Barron and Woodburn, 1995). Many previous studies have indicated that toxicity of chlorpyrifos to fish varies by species, and that the same species of fish may show different susceptibility to the pesticide depending on the ambient environmental conditions (Mayer and Ellersieck, 1986; Barron and Woodburn, 1995; Deb and Das, 2013).

Most of the toxicity studies to date have dealt with the effects of particular pollutants on model test organisms under laboratory conditions. However, the decisive role of ambient environmental variables on toxicity of pollutants in natural ecosystems should be considered; otherwise, results based on a limited range of parameters may be ambiguous. Vegetation and soil or sediment present in freshwater ecosystems often remove or reduce pollutant load, and thereby decrease the pollutant concentration to an acceptable level (Moore *et al*., 2011). Therefore, important physical and biological parameters, namely turbidity and the presence of the aquatic fern *Azolla pinnata*, were considered in the present assessment of chlorpyrifos risk to fish. *Azolla pinnata* is a free-floating aquatic fern found in confined water bodies of Asia, Africa and in few parts of Australia. It has a symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena azollae*. The efficiency of *A. pinnata* and its associated bacteria in converting atmospheric nitrogen into ammonia makes them suitable as a biofertilizer in paddy fields (Prasad *et al*., 2015). Use of green azolla manure in agriculture is currently a popular way to increase yield. This fern is also used as a source material to form compost for application in pisciculture ponds. Its free-floating nature, ability for growth in nitrogen-deficient sites, tolerance to a wide range of pollutants, and capability for heavy metal accumulation make this aquatic fern a promising candidate for phytoremediation (Sood *et al*., 2012). On the other hand, more than 80 % of the pesticides are applied in paddy fields (Prasad *et al*., 2015). Therefore, the aquatic weed *A. pinnata* was used in the present study to determine whether its presence in water bodies can become an eco-friendly alternative to restore chlorpyrifos level in receiving water body or not. Turbidity of water is also another important physicochemical parameter that influences productivity of a freshwater ecosystem and toxicity of incoming pollutants to the ecosystem (Dabrowski *et al*., 2005; Majumder and Kaviraj, 2021).

Nile tilapia *Oreochromis niloticus* is one of the most widely cultured freshwater fish in tropical and sub-tropical countries. It is exotic to India but is widely cultured throughout the country. This species' ability to adapt to local conditions, efficiency in utilizing diverse diets, rapid growth, prolific breeding habit, great resistance to diseases and handling, tolerance to adverse environmental conditions, easy production of fingerlings in captivity, and consumer acceptance are all reasons for their increased demand in aquaculture (Majumder and Kaviraj, 2019). On the other hand, there is growing concern over the effects of pesticides, particularly

chlorpyrifos, on non-target organisms; these agrochemicals continue to be a problem across Asia. Therefore, Nile tilapia has been chosen as a test organism in the present study. Several studies have already identified both acute and chronic harmful effects of chlorpyrifos to Nile tilapia (Zahran *et al*., 2018; Majumder and Kaviraj, 2019). The present study aimed to investigate whether the aquatic fern *A. pinnata* or water turbidity have any effect on the toxicity of chlorpyrifos (20% EC) to Nile tilapia. This study can help guide the appropriate implementation of pesticide monitoring and control programs based on physicochemical and biological conditions of the receiving water.

MATERIALS AND METHODS

Test fish, aquatic weed and chemicals

Fingerlings of Nile tilapia (total length 5.18±0.34 cm, weight 3.02±0.56 g) were obtained from a local fish farm (Naihati, West Bengal, India). The fingerlings were transported to the laboratory in well-aerated polythene packets containing water, then unpacked and stocked in outdoor cement tanks for acclimatization for one week before being used in bioassays. Each cement tank (diameter 90 cm and depth 75 cm) had a 3-cm layer of soil at the bottom and was filled with 300 L of deep tube-well water (temperature 27±1 °C, pH 7.3±0.1; free CO_2 3.45±0.35 mg·L^{-1}; dissolved oxygen 7.1±0.2 mg·L^{-1}; total alkalinity 126.21±3.21 mg·L^{-1} as $CaCO_3$; total hardness 138.24±6.48 mg·L^{-1} as $CaCO_3$) stored in an overhead tank. Each cement tank was stocked with 30 fingerlings. The fish were fed a balanced diet containing 30 % crude protein throughout the acclimatization period. Air pumps were used to ensure aeration of the water tanks. On alternate days, water was partially exchanged (30-35 %) to maintain suitable water quality. The aquatic fern *A. pinnata* was procured from unpolluted local ponds and was also kept in separate tanks for acclimatization. A commercial formulation (20% EC) of chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl-phosphorothioate) available in the market as Dursban® was used in the experiments.

Static bioassays

Static bioassays were carried out according to the standard protocol of APHA (1995) in 15 L glass aquaria which contained 10 L of deep tube-well water (temperature 27±1 °C, pH 7.2±0.1; free CO_2 3.45±0.35 mg·L^{-1}; dissolved oxygen 7.1±0.2 mg·L^{-1}; total alkalinity 128.21±3.21 mg·L^{-1} as $CaCO_3$; total hardness 135.24±6.48 mg·L^{-1} as $CaCO_3$) and five Nile tilapia.

Bioassay with aquatic vegetation

Two separate bioassays were conducted to investigate acute toxicity of chlorpyrifos in the presence of aquatic vegetation: one with only chlorpyrifos (20% EC) and another with chlorpyrifos (20% EC) and the floating aquatic weed *Azolla pinnata* (150 g·aquarium^{-1}). A stock solution of 100 mg·L^{-1} chlorpyrifos was prepared by dissolving the appropriate amount of the pesticide in 10 mL of distilled water. Then, the appropriate amount of stock solution was pipetted out for each treatment and diluted with the test water in the aquaria. Several test concentrations of chlorpyrifos were prepared from stock solution for use in bioassays, each with three replicates. Chlorpyrifos concentrations used for the bioassays ranged from 0 to 160 µg·L^{-1}. For each concentration of chlorpyrifos examined, there was a control with three replicates. Every 24 h, fish mortality was recorded, and any dead fish were removed. To avoid excretory products interfering with the test chemical, no food was supplied throughout the bioassay. Probit analysis (Finney, 1971) was used to estimate the median lethal concentration (LC_{50}) with 95 % confidence limit for chlorpyrifos for 24, 48, and 72 h using a computer program developed by the US EPA. The LC_{50} values of chlorpyrifos (20% EC) with and without aquatic weed were compared according to the criteria of Mayer and Ellersieck (1986), Schmuck *et al*. (1994) and Demetrio *et al*. (2014).

Analytical methods

Chromatographic methods were used to quantify chlorpyrifos concentrations in water after 2 h of exposure. A 250-mL sample of water from

each aquarium was added to a 500 mL conical flask, and 25 g sodium chloride (NaCl) was added. The solution was partitioned three times with 50 mL of a mixture of hexane and dichloromethane (80:20). The organic phase was collected over anhydrous sodium sulfate in a conical flask and evaporated in a rotary evaporator. The volume was made up to 10 mL with ethyl acetate. The extract was filtered with a syringe filter using 25 mm, 0.22 µm nylon filter paper and transferred into vials for chlorpyrifos estimation in a gas chromatograph equipped with ECD detector (Agilent 6890N) with wide-bore HP column (HP-5, 30 m, 0.32 mm ID, 0.25 µm film thickness) and a 7683 B Series auto injector. As a carrier, N_2 gas was used. The concentration of chlorpyrifos was determined from the calibration curve prepared from standard chlorpyrifos concentrations, using ChemStation software. The instrument's limit of detection (LOD) and limit of quantification (LOQ) were 0.005 and 0.15 ppm, respectively.

Bioassays to determine effects of turbidity on toxicity of chlorpyrifos

In order to study the effects of water turbidity on mortality of *Oreochromis niloticus* caused by chlorpyrifos exposure, static bioassays were carried out again in 15 L glass aquaria, similar to the procedure described above. The fixed concentration for this bioassay was set at 25 $\mu g \cdot L^{-1}$, which represents 50 % of the 72 h-LC_{50} value of chlorpyrifos on *O. niloticus*. Two different bioassays were carried out. In one assay, turbidity was created using compost of the aquatic weed *Azolla pinnata*; in the second, suspended clay particles were used. In total, eight turbidity concentrations (0, 10, 15, 20, 30, 35, 40 and 50 $g \cdot L^{-1}$) were used in each of the two assays. For each turbidity concentration and control, three replicates were made. A separate set of experimental setups were also maintained in triplicate at eight turbidity concentrations as described above, both with (clay and compost) and without turbidity (control), but without any chlorpyrifos treatment. The experiment lasted for 24 h, and then fish mortality was recorded.

Statistical analysis

Mean mortality values were compared by Duncan's multiple range test (DMRT) after calculating the residual variance using repeated measures ANOVA (Winer, 1971) for arcsine-converted mortality data (dead individuals/initial number of individuals). The repeated measure factor was time of exposure, while the second factor was treatment. Furthermore, the APHA (1995) method was used to compare LC_{50} values. One-way ANOVA and least significant difference (LSD) tests were carried out on mortality data to test variation in mortality with the doses of compost and turbidity of water used (Gomez and Gomez, 1984). The tests were considered significant at $p<0.05$.

RESULTS

Effects of aquatic vegetation

Average recovery (%) of chlorpyrifos in water samples with and without *Azolla pinnata* was 83.99±0.42 $\mu g \cdot L^{-1}$ and 88.56±1.90 $\mu g \cdot L^{-1}$, respectively. Table 1 shows the 72 h-LC_{50} of chlorpyrifos (20% EC) on Nile tilapia with and without *A. pinnata* as 80 $\mu g \cdot L^{-1}$ and 52 $\mu g \cdot L^{-1}$, respectively. Comparing the two treatments, it was found that in the presence of the aquatic weed, the LC_{50} value of chlorpyrifos on Nile tilapia was 1.54 times higher than without the aquatic weed. Log-probit line regression parameters for Nile tilapia along with 72 h toxicity data of chlorpyrifos (20% EC) in the presence and absence of *A. pinnata* are presented in Table 1. When the slopes of the log concentration and probit mortality regression lines were compared, the lines were not precisely parallel (Figure 1). Chlorpyrifos toxicity decreased steadily with exposure time, however after 72 h, either there was no change or there was a marginally reduced toxicity of the pesticide in the presence of aquatic weed (Figure 2).

Effects of turbidity of water

The results showed that turbidity generated by compost of *Azolla pinnata* reduced Nile tilapia

mortality due to 25 $\mu g \cdot L^{-1}$ of chlorpyrifos exposure. Maximum mortality was recorded after 24 h of chlorpyrifos exposure in test aquatic medium without any compost (0 $g \cdot L^{-1}$ compost). Mortality decreased with the increase in concentration of compost from 5 $g \cdot L^{-1}$ to 30 $g \cdot L^{-1}$. There was no mortality at compost levels of 40 $g \cdot L^{-1}$ or higher (Figure 3). The results revealed no significant difference between control and 5 $g \cdot L^{-1}$ of compost ($p<0.05$). Similarly, there was no difference in mortality between treatments with 15 and 20 $g \cdot L^{-1}$ of compost.

Like with the compost, turbidity due to suspended clay materials also reduced mortality of fish exposed to 25 $\mu g \cdot L^{-1}$ of chlorpyrifos. Mortality decreased gradually from turbidity of 5 to 20 $g \cdot L^{-1}$, but remained unaltered thereafter (Figure 4).

No fish mortality was recorded in the separate experimental setups, both with and without turbidity (control), where no chlorpyrifos was added to the water.

Table 1. 72 h-LC_{50} values ($\mu g \cdot L^{-1}$) of chlorpyrifos on Nile tilapia (with 95 % confidence limit in parentheses) based on actual concentration measured after 2 h of treatments with and without aquatic weed *Azolla pinnata*.

	Chlorpyrifos (CPF)	**Chlorpyrifos with aquatic weed (CPF+AW)**
LC_{50}	52^{a} (37-67)	80^{b} (71-99)
Slope	1.52	2.23
Intercept	2.47	0.88
n	9	7
R^2	0.91	0.91

Note: Different superscripts indicate significant difference in LC_{50} values at $p<0.05$.

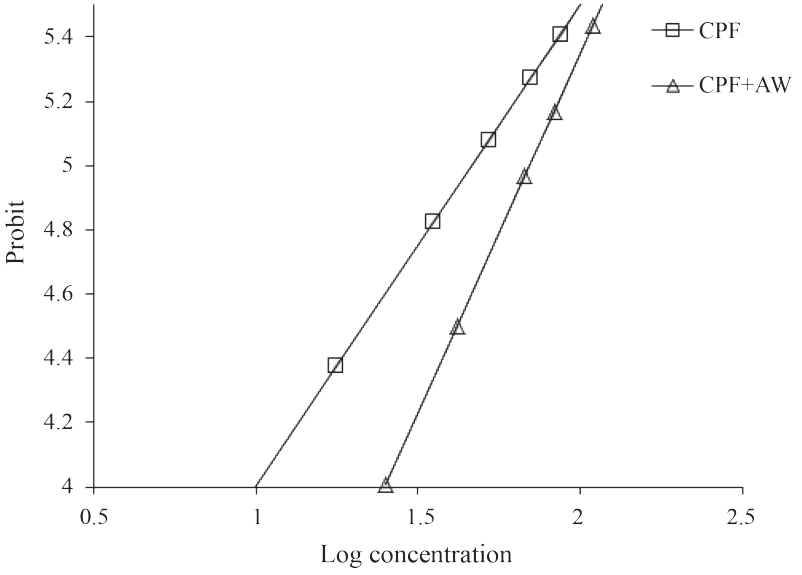

Figure 1. Regression lines in the log-probit model for log concentration versus probit values for Nile tilapia after 72-h exposure to chlorpyrifos 20% EC (CPF) and chlorpyrifos 20% EC with the aquatic weed *Azolla pinnata* (CPF+AW).

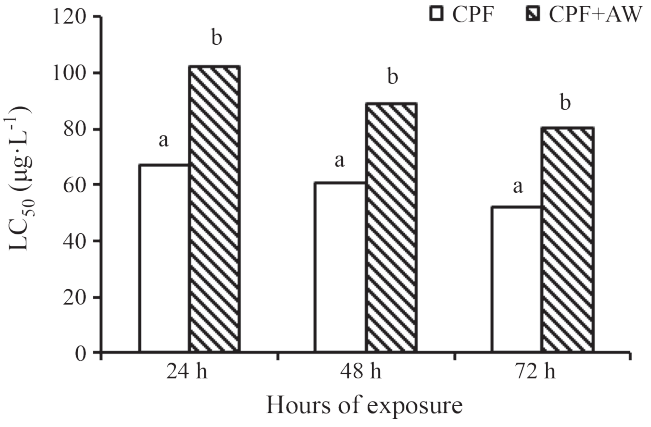

Figure 2. Change in LC_{50} value of chlorpyrifos 20% EC alone (CPF) and with *Azolla pinnata* (CPF+AW) on Nile tilapia over time. Different lowercase letters above bars indicate significant ($p<0.05$) difference in LC_{50} values at each time point.

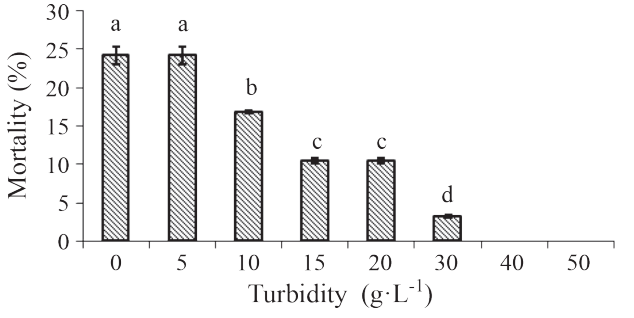

Figure 3. Mortality (%) of Nile tilapia with exposure to 25 µg·L^{-1} chlorpyrifos 20% EC for 24 h with the addition of organic compost to increase water turbidity. Histogram bars show mean±SD (n = 5); different lowercase letters above bars indicate significant ($p<0.05$) differences among means.

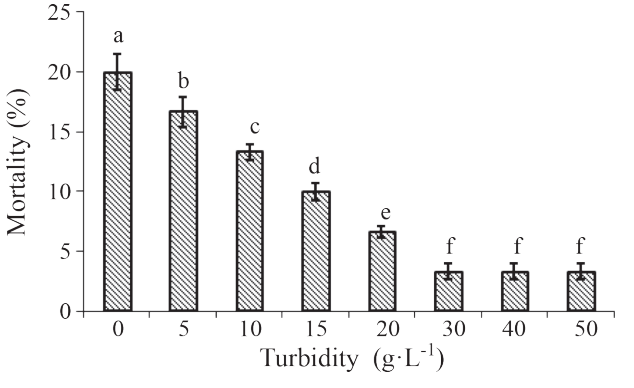

Figure 4. Mortality (%) of Nile tilapia exposed to 25 µg·L^{-1} chlorpyrifos 20% EC for 24 h with the addition of suspended clay to increase water turbidity. Histogram bars show mean±SD (n = 5); different lowercase letters above bars indicate significant ($p<0.05$) differences among means.

DISCUSSION

Role of aquatic vegetation on Chlorpyrifos toxicity

The results of the present study indicate that the presence of the aquatic plant *Azolla pinnata* (150 g·10 L^{-1}) substantially removes chlorpyrifos from water, thereby reducing toxicity of chlorpyrifos (20% EC) to Nile tilapia. Similarly, other plants (*Populus* sp. and *Salix* sp.) have been shown to mediate chlorpyrifos uptake from hydroponic solution, and accumulation within plant tissues has been reported by Lee *et al*. (2012), whereby accumulation of chlorpyrifos was much higher in roots than shoots. Chlorpyrifos never persisted in the plants, but rather underwent metabolism in plant tissues (Lee *et al*., 2012). Capacity of the aquatic macrophyte *Acorus calamus* for removal of chlorpyrifos at low concentrations from nutrient solution was also documented by Wang *et al*. (2016). Uptake of chlorpyrifos by plants from hydroponic solution decreased gradually with increase in chlorpyrifos concentrations. Root and foliar uptake are the two most common pathways for chlorpyrifos entry into plants (Muñoz *et al*., 2009; Lee *et al*., 2012). A study of sorption and photo-degradation of chlorpyrifos in aquatic and riparian macrophytes by Muñoz *et al*. (2009) showed that epicuticular waxes of leaves help in photo-degradation of chlorpyrifos. In another study made by De Souza *et al*. (2017), it was found that constructed wetlands planted with *Polygonum punctatum*, *Cynodon* spp. and *Mentha aquatica* acted as suitable means of bioremediation of chlorpyrifos from water bodies due to root-mediated adsorption and microbial degradation. In the present study, the higher LC_{50} values of chlorpyrifos on Nile tilapia in the presence of *A. pinnata* versus chlorpyrifos alone may be due to plant-mediated absorption of chlorpyrifos, rendering it less toxic to fish.

Using a quotient of two LC_{50} values (higher LC_{50} value/lower LC_{50} value), Mayer and Ellersieck (1986) proposed that a toxicant is more toxic when the quotient is more than 1. However, Schmuck *et al*. (1994) assumed that natural variability causes the quotient to vary between 0.5 and 2.0, and suggested that a toxicant is more harmful when the quotient value reaches 2. When the results of the present study (72 h-LC_{50}CPF+AW/72 h- LC_{50}CPF = 1.54) were compared, it was revealed that CPF alone was more toxic than CPF+AW according to Mayer and Ellersieck's (1986) criterion, but CPF alone was similar to CPF+AW according to Schmuck *et al*. (1994). Demetrio *et al*. (2014) advocated accepting a criterion as valid provided the concentration effect lines were parallel, since both of the criteria employ only a single value (LC_{50}) to describe the concentration-effect function. In the present study, the slopes of the regression lines for log concentration and probit mortality were not precisely parallel. As there is no overlap in confidence limits of the LC_{50}, LC_{50} values of CPF alone and CPF+AW differ at each time point. Therefore, it can be concluded that acute toxicity of chlorpyrifos is influenced by vegetation present in water.

Role of water turbidity on Chlorpyrifos toxicity

The results of the present study also indicated that water turbidity, created by adding either compost or suspended clay materials to the water, decreased mortality of Nile tilapia exposed to chlorpyrifos (25 µg·L^{-1}). Suspended materials in water bodies frequently adsorb pesticides, and consequently their bioavailability to aquatic organisms becomes reduced. While studying toxicity of chlorpyrifos in the presence of compost humic substances (CHS), Jones and Huang (2003) found that there was a 4.4-100 % decrease in chlorpyrifos toxicity, and they reported that it may be due to interaction of chlorpyrifos with CHS. In natural water, CHS have the ability to cause remediation of chlorpyrifos. Application of organic amendments can also be a useful strategy for reducing chlorpyrifos bioavailability in soils (Tejada *et al*., 2011). Because of the high humic acid content in organic waste, soil adsorption of chlorpyrifos becomes high, resulting in less chlorpyrifos being freely available to organisms. The effectiveness of the organic amendments in accelerating the degradation of cypermethrin and chlorpyrifos was also reported by Amin *et al*. (2021). Again, Aziz *et al*. (2018) studied the capacity of organic amendments to sorb chlorpyrifos. Application of

organic matter in soil induces microbial activity by working as a co-substrate as well as by sorption of pesticides, thereby reducing pesticide contamination in adjacent water bodies (Siedt *et al*., 2021). Compost bioremediation is now one of the most innovative strategies for the degradation of environmental contaminants (Cole, 1998). Post-remediation application of macrophytes (*Phragmites australis* or *Typha angustifolia*) as composting substances was found to be beneficial, eco-friendly, more suitable for sustainable management, and cheaper than commercial compost (Song, 2017). In the present study, microbes present in organic compost were probably responsible for chlorpyrifos degradation, resulting in less chlorpyrifos toxicity to Nile tilapia.

In the environment, natural clay can function as a pollutant scavenger (Srinivasan, 2011). Clays can reduce the bioavailability of different contaminants in the receiving ecosystem due to their adsorbent capacity. In another study by Suciu and Capri (2009), it was found that clay and its different modified forms can effectively remove pesticide residues from contaminated water. The micelle-clay complex mediated removal of chlorpyrifos and TCP from water samples was documented by Qurie *et al*. (2015). Osman and Abdelbagi (2018) reported that clay from El-gash River, Kassala state, Sudan, was used to adsorb chlorpyrifos from aqueous solution. In this study also, clay particles suspended in water adsorbed chlorpyrifos, making it less toxic to Nile tilapia.

The ambient environmental factors of recipient ecosystems should be addressed when setting safety standards for pest control programs in agriculture. Changes in parameters such as temperature, pH, and salinity influence the sensitivity of non-target aquatic organisms (Patra *et al*., 2015; Amiri *et al*., 2018; Pawar *et al*., 2020). Furthermore, while correlating the safety of non-target aquatic organisms with advanced management options for pest control, physicochemical and biological criteria, as documented in this study, should also be given due consideration; otherwise, any conclusion based on limited data generated from laboratory study in a confined test medium may be ambiguous.

CONCLUSION

It is concluded from this study that biological and physicochemical conditions of aquatic ecosystems can play important roles in influencing toxicity of chlorpyrifos to fish. Susceptibility of Nile tilapia to chlorpyrifos is reduced in the presence of the aquatic weed *Azolla pinnata* and also with increased turbidity of water. Determination of LC_{50} of a pesticide on non-target aquatic organisms is essential when adopting strategies for pest control. This study reveals that protocols for LC_{50} determination should consider biological and physicochemical conditions of the receiving water.

ACKNOWLEDGEMENTS

I am grateful to Professor Anilava Kaviraj, Department of Zoology, University of Kalyani, for giving me invaluable suggestions and encouragement during this study. I would like to express my gratitude to the Head, Department of Zoology, University of Kalyani for providing required laboratory facilities for this study. I am also thankful to the Principal, Vivekananda Mahavidyalaya, Haripal.

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