Research article

Behavioural incongruities in juvenile *Cyprinus carpio* exposed to organophosphate compounds

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ARTICLE INFO

Keywords:
Organophosphates chlorpyrifos
Dimethoate
*Cyprinus carpio*
Behaviour
Genotoxicity

ABSTRACT

For the ever increasing human population, the necessity to produce the food in large quantities has become the main goal internationally which has led to increase the practice of pesticides globally. Presence of pesticides in aquatic water bodies is largely due to the runoff from agricultural fields causing to deteriorate the healthy characteristics of an aquatic environment system leading to the toxic impact on non-target aquatic organism such as fish.

Approach: In fish, there are various portal of entry through which the contaminants enter. Via various routes, the contaminants reach into the blood and subsequently to different organs or systems. Since Pesticides are known to modify the behavior of animals when exposed to toxic levels. The behavioral changes may be caused by the changes in the nervous system triggered directly or through metabolic or physiological activities. However, the effects have been found to be multifarious and known to differ at different concentrations. Also, Blood is the most accessible component of the vertebrate body fluid system and consequences of direct and indirect damage to blood cells and their precursors are predictable and potentially life threatening. Therefore, behavioural and genotoxicological studies have been considered and used as diagnostic tool in order to investigate behavioural and genotoxicological alterations. This study was undertaken to investigate behavioural changes in *Cyprinus carpio* exposed to two organophosphate compounds, chlorpyrifos (cpf) and dimethoate (dim). Fishes weighing 10 ± 2 g were exposed to sub-lethal concentrations of cpf (0.76 ppb, 1.52 ppb, 2.28 ppb, 3.04 ppb and 3.8 ppb) and dimethoate (0.22 ppm, 0.44 ppm, 0.66 ppm, 0.88 ppm and 1.1 ppm) for the period of 96 h and various behavioural indices were evaluated during that period. Both the pesticides were found to induce behavioral

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https://doi.org/10.1016/j.heliyon.2022.e11227
Received 18 May 2022; Received in revised form 9 August 2022; Accepted 19 October 2022
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Pesticide is one of the aquatic pollutants that contribute to the environmental problems all over the world. Significantly large amount of the applied pesticides in farmlands and agricultural fields find their way back into aquatic habitats such as rivers, lakes and ponds as reported by Werimo et al. (2009) and Ensminger et al. (2011). On annual basis several conventional pesticides are used in different areas all over the world as reported by EPA (2001) which indicates that pesticides are spread across the ecosystem worldwide on a yearly basis as per the evaluation revealed by Piemental and Levitan (1986). Practice of organophosphates is on rise as their persistence in the environment is less due to their quick transformation into nontoxic compounds in less amount of time, yet their effects on the growth and survival in aquatic animals are adverse (Candioti et al., 2010; Bolognesi, 2003; Cavas and Ergene-Gözükar, 2005; Mohanty et al., 2011). Owing to high non-specific acute toxicity may lead to frequent intoxication of non-target organisms (Nunes, 2011) contributing extensively to the food chain and water bodies (Bagheri, 2007).

Fish are very sensitive organism to the environmental contaminants and as such, pollutants such as pesticides may initiate a significant disruption of certain metabolic activities in fish as a result of contact between the contaminants and fish organs (John, 2007). Many studies that were conducted in recent times have shown that pesticides can impair the health of fish (Siang et al., 2007; Nwan et al., 2010). Behavioural anomalies are the earliest of all shown by fishes even if they witness pesticide exposure in minute quantities. In most of the studies, the behavioural changes may be detectable before the occurrence of apparent physiological alterations (Marigoudar and Ahmed, 2009; Nagaraju et al., 2011; Devi and Mishra, 2013; Qayoom et al., 2016a, Qayoom et al., 2016b). Organophosphate pesticides are known to induce behavioural toxicity in fishes by targeting specific physiological systems and exert their effects on behaviour via physiological pathways. Popularly known as neurotoxins, they are key factors to cause damage in the brain muscles to cause Organophosphate Induced Delayed Neurotoxicity (OPIDN) (Hudson, 2004). This gives an indication, that their neuro-toxic nature may be secondarily involved in inducing neuro-behavioural toxicity in fishes to cause ostensible behavioural changes like expressions of mood, changes in behaviour and thinking attrition of scales, imbalance in swimming, quiescent and frenzied movements and uncoordinated movements (Qayoom et al., 2014; Qayoom et al., 2016a, Qayoom et al., 2016b; Uner et al., 2006). There are some other apparent and quantifiable behavioural changes like Tail Beat Frequency (TBF), Swimming Velocity (SV), and Opercular Beat Frequency (OBF) reported to get altered due to chemical insults of organophosphorous pesticide in fishes (Levin et al., 2003; Kane et al., 2005; Xia et al., 2010; Devi and Mishra, 2013). Ecotoxicological risk assessment of pesticides is based on available toxicity data and the effects resulting from actual exposure to a chemical in non-target organisms like fish serving both as targets and as models.

Common carp (Cyprinus carpio) is one of the most important cultured fish in India. Common carp and other species belonging to the family Cyprinidae are found in most rivers and lakes, making it a proper model species to study eco-toxicity of pesticides. Therefore, the aim of this study was to identify the changes in behaviour of carp exposed to sub-lethal concentrations of technical grade of organophosphate pesticides. In addition to the behavioural alterations, genotoxic changes have also been reported by number of researchers in terms of micronucleus formation in fish hematocytes. Micronuclei (MN) are extra-nuclear damaged chromosomes or their fragments which fail to transfer to daughter nuclei during cell division. These chromatin containing bodies fail in anaphase due to delay caused by xenobiotic exposure of pesticides leading to genetic injury in fishes. Permanent genetic damage due to MN formation leads to cell apoptosis, genomic instability or cancer development. However, it is found from the literature that a significant work has not yet been done in the field of behavioural toxicology of fishes with respect to organophosphate pesticides and need to be carried out extensively to attain a proper understanding behind the mechanisms involved in it. Similarly, genotoxic responses due to MN formation in fish hematocytes need to be studied with more deliberation to evaluate their intensity of toxicity of different pesticides in fishes. Keeping the importance of this research in consideration, the study was taken to assess the behavioural and genotoxic responses in C. carpio juveniles under in-vivo exposure of two organophosphorus compounds used worldwide, dimethoate and chlorpyrifos. The study was carried out to observe the elicited responses under controlled bioassays.

2. Material and methods

The whole experimental design is described in Figure 1.

2.1. Test organisms

Juveniles of common carp, C. carpio var. comminis weighing 10±2g were collected from a local hatchery in oxygenated plastic bags and immediately disinfected in 0.05% KMnO4 solution for 2 min. The weight of the fishes was selected same as used by Qayoom et al. (2016a, 2016b) to measure the exact sub-lethal concentrations used in this study. Soon fishes were transferred in aquaria with the size of 60 × 30 × 40 cm which also were also disinfected with KMnO4 before the introduction of fishes. Fish samples were kept for two weeks for acclimation and fed with artificial diet @ 3% body weight. During this period adequate oxygen was supplied through aerators and aquarium water was changed every morning.

2.2. Test design

Methodology from Manual of Methods in Aquatic Environment Research (Part 10, Short-term Static Bioassay), Technical Manual of FAO was applied in the study (Reish and Oshida, 1987). All the ethical guidelines for using and handling of test animals, chemicals for euthanasia and anesthesia, and drawing of blood from fishes were used in accordance with the Canadian Council of Animal Care (Nickum, 2004) opted by Canadian Department of Fisheries and Oceans Animal user training template (CDFOA, 2004). Ten fishes were placed in one aquarium in which bioassays were carried out in triplicates using double distilled water as dilution medium. Some Physical and chemical parameters like temperature, pH, CO2 and dissolved oxygen were analyzed daily to check the water quality (A. P. H. A., 2012).
2.3. Test concentration

Fishes were exposed to sub-lethal concentrations of dimethoate and chlorpyrifos under static bioassay tests. Technical grade of CPF and dimethoate were used in this study were procured from Gharda Chemicals Ltd. with 98.2% purity. The stock solution was prepared in methanol and subsequent concentrations in deionized water (Reish and Oshida, 1987). Sub-lethal concentrations of both the pesticides were chosen as per the available literature with 20%, 40%, 60%, 80% and 100% of LC50 (Qayoom et al., 2016a, Qayoom et al., 2016b). Behavioural responses were estimated within 96 h of the experiment while as for geno-toxicological studies, blood was withdrawn after 21 days of exposure of pesticides to fishes. All the experiments were static bioassay run in triplicates along with control.

2.4. Estimation of behavioural indices

For estimation of behavioural changes in fishes, the behavioural indices like Swimming Activity Index (Ai), Swimming Velocity (Vs), Opercular Beat Frequency (OBF), Tail Beat Frequency (TBF), Eye (Eo) and Fin deformities (Fd) were calculated as per the formulae given in Table 1.

2.5. Blood collection

For experimental study, blood was collected individually from each fish. Before blood collection fish were dipped in a solution of clove oil (75 mg/l) for anesthesia each time. Blood was withdrawn from caudal peduncle as per the guidelines provided by Canadian Council of Animal Care (Nickum).

2.6. Estimation of micro-nuclei for cytogenetic studies

The micro-nucleus (MN) test was done using methodology of Barsiene et al. (2013) Figure 2A drop of blood was immediately smeared on glass slide and air dried after collection. Then fixed in absolute methanol for 10 min, the slide was stained using 5% Giemsa for half an hour for the calculation of MN frequency using 100x magnifications and calculated using the following formula:

\[
\frac{\text{No. of micronuclei}}{1000} = \text{MN Frequency} \text{Cell}
\]

2.7. Ethical statement

The Aquatic Animal Ethics Committee of Faculty of Fisheries, SKUAST-K, J&K, India, reviewed the scientific procedures and protocols which were to be employed throughout the experimental study and whether they follow the aquatic animal ethical guidelines of the Committee For The Purpose Of Control And Supervision Of Experiments On Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC). After a review, the approval for conducting the study was granted (No: 2017-F-60-M: dated 14/03/2019).

2.8. Statistical analysis

The dataset obtained in behavioural indices was analysed using Two-Way ANOVA with the calculation of Critical Difference (C.D.) at 5%. The CD is the smallest difference between the inferences indicating a true change in the parameters. For the calculation of micronuclei and water quality, descriptive statistics for calculation of means, standard deviations and frequency was carried out using SPSS 20.0 version software.

3. Results

Five concentrations of CPF, i.e. 20%, 40%, 60%, 80% and 100% of LC50 (3.8 ppb) (0.76 ppb, 1.52 ppb, 2.28 ppb, 3.04 ppb and 3.8 ppb) and dimethoate (LC50 = 1.1 ppm) (0.22 ppm, 0.44 ppm, 0.66 ppm, 0.88 ppm and 1.1 ppm) were calculated from the available literature (Qayoom et al., 2016a, Qayoom et al., 2016b) and labeled from C1 to C5 with increasing order of concentration. In addition, development of
3.1. Behavioural indices

3.1.1. Tail Beat Frequency (TBF/min.)

Effects of chlorpyrifos and dimethoate on Tail Beat Frequency are presented in Tables 1 and 2. The tail beats decreased with the increase of exposure of pesticide dose and exposure time. TBF significantly (p < 0.001) decreased with the increase in concentration of CPF from C1 to C5 with an average values of 93.633/m to 53.417/m in E1, 61.436/m to 53.45/m in E2 and 25.75/m to 53.833/m in E3. The calculated critical difference (C.D.) in E1, E2 and E3 was found 17.417, 11.876 and 14.606 respectively (Table 2) indicating a significant reduction in the tail beats in CPF exposed fishes when compared with the control specimens. In dimethoate exposed fishes; TBF was also found diminishing with increasing concentrations of pesticide and the exposure time to fishes. The average values of TBF dropped down in C1 to C5 from 63.690/m to 50.400/m in E1, 75.092/m to 50.217/m in E2 and 76.283/m to 52.700/m in E3 with calculated C.D. of 8.178, 50.400/m in E1, 75.092/m to 50.217/m in E2 and 76.283/m to 52.700/m in E3 with calculated C.D. of 9.982, 10.097 and 10.088 respectively (Table 3). The results indicated that CPF had a profound effect on TBF of fishes suggesting that CPF is more toxic to common carps than dimethoate.

3.1.2. Opercular Beat Frequency (OBF/min.)

Chlorpyrifos and dimethoate were found to induce toxic effects on the C. carpio var. communis which caused change in the OBF in fishes exposed to both the pesticides. OBF was significantly (p < 0.001) found decreased with increasing concentrations of CPF and dropped down with the increase in concentration from C1 to C5 from the average values of 64.467/m to 32.667/m in E1, 64.183/m to 35.90/m in E2 and 59.813/m to 42.417/m in E3 with calculated C.D. of 8.178, 7.39 and 9.858 respectively (Table 4). In dimethoate experiments, OBF was also found diminishing as the concentration of pesticides increased from C1 to C5 with the average values of 77.667/m to 64.883/m in E1, 94.033/m to 71.267/m in E2 and 91.183/m to 67.85/m in E3 with calculated C.D. of 0.086, 10.903 and 10.881 respectively (Table 5). The profound decrease in CPF exposed fishes than dimethoate is indicative of its potential toxicity towards fishes. A steep decrease in TBF from C1 to C5 in both CPF and dimethoate bioassays indicate that with the increase in concentrations of pesticides, the sluggish movements recorded in fishes was found increased which caused decrease in OBF as well. Thus OBF was also found dependent on exposure of pesticide and time.

### Formulae for calculation of behavioural responses.

| S. No | Behavioural Response | Formula | Reference |
|-------|----------------------|---------|-----------|
| 1     | Swimming Activity Index (Ai) | Average number of total moves registered in the period number of total moves on day 1 | Eissa et al. (2009) |
| 2     | Swimming Velocity (Vd) | V = d (n - m)/dt | Eissa et al. (2009) |
| 3     | Opercular Beat Frequency (OBF) | Counted per minute in each individual in aquarium | Tanterpale et al. (2012) |
| 4     | Tail Beat Frequency (TBF) | Counted per minute in each individual in aquarium | Devi and Mishra (2013) |
| 5     | Eye deformities (Ed) | Microphthalmia Exophthalmia Unilateral Anophthalmia | Devi and Mishra (2013) |
| 6     | Fin deformities (Fd) | Necrosis Split fins | Devi and Mishra (2013) |

3.1.3. Swimming velocity (cm/sec)

The daily mean values of swimming velocity were calculated for the entire bioassay which was found fluctuating and got decreased with the increase in the concentrations of pesticides. Average mean values of swimming velocity dropped from C1 to C5 in CPF exposed fishes and ranged between 1.353 cm/s to 0.988 cm/s in E1, 1.112 cm/s to 0.817 cm/s in E2 and 2.656 cm/s to 0.872 cm/s in E3 with a non-significant critical difference (Table 6) in all the experiments. In dimethoate challenged fishes, the average mean values of swimming velocity decreased from C1 to C5 from 9.865 cm/s to 3.599 cm/s in E1, 6.146 cm/s to 2.367 cm/s in E2 and 5.518 cm/s to 2.169 cm/s in E3 with a calculated C.D. of 4.602, 4.432 respectively. The C.D. in E3 of CPF was found insignificant with the value of (Table 7).

3.1.4. Swimming activity index (Ai/day)

The mean swimming activity index (Ai) decreased in pesticide exposed fishes as compared to control. The activity was found to decrease in the bioassays with the passage of time and increase in pesticide concentrations. In CPF experiments, the average Ai values from C1 to C5 decreased from 0.235 ± 0.084 to 0.115 ± 0.041 in E1, 0.295 ± 0.046 to 0.0975 ± 0.016 in E2 and 0.4025 ± 0.033 to 0.195 ± 0.019 in E3 (Table 8). In dimethoate experiments the average swimming activity index (Ai) was also found to get decreased from C1 to C5 and ranged from 0.147 ± 0.006 to 0.0725 ± 0.013 in E1, 0.18 ± 0.008 to 0.1115 ± 0.007 in E2 and 0.18475 ± 0.003 to 0.1055 ± 0.003 in E3 (Table 9).

3.1.5. Eye deformities

No microphthalmia, unilateral anophthalmia and exophthalmia were observed in this study. However, clouding of eyes which initially turned the pupil into a white ball and whole eye ball was covered later on was noticed in both, CPF and dimethoate challenged fishes. The white mass of cloud turned dense with the increase in exposure time of pesticides to fishes and was reported early in the chlorpyrifos exposed samples. However, both the pesticides were equally seen to induce clouding of eyes in fishes (Figures 3, 4, and 5).

3.1.6. Fin deformities

During present investigation, none of the experimental fishes were found to develop fin necrosis in any of the experiments conducted for dimethoate and chlorpyrifos. However, there were various instances of split fins found at the termination of bioassays of both the pesticides. In dimethoate exposed fishes, not more than 20% of the fishes in C5 developed split fins at 96 h of the experiment indicating that this concentration can be lethal for fishes leading to the morphological deformities (Table 10). Fishes exposed to chlorpyrifos started to show the morphological deformities in fins early in the 48 h of experiment in higher concentrations of C4 and C5 (Table 10) which suggests that even small quantities of chlorpyrifos are lethal and potentially toxic to common carps. The maximum number of 20% of fishes was found to develop split fins with chlorpyrifos exposure (Figures 6 and 7).

3.1.7. Micro-nuclei formation

Fish exposed to chlorpyrifos and dimethoate was found to get intoxicated genetically which was confirmed by the incidence of micro-nuclei development in the fish erythrocytes exposed to both pesticides. The incidence of micronuclei was found both, time and dose dependent in fishes exposed to both pesticides. The frequencies of micronuclei are presented in Table 11 for chlorpyrifos and dimethoate respectively.

In chlorpyrifos exposed fishes, the percentage development of micronuclei increased with the increase in pesticide concentration of with the average values of 1.67 ± 0.66 in C1 to 10.67 ± 0.88 in C5 with none recorded in the control (Table 11). In dimethoate treated fishes, more or less same trend was observed with least micronucleus frequency of 1.00 ± 0.00 in C1 and the maximum frequency of 9.33 ± 0.33 in C5 (Table 11). No micronuclei were found in the control samples. The progressive increase in micronuclei incidence with increasing toxicant concentrations.
suggest that the genotoxicity due to chlorpyrifos and dimethoate in fish erythrocytes is dependent on the dose of the pesticide (Figures 8 and 9).

4. Discussion

When fish are exposed to aquatic pollutants, it metabolizes and stores aquatic pollutants (Daoud et al., 2009). The toxicants like pesticides gets accumulated within the tissue of exposed fish due to its lipophilic nature thereby resulting into impairment in the biology of fish. The aim of this study is to evaluate the behavioural and genotoxicological impairments in the fish exposed to the organophosphate pesticides. Behavioural toxicology is a primary tool to observe changes in fishes exposed to pesticides. The brain functions that are compromised on account of toxic insults directly influence fish behaviour and this is more conspicuous in fish, particularly with reference to aquatic pollution (Rao, 1999). In this study, organophosphate compounds were found detrimental to fish physiology and induction of behavioural and genotoxicity. Comparatively, CPF was found more hazardous than dimethoate and induced severe toxicity.

Tail Beat Frequency (TBF) is the quantitative estimation of beats per minute. Same stands true for Opercular Beat Frequency (OBF). Any change in TBF and OBF indicates the condition of stress, pathology or toxicity in fishes which make them lethargic with the onset of sluggish, uncoordinated and erratic movements. Induced toxicity by organophosphate pesticides is known to alter the TBF and OBF in fishes. In this study, the TBF and OBF of common carps were found significantly (P < 0.001) reduced with increasing exposure time and pesticide concentrations in all the replicates conducted for dimethoate and chlorpyrifos (Tables 4 and 5). Similar rise in OBF values in C2

![Figure 2. Protocol adopted for Geno-toxicological studies.](image)
concentrations were observed followed by a gradual decrease in their values from C3 to C5 in both, CPF and dimethoate bioassays. The reason for this sudden increase may be attributed to the fact that fishes initially experience a sudden shock due to the pesticide exposure due to which they show quiescent movements which leads to the increase in TBF. Similarly the sudden shock due to pesticide exposure leads to increased respiratory rates to avoid the toxic medium leads to increased values of OBF. Our results are in consonance with the results reported by Omorogie (1995), Grillitsch et al. (1999) and Chindah et al. (2004) who argued that chemical stress elicits behavioural changes under both acute and sub-lethal toxicity which causes reduction in TBF and OBF values as well. Devi and Mishra (2013) reported increase in the TBF of *Channa punctatus* exposed to chlorpyrifos up to 24th hour of pesticide exposure which declined afterwards with the termination of experiment. Their

| Time | C1    | C2    | C3    | C4    | C5    | Control | Mean for time | C.D. at 5% |
|------|-------|-------|-------|-------|-------|---------|--------------|-----------|
| E1   | 06    | 60.340| 64.100| 48.900| 48.300| 45.500  | 48.200       | 52.557    |
|      | 12    | 59.900| 59.900| 57.400| 54.400| 48.800  | 49.300       | 54.950    |
|      | 24    | 70.500| 73.400| 58.800| 50.100| 51.600  | 42.300       | 57.783    |
|      | 48    | 49.600| 48.200| 43.400| 39.000| 31.300  | 28.600       | 40.017    |
|      | 72    | 45.600| 47.000| 37.400| 24.600| 25.300  | 16.700       | 32.776    |
|      | 96    | 96.200| 102.60| 103.90| 102.80| 99.900  | 100.700      | 101.017   |
| Mean for concentrations | 63.690| 65.867| 58.300| 53.200| 50.400 | 47.633  |              |           |
| E2   | 06    | 70.750| 74.000| 66.200| 61.400| 50.800  | 49.300       | 52.557    |
|      | 12    | 77.400| 76.600| 67.300| 55.300| 48.500  | 47.700       | 50.033    |
|      | 24    | 72.800| 71.000| 68.700| 49.500| 39.000  | 14.800       | 50.683    |
|      | 48    | 73.100| 70.900| 58.400| 52.100| 29.000  | 9.600        | 39.983    |
|      | 72    | 61.200| 62.800| 48.300| 34.100| 24.600  | 6.200        | 44.417    |
|      | 96    | 95.300| 104.60| 104.00| 102.40| 102.80  | 103.900      | 101.967   |
| Mean for concentrations | 75.092| 77.617| 68.817| 59.133| 50.217 | 45.300  |              |           |

| Time | C1    | C2    | C3    | C4    | C5    | Control | Mean for time | C.D. at 5% |
|------|-------|-------|-------|-------|-------|---------|--------------|-----------|
| E3   | 06    | 72.900| 81.500| 79.000| 68.900| 62.100  | 50.400       | 52.557    |
|      | 12    | 77.900| 76.000| 72.000| 62.100| 56.300  | 47.600       | 65.217    |
|      | 24    | 74.100| 68.900| 50.500| 49.100| 42.500  | 31.800       | 51.767    |
|      | 48    | 75.700| 68.400| 62.900| 54.500| 29.000  | 20.100       | 51.767    |
|      | 72    | 66.500| 66.700| 53.700| 42.800| 24.600  | 9.600        | 44.417    |
|      | 96    | 90.600| 102.50| 102.20| 102.80| 99.800  | 99.817       | 99.817    |
| Mean for concentrations | 76.283| 77.333| 70.050| 63.267| 52.700 | 43.633  |              |           |

| Time | C1    | C2    | C3    | C4    | C5    | Control | Mean for time | C.D. at 5% |
|------|-------|-------|-------|-------|-------|---------|--------------|-----------|
| E1   | 06    | 58.400| 53.400| 45.900| 37.100| 26.800  | 26.200       | 41.300    |
|      | 12    | 46.600| 43.000| 37.100| 26.100| 26.100  | 17.000       | 32.200    |
|      | 24    | 42.000| 41.200| 27.600| 21.300| 16.500  | 10.300       | 26.483    |
|      | 48    | 38.900| 37.000| 16.000| 19.000| 13.900  | 7.600        | 22.067    |
|      | 72    | 36.400| 25.100| 17.700| 14.500| 10.600  | 6.200        | 18.417    |
|      | 96    | 104.500| 103.400| 102.800| 102.700| 104.800 | 99.700       | 102.983   |
| Mean for concentrations | 54.467| 50.517| 41.183| 36.783| 32.667 | 27.833  |              |           |
| E2   | 06    | 57.400| 58.200| 52.000| 48.300| 32.600  | 32.500       | 46.833    |
|      | 12    | 59.500| 54.600| 44.500| 40.700| 27.300  | 25.000       | 41.933    |
|      | 24    | 55.600| 55.400| 44.700| 32.700| 21.600  | 13.400       | 37.233    |
|      | 48    | 55.100| 50.200| 36.400| 21.100| 15.800  | 10.700       | 31.550    |
|      | 72    | 51.200| 42.800| 29.800| 18.800| 12.500  | 7.000        | 27.017    |
|      | 96    | 106.300| 106.000| 104.300| 105.400| 105.600 | 103.000      | 105.150   |
| Mean for concentrations | 64.183| 61.200| 51.950| 44.500| 35.900 | 31.983  |              |           |

Table 3. Tail beat frequency elicited by the fish when exposed to dimethoate.

Table 4. Opercular beat frequency elicited by the fish when exposed to CPF.
results are in accordance with the results obtained in the present study. Other studies indicate that toxicity due to petroleum related hydrocarbon compounds cause damage to epithelial cells of the gill chamber (Omoregie, 1995) which leads to retardation of OBF in common carps. Our results are in accordance with Chindah et al. (2004); Pandey et al. (2008); Woke and Wokoma, 2009; Devi and Mishra (2013); Misha and Verma (2016); Harit and Srivastava (2018) and Banjara and Singh (2019) who reported decrease in opercular beats of various fishes exposed to organophosphate pesticides. One of the primary reasons for the decrease in Tail Beat and Opercular Beat Frequencies in all the dimethoate and CPF exposed fishes is resultant of AChE inhibition in muscles which results in the blockade of neural transmission (Devi and Mishra, 2013) which is indicative of paralysis and pending death and causes retarded physiological processes in fish physiological functions (Omoregie, 1995;

| Time | C1 | C2 | C3 | C4 | C5 | Control | Mean for time | C.D. at 5% |
|------|----|----|----|----|----|---------|---------------|----------|
| E1 06 | 104.600 | 105.800 | 99.200 | 89.500 | 92.100 | 82.400 | 95.600 | 4.1176 |
| 12 | 71.900 | 70.400 | 61.500 | 58.200 | 53.800 | 50.500 | 63.667 | 4.1176 |
| 24 | 73.500 | 65.400 | 62.900 | 61.500 | 58.200 | 53.800 | 62.550 | 4.1176 |
| 48 | 70.200 | 70.800 | 69.500 | 61.500 | 58.200 | 53.800 | 63.667 | 4.1176 |
| 72 | 52.300 | 55.400 | 54.500 | 33.700 | 21.900 | 42.283 | 4.1176 |
| 96 | 93.500 | 96.000 | 97.400 | 97.300 | 98.000 | 96.783 | 4.1176 |
| Mean for concentrations | 77.667 | 77.633 | 74.483 | 69.017 | 64.883 | 60.283 | 4.1176 |
| E2 06 | 100.800 | 91.700 | 90.700 | 91.300 | 87.900 | 82.800 | 90.867 | 10.903 |
| 12 | 97.300 | 89.400 | 88.100 | 85.300 | 81.800 | 79.000 | 86.817 | 10.903 |
| 24 | 95.000 | 91.400 | 90.300 | 83.000 | 75.100 | 87.450 | 10.903 |
| 48 | 97.100 | 97.900 | 90.700 | 91.400 | 91.300 | 91.300 | 97.800 | 10.903 |
| 72 | 84.600 | 86.700 | 53.700 | 42.800 | 24.700 | 44.417 | 10.903 |
| 96 | 90.600 | 100.000 | 98.900 | 99.800 | 98.000 | 99.800 | 99.800 | 10.903 |
| Mean for concentrations | 91.183 | 92.333 | 83.683 | 76.417 | 67.850 | 62.767 | 10.903 |
| E3 06 | 102.500 | 97.900 | 89.500 | 78.100 | 78.800 | 76.417 | 87.183 | 10.881 |
| 12 | 96.500 | 98.800 | 82.400 | 77.300 | 79.000 | 84.233 | 10.881 |
| 24 | 95.200 | 96.600 | 84.200 | 79.200 | 75.100 | 83.467 | 10.881 |
| 48 | 95.800 | 94.500 | 90.100 | 78.900 | 70.600 | 83.467 | 10.881 |
| 72 | 66.500 | 66.700 | 53.700 | 42.800 | 24.700 | 44.417 | 10.881 |
| 96 | 90.600 | 102.500 | 102.200 | 102.200 | 101.600 | 99.800 | 99.800 | 10.881 |
| Mean for concentrations | 91.183 | 92.333 | 83.683 | 76.417 | 67.850 | 62.767 | 10.881 |

**Table 5.** Opercular beat frequency elicited by the fish when exposed to dimethoate.

**Table 6.** Swimming velocity-cm per sec elicited by the fish exposed to CPF bioassays.
Table 7. Swimming velocity-cm per sec elicited by the fish exposed to dimethoate bioassays.

| Factor (A) | Time | C1 | C2 | C3 | C4 | C5 | Control | Mean for time |
|-----------|------|----|----|----|----|----|---------|---------------|
| E1        | 06   | 16.333 | 4.438 | 3.547 | 3.810 | 2.777 | 1.965 | 5.478 | 1.8786 |
| 12        | 6.186 | 4.852 | 5.181 | 3.620 | 2.800 | 2.185 | 4.137 | 1.8092 |
| 24        | 11.272 | 9.600 | 3.446 | 2.574 | 2.045 | 2.014 | 5.158 | 4.432 |
| 48        | 8.176 | 6.406 | 2.848 | 2.234 | 1.539 | 1.229 | 3.795 | 2.012 |
| 72        | 8.750 | 6.753 | 2.552 | 1.409 | 2.482 | 0.824 | 3.795 | 4.432 |
| 96        | 8.475 | 8.600 | 10.225 | 11.850 | 9.950 | 8.175 | 9.546 | 2.012 |
| Mean for concentrations | 9.865 | 6.775 | 4.633 | 4.250 | 3.599 | 2.732 | 1.8092 |
| E2        | 06   | 15.525 | 3.633 | 2.988 | 2.682 | 2.134 | 1.851 | 4.432 |
| 12        | 4.738 | 3.083 | 2.567 | 2.642 | 2.390 | 2.037 | 2.895 | 4.432 |
| 24        | 4.569 | 3.172 | 2.600 | 1.993 | 1.873 | 1.871 | 2.680 | 4.432 |
| 48        | 3.372 | 3.300 | 2.689 | 2.078 | 1.038 | 1.034 | 2.252 | 4.432 |
| 72        | 3.747 | 4.067 | 2.226 | 1.529 | 1.196 | 0.670 | 2.239 | 4.432 |
| 96        | 4.925 | 5.353 | 6.378 | 6.581 | 5.659 | 5.437 | 5.722 | 4.432 |
| Mean for concentrations | 6.146 | 4.378 | 3.241 | 2.918 | 2.367 | 2.150 | 4.432 |

Table 8. Swimming activity index elicited by the chlorpyrifos exposed fishes during three trials at different concentrations.

| Days | C1 | C2 | C3 | C4 | C5 | Control | Mean for time |
|------|----|----|----|----|----|---------|---------------|
| E1   | Day 1 | 0.48 | 0.31 | 0.25 | 0.43 | 0.23 | 1.02 | 0.8786 |
| Day 2 | 0.19 | 0.27 | 0.19 | 0.17 | 0.07 | 1.03 | 1.8786 |
| Day 3 | 0.18 | 0.26 | 0.15 | 0.12 | 0.12 | 1 | 4.602 |
| Day 4 | 0.09 | 0.09 | 0.1 | 0.06 | 0.04 | 1.09 | 4.602 |
| Mean ± SE | 0.235 ± 0.0084 | 0.232 ± 0.0048 | 0.172 ± 0.0031 | 0.195 ± 0.0041 | 0.135 ± 0.0019 | 4.602 |
| E2   | Day 1 | 0.42 | 0.3 | 0.25 | 0.27 | 0.07 | 1.02 | 1.8092 |
| Day 2 | 0.3 | 0.25 | 0.01 | 0.17 | 0.07 | 1.03 | 4.432 |
| Day 3 | 0.25 | 0.21 | 0.17 | 0.14 | 0.12 | 1 | 4.432 |
| Day 4 | 0.21 | 0.18 | 0.14 | 0.14 | 0.13 | 1.09 | 4.432 |
| Mean ± SE | 0.295 ± 0.0046 | 0.235 ± 0.0026 | 0.1425 ± 0.0049 | 0.18 ± 0.0031 | 0.135 ± 0.0016 | 4.432 |
| E3   | Day 1 | 0.36 | 0.29 | 0.27 | 0.23 | 0.22 | 1.02 | 1.8617 |
| Day 2 | 0.39 | 0.36 | 0.26 | 0.22 | 1.03 | 4.432 |
| Day 3 | 0.36 | 0.36 | 0.25 | 0.18 | 1.04 | 4.432 |
| Day 4 | 0.5 | 0.42 | 0.39 | 0.25 | 0.2 | 1.09 | 4.432 |
| Mean ± SE | 0.4025 ± 0.003 | 0.3575 ± 0.006 | 0.32 ± 0.003 | 0.23 ± 0.0018 | 0.195 ± 0.0019 | 4.432 |

It is important to mention that during initial hours of pesticide exposure, the opercular movements were found to get increased which compensated the need of required oxygen demand in fish body. But in the later hours of experiments, when the opercular movements got reduced due to the induction of paralysis, fishes tried to escape the toxic medium, thereby depicting the surfacing and gulping movements.

Swimming Velocity ($V_s$) is the rate of change of distance from one point (n) to another (m) in a given test chamber. Swimming Activity Index ($A_s$) is the average number of total moves registered in the period divided by the number of total moves recorded on each particular experimental day $i$ of the period (Eissa et al., 2003, 2006). Increase in Swimming Velocity indicates hyperactivity while as its reduction depicts lethargy, paralysis and death in fishes. Similarly the Swimming Activity describes whether the fishes are moving normally without any indication of disease, stress and toxicity. Hence, any deviations of these indices are indicative abnormal behaviour in fishes.

In this study, the Swimming Velocity and Swimming Activity indices decreased in common carps exposed to dimethoate and CPF. In both experimental setups, the Swimming Velocity decreased in all the replicates (E1, E2 and E3) with the exposure of time and increase in the pesticide concentration. From C1 to C5 the gradual decrease in the Swimming Velocity values significantly ($p \leq 0.001$) decreased when compared with control (Tables 6 and 7). The Swimming Activity also decreased significantly ($p \leq 0.001$) compared to the control (Tables 8 and 9). Our results are in agreement with the findings of Rao et al. (2005) who found reduced Swimming Velocity in Gambusia affinis after chlorpyrifos exposure. Similar results were reported by Kavitha and Rao (2008) who reported decrease in Swimming Velocity of Gambusia affinis exposed to chlorpyrifos with the increase in the pesticide concentration which are in accordance with the results obtained in this study. Devi and Mishra (2013) obtained similar results in Channa punctatus under chlorpyrifos intoxication while as Verma et al. (2017) also obtained the similar results against Hilban on Heteropneustes fossilis which are in accordance with the results of this investigation. Decrease in Swimming Activity is also reported by Levin et al. (2003); Ramesh and Saravanan (2008), Dogan and Can (2011) and Harit and Srivastava (2018) who also reported reduction Swimming Activity Index in different fishes after sub-lethal exposure to different pesticide concentrations.

The reduction in the values of swimming indices is the outcome of neurotoxic stress induced by toxicants. Accumulation of Ach at synaptic junctions due to inhibition of AChE enzyme leads to sluggish movements in fishes, causing lowering of swimming indices. Hence, change in fish locomotor behaviour is resultant of Ach accumulation causing interruption in coordination between the nervous and muscular junctions. Similar reduction in locomotor activity has been reported by Rao et al. (2005); Begum et al. (2006) and Kavitha and Rao (2008) who linked depressed locomotor activity with the inhibition of AChE enzyme in fishes.
It is pertinent to mention that no microphthalmia, unilateral anophthalmia and exophthalmia were reported in this study. Our results differ with those obtained by Devi and Mishra (2013) who reported various eye deformities including microphthalmia, unilateral anophthalmia and exophthalmia in *Channa punctatus* exposed to chlorpyrifos. Misha and Verma (2016) and Verma et al. (2017) also reported several eye deformities in *Heteropneustes fossilis* under CPF intoxication. In this study, however, clouding of eyes which initially turned the pupil and later on the whole eye in to a white ball of cloud was noticed in both, dimethoate and chlorpyrifos exposed fishes. The white mass of cloud turned dense

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### Table 9. Swimming activity index elicited by the dimethoate exposed fishes during three trails at different concentrations.

| Days  | C1   | C2   | C3   | C4   | C5   | Control |
|-------|------|------|------|------|------|---------|
| E1 Day 1 | 0.155 | 0.155 | 0.13 | 0.11 | 0.1  | 1.06    |
| Day 2 | 0.144 | 0.1  | 0.11 | 0.09 | 0.09 | 1       |
| Day 3 | 0.13  | 0.12 | 0.09 | 0.07 | 0.04 | 1.04    |
| Day 4 | 0.159 | 0.14 | 0.11 | 0.09 | 0.06 | 1       |
| Mean ± SE | 0.147 ± 0.006 | 0.12875 ± 0.012 | 0.11 ± 0.008 | 0.09 ± 0.008 | 0.0725 ± 0.013 | 1.025 ± 0.015 |

| E2 Day 1 | 0.183 | 0.165 | 0.134 | 0.125 | 0.116 | 1.06    |
| Day 2 | 0.17  | 0.157 | 0.14  | 0.097 | 0.097 | 1       |
| Day 3 | 0.167 | 0.157 | 0.157 | 0.123 | 0.103 | 1.04    |
| Day 4 | 0.17  | 0.18  | 0.18  | 0.13  | 0.13  | 1       |
| Mean ± SE | 0.18 ± 0.008 | 0.16475 ± 0.005 | 0.15275 ± 0.010 | 0.11875 ± 0.007 | 0.1115 ± 0.007 | 1.025 ± 0.015 |

| E3 Day 1 | 0.188 | 0.184 | 0.142 | 0.142 | 0.108 | 1.06    |
| Day 2 | 0.183 | 0.175 | 0.135 | 0.118 | 0.1   | 1       |
| Day 3 | 0.178 | 0.16  | 0.141 | 0.12  | 0.114 | 1.04    |
| Day 4 | 0.19  | 0.173 | 0.141 | 0.135 | 0.1   | 1       |
| Mean ± SE | 0.18475 ± 0.003 | 0.173 ± 0.005 | 0.13975 ± 0.002 | 0.12875 ± 0.006 | 0.1055 ± 0.003 | 1.025 ± 0.015 |

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**Figure 3.** Clouding of eyes at initial hours of pesticide exposure.

**Figure 4.** Clouding of eyes of common carps exposed to dimethoate initial hours of pesticide exposure.

**Figure 5.** Clouding of eyes of common carps exposed to CPF initial hours of pesticide exposure.
with the increase in exposure time of pesticides to fishes and was reported early in the chlorpyrifos exposed samples. The same results were reported by Dey and Saha (2014).

In present study none of the experimental fishes were found to develop fin necrosis in any trials of dimethoate and chlorpyrifos which are not in accordance with the results reported by Pandey et al. (2008); Misha and Verma (2016) and Verma et al. (2017) who reported fin necrosis in various fish species exposed to different pesticides. However, there were various instances of split fins found at the termination of bioassays of dimethoate and chlorpyrifos. In dimethoate exposed fishes, up to 20% of the fishes developed split fins at 96 h of the experiment in C5 indicating that this concentration can be lethal for fishes leading to the morphological deformities (Table 10). Fishes exposed to chlorpyrifos started to show the morphological deformities in fins early in the 48 h of experiment in C4 and C5 (Table 11) which suggests that even small quantities of chlorpyrifos are lethal and potentially toxic to common

Table 10. Fin deformities developed in Cyprinus carpio var. communis exposed to different concentrations of dimethoate and chlorpyrifos.

| Pesticide | Time in hours | C1 | C2 | C3 | C4 | C5 | CT |
|-----------|---------------|----|----|----|----|----|----|
| Dimethoate | 6             | -  | -  | -  | -  | -  | -  |
|           | 12            | -  | -  | -  | -  | -  | -  |
|           | 24            | -  | -  | -  | -  | -  | -  |
|           | 48            | -  | -  | -  | -  | -  | -  |
|           | 72            | -  | -  | -  | -  | -  | -  |
|           | 96            | -  | -  | -  | -  | -  | -  |
| Chlorpyrifos | 6          | -  | -  | -  | -  | -  | -  |
|            | 12           | -  | -  | -  | -  | -  | -  |
|            | 24           | -  | -  | -  | -  | -  | -  |
|            | 48           | -  | -  | -  | -  | -  | -  |
|            | 72           | -  | -  | -  | -  | -  | -  |
|            | 96           | -  | -  | -  | -  | -  | -  |

T denotes trial, CT = control.

Figure 6. Split fins in common carps exposed to dimethoate.

Figure 7. Split fins in common carps exposed to CPF.
Table 11. Incidence of Micro-Nucleus (MN) after 21 days of chlorpyrifos exposure to *Cyprinus carpio* var. *communis* (R1-R3).

| Pesticide | S. No. | Conc. of pesticide (ppb) | No. of Fishes Observed | No. of cells counted | No. of cells with MN (R1, R2, R3) | MN Frequencies Mean ± S.E. |
|-----------|--------|--------------------------|------------------------|---------------------|-----------------------------------|-----------------------------|
| Chlorpyrifos | 1      | 0.76                     | 5                      | 100                 | 1, 3, 1                           | 1.67 ± 0.66                 |
|           | 2      | 1.52                     | 5                      | 100                 | 3, 2, 2                           | 2.33 ± 0.33                 |
|           | 3      | 2.28                     | 5                      | 100                 | 4, 7, 7                           | 6.00 ± 1.00                 |
|           | 4      | 3.04                     | 5                      | 100                 | 7, 7, 9                           | 7.67 ± 0.67                 |
|           | 5      | 3.8                      | 5                      | 100                 | 12, 11, 9                         | 10.67 ± 0.88                |
|           | 6      | 0.00*                    | 5                      | 100                 | 0, 0, 0                           | 0.0 ± 0.0                   |
| Dimethoate | 1      | 0.22                     | 5                      | 100                 | 1, 1, 1                           | 1.00 ± 0.00                 |
|           | 2      | 0.44                     | 5                      | 100                 | 1, 2, 1                           | 1.33 ± 0.33                 |
|           | 3      | 0.66                     | 5                      | 100                 | 2, 2, 1                           | 1.67 ± 0.33                 |
|           | 4      | 0.88                     | 5                      | 100                 | 7, 4, 6                           | 5.67 ± 0.88                 |
|           | 5      | 1.10                     | 5                      | 100                 | 9, 10, 9                          | 9.33 ± 0.33                 |
|           | 6      | 0.00*                    | 5                      | 100                 | 0, 0, 0                           | 0.0 ± 0.0                   |

a = zero conc. for control.

Figure 8. Micronuclei formation due to dimethoate.

Figure 9. Micronuclei formation due to CPF.
carps. The maximum number of 20% of fishes was found to develop split fins. The reason behind it might be the dermal absorption of the toxicant being one of the portal of entry. These results are in accordance with the results of Devi and Mishra (2013).

The instances of genotoxicity in this study were confirmed by the incidence of micro-nuclei development in the fish erythrocytes. The incidence was found both, time and dose dependent in both the pesticides. In chlorpyrifos treated fishes, same trend was observed with least frequencies recorded in C1 and highest in C5 (Table 11). In dimethoate exposed fishes, the percentage of development of micro-nuclei was found to get increased from C1 to C5 with none recorded in the control samples (Table 11). However, the incidence of micronuclei in chlorpyrifos exposed fishes was found higher than that of dimethoate confirming its severe induction of toxicity. Our findings are in agreement with Naqvi et al. (2016) who found that increase in frequencies of micronuclei are significantly dose dependent in Oreochromis mossambicus, Malik and Ganaie (2011); Malik et al. (2011); Ali et al. (2014); Dar et al. (2014); Anita et al. (2016), Bhatnagar et al. (2016) who found that increase in frequencies of micronuclei are severe induction of toxicity. Our findings are in agreement with Naqvi et al. (2016) who found that increase in frequencies of micronuclei are significantly dose dependent in Oreochromis mossambicus, Malik and Ganaie (2011); Malik et al. (2011); Ali et al. (2014); Dar et al. (2014); Anita et al. (2016), Bhatnagar et al. (2016) who reported micronuclei frequencies associated with the dose of different pesticides in fishes. Increase in the micronuclei frequency in organophosphate exposed fishes is indicates damage in the chromatin material, although the exact mechanism of genotoxicity is still not understood fully. Oxidative damage is thought to be an important mechanism in the DNA damage caused by organophosphate pesticides (Hodgson and Levi, 1996).

5. Conclusion

The alterations reported in the behavioural and genotoxicological responses of C. carpio communis juveniles exposed to sublethal concentrations of organophosphate pesticides in this study indicates that organophosphate pesticides have direct impact in alteration of behaviour of fishes and inducing Genotoxicity. Chlorpyrifos is more hazardous than dimethoate for inducing behavioural and genotoxicity in fish hemocytes. Behavioural and genotoxicological responses analysis maybe useful approach for monitoring the long-term effects of pesticides on cultured fish. This in turn will affect the growth and fitness, fecundity of the fish population and other non-targeted organisms such as man through the food chain. Therefore, the toxic hazard of organophosphate pesticides should be taken into consideration during its use in adjacent aquatic habitat.

Declarations

Author contribution statement

Sameena Khan: Performed the experiments; Wrote the paper. Imitiyaz Qayoom: Analysed and Interpreted the data; Conceived and designed the experiments. Masood H. Balkhi: Conceived and designed the experiments. Adnan Abubakr: Analyzed and interpreted the data. Sumyaa Rashid: Contributed reagents, materials, analysis tools or data. Rana M Alsaffar: Contributed reagents, materials, analysis tools or data; Analysed and interpreted the data. Muneeb U Rehman: Conceived and designed the experiments; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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