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Quinalphos (EC 25) Induced Behavioural Changes in *Cyprinus carpio* (Linnaeus 1758)

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Abstract:
For pest eradication in agriculture the commonly used organophosphate in Kashmir is Quinalphos (QP). Prolonged usage of QP finally reaches to the aquatic environment, wherein it results in considerable threat to the resident organisms. Therefore, the current endeavour was performed to assess acute toxic effects of quinalphos. Moreover, changes in the behaviour, differential leucocyte count (DLC) and genotoxic effects in common carp (*Cyprinus carpio* var. *communis*) were also observed. Weight of the fish taken ranged between 10± 2 gms and data of mortality was carried using Finney's Probit Method. For definitive tests, doses were chosen, based on pre- determined range finding tests, run in triplicates. In static bioassay system after 96 h, LC_{50} value for common carp was observed 13.35 ppm. The fish elicited clinical signs like excessive mucus secretion, convulsions, scale erosions, hemorrhagic patches and uncoordinated movements, change in body colour and surfacing and gulping onto air-water interface was also noticed at 96 h after exposing them to different sub-lethal concentrations of QP i.e., C_1 ($1/80^{th}$ of LC_{50}), C_2 ($1/40^{th}$ of LC_{50}) and C_3 ($1/20^{th}$ of LC_{50}). The results also revealed significant decrease in various parameters such as Tail Beat Frequency (TBF), Opercular Beat Frequency (OBF), Swimming Velocity (V_S), Swimming Activity Index (A_i) and Fin Deformities (F_d) with the increase in time and pesticide concentration. Fish also elicited genotoxic response which was evaluated by calculating the frequency of micronuclei formation in their hematocytes at different intervals of 7, 14 and 21 days of exposure. Moreover, variation in the differential leukocyte count was also observed in fishes exposed to different concentrations of the pesticide.

Keywords: Quinalphos; toxicity; behavioural changes; common carp

Introduction

Organophosphates (OPs) are widely used broad-spectrum pesticides in agriculture due to their potent pesticidal activity and biodegradability **(Amin et al., 2021)**. The primary mode of action of OPs is the inhibition of acetylcholinesterase (AChE), an enzyme crucial for regulating neurotransmitter acetylcholine signaling at cholinergic synapses in the central and peripheral nervous systems of fish and other animals **(Fulton and Key, 2001)**. This inhibition leads to the accumulation of acetylcholine at the neuromuscular junction, causing continuous nerve impulses that eventually result in the death of the target organism **(Ecobichon, 1991; Li et al., 2012)**.The inhibitory effects of OPs are dependent on their ability to bind to the active site of AChE and their phosphorylation behaviour **(Kavitha and Rao, 2006).** The enzyme inhibition causes acetylcholine to accumulate in the synapse, leading to increased firing of the postsynaptic neuron and heightened neuro-effector activity. Enhanced cholinergic activity in the parasympathetic autonomic nervous system (muscarinic receptors) may lead to symptoms such as increased mucous secretion, loss of balance, erratic movements alternating with quiescent periods, hypoxia, and gill erosion **(Silman and Sussman, 2005).** OPs are known to induce behavioural toxicity in fish by targeting specific physiological systems, affecting behavior through various physiological pathways **(Nagaraju et al., 2011; Yogita & Mishra, 2013)**. Recognized as neurotoxins, OPs can cause significant damage to the brain and muscles, leading to Organophosphate Induced Delayed Neurotoxicity (OPIDN) **(Hudgson, 2004).**

Insecticides, especially those belonging to the organophosphate (OP) class of compounds, are generally highly toxic to fish and aquatic invertebrates. Quinalphos(QP) being an insecticide, has been placed in group 2nd (Moderately Hazardous pesticide) by WHO **(Mostakim et al., 2015)**. It is among the most frequently used pesticide on wide range of pests such as scale insects, aphids, thrips, mites, caterpillars etc which are present on different crops such as soyabean, tea, paddy and cotton **(Hayes and Laws, 1991; Talwar et al., 2014).** The decomposition of the pesticide takes place by the process of hydrolysis, photolysis, oxidation, de-alkylation and isomerization that results in the formation of various intermediate compounds which can have a number of effects (both positive or negative) and these effects are similar to that of their parent compounds **(Kralj *et al*., 2007 and Zeinat *et al*., 2008)**, and ultimately carbon-dioxide is produced as end product on breakdown of these compounds. Some of these intermediate products result in enhanced inhibition of AChE than that of the principal compound **(Gaelli *et al*., 1994)**. Quinalphos is reported to have the half-life of 2 weeks, undergoes hydrolytic breakdown and quickly degrades in soil where aerobic conditions prevail **(Babu *et al*., 1998 and Goncalves *et al*., 2006).** Even after 20 days of the complete breakdown of the parent compound, one of the degradation products i.e., 2-hydroxyquinoxaline has been still reported to be present in soil **(Babu *et al*., 1998**). The other degradation products include quinalphosoxan, O-ethyl-O-quinoxalin-2-yl phosphoric acid and quinoxaline-2- thiol. The temperature, pH and dissolved organic matter are reported to be directly proportional to the degradation of the quinalphos (**Gupta *et al*., 2011**). Moreover, Quinalphos is reported to persist in water for longer time **(Prasad *et al*., 1985)**.

The widespread and unregulated use of pesticides in farming, livestock management, and post-harvest processes poses a significant threat to natural water systems, public health, and human welfare **(Tilak et al., 2007). Cessna and Allan (2009)** observed that not all pesticides hit their intended targets. Indeed, over 98% of sprayed insecticides end up in places other than their intended targets, as they are distributed across entire agricultural fields **(George, 2004). Tang et al. (2012)** noted that runoff can transport pesticides into aquatic ecosystems, while Tashkent (1998) highlighted that wind can carry them to other fields, grazing lands, or human settlements, potentially impacting other species. Among aquatic organisms, fish occupy the top tier of the aquatic food chain, leading to frequent biomagnification of pesticides **(Henny et al., 2003).** Furthermore, the exposure of fish to pesticides is alarming, given that fish are a key group of aquatic organisms consumed by humans. Approximately 50% of pesticides persist in fish for over 30 days, making them indicators of environmental pollutants **(Guo et al., 2008a; Guo et al., 2008b).** Consequently, there is increasing concern globally about pesticide contamination in aquatic environments. Fish species are currently regarded as suitable bio-indicators of aquatic pollution. They can accumulate various chemical substances and toxins in their bodies, revealing a range of biochemical, neurotoxic, hematological, cellular, and genotoxic effects (Wang et al., 2015b). Among aquaculture species, carps have the longest history **(Ensibi et al., 2012).** As a marker species, *Cyprinus carpio* will be chosen due to their ecological traits, wide distribution in Kashmir's freshwater environments, year-round availability, and commercial significance, making them an excellent test specimen (**Meraj et al., 2017**). In addition to this, *Cyprinus carpio* var. *communis* has been reported to be the most presiding fish in the water bodies of Kashmir **(Mohamad *et al*., 2020)**.

Various classes of pesticides are reported to exist in the water bodies of Kashmir **(Shah *et al*., 2012; Banday *et***

***al*., 2012; Qayoom, 2016 and Qayoom *et al*., 2018)**. Since, we have found enough literature supporting the toxicity caused by Quinalphos in different fish species **(Chebbi & David 2009, 2010a & b; Sadiqul *et al*., 2016 & 2017; Hemalatha *et al*., 2016 & 2019 and Mishu *et al*., 2020)** and as the same pesticide is also being used in the different fruit orchards of Kashmir valley as a control measure against well-known pests **(Plant protection Schedule -2018, 2020, 2021, 2022, 2023 & 2024 SKUAST-K, Khan, A. A. 2013 and Rather *et al*., 2020)**. Since, pesticides find their way into lakes and rivers by different ways such as pesticide drift, precipitation, discharge, percolation and agricultural runoff **(Honarpajouh, 2003; Vryzas et al., 2009)** thereby inducing several alterations. Genotoxic changes in terms of micronucleus formation in fish erythrocytes on exposure to organophosphate pesticide have also been reported by number of researchers (**Hemalatha *et al*., 2016 & 2019; Nwani et al., 2009; Mohanty et al., 2010; Ali et al., 2015; Hussain et al., 2017; Costa et al., 2018)**. Micronuclei (MN) are extra-nuclear damaged chromosomes or their fragments which fail to transfer to daughter nuclei during cell division **(Sameena et al., 2022).** They are formed by condensation of chromosomal fragments or whole chromosome that are not included in the main nucleus following anaphase. The micronucleus test in fish also has potential in detecting clastogenic substances in aqueous media **(Al sabti and Metcalfe., 1995).** Exposure to organophosphate pesticides have also reported to cause changes in the blood cells in fishes **(Qayoom et al., 2018; 2019**). As the fish blood in gills is in direct contact with water medium, any adverse change in the aquatic environment could be revealed in the circulatory system. Therefore, fish blood has been widely used in toxicological and environmental monitoring as a possible indicator of physiological changes in fisheries **(Mulcahy, 1975).** So the hematological studies of fish could be used to indicate the health status of fish and water quality. 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, genotoxic responses and hematological alterations in *C. carpio* juveniles under in-vivo exposure of Quinalphos. The study was carried out to observe the elicited responses under controlled bioassays.

1. MATERIAL AND METHODS:

2.1 Fish and Husbandry

The present trial was undertaken as per the methodology by **(Reish and Oshida, 1987)**. The specimens of experiment (juveniles of *Cyprinus carpio* var. *communis*) weighing 10 ± 2 g, brought from National Fish Seed Farm, Manasbal and were taken to the lab in oxygenated bags (plastic) with sufficient water in order to prevent them from stress and injury. The total number of 360 juveniles were used and it was ensured that the size of the largest fish should not exceed the size of the smallest fish by 1.5 folds. Once the fishes were transported to laboratory they were dipped in 0.5% potassium permanganate(KM_nO_4) for two minutes so as to disinfect them **(Ismail et al., 2017)**. Then the specimens were acclimatised in aquarium (60× 30×40 cm) for the period of two weeks **(Sadiqul et al., 2016).** Juveniles were fed with the grinded artificial feed once per day @ 3% body weight during this period **(Woo and Chung, 2020)**.The aquarium water was changed in next morning with the help of hand motor so as to remove the excreta or remaining feed from the aquarium. During acclimatization and test periods, 12:12 h photoperiod was maintained before carrying out the bioassays, 2 groups of specimens were taken with equal number of fish in each to be taken in range finding and definitive tests with 180 specimens in each **(Qayoom et al., 2016)**. Bioassays were carried out in triplicates of 10 fish per aquarium ((60× 30×40 cm) so as to maintain the stress free environment and prevent fish from getting choked **(Chebbi and David, 2010)**.

2.1.1 Test Concentration

Commercial grade of Quinalphos (EC-25) under the trade name of (Celquin) was used during the investigation which was obtained from Sumitomo Chemicals, India (A Subsidary of Sumitomo Chemical Co. Ltd. Japan). Stock solutions was prepared in acetone and range finding tests were performed for determination of concentration for definitive tests **(Hemalatha et al., 2016)**. In definitive tests a range of 8 ppm, 16 ppm, 32 ppm, 64 ppm and 128 ppm concentrations were taken and administered in triplicates. A negative control group was also taken in parallel to ensure whether the mortality of the specimens was due to solvent or toxicant.

2.1.2 Biological assay

During the present study the protocol of US-EPA EPA-660/3-75-009 was used to carry out the bioassay. The laboratory experiments and procedures were performed by following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Experimentation and Ethics in India.

For 96 hours static bioassay was performed. Before 24 h of experiment, feeding was stopped. The fish in the bioassay as well as control were starved during the experiment. The Quinalphos was added to the water and then fish were introduced in it within one hour. The mortality was recorded at the intervals of 6, 12, 24, 48 and 96 h. In definitive test, five concentration per toxicant were used and all concentrations were in triplicates with 10 specimens in each **(Hemalatha et al., 2016).** Fish were declared dead if any of the signs such as immobilization, no opercular movement, inequillibrium or morbidity was seen. This shows an reflection of awaiting death **(Mohanpatra and Rangarajan, 1995)**. Dead fish were removed from aquarium once observed. Test solution was discarded and aquarium was cleaned with HCl (10%) for disinfection after the experiment was over **(Reish and Oshida, 1987)**.

2.2 Water quality parameters of aquaria during bioassay

Different physico-chemical parameters such as laboratory and water temperature, pH, carbon-dioxide (CO_2), dissolved oxygen (DO), and total dissolved solids (TDS) were monitored regularly **(APHA, 2012)** at intervals of 24 hrs.

2.3 Clinical signs and behavioural responses

Qualitative and quantitative behavioural responses were noted during the static bioassay in different concentrations i.e C_1 (1/80th of LC_{50}), C_2 (1/40th of LC_{50}) and C_3 (1/20th of LC_{50}) at the intervals of 7, 14 and 21 days. The experiment was performed in triplicates (T_1, T_2 and T_3) for each parameter. During the first few days the movement of fish was slowed down which increased with advent of time. Qualitative behavioural response such as surplus mucus secretion, convulsions, uncoordinated movements, hemorrhagic patches, scale erosions, changes in body colour, surfacing and gulping were analysed and marked as feeble (+), progressive (++), intense (+++) and severe (++++) as adopted from **Qayoom et al., 2016a, b**. Quantitative behavioural response such as change in TBF, OBF, V_S, A_i and F_d were also observed as adopted from **Khan, 2020** and **Khan et al., 2022**.

Micronucleus test

Prior to blood collection, fish were anaesthetized with 0.12 g L^{-1} benzocaine **(Marques de Miranda Cabral Gontijo *et al*., 2003)**. Blood samples were withdrawn by cardiac puncture and peripheral blood smears were immediately made by applying two micro drops of blood on precleaned, grease free slides, using the standard fish micronucleated erythrocytes method of **Al-Sabti & Metcalfe (1995)**. The frequency of MN/fish were calculated per 1000 cells **(Raisuddin & Jha, 2004)**, and were evaluated by scoring the slides under oil immersion at 1000X magnification. Coded and randomized slides were scored using blind review by a single observer to avoid any technical variation. The criteria for the identification of micronuclei was according to standard procedures **(Fenech *et al*., 2003).**

Differential Leukocyte Count (DLC)

DLC was performed as per the methodology of **Hudson and Hay (1991)**. A small drop of fresh, anticoagulated non haemolysed blood was poured on the grease free slide and using a spreader slide, a thin tongue shaped smear was made. The blood smear was air dried and stained with Leishman's stain for 5 minutes. The stain was diluted with distilled water and again kept undisturbed for 10 minutes. Then the slides were washed, dried and observed under oil immersion objective (100X).

Statistical Analysis

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

Results & Discussion

3.1 Median Lethal Concentration (LC_{50}) of Quinalphos and mortality

Lethal concentration (LC_{50} was found to be 13.35 ppm with 17.19 and 9.49 ppm as upper and lower fiducial limits respectively **(Table 1**). At the lowest concentration of pesticide (8 ppm) no mortality was observed by the end of 96 h. At (16 ppm) concentration of the pesticide, 10% mortality was seen at 24 h, 30% at 48 h, 30%, at 72 h and 10% at 96 h. However, at higher concentrations of 32, 64, and 128 ppm of pesticide complete mortality (100%) was recorded by the end of 96 h. No mortality was reported in the control groups **(Fig 1**). Various studies have been conducted on different fish species exposed to various organophosphorus pesticides (**Chebbi and David 2010; Pandey et al., 2009; Hussain et al., 2015; Qayoom et al., 2016; Saha et al. 2016; Verma et al., 2017; Khan et al., 2018, 2021)**. LC_{50} for 96 h of QP in freshwater fish *Cyprinus carpio* was found to be 10.9 ml/l, while as LC_{50} in the same fish was reported to range between 7.5± 0.02 ml/l **(Chebbi and David, 2009, 2010)** which are lower from the values of the present study. The probable difference obtained during present study may be on account of the large size of fish (10±2 g). Moreover, as the pesticides are distributed quickly in the body of small sized fish, it causes the faster initiation of behavioural changes in them, since the consumption of a toxicant is directly proportional to the fish size **(Lewin et al., 2019).**

Table 1: LC_{50}

LC50 Value (ppm)	95% Confidential limits Upper Fiducial Limit	Lower FiducialLimit	Regression Equation Y=a-bX Co-efficient of regression(R^2)
13.35	17.19	9.49	Y=7.3082 – 3.4X R^2= 0.6436

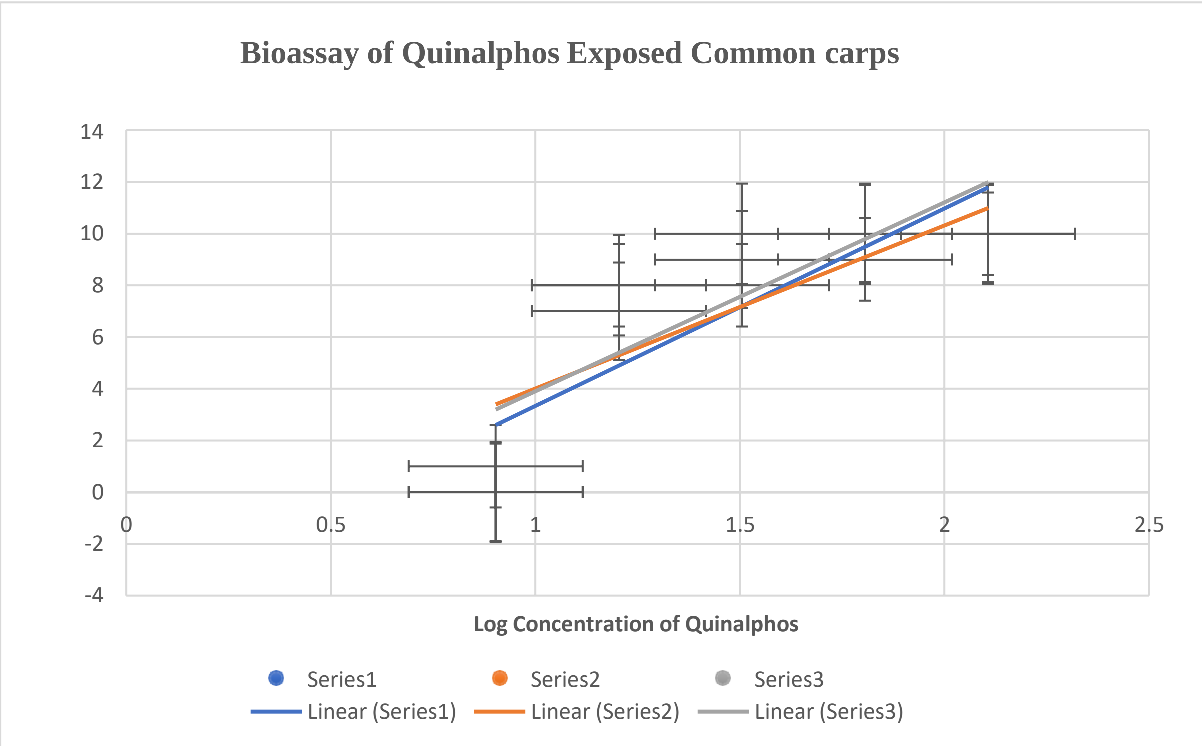

Fig 1: Graphical Representation of mortality estimate obtained in range finding test of Quinalphos

3.2 Water quality of aquarium during bioassay

During the static bioassay, very less difference was recorded in the water quality parameters. This perhaps may be due to the reason that fish were starved prior to 24 h of the start of the bioassay, as a result no contamination was found in the aquarium. Lab and water temperatures ranged from 16.10±0.11 to17.00±0.11°C and 12.80±0.11 to 13.40±0.011°C respectively. CO_2 values remained constant throughout the experiment with the average value of 2.00±0.11 to 2.00±0.11 indicating non hypoxic conditions. Dissolved oxygen ranged between 4.60±0.11 to

4.60±0.11mg/L, pH from 6.90±0.11 to 7.00±0.115 and TDS from 2.40±0.11 to 2.40±0.11**(Fig 2).** Thus, it could be conclude that in the present study, the mortality of fish was solely due to the intoxication of QP and not due to depletion of oxygen. Similar finding was observed in the juveniles of *Cyprinus carpio* var. *communis* on exposure to dichlorvos **(Khan et al., 2018).**

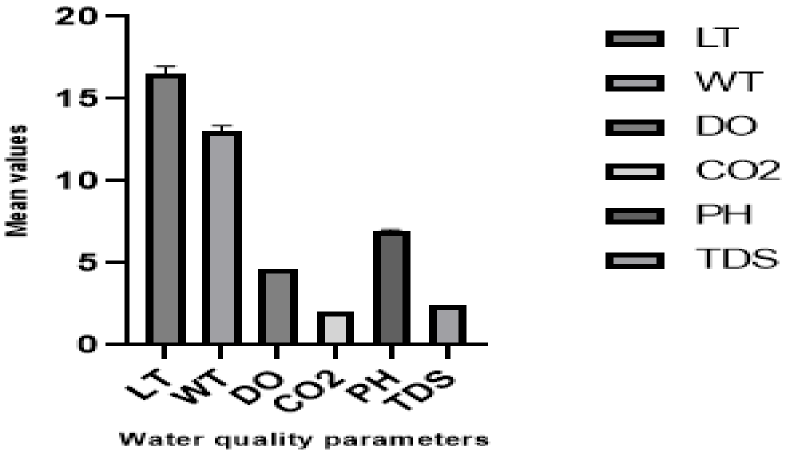

Fig 2: LT= Lab Temperature; WT= Water Temperature; DO= Dissolved Oxygen; CO_2 = Carbon dioxide; TDS= Total Dissolved Solids

3.3 Behavioural Responses in Fish Exposed to QP

Organophosphate pesticides are known to target various physiological pathways and show their effects on behaviour of fish. In many investigations, changes in behaviour have been observed before the visible physiological changes as reported by different workers **(Nagaraju et al., 2011; Yogita and Mishra, 2013; Qayoom et al., 2014, 2016a, b; Khan et al., 2022) (Table 2).**The mechanism of action of organophosphates is to obstruct the activity AChE, since quinalphos is an OP pesticide, suggesting the deposition of acetylcholine in the synapse, resulting in the enhanced cholinergic activity in parasympathetic autonomic nervous system (muscarinic receptors) which results in excess secretion of mucus, unbalance, erratic movements with alternate dormant stages, depletion of oxygen and gill erosion **(Binukumar and Gill, 2010; Qayoom et al., 2014)**.

Table 2: Formulae for calculation of behavioural responses

S.No	Behavioural Response	Formula	Refrences
1.	Tail Beat Frequency (TBF)	Counted per minute in each individual in aquarium	Devi and Mishra (2013)
2.	Opercular Beat Frequency (OBF)	Counted per minute in each individual in aquarium	Tanterpale et al. (2012)
3.	Swimming Velocity (S_V cm/sec)	V=d (n-m)/dt	Eissa et al. (2009)
4.	Swimming Activity (Index A_i)	Average number of total moves recorded in the period/ number of total moves on day 1	Eissa et al. (2009)
5.	Fin Deformities	Necrosis Split fins	Devi and Mishra (2013)

3.3.1 Qualitative Response

Excessive mucus secretion

Among qualitative responses (clinical signs), excessive mucus secretion was observed at higher concentration (C1and C2) which range between feeble (+) progressive (++) to intense at intervals of 7, 14 and 21 days while as at low concentration (C3) as well as in control samples no excessive mucus secretion was seen. Excessive mucus secretion in fish to pesticide exposure serves a defense mechanism (non- specific response) **(Mezin and Hale, 2000)** (**Table 3**). Our findings are in agreement researchers who worked on different fish species exposed to different organophosphate pesticides. Excessive mucus secretion was reported in *Cyprinus carpio* (**Chebbi and David 2010)** on exposure to QP, *Heteropneustes fossilis* (**Pandey et al., 2009)** *Catla catla* (**Hussain et al., 2015)** and *Cyprinus carpio* **(Qayoom et al., 2016)** on exposure to dimethoate, *Oreochromis mossambicus* and *Cyprinus carpio* (**Saha et al. 2016; Khan et al., 2018, 2021)** when exposed to dichlorvos respectively. Surplus mucus secretion was also reported in *Heteropneustes fossilis* **(Verma et al., 2017)** against Hilban intoxication.

Convulsions

Convulsive fits were observed on the 7th day till the end of the experiment varying between feeble (+) to progressive (++) in first 7 days, while as fish manifested intense(+++) convulsive fits on day 14 in C1. Fish in C3 and control did not elicit any fits at any stage of the bioassay (**Table 3**). Convulsions were reported in *Oncorhynchus mykiss* (**Dogan and Can, 2011),** *Catla catla* **(Hussain et al., 2015),** *Cyprinus carpio* var. *communis* (**Qayoom et al., 2016a, b)** on exposure to dimethoate and *Channa punctatus* **(Devi and Mishra, 2013)** on exposure to chlorpyrifos.

Uncoordinated movements

Uncoordinated movements were recorded simultaneously in exposed fish. As these movements were found to be directly proportional to time and concentration of pesticide. Feeble(+) movements were recorded in C1 and C2 on day 7, while as on day 14 feeble(+) to intense (+++) movements were recorded in the same concentrations. In C3 concentration (1/80th of LC_{50}) and control, no movements were observed (**Table 3**). Similar results were reported in *Heteropneustes fossilis (***Pandey et al., 2009)** and *Cyprinus carpio* **(Chebbi and David, 2010)** against dimethoate exposure. These movements were also noticed in common carp **(Banae et al., 2013)** on chlorpyrifos exposure. Uncoordinated movements were also reported in *Mystus singhala* **(Banjara and Singh, 2019)** following exposure to endosulphan, dichlorvos, phorate and carbofuran. Convulsions and uncoordinated movements were recorded because of the inhibition of acetylcholinesterase activity thereby causing the deposition of acetylcholine in the cholinergic synapses **(Hemalatha et al., 2016)** ensuring hyperstimulation, which was directly proportional to the time of exposure to the pesticide.

Scale erosion and Hemorrhagic Patches

On day 7, intense(+++) scale erosion was observed in C1 while in C2 feeble scale erosion was seen. Scale erosion increased progressively (++) on day 14 in both C1 and C2 concentrations while as on 21st day intense(+++) scale erosion was observed in both C1 and C2 concentrations. No change was observed in C3 and control on any of the days(i.e 7, 14 and 21st day). Moreover, hemorrhagic patches on skin were reported in fish with scale erosion.The fish in which no or feeble (+) scale erosion was present were hardly seen to show hemorrhages on their skin (**Table 3**) (**Fig 3**). Scale erosion was observed in *Clarias batrachus* (**Woke and Aleleye-Wokoma, 2009)** against chlorpyrifos-ethylon intoxication, *Channa punctatus* (**Devi and Mishra, 2013)**) against chlorpyrifos exposure as well as in *Cyprinus carpio* (**Khan et al., 2020)** on exposure to dimethoate. Erosion of scales and hemorrhagic patches could be on account of cutaneous absorption through skin after prolonged exposure to pesticide **(Qayoom et al., 2016a, b)**.

Surfacing and Gulping and Change in Body Colour

Fish exposed to quinalphos bioassays were seen to come on the surface so as to inhale the oxygen due to extended need of energy for survival, and this behaviour was reported to range from feeble (+), progressive(++) to intense(+++) in higher concentration (C1)(**Table 3**). Similar results have been assessed in *Anabas testudineus* **(Santhan kumar et al., 2000)** when exposed to sublethal concentrations of monocotrophs, *Silurus glanis* **(Ural and Simsek, 2006)** against dichlorvos intoxication, *Heteropneustes fossilis* **(Pandey et al., 2009; Verma et al., 2017)** on exposure to dimethoate and CPF respectively. Nuvan induced stress was also reported in *Cirrhinus mrigala* **(Srivastava et al., 2012)**. Surfacing phenomenon could be due to higher demand of oxygen in pesticide contaminated water in order to avoid suffocation throughout the exposure period **(Katja et al., 2005).** Body colour of fish exposed to quinalphos was seen to change feebly (+) in C2 on day 7 and then progressively (++) with the beginning of 14th and 21st day in C1. No change was seen in the control and C3 on any of the day during the trail of 21 days (**Table 3) (Fig 4**). Same results were observed in *Nemacheilus botia* **(Vasait and Patil, 2005)** intoxicated with sublethal concentrations of monocotrophos, *Channa punctatus* **(Pandey et al., 2011; Devi and Mishra, 2013)** against profenos and CPF exposure respectively. The pale colouration in fish on exposure to pesticide is due to the fact that stress conditions results in stimulation of adrenal glands which in turn causes hyper secretion of epinephrine thereby causing inhibition of Melanocyte Stimulating Hormone that ultimately resulted in pale colouration of the body **(Tyagi 2004)**.

Table 3: Qualitative behavioural parameters

EMS = Excessive Mucus Secretion, Conv = Convulsions, UM = Uncoordinated Movements, SE =Scale Erosion,
HP = Haemorrhagic Patches, S&G = Surfacing and Gulping, CBC = Change in Body Colour

+ = Feeble, ++ = Progressive, +++ = Intense

Response	Day 7			Day 14			Day 21			Control
	(C1)	(C2)	(C3)	(C1)	(C2)	(C3)	(C1)	(C2)	(C3)	
EMS	++	+	-	++	++	-	+++	+++	-	-
Conv	++	+	-	+++	+	-	++	++	-	-
UM	++	++	-	++	+++	-	+	+	-	-
SE	+++	+	-	++	++	-	+++	+++	-	-
HP	++	++	-	+++	+++	-	++	++	-	-
S&G	++	+	-	++	++	-	+++	+++	-	-
CBC	+++	+	-	++	+++	-	++	++	-	-

Fig 3: Scale erosion with hemorhaggic patch on tail of fish exposed to Quinalphos

Fig 4: Change in body colour of fish exposed to Quinalphos

Fig 5: Split fins in fish exposed to Quinalphos

Quantitative Behavioural Responses

Tail Beat Frequency and Opercular Beat Frequency

Among quantitative responses, TBF and OBF were found to be significantly declined with increase in the concentration of quinalphos from C1 to C3 (**Table 4)** at exposure of 7^{th}, 14^{th} and 21^{st} days with the p value of >0.05 and <0.05 for TBF and OBF respectively. This steep decrease in TBF and OBF indicated that the sluggish movements in fish are directly proportional to pesticide concentration. In C2 a slight increase in the TBF and OBF values were observed than C1 followed by a gradual decrease in the higher concentration (C3) (**Table 4**). The sudden increase may be due to the fact of initial exposure of fish to pesticide thereby resulting in quiescent movements which lead to the increase in TBF. Similarly, the pesticide exposure shock leads to increased respiratory rates to avoid the toxic medium thereby causing increased in the values OBF. Our results are in accordance with **Omoregie, 1995; Chindah et al., 2004; Devi and Mishra, 2013** who observed initial increase followed by decline in TBF and OBF in *Channa punctatus* exposed to chlorpyrifos. Acute toxicity was also reported in *Heteropneustes fossilis* **(Pandey et al., 2009)** against dimethoate**,** *Clarias gariepinus* **(Woke and Aleleye- Wokoma, 2009)** once exposed to chlorpyrifos, *Channa punctatus (***Harit and Srivastava, 2018**) following endosulfan exposure. Subjection to Chlorpyrifos based pesticide- Termifos also revealed similar results in *Clarias gariepinus* **(Nwani et al., 2013)**. Change in TBF and OBF were reported in *Labeo rohita* and *Oreochromis mossambicus* **(Bhavika, 2013)** against curzate and imidocloprid exposure respectively. One of the primary reasons for the decrease in Tail Beat and Opercular Beat Frequencies in all the concentrations of QP may be on account of inhibition of AChE activity due to which neural transmission is blocked. These results are in accordance with **Devi and Mishra, 2013** who also reported inhibition of AChE, which is indicative of paralysis and pending death resulting in retardation of the physiological processes in fish **(Omoregie, 1995; Fryday et al., 1996)**.

Swimming velocity and Swimming Activity index

The calculated value of swimming velocity(S_V cm/sec) and swimming activity index (A_i) was found to decrease with the rise in pesticide concentration when recorded at intervals of day 7, day 14 and day 21.Average mean values of swimming velocity dropped from C1 to C3 (**Table 4**) with P value of <0.05 and >0.05 for swimming velocity and swimming activity index .The outcome of the present work are in consonance to that of **Rao et al., 2005**, **Levin et al., 2003**, **Ramesh and Sarvanan, 2008 Devi and Mishra 2013** and **Verma et al., 2017** who found reduced Swimming Velocity in *Gambusia affinis*, zebra fish (*Danio rerio*) and *Cyprinus carpio, Channa punctatus* and *Heteropneustes fossilis* following exposure to chlorpyrifos and Hilban respectively. Decrease in the Swimming Activity Index was reported in *Channa punctatus* (**Dogan and Can, 2011),** *Oncorhynchus mykiss* (**Harit and Srivastava, 2018)** against dimethoate and endosulfan exposure respectively. Similar decline in locomotory activity was seen in *Gambusia affinis* **(Begum et al., 2006; Kavitha and Rao, 2008)** when exposed to chromium and chlorpyrifos respectively. The deposition of acetylcholine at the neuromuscular junctions is because of the inhibition of AChE activity which causes alterations in the locomotory behaviour of the fish are observed **(Hernandez-Moreno et al., 2011)**.

Table 4: Quantitative behavioural parameters

TBF= Tail Beat Frequency; OBF= Opercular Beat Frequency;

Sv= Swimming Velocity; Si= Swimming Activity Index

Response	Day 7			Day 14			Day 21			P value
	(C1)	(C2)	(C3)	(C1)	(C2)	(C3)	(C1)	(C2)	(C3)	
TBF	82.66	83.33	88.33	89.00	78.33	89.00	83.33	75.33	68.00	>0.05
OBF	68.00	63.66	66.00	63.66	64.66	54.66	56.33	53.66	54.33	<0.05
Sv	1.38	1.20	1.58	1.30	1.06	0.67	0.53	0.32	0.24	<0.05
Si	0.79	0.55	0.35	0.51	0.51	0.51	0.52	0.50	0.89	>0.05

In quinalphos exposed fish, none of the experimental fishes were found to develop fin necrosis. However, various instances of split fins found at the end of the 21st day of the bioassay. The maximum number of 20% of fish was found to develop split fins with quinalphos exposure(**Table 5**) in C3 which suggests that the toxic nature of quinalphos (**Fig 5).** These results are in accordance with **Sunanda et al., 2016** who reported morphological deformities such as split fins and fin necrosis in fish exposed to chlorpyrifos at different exposure periods.

Table 5: Fin Deformities developed in *Cyprinus carpio* when exposed to different concentrations of QP

Response	Day 7			Day 14			Day 21		
	(C1)	(C2)	(C3)	(C1)	(C2)	(C3)	(C1)	(C2)	(C3)
Split fins	20%	20%	17%	4%	5%	4%	13%	10%	12%

Micronucleus test

The instances of genotoxicity in this study were recorded at intervals of 7, 14, 21 days and were validated by the incidence of micro-nuclei development in the fish erythrocytes The incidence was found dependent on both, time and dose of the pesticide. Significant changes($P<0.05$) were recorded in the frequency of micronuclei in all the three concentrations (0.66, 0.33 and 0.16 ppm) at different time intervals of 7, 14 and 21st day with the Mean ± SE of 2.33±0.88, 2.33±0.33 and . 4±1.52. (**Table 6**). Our findings are in agreement with **Turan and Ergenler (2022); Ucar et al., 2021; Ghaffar et al., (2019); Naqvi et al. (2016) and Khan et al., 2022** who found that increase in frequencies of micronuclei are significantly dose dependent in *C. carpio*, *Oncorhynchus mykiss*, *Labeo rohita*, *Oreochromis mossambicus, Cyprinus carpio.* **Malik and Ganaie (2011); Malik et al. (2011); Ali et al. (2014); Dar et al. (2014); Anita et al. (2016), Bhatnagar et al. (2016); Kurteshi and Shaqiri (2019)** also reported micronuclei frequencies associated with the dose of different pesticides in fish.

Table 6: Incidence of micronucleus (MN) after 7,14 and 21 days of Quinalphosexposure test Quinalphos

Conc. of pesticide	No. of fishes observed	No. of cells counted	No. of cells with MN			Mean ± SE	P value
			Day 7	Day 14	Day 21		
0.16	10	100	1	2	4	2.33±0.88	<0.05
0.33	10	100	2	2	3	2.33±0.33	<0.05
0.66	10	100	2	3	7	4±1.52	<0.05

An increase in micronuclei frequency in fish exposed to organophosphates suggests damage to chromatin material, though the precise mechanism of genotoxicity is not yet completely understood. Oxidative damage is believed to be a crucial mechanism in the DNA damage caused by organophosphate pesticides (Hodgson and Levi, 1996).

Differential Leucocyte Count

The number of leukocytes was found to be directly dependent on the concentration of the pesticide when observed at different intervals of 7, 14 and 21 days respectively. The increase in the number of leukocytes (leucocytosis) is attributed to the enhanced defence activity of fishes under stress on exposure to pesticide. **Das and Mukherjee (2001)** related the increase in the TLCs in *Labeo rohita* due to stimulated lymphopoiesis and/ or enhanced release of lymphocytes from lympho-mycloid tissues and could be attributed to the presence of toxic substances or may be related to the tissue damage due to increased pollutant levels as suggested by **Haniffa (1990)**. Insignificant difference (($P =<0.05$) was observed among the different sub-lethal concentrations, parameters and interval of 7, 14 and 21[st] days with Mean ± SE= 4.66±1.20, 79±3.78, 5±1, 0.33±0.33 and 0±0; 7.66±2.60, 95.33±11.89, 5.33±1.33, 0.66±0.33 and 0.33±0.33; 9.66±1.76, 113.66±6.56, 7.66±2.60, 1±1 and 0.66±0.33 of monocyte, lymphocytes, neutrophil, eosinophil and basophils respectively. The P value is all cases was found to be < 0.05 (**Table 7**).

Table 7: Differential leukocyte count in fish exposed to different concentrations of QP

S. NO	DLC Parameters	Day 7			Mean± S.E	Day 14			Mean± S.E	Day 21			Mean± S.E	P value
		C_1	C_2	C_3		C1	C2	C 3		C1	C2	C3		
1	Monocyte (%)	7	4	3	4.66±1.20	12	8	3	7.66±2.60	13	7	9	9.66±1.76	<0.05
2	Lymphocyte (%)	80	72	85	79±3.78	103	111	72	95.33±11.89	123	117	101	113.66±6.56	<0.05
3	Neutrophil (%)	6	6	3	5±1	8	4	4	5.33±1.33	12	8	3	7.66±2.60	<0.05
4	Eosinophil (%)	1	0	0	0.33±0.33	1	1	0	0.66±**0.33**	1	1	1	1±1	<0.05
5	Basophil (%)	0	0	0	0±0	1	0	0	0.33±**0.33**	1	1	0	0.66±0.33	<0.05

Ghayyur et al., 2021, reported that the values of WBCs and platelets of *C. mrigala* exposed to pesticides (Chlorfenapyr, Dimethoate and Acetamiprid) exhibited significant increase as compared to control for 10th, 20th and 30th days of exposure. The raise in WBCs number can be linked with more antibodies formation, which aids in recovery and survival of the fish exposed to different pesticides **(Malik and Maurya, 2014).**

Kole et al., 2022 evaluated the effect of sumithion pesticide on the hematological parameters of *Barbonyms gonionotus* on exposure to two sub lethal concentrations of pesticide. The results showed increase in the number of WBC's when noted at different intervals of 1, 7, 14, 21 and 28[th] day. During the exposure period to sumithion, enhanced WBC count indicates that fish can overcome toxic stress by developing a defensive mechanism. In the presence of foreign substances, leucocytosis in fish stimulates immunological defense **(John., 2007).** High WBC counts suggest damage to body tissues from infection, extreme physical stress, or even leukemia **(Verma et al., 2019)**. Increased WBC counts are linked to increased antibody synthesis, which aids in the recovery and survival of pesticide-exposed fish **(Salam et al. , 2015; Mostakim et al., 2015; Joshi et al., 2022).**

Lutfiyah et al., 2020 observed the effect of organophosphate pesticide on hematological parameters of Silver Rasbora (*Rasbora argyrotaenia).* The results of this study showed decrease in total erythrocytes (0.59±0.004) and hemoglobin (2.5±0.1) while total leukocytes increased (245.35±15.78). Also, there are differential changes in leukocytes, i.e, increased in the number of monocytes (5±1) and neutrophils (24±3), but lymphocytes have decreased in number (72±1). **Akhtar et al.,2021** observed an increase in the levels of neutrophils and monocytes while as lymphocytes were found to decrease in *Schizothorax esocinus* on exposure to Cypermethrin. Several workers observed initial increase in leukocyte count of fish species exposed to pesticides followed by the a drastic decrease which is generally associated to the fact that the initial increase in the TLC count is to combat the infection due to pesticide exposure. Increase in time of exposure of pesticide, generally in acute levels is associated with the complete destruction of hematopoietic system as an indicative of hematotoxicity in fishes **(Ali and Rani, 2009)**. **Ahamad (2011)** observed decrease in TLC of *Cyprinus carpio* to malfunctioning of the haematopoietic system caused by exposure to organophosphates. He suggested that the changes in leukocyte system manifest in the form of leukocytosis with heterophilia and lymphopenia, which are characteristics of leukocytic response in animals exhibiting stress.

Conclusion

The organophosphate compound (Quinalphos) is not only toxic to common carp but also could induce various morphological, behavioural, genotoxic and hematological changes in them. Therefore, it must be ensured that there should be less in flow of pesticides in the aquatic ecosystems with particular reference to quinalphos. As the ingression of QP could lead to the waterbody contamination as well as other economically important flora and fauna (especially fish) which could eventually lead to toxicity of human beings. Moreover, if the relentless application of pesticide is continued, it will have adverse effects on fish health as well as on livelihood of the people who are dependent on it directly or indirectly.

Conflict of interest

The authors declare that there is no conflict of interest

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