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Phosalone (35%EC) an organophosphate Induced toxicity and behavioural changes of fresh water fish *Ctenopharyngodon idella*

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Abstract
Background: Pollutants such as insecticides may affect significantly certain physiological and biochemical processes that different kinds of insecticides can cause serious impairment to health status of fishes. Fishes are very sensitive to a wide variety of toxicants in water; various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides. Toxicity studies have played an important role in man's efforts to monitor and modify the effects of his activities on the biota.
Aim and Objective: To study the Phosalone (35%EC) an organophosphate Induced toxicity and behavioural changes of fresh water fish *Ctenopharyngodon idella*
Results: In the present study**,** we observed percent mortality along with exposure concentrations of freshwater fish *Ctenopharyngodon idella* (Grass carp), to Phosalone (35 % EC) formulation, for 24,48,72,96 hrs in static system along with percent mortality and probit mortality were presented. The LC_{50} values of Phosalone (35% EC) for the fish *Ctenopharyngodon idella* (Grass carp), after 24,48,72,96 hrs, in static system were 2.8, 2.4, 2.0 and 1.6 ppm respectively. The LC_{50} value was high for 24 hrs i.,e 2.8, the LC_{50} value was low for 96 hrs i.,e 1.6 ppm but for 48 hrs the LC_{50} value 2.4 ppm and for 72 hrs 2.0 ppm only. The low value for 96 hrs may be due to accumulated toxicant accumulates effect on the fish. The LC_{50} value regression value (R^2) of Phosalone (35%EC) for exposed fish *Ctenopharyngodon idella* (Grass carp), after 24,48,72,96 hrs, in static system were observed.
Conclusion: The present study LC_{50} value of Phosalone (35% EC) exposed to the fish, *C. idella* is agreed with the earlier reports and it is evidence from the above started LC_{50} values that organochlorines, organophosphates, carbamate and synthetic pyrethroids are more toxic to fish based on LC_{50} values. Due to their persistence and high toxicity on fish and mammals, a restricted on their use is imposed, based on the above results the selected test compound was rated in toxicity.

Keywords: *Ctenopharyngodon idella, toxicity,* organophosphates, phosalone, mortality

Introduction

Fisheries and aquatic resources (ponds, rivers, streams, seas and the oceans) are supplying people with long term benefits can be the direct financial ones that provide employment, profit, and save money. For example, the sea food industry provides hobs for commercial fisheries, wholesalers and retailers. More indirect, but equally valuable, benefits of fish and aquatic ecosystems include recreational boating, sports like fishing, swimming, relaxation, and natural beauty, Science daily (2008a). There are occupational hazards and safety in the aquaculture industry. Some practices have caused environmental degradation. Public perception to farmed fish is that they are "cleaner" than comparable wild fish. However, some farmed fish have much higher body burden of natural and manmade toxic substances e.g., antibiotics, pesticides, and persistent organic pollutants than wild fish. These contaminants in fish can pose health concerns to unsuspecting consumers, in particular pregnant or nursing women.

Hence, pollutants such as insecticides may affect significantly certain physiological and biochemical processes that different kinds of insecticides can cause serious impairment to health status of fishes, Banaee (2013) [1]. The increasing population has put a stress on resources, resulting in the excessive use of organophosphorous pesticides and fertilizers to meet the demand. These substances ultimately pollute the aquatic environment and cause severe damage to the aquatic life especially to the fish, prawn and crab species Kumar and

Prasad (2010) [2]. Among the different groups of pesticides organophosphates are being used commonly as insecticides due to their facilitation properties like less persistence and rapid biodegradability in nature. Singh and Pandey (2010) [3].

Organophosphates and carbamate compounds are among the most widely used pesticides. Contamination of surface water by these compounds is concern because of potential toxicity to aquatic organisms, especially those at lower levels. After exposure to different concentrations of insecticides in water, the fish absorbs them in its gill, skin or gastrointestinal tract. Due to their lipophilicity, most insecticides are rapidly metabolized and extracted and may be bio-concentrated in various tissues of fish. Bio-accumulation occurs if the insecticides increases, it becomes more harmful to the consumer or animals.

Pesticides are able to move from one ecosystem to another through processes such as transfer (mobility) and transformation (degradation). Transfer may occur through surface run off, vaporization to atmosphere, sorption (adsorption/desorption), plant uptake or soil water fluxes. Transformation occurs through chemical, microbial and photo degradation. The transportation of pesticides to their final destination in the aquatic ecosystem may result in adverse health effects on the organisms found there.

The study suggests that freshwater arthropods very less in their immobility response than in their mortality response. The suggested immobility as the relevant endpoint for SSDs and ERA (Environmental Risk Assessment) because they found it was a more sensitive endpoint than mortality, with less variability across tested species. Generally, effect concentrations for immobility and mortality will converge to the same value with time, but this does not occur with the same speed for all species Rubach and Crum (2011) [4]. However, a good match between effective (immobility) and lethal (mortality) concentrations can exist right from the start of toxicity test where LC_{50}/EC_{50} ratios equal one, approximately.

Materials and methods

Animal selected

Ctenopharyngodon idella (Cuvier and valenciennes, 1844, Franz Steindachner, Austrian Zoologist, 1866) is commonly known as Grass Carp. It is a freshwater fish, are native to the Amur River in china and Russia. Grass Carp is cultivated in china for food, but was introduced in Europe and United States for Aquatic weed control. Grass Carp has a wide degree of temperature tolerance; it will enter reproductive condition and spawn at 20^0 to 30^0 c. In United States it is commonly known as "White Amur". In Andhra Pradesh, especially in Guntur Dist. Grass Carp is widely cultivated for food.

Fig 1: Experimental fish *Ctenopharyngodon idella*

Scientific Classification of *Ctenopharyngodon idella*:

Kingdom : Animalia
Phylum : Chordata
Class : Actinopterygii
Order : Cypriniformes
Family : Cyprinidae
Sub-Family : Squaliobarbinae
Genus : *Ctenopharyngodon*
Species : C. *idella*

Procurement and maintenance of fish:

Healthy freshwater fish, *Ctenopharyngodon idella* (Valenciennes) size (6±7cm total length (TL) and 6.5±7.5g body weight) were immediately transported in large plastic tanks with required aeration and brought to the laboratory. Then the fish acclimatized to the laboratory conditions in large cement (200L) tanks with sufficient dechlorinated ground water for 15 days at room temperature 28 ± 2^0C.

Then the fish were separated into the batch of having the length of the fish 6±7 and body size 6.5 to 7.5g were maintained in static water without any flow. All the precautions were laid by APHA *et al.*, (1998) [5]. As the level of toxicity was reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of the animals, size and sex etc. Precautions were taken throughout this investigation to control all these factors as far as possible. As a part of it, water from the same source has been used for maintenance of the fish. The size of the animals selected was also maintained strictly throughout the investigation.

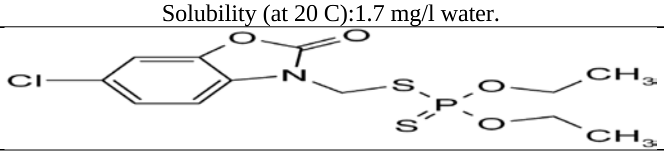
Selection of Organophosphate Pesticide

The test compound for the study belonged to Organophosphate pesticide Phosalone (35%EC) it was widely used in agriculture field.

Phosalone

Phosalone is an organophosphate insecticide and acaricide. It is developed by Rhone-Poulene (a French chemical and pharmaceutical company) in France, but EU (European Union) eliminated it from pesticide registration on Dec2006. Because of carcinogenic properties, then after completion of the tolerance re-assessment, they found that it is not likely to be carcinogenic to humans and registration eligibility was obtained in June 20th 2007. Toxicity of Phosalone was established in 1993 by JMPR (Joint FAO/WHO meeting on pesticide residues). The toxicant was purchase from the local pesticide market in Guntur of Andhra Pradesh, India.

Table: Physico-chemical properties of phosalone

Common Name	Phosalone
Chemical Name	IUTPAC;S-6-chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl O,O-diethly phosphorodithiate. S.[(6-CHLORO-2-OXO-3(2h) benzoxazolyl)methyl] O,O-diethyl Phosphorodithioate,(CA):
Chemical formula	C12H15CINO4PS2
Melting point	46.2C Purity:995g/kg
Purity:	94.61%(one determination of one lot) (Charbassol,1988)
Appearance	Colorless crystals with a garlic-like colour
Stability	(<10%degradation after 4 weeks)in water at PH 5 and 7
Vapour pressure	5.03*10 to the minus 7mm Hg at 25 degrees C
Solubility in other solvents:	Soluble (ca.1000g/l) in ethyl acetate, acetone, acetonitrile, benzene, chloroform, methylenechloride, cyclohexane, dioxane, methylethyl ketone, toluene, xylene (all at 20 degrees C) (The Agrochemicals handbook. 1983).In methanol and ethanol, ca. 200g/l at 20 degrees C.
Solubility in water	Solubility (at 20 C):1.7 mg/l water.
Chemical Structure	

Preparation of stock solution
Phosalone stock solution was prepared by dissolving 1gram of phosalone pesticide in 100ml of acetone (stock solution) and the required quantity of acetone was drawn in the stock solution to maintain the suitable concentration of 1mg/l in the container. The fish were separated into several groups and each containing 10 individuals and pilot experiments were conducted to drive the LC_{50} determinations.

Studies on lethal toxicity
Experiments were carried out to assess the lethal response of phosalone by the experimental animals. The acute toxicity (96 hr LC_{50}) of test toxicants for the freshwater fish, *Ctenopharyngodon idella* was determined in the laboratory using the static renewal method according to OCED (1996). The containers of the last media are of 15 litres capacity; where in for each test five containers were used in each container 10 fish were introduced.

The fish were exposed to different concentrations of phosalone with five replicates for each concentration. Maximum 10 fish were used per each concentration of the test toxicant and 10 fish were also maintained in separate container along with experimental group, these were served as control. Water was renewed every day of the test medium for every 24hr with respective concentrations of the phosalone without oxygen (aeration).The data on the mortality rate of the fish was recorded and the dead fish were removed. Toxicity tests were conducted to choose the mortality range from 10% to 96% for 96 hr in static renewal systems. Finney probit analysis (Finney, 1971) [7] as recorded by Roberts and boyce (1972) was followed to calculate the median lethal concentration (LC_{50}) values and its 95% confidence limits. The mean values were derived following the method of Finney probit kill theory.

The data was subjected to the following statistical equations for arriving at LC_{50} values.

$$\text{Log } LC_{50} = \frac{\text{Log } A + 50 - a}{b - a \text{ Log}^2}$$

Where:
A=concentration of pesticide at 50% mortality.
a=percent kill just below 50% mortality
b=percent kill just above 50%mortality

Selection of sub lethal and lethal concentrations
Toxicants may exist in the aquatic system at concentrations too low to cause rapid death directly; but they may impair the functioning of organisms. Through pesticide may not be presently in lethal concentration, accidental spoilages may result in toxic concentrations. Hence, in present investigation, 96hr LC_{50} and $1/10^{th}$ of 96hr LC_{50} was selected as lethal and sub lethal concentrations for toxicants to study the behavioural responses and physiological alterations in experimental animal.

Calculation of the 95% confidence intervals
Method A: Hand calculates using the following equation: The standard error is approximately: ± 1/b√(snw): b= estimate of the slope of the line, Snw = summation of nw w= weighted coefficient from table no $=Z^2/PQ$ Finney,1972.

Method B: SPSS and other computer programs were applied to calculate this automatically. By using second method B, calculated the 95% confidence limits for toxicant Phosalone (35% EC) induced to *Ctenopharyngodon idella* (Grass carp), for exposed periods 24, 48, 72, 96 hrs.

Results and Discussions
The observed percent mortality along with exposure concentrations of freshwater fish *Ctenopharyngodon idella* (Grass carp), to Phosalone (35% EC) formulation, for 24,48,72,96 hrs in static system along with percent mortality and probit mortality were presented in Table respectively. The 95% confidence limits of the LC_{50} values were presented and consolidate LC_{50} value range with regression values were presented.

The LC_{50} values of Phosalone (35% EC) for the fish *Ctenopharyngodon idella* (Grass carp), after 24,48,72,96 hrs, in static system were 2.8, 2.4, 2.0 and 1.6 ppm respectively. The LC_{50} value was high for 24 hrs i.,e 2.8, the LC_{50} value was low for 96 hrs i.,e 1.6 ppm but for 48 hrs the LC_{50} value 2.4 ppm and for 72 hrs 2.0 ppm only. The low value for 96 hrs may be due to accumulated toxicant accumulates effect on the fish. The LC_{50} value regression value (R^2) of Phosalone (35%EC) for exposed fish *Ctenopharyngodon idella* (Grass carp), after 24,48,72,96 hrs, in static system were presented.

Table 1: Observed percent mortality, probit mortaliy and LC_{50} value of the fish *Ctenopharyngodon idella* exposed to Phosalone (35% EC) for static 24 hrs.

S. No	Dose In ppm	Mortality	Probit value (y)	Log(100* Dose)=x	X*X	X*Y	Y*Y
1	2.4	10	3.72	2.380211242	5.665405555	8.85438582	13.8384
2	2.6	30	4.48	2.414973348	5.832096271	10.8190806	20.0704
3	2.8	50	5.00	2.447158031	5.98858243	12.23579016	25
4	3.0	70	5.52	2.477121255	6.136129711	13.67370933	30.4704
5	3.2	90	6.28	2.505149978	6.275776414	15.73234186	39.4384
			25	12.22461385	29.89799038	61.31539777	128.8176

X 2.44492277
Y 5
SXX 9.7536236
SYY 3.8176
SXY 0.19223852
SLOPE B 6286.413161

VARIANCE B 0.010252599
VARIANCE A 0.161286421
M 2.44492277
Anti-Log 2.780
LC_{50} 2.8 ppm
R^2 Value 0.992

Table 2: Observed percent mortality, probit mortaliy and LC_{50} value of the fish *Ctenopharyngodon idella* exposed to Phosalone (35% EC) for static 48hrs.

S. No	Dose in ppm	Mortality	Probit value (y)	Log (100*dose)=x	X*X	X*Y	Y*Y
1	2.0	10	3.72	2.301029996	5.294739041	8.559831585	13.8384
2	2.2	30	4.48	2.342422681	5.486944016	10.49405361	20.0704
3	2.4	50	5.00	2.380211242	5.665405555	11.90105621	25
4	2.6	70	5.52	2.414973348	5.832096271	13.33065288	30.4704
5	2.8	90	6.28	2.447158031	5.98858243	15.36815243	39.4384
			25	11.8857953	28.26776731	59.65374672	128.8176

X 2.37715906
Y 5
SXX 0.013341327
SYY 3.8176
SXY 0.22477022
SLOPE B 16.8476659

VARIANCE B 7.495506257
VARIANCE A 42.45624535
M 2.37715096
Anti-Log 2.382
LC_{50} 2.4 ppm
R^2 Value 0.991

Table 3: Observed percent mortality, probit mortaliy and LC_{50} value of the fish *Ctenopharyngodon idella* exposed to Phosalone (35% EC) for static 72 hrs.

S. No	Dose In ppm	Mortality	Probit value (y)	Log (100*dose)=x	X*X	X*Y	Y*Y
1	1.6	10	3.72	2.204119983	4.858144898	8.199326331	13.8384
2	1.8	30	4.48	2.255272505	5.086254072	10.10362082	20.0704
3	2.0	50	5.00	2.301029996	5.294739041	11.50514998	25
4	2.2	70	5.52	2.342422681	5.486944016	12.9301732	30.4704
5	2.4	90	6.28	2.380211242	5.665405555	14.9477266	39.4384
			25	11.48305641	26.39148758	57.68599694	128.8176

X 2.296611282
Y 5
SXX 0.019370677
SYY 3.8176
SXY 0.27071494
SLOPE B 13.97550225

VARIANCE B 5.16244218
VARIANCE A 27.32890574
M 2.296611282
Anti-Log 1.977
LC_{50} 2.0 ppm
R^2 Value 0.991

Table 4: Observed percent mortality, probit mortaliy and LC_{50} value of the fish *Ctenopharyngodon idella* exposed to Phosalone (35% EC) for static 96 hrs.

S. No	Dose in ppm	Mortality	Probit value (y)	Log (100*dose)=x	X*X	X*Y	Y*Y
1	1.2	10	3.72	2.079181246	4.322994654	7.734554235	13.8384
2	1.4	30	4.48	2.146128036	4.605865546	9.614653601	20.0704
3	1.6	50	5.00	2.204119983	4.858144898	11.02059992	25
4	1.8	70	5.52	2.255272505	5.086254072	12.44910423	30.4704
5	2.0	90	6.28	2.301029996	5.294739041	14.45046837	39.4384
			25	10.98573177	24.16799821	55.2693806	128.8176

X 2.197146354
Y 5
SXX 0.0307377
SYY 3.8176
SXY 0.34072151
SLOPE B 11.08480791

VARIANCE B 3.253333854
VARIANCE A 15.80531335
M 2.197146354
Anti-Log 1.574
LC_{50} Value = 1.6 ppm
R^2 Value = 0.989

Table 5: Confidence Levels of 95% for Phosalone (35% EC) exposed to fish *Ctenopharyngodon idella* at different exposed periods.

Hours of exposure(hrs)	Lower and Upper confidence limits
24	2.407 ± 3.193
48	2.007±2.793
72	1.607±2.393
96	1.207±1.993

Table 6: Regression values for toxicant Phosalone (35% EC) exposed to fish *Ctenopharyngodon idella* at different exposed periods.

S.no	Exposure time in hrs	LC_{50} in % Concentration	No. of fish exposed	% Mortality	Regression equation Y=(y-bx) + bx
1	24	2.8	10	50	Y=19.71x43.19, R^2=0.992
2	48	2.4	10	50	Y=16.85x35.05, R^2=0.991
3	72	2.0	10	50	Y=13.98x27.10, R^2=0.991
4	96	1.6	10	50	Y=11.08x-19.35, R^2=0.989

The LC_{50} values and their regression values of Phosalone (35 % EC) were moderately toxic to exposed fish *Ctenopharyngodon idella* and have been calculated to support present observations in Table 6.

Fishes are very sensitive to a wide variety of toxicants in water; various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides. Toxicity studies have played an important role in man's efforts to monitor and modify the effects of his activities on the biota (Bhandare *et al.*, 2011) [11]. In aquatic toxicology LC_{50} may be defined as the concentration of a compound that causes lethality of 50% of the exposed individuals. Fish serves as a bio indicator species as it responds with great sensitivity to changes in the aquatic environment and thus, has an important role in the monitoring of water pollution (Srivastava *et al.*, 2010) [9].

The ecological effects of pollutants in aquatic ecosystems and their bio availability and toxicity are closely related to species distribution, both in the liquid phase of the aquatic ecosystem. (Ilavazhahan *et al.*, 2010) [10].

The LC_{50} value for fresh water fish *Puntius stigma* exposed to Roger for 96 hrs was recorded at 7.1 ppm (Bhandare *et al.*, 2011) [11], at 11.34 mg/L for *Heteropneustes fossilis* (Srivastava *et al.*, 2010) [14]. The value for monocrotophos on the juveniles of rohu and mrigal were found to be 46.34 and 42.33 ppm respectively. The LC_{50} of dimethoate for 96th exposure to the fish Nile tilapia (*Orechromis niloticus*) was 40 mg/L. Schimmel *et al.*, (1976) concluded that it is difficult to compare the toxicity of individual insecticides to different species of fish because they are influenced by several factors like temperature, hardness, PH and dissolved oxygen contest of the test water. The LC_{50} values obtained for mesasytox on *Nemacheilus botia* exposed for 96h was at 7.018 ppm (Nikam *et al.*, 2011) [11]. The median lethal concentration (LC_{50}) values of Diazion 60 EC on A. *testudineus, C. punctatus B. goniontus* were reported at 6.55,3.09 and 2.72 ppm for 96 hrs of exposure (Rahman *et al.*, 2002) [12].

Muthukumaravel *et al.*, (2013) reported that medium lethal concentration (LC_{50}) of monocrotophos for 24,48,72,96 hr were 0.0041, 0.0039,0.0037 and 0.0036ppm reasspectively, where as the LC_{50} values of synthetic pyrethoid lambda cyhalothrin for 24,48,72,96hr were 0.0026, 0.0024, 0.0024, 0.0022 and 0.0021 ppm, to fish *Labeo rohita* the LC_{50} value of malathion an organophosphate pesticide found to 9.0µl l^{-1}, reported by. Bilal Ahmad Bhat (2012) reported that the 96 hr LC_{50} values of botanical pesticide Kethrin and an Organohophate pesticide Dichlovos was found to be 21.68 ppm and 16.71 ppm to fresh water fish *Labeo rohita*, respectively.

The LC_{50} values of phosalone for the freshwater fish, *Ctenopharyngodon idell*a at 24, 48, 72 and 96h were 2.8, 2.4, 2.0 and 1.6 ppm/L, respectively. It is important to consider the physico-chemical characteristics of test medium along with biotic factors to know the mechanisms affecting LC_{50} concentrations of fish in toxicity test.

The median lethal concentration of Neem-On to the freshwater fish *Labeo rohita* was found to be 42.66ppm.The variation in the LC_{50} values is due to its depending upon various factors *viz*., sensitivity to the pesticide concentration and duration of exposure, reported by Bhat *et al.*, (2012), in the present study results supported by many authors.

Behavioural changes as a result of stress are further accepted as the most sensitive indication of potential toxic effects (Nwani *et al.*, 2010) [14]. In the present investigation, fish were observed moving fast and trying to escape or avoid the toxic water. They frequently came into the upper surface and sometimes jumped. Faster opercular activity, erecting dorsal and ventral fin were observed. Hassanein & Okali (2008) [15] observed loss of activity, erecting dorsal and balance, with an initial increase in the opercular ventilation rate which then decreased significantly in *Ctenopharygodon idella* after exposure to neem biopesticide.

The LC_{50} values of hr oscillate frame 0.007mg/ l. Depending on the sensitivity of the species. The 96 hr LC_{50} value of 1.75 mg/l for blue gill sunfish and less toxic as compared the LC_{50} (96hr) is 0.60,0.231 and 0.122 mg/l (Boran *et al.*, 2007) [16] for rainbow trout (*oncorhincus nykiss*) and guppy (*poecillia reticulate*) for the same pesticide. The opercular movements in the grass cape expose to Malathion may be due to hypoxic stress accompanied by a sub sequential inhibitory influence of OP coumpound on the respiratory mechanism. Dubey and Hosetti, (2010) [17], reported the LC_{50} values of endosulfan, malathion and carbaryl exposed to freshwater fish, *L. rohita* was found to be 320µ/g.

The present study LC_{50} value of Phosalone (35% EC) exposed to the fish, *C. idella* is agreed with the earlier reports and it is evidence from the above started LC_{50} values that organochlorines, organophosphates, carbamate and synthetic pyrethroids are more toxic to fish based on LC_{50} values. Due to their persistence and high toxicity on fish and mammals, a restricted on their use is imposed. Based on the above results the selected test compound was rated in toxicity.

Behavioural changes in fish due to acute toxicity

During the course of experimental, the fish have exhibited different behavioural alterations to the Phosalone concentrations. In the initial stage, fish stopped swimming and remained in static position by sudden change in the surrounding water. Then rapid swimming activities were observed followed by vertical hanging in the water and hitting to the container walls. Then the fish slowly become restless and excessive mucus formed all over the body, surfacing frequency, gulping of surface water and opercular activity increased remarkably in exposed fishes. Loss of balance increased and more fecal matter was found at the bottom of the circular tubs than control group fish. Finally fish was swimming at the bottom due to complete loss of balance and sank to the their ventral side facing upwards due to organophosphate phosalone stress.

In the present study, the impact of the pesticide could be observed by the behavioral changes like surfacing, erratic movement, increased mucous secretion, decreased opercular movement and loss of balance. Similar observations were reported on Shiva kumar *et al.*, (2005) [18] in *L. rohita* when exposed to endosulfan. The erratic swimming of the treated fish indicates the loss of physiological equilibrium and the hyper-excitability of the fish invariably in the lethal and sub lethal exposure of chemical may be used to the inhibition of cholinesterase Anita and Sobha (2010) [19]. Abnormal swimming and loss of balance was caused by the defiency in nervous and muscular coordination. Opercular movement has been decreased with increase in toxicant concentration and accumulation of more fecal matter was observed in the container and similar results were observed by imtiyaz *et al.*, (2012) [20].

The skin shows marked decrease in the pigmentation associated with appearance of dark patches and rashes. Pandey *et al.*, (1990) [21] also reported depigmentation in toxicant exposed fish and attributed it to reduction in number and size of chromatophores. Behaviour is considered a promising tool in ecotoxicology (Drummond and Russom, 1990) [22] and these studies are becoming prominent in toxicity assessments in unicellular organisms. Most physiologic and environment changes induce variations in fish behaviour.

In the present study, the control fish behaved in natural manner i.e. they were active with their well-coordinated movements. They are alert at the slightest disturbance, but in the toxic environment, fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium which is due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic, synapses ending up with hyper stimulation **(**Mushigeri and David, 2005) [18]. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over the body. Mucus secretion in fish forms a barrier between body and toxic media thereby probably reduces contact of toxicant so as to minimize its irritating effect or to eliminate it through epidermal mucus; opercular movements increased initially in all exposure periods but decreased further steadily in lethal exposure compared to sub lethal exposure periods.

Present study, *ctneophoryngodon idella* exposed to lethal and sub lethal concentration of Phosalone. Gulping air at the surface, swimming at the water surface, disrupted shoaling behaviour and easy predation was seen on the firstday itself in lethal and sublethal exposure period of phosalone made by Ural and Simsek (2006). Gulping 0f of air may help to avoid of toxic medium. Surfacing phrnomenon i.e., significant preference of upper layer exposed (Katja *et al.*, 2005) [24]. Fainally fish sunk to the bottom with the least opercular movements and died with their mouth opened. It is clear those earlier studies that LC_{50} of insecticides for a freshwater varies from species under influence of number of factors including size and time of exposure. The response was initiated at the threshold dose when increased intensity of dose relationship.

Disruptind of schooling behaviour of the fish, due to the lethal and sublethal stress of pesticides, result increased swimming activity and entails increased expenditure of energy. The erradic swimming of the treated fish indicates loss of equilibrium. This leads to the abnormal functioning of the body including loss of balance, moving in circular from (convulsions) and at higher concentrations of insecticides resulting in death of the organism. Fish kills occur when pesticides are improperly applied to or otherwise end up in bodies of water through either misapplication or drift. The response was initiated at the threshold dose when increase intensity of dose and exposure time is increased.

Fish in the experimental group applied with the highest concentration of pesticide were convulsions laterally at the bottom with loss of balance, swimming down in a spiral movement with jerks. Thus the behavioral changes of the fish under insecticidal habit and may affect the stability of the pollution. In sublethal exposure of *Ctenopharaygodon idella*, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Leaning of fish indicate reducdamount of diretary protein consumed by the fish at pesticide stress, which was immediately utilized and was not stored in the body weight.

Conclusion

In the present study of test organism *Ctenopharyngodon idella* showed jerky movements, hyper secretion movements, hyper secretion of mucus, opening mouth for gasping, loosing scales, hyperactivity were observed in increase the toxicant concentration. Behavioral characteristics are obviously sensitive indicators of toxicants effect. It is necessary, to select behavioural indices a more accurate assessment of the hazards that a contaminant may pose in natural systems. After observing and all autor reports the present study showed that phosalone also highly toxic to the fish *Ctenpharyngodon idella.*

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Conflict of Interest

None

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