Determination of LC50 of Chlorpyrifos with special reference to Behavioral and Morphological changes in zebra fish, *Danio rerio*

[1]Shadab Ahmad, [2]Vineeta Yadav, [3]Kaneez Zahra

[1] Research Scholar, [2]Research Scholar, [3]Associate Professor

[1 2 3] Department of Zoology,

Bipin Bihari P. G. College, Bundelkhand University, Jhansi, U. P., India

ABSTRACT

Chlorpyrifos is most widely used chlorinated organophosphate insecticide in agricultural fields to protect the crops from injuries caused by different types of insects and pests. Chemical discharge in water resources from surrounding area led to deleterious effects for inhabiting aquatic organism including fishes. The present study was aimed to determine the acute toxicity of Chlorpyrifos (Alldrin 20% EC) and its effect on behavior of zebra fishes. Adult zebra fishes were randomly selected and toxicity bioassay was performed to estimate LC50 values at different exposure periods. LC50 values were determined by direct interpolation method by plotting graphs between % mortalities and concentrations of toxicant at 24, 48, 72 and 96 h which were 0.013 ml/l, 0.010 ml/l, 0.008 ml/l and 0.007 ml/l respectively. Then, obtained data were evaluated by the probit analysis (statistical method) using Finney's table and the values were found to be 0.0122 ml/l, 0.0093 ml/l, 0.0073 ml/l and 0.0063 ml/l. During all experimental periods, ethological and morphological responses were also observed. Fishes showed respiratory troubles, abnormal opercular movement, erratic and jerky movement, decrease locomotion, hyper excitability, loss of equilibrium and sinking to the bottom prior to mortality. Discoloration, destruction of scales and copious mucus secretion all over the body were the chief morphological changes. The behavioral changes observed in chlorpyrifos induced zebra fishes indicate that this pesticide is very toxic and zebra fishes are highly sensitive at very low concentration of chlorpyrifos.

KEYWORDS: Acute toxicity bioassay, Chlorpyrifos, ethological and morphological responses, zebra fish

INTRODUCTION

Currently synthetic pesticides are most suitable and dominant agent for eradication of insects/pests. These are considered quick and cheap means of controlling pests. In contrast the indiscriminate use of pesticides causes serious problems to the environment. Pesticides enter the aquatic environment through runoff water or may be applied directly, causing toxicity to non target organisms especially fishes (Nwani *et al.*, 2013; Tiwari and

Ansari, 2014; Sunanda *et al.*, 2016). Exposure of toxicants causes fish poisoning which damage their vital organs, altering biochemical parameters and decline in reproductive ability.

Chlorpyrifos (O, O-diethyl O-3, 5, 6 trichloro-2-pyridyl – phosphoro thioate) is a broad spectrum chlorinated organophosphate insecticide. It affects the nervous system of insects by inhibiting the breakdown of acetylcholine (ACh) which is a neurotransmitter. It has been reported that it inhibits acetylcholinesterase (AChE) activity in non- target animals especially fishes (Sun and Chen, 2008; Black and Read, 2013). After exposure chlorpyrifos binds with the enzyme cholinesterase that prevents breakdown of acetylcholine at synapse. Restriction of AChE activity causes over stimulation of neuron tends to neurotoxicity (Miron *et al.*, 2005; Yen J *et al.*, 2011; Jin *et al.*, 2015).

Toxicity tests are the principal indicator to evaluate the effect of pesticides on aquatic fauna such as fishes. The effects of pesticides on fish population and other organisms depend on concentrations and exposure periods (Khare, 2015; Mishra and Verma, 2016). Toxicity is species- specific having different levels of responses to the same dose and time interval of a toxicant (Bridges and Semlitsch, 2000). Many chemical contaminants target physiological system and exert their effects on behavior. The investigation of morpho-behavioral markers are becoming the most potent and sensitive tool of ecotoxicology to evaluate the toxic effects (Cong *et al.*, 2009; Onyedineke *et al.*, 2010). Behavior is the cumulative manifestation of genetical, physiological and biochemical processes (Kane *et al.*, 2005; Dube and Hosetti, 2010). It allows an organism to adjust itself to external and internal stimuli in most challenging environment (Halappa and David, 2009). Some authors have been reported abnormal behavioral and morphological changes in fishes due to different pesticide exposure (Reza and Gholamreza, 2012; Ishi and Patil, 2017; Bridi *et al.*, 2017). Several researchers have also been reported the toxicity and behavioral alterations in fishes induced by organophosphate compounds (Mishra and Poddar, 2014; Misha and Verma, 2016; Singh *et al.*, 2017; Majumder and Kaviraj, 2018).

Zebra fishes are used as laboratory model for molecular, endocrinological, developmental and toxicological research due to its genomic similarities with human beings (Hill *et al.*, 2005; Bambino and Chu, 2017; Tanguay, 2018). It is very sensitive to the external changes in environment caused by different toxicants. Therefore, the current investigation was designed to assess the acute toxicity of commercial formulation of chlorpyrifos (Alldrin-20) with special emphasis on behavioral and morphological alterations on zebra fish, *Danio rerio*.

MATERIALS AND METHODS

i. Collection and maintenance of fishes-

For acute toxicity bioassay 120 healthy, live and sexually mature specimens of both sexes of zebra fishes were selected as an experimental model. The zebra fishes of almost similar age group were purchased from aquarium shop of Jhansi district, U.P. India and acclimatized for 10-15 days. Fishes were rinsed in 0.1%

potassium permanganate solution for 2-4 minutes to check injuries and elimination of bacterial contamination during transport. They were fed on commercial diet twice daily. Feeding was stopped 24 hrs before experiment.

ii. Toxicant used-

Alldrin 20 (Chlorpyriphos 20% EC) insecticide is used in this study. It is manufactured by Kingtech Bio Chem Pvt. Ltd. District Kheda, Gujarat, India. The chemical formula of Alldrin 20 is $C_9H_{11}cl_3NO_3PS$.

iii. Experimental design for estimation of LC50-

a. Direct Interpolation Method

Estimation of LC50 values were done by direct interpolation method in which two exploratory tests and one definitive test were carried out. During these tests, the mortality was recorded after 24, 48, 72 and 96 hrs exposure. Stock solution of chlorpyrifos was prepared by dissolving 1ml Alldrin-20 in one litre of distilled water. For the 1st exploratory test, one lower concentration 0.001 ml/l and one higher concentration 0.03 ml/l were implemented from prepared stock solution into rectangular glass aquaria (2′×1′×1′) separately. Five fishes were taken into each aquarium to record mortalities between 0% to 100%. In 2nd exploratory test, four concentrations (0.003, 0.009, 0.015 and 0.021 ml/l) were considered to determine the narrow range of concentrations to be used in definitive test. Seven concentrations (0.004, 0.006, 0.008, 0.010, 0.012, 0.014 and 0.016 ml/l) were selected for definitive test. In 2nd exploratory and definitive test 10 fishes were exposed to each concentration. The mortality was recorded after 24, 48, 72 and 96 hrs exposure period. The concentrations from definitive test were used to evaluate the LC50 value by plotting a dose response curve between % mortalities and concentrations of toxicant.

b. Probit analysis-

For statistical analysis obtained values of definitive test were converted into log concentrations, correct % mortalities and probit using Finney' table (1971). Graphs (regression line) were plotted between the log concentrations and probit values. For different time intervals, LC50 were obtained by drawing a perpendicular at 5 probit corresponding to the 50 % mortality from the regression line. Calculations were also made with regression equation and by taking the antilog, the actual LC50 values were determined. For evaluation of SE of LC50 the probit values of LC84 and LC16 were taken from Finney's table, which were almost equal to probit 6 and 4. Log concentrations were obtained by plotting a perpendicular on the regression line at these probit. SE of LC50 was calculated by taking antilog of the log values obtained after dividing the difference of log LC84 and log LC16 by root value of 2N (2×Number of fishes).

RESULTS AND DISCUSSION

After 24 hrs of initial exploratory test for Alldrin 20, 100% mortality was obtained at 0.03 ml/l while all fishes were remained alive at 0.001 ml/l (Table 1). In IInd exploratory test, 20% mortality was recorded after 96 hrs at 0.003 ml/l conc. and 100% mortality occurred after 48 hrs at 0.021 ml/l of Alldrin exposure (Table 2). On the basis of IInd exploratory test seven concentrations (0.004, 0.006, 0.008, 0.010, 0.012, 0.014 and 0.016 ml/l) were selected for definitive test and mortality rate is illustrated in Table-3. A dose response curves were plotted between concentrations and % mortalities given in Table 3. LC50 values were obtained by drawing a perpendicular on each curve at 50% mortality which are 0.013 ml/l, 0.010 ml/l, 0.008 ml/l and 0.007 ml/l respectively after all exposure periods (Fig. 1).

Table 1. Ist Exploratory Test

Conc. ml/l	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
		M	% M	M	% M	M	% M	M	% M
0.001	5	-	-	-	-	-	-	-	-
0.03	5	5	100%						

Table 2. IInd Exploratory Test

Conc. ml/l	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
		M	% M	M	%M	M	%M	M	%M
0.003	10	-	-	-	-	1	10	1	20
0.009	10	2	20	2	40	2	60	1	70
0.015	10	6	60	2	80	2	100		
0.021	10	8	80	2	100				

Table3. Definitive test for Direct Interpolation Method

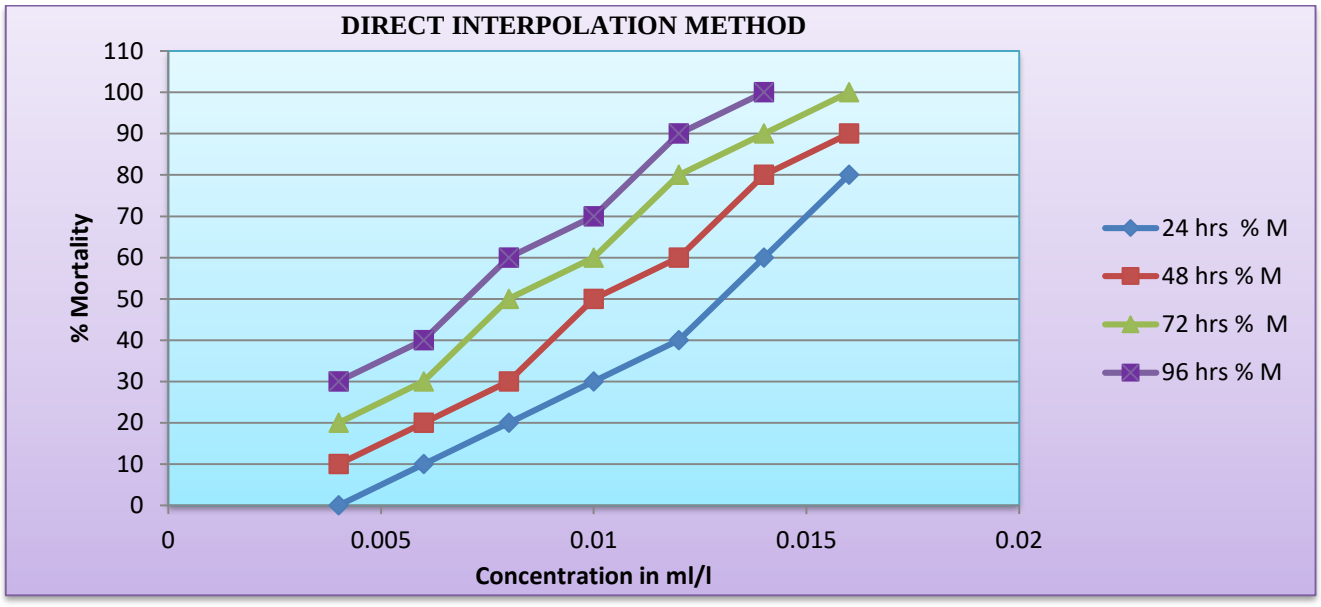


Fig. 1. Determination of LC50 at different exposure periods

Fig. 2. Plot of log concentrations versus probit from table (4) following 24 and 48 hrs intoxication of Chlorpyrifos.

S.N.	Conc. (ml/l)	No. of fishes	24 hrs		48 hrs		72 hrs		96 hrs	
			M	% M	M	% M	M	% M	M	% M
1	0.004	10	0	0	1	10	1	20	1	30
2	0.006	10	1	10	1	20	1	30	1	40
3	0.008	10	2	20	1	30	2	50	1	60
4	0.010	10	3	30	2	50	1	60	1	70
5	0.012	10	4	40	2	60	2	80	1	90
6	0.014	10	6	60	2	80	1	90	1	100
7	0.016	10	8	80	1	90	1	100		

The log values of concentrations obtained in definitive test, % mortalities into correct % and their corresponding probit values are shown in Table 4 and 5 according to the Finney's table (Finney, 1971).

Table 4. Log concentrations and probit values of Chlorpyrifos in zebra fishes after 24 hrs and 48 hrs exposure

S.N.	Conc. (ml/l)	Log conc.	No. of fishes	24hrs			48 hrs		
				% dead	Correct %	Probit	% dead	Correct %	Probit
1	0.004	-2.39	10	0	2.5	3.04	10	10	3.72
2	0.006	-2.22	10	10	10	3.72	20	20	4.16
3	0.008	-2.09	10	20	20	4.16	30	30	4.48
4	0.010	-2	10	30	30	4.48	50	50	5
5	0.012	-1.92	10	40	40	4.75	60	60	5.25
6	0.014	-1.85	10	60	60	5.25	80	80	5.84
7	0.016	-1.79	10	80	80	5.84	90	90	6.28

Table 5. Log concentrations and probit values of Chlorpyrifos in zebra fishes after 72 hrs and 96 hrs exposure

S.N.	Conc. (ml/l)	Log conc.	No. of fishes	72 hrs			96 hrs		
				% dead	Correct %	Probit	% dead	Correct %	Probit
1	0.004	-2.39	10	20	20	4.16	30	30	4.48
2	0.006	-2.22	10	30	30	4.48	40	40	4.75
3	0.008	-2.09	10	50	50	5	60	60	5.25
4	0.010	-2	10	60	60	5.25	70	70	5.52
5	0.012	-1.92	10	80	80	5.84	90	90	6.28
6	0.014	-1.85	10	90	90	6.28	100	97.5	6.96
7	0.016	-1.79	10	100	97.5	6.96	100	97.5	6.96

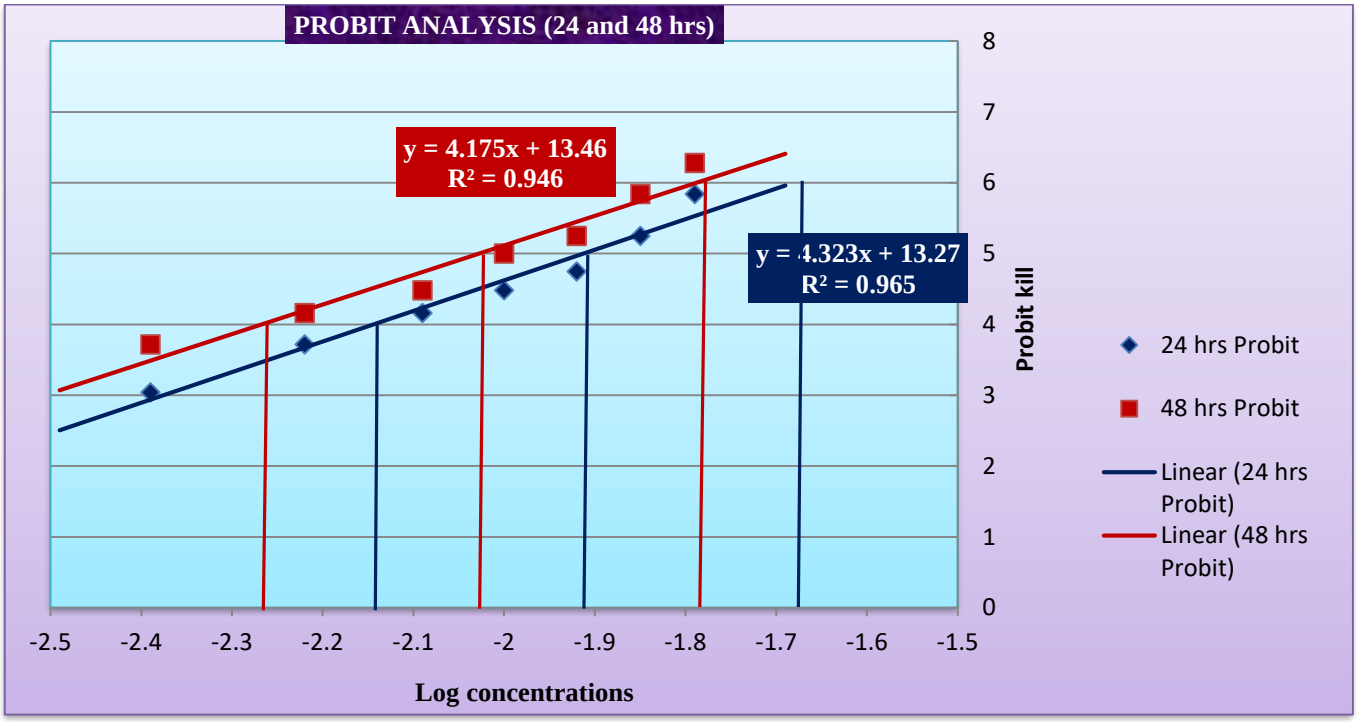
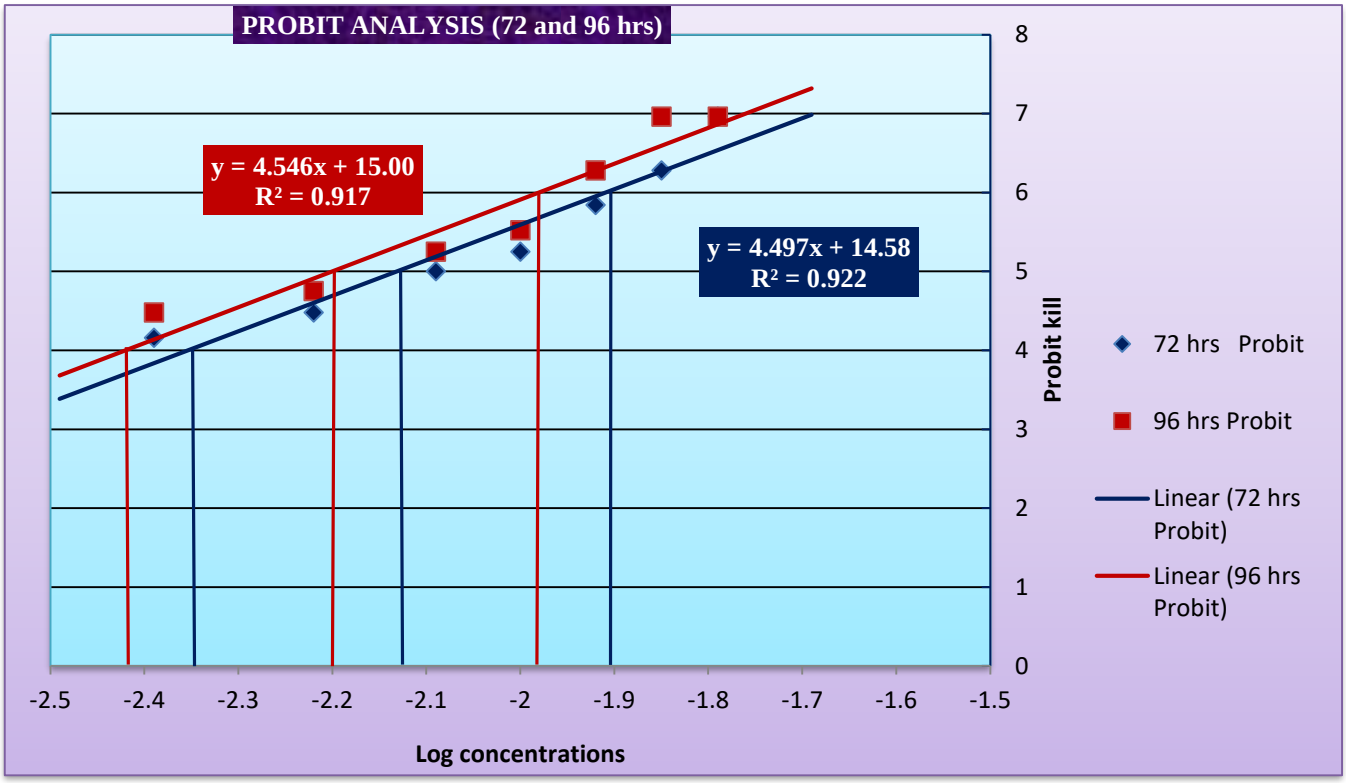


Fig. 4. Plot of log concentrations versus probit from table (5) following 72 and 96 hrs intoxication of Chlorpyrifos.



At different exposure period of Alldrin intoxication, the log concentrations at 5 probit were -1.91, -2.02, -2.13 and -2.20 ml/l (Fig 2 and 3). After taking antilog the actual LC50 values were found to be 0.0122, 0.0093, 0.0073 and 0.0063 ml/l following 24, 48, 72 and 96 hrs exposure. The values of probit 6 and 4 were obtained by plotting a perpendicular on graphs as shown in Fig 2 and 3 (Singh and Zahra, 2017) and SE of LC50 was calculated as 0.0122 ± 0.0030, 0.0093 ± 0.0024, 0.0073 ± 0.0017 and 0.0063 ± 0.0014 after 24, 48, 72 and 96 hrs exposure period respectively.

The estimated LC50 values in fishes from 24-96 hrs exposure periods suggest that the susceptibility of fishes to various concentrations of Chlorpyrifos was duration and concentration dependent as mortality increased with an increase in its concentration and exposure periods. Tiwari and Ansari (2014) reported the LC50 values of Chlorpyrifos after 24, 48, 72 and 96 hrs in zebra fishes which were found to be 0.40, 0.29, 0.21 and 0.16µg/l respectively. On the contrary, some researchers reported LC50 values of Chlorpyrifos to zebra fishes which are higher than the values obtained in the present study (Jeon *et al.*, 2016; Singh *et al.*, 2017). The lethal concentration (50%) has been evaluated for Chlorpyrifos to different fish species by earlier workers (Ramesh and Saravanan, 2008; Hallappa and David, 2009; Khare, 2015; Nwani *et al.*, 2013; Misha and Verma, 2016). LC50 values of rainbow trout intoxicated by Chlorpyrifos were estimated as 2.25 and 6.75 µg/l after 24 and 96 hrs exposure periods (Topal *et al.*, 2014). Devi and Mishra (2013) reported a 24 and 96 hrs LC50 of Chlorpyrifos in *Channa punctatus* which is comparable to the findings of our study. All the results reveal that Chlorpyrifos is extremely toxic to fishes.

The toxicity of pesticides in fishes can be observed by behavioral and morphological alterations which are the most sensitive indicator of aqua-toxicity. The changes observed during intoxication are known as the first sign of toxic stress. Tables 6-9 show the morpho-behavioral changes following 24-96 hrs Chlorpyrifos exposure. Behavioral changes were started just after addition of toxicant into the aquarium in treated group whereas the fishes of control groups were very active and no changes were seen during experiment. On exposure of different concentration of Chlorpyrifos, zebra fishes showed disrupted schooling behavior as hyper-excitability, abrupt swimming, jerky movement and opercular movement. Within 1-3 hours of exposure the swimming rate was abrupted as result of loss of co-ordination. The jerky movement was also observed immediately after addition of toxicant which was concentration and time dependent. Higher the concentration and exposure period resulted in more jerky movement and swimming rate. The opercular movements of fishes were increased as a sudden response to shock. It was directly proportional to exposure periods and concentrations of chlorpyrifos. All the observations of present work are supported by several authors (Fulton and Key, 2001; Pandey *et al.*, 2005; Misha and Verma, 2016). Fishes came to the surface frequently for gulping air. These responses may be due to more oxygen demand in severe stressed condition. The observed alterations may be attributed to the inhibition the activity of acetylcholinesterase (AChE) which leads to accumulation of acetylcholine (ACh) in cholinergic synapses ensuing hyperstimulation (Hulya *et al.*, 2006; Chawanrat *et al.*, 2007; Ismail *et al.*, 2014). It has been proved that Chlorpyrifos acts as nerve poison which is reflected in

uncoordinated disrupt behavior and decreased mobility of fresh water fishes after toxicant exposure (Devi and Mishra, 2013).

Ethological and morphological responses during 24 hrs exposure	Concentration of chlorpyrifos in ml/l							
	control	0.004	0.006	0.008	0.010	0.012	0.014	0.016
Hyper activity	-	-	-	+	+	+	++	+++
Opercular movement	-	-	-	-	+	+	+	++
Forward pectoral fin	-	-	-	-	-	+	+	+
Abrupt Swimming	-	-	-	+	+	++	++	+++
Jerky movement	-	-	-	-	+	+	+	++
Escaping tendancy	-	-	-	+	+	+	+	++
Loss of buoancy	-	-	-	-	+	+	+	+
Discoloration of skin	-	-	-	-	-	-	+	+
Destruction of scales	-	-	-	-	-	-	-	+
Mucus secretion	-	-	-	-	-	-	-	+

Table 6. Effects of chlorpyrifos on the ethological and morphological responses of zebra fish at 24 hrs

Table 7. Effects of chlorpyrifos on the ethological and morphological responses of zebra fish at 48 hrs

Ethological and morphological responses during 48 hrs exposure	Concentration of chlorpyrifos in ml/l							
	control	0.004	0.006	0.008	0.010	0.012	0.014	0.016
Hyper activity	-	-	-	+	+	++	++	+++
Opercular movement	-	-	-	+	+	+	++	++
Forward pectoral fin	-	-	-	-	-	+	+	++
Abrupt Swimming	-	-	-	+	+	++	++	+++
Jerky movement	-	-	-	-	+	++	++	+++
Escaping tendancy	-	-	+	+	++	++	+++	+++
Loss of buoancy	-	+	++	++	++	++	+++	+++
Discoloration of skin	-	-	-	-	-	-	+	+
Destruction of scales	-	-	-	-	-	-	+	+
Mucus secretion	-	-	-	-	+	+	+	++

Table 8. Effects of chlorpyrifos on the ethological and morphological responses of zebra fish at 72 hrs

Ethological and morphological responses during 72 hrs exposure	Concentration of chlorpyrifos in ml/l							
	control	0.004	0.006	0.008	0.010	0.012	0.014	0.016
Hyper activity	-	-	+	+	++	++	+++	+++
Opercular movement	-	+	++	++	++	+++	+++	+++
Forward pectoral fin	-	-	+	+	++	++	++	+++
Abrupt Swimming	-	-	+	+	++	++	+++	+++
Jerky movement	-	+	++	++	+++	+++	+++	+++
Escaping tendancy	-	+	+	++	++	+++	+++	+++
Loss of buoancy	-	+	++	++	++	+++	+++	+++
Discoloration of skin	-	++	++	++	++	+++	+++	+++
Destruction of scales	-	-	+	+	+	++	++	+++
Mucus secretion	-	+	++	++	++	++	+++	+++

Table 9. Effects of chlorpyrifos on the ethological and morphological responses of zebra fish at 96 hrs

Ethological and morphological responses during 96 hrs exposure	Concentration of chlorpyrifos in ml/l							
	control	0.004	0.006	0.008	0.010	0.012	0.014	0.016
Hyper activity	-	-	+	++	++	++	+++	+++
Opercular movement	-	+	++	++	++	+++	+++	+++
Forward pectoral fin	-	-	+	+	++	++	++	+++
Abrupt Swimming	-	-	+	++	++	+++	+++	+++
Jerky movement	-	+	++	++	+++	+++	+++	+++
Escaping tendancy	-	+	+	++	++	+++	+++	+++
Loss of buoancy	-	+	++	++	++	+++	+++	+++
Discoloration of skin	-	++	++	++	++	+++	+++	+++
Destruction of scales	-	-	+	+	+	++	++	+++
Mucus secretion	-	+	++	++	++	++	+++	+++

Timchalk *et al.* (2002) also reported the inhibition of Acetylcholinesterase activity in Chlorpyrifos induced fishes. Discoloration of body color was observed in fishes considered as the sign of stressed conditions after Chlorpyrifos intoxication. This alteration may be due to manipulation of number and size of chromatophores by pesticide (Pandey *et al.,* 2005; Ashraf *et al.,* 2010). An excess amount of mucous secretion all over the body was shown in current study. Fishes were secreted an excess amount of mucous which is non- specific responses against toxicants. Mucous coating makes a barrier between skin of the body and toxic surrounding medium suggesting to minimize its irritating effect or to scavenge it through epidermal mucous. These findings are in agreement with several authors (Rao, 2006; Halappa and David, 2009; Kristen *et al.,* 2009; Parithabhanu, 2013). Chlorpyrifos has the potential to impair physiological and biochemical activities of zebra fishes leading to altered behavioral responses and mortality (Watson *et al.,* 2014; Richendrfer and Creton, 2015). The changes in behavioral pattern after chlorpyrifos toxication may also be associated with physiological and biochemical alterations as observed by several researchers (Ramesh and Munniswamy, 2009; Sharbidre *et al.,* 2011)

CONCLUSION

To evaluate acute toxicity bioassay zebra fishes were used as an experimental model for the first time in Bundelkhand region, Uttar Pradesh, India. From the present study it is concluded that the commercial formulation of Chlorpyrifos (Alldrin 20) is highly toxic to fishes. Alldrin 20 has a great potential to disturb endocrinal secretions altering the hormone level in serum. The hormones studied in this investigation can be used as potential biomarkers in evaluating toxic effect of Chlorpyrifos. Therefore the use of Chlorpyrifos in aquatic environment and terrestrial habitat should be strictly monitored.

ACKNOWLEDGEMENTS

The authors are very grateful to thank the Head of the Department of Zoology and institutional authorities for providing necessary facilities to carry out this research work.

REFERENCES

Ashraf, M.M., Ayub, M., Sajjad, T., Elahi, N., Ali, I., Ahmed, Z. (2010). Replacement of rotenone by locally grown herbal extracts. *Int. J. Agric. Biol.,* 12: 77-80.

Bambino, K., Chu, J. (2017). Zebrafish in Toxicology and Environmental Health. *Curr Top Dev Biol.,* 124: 331-367.

Bridges, C.M., Semlitsch, R.D. (2000). Variation in pesticide tolerance of tad-poles among and within species of Ranidae and pattern amphibian decline. *Conserv. Biol.,* 14: 1490-1499.

Bridi, D., Altenhofen, S., Gonzalez, J.B., Reolon, G.K., Bonan, C.D. (2017). Glyphosate and Roundup® alter morphology and behavior in zebrafish. *Toxicol.*, 392: 32-39.

Black, R.M., Read, R.W. (2013). Biological markers of exposure to organophosphorus nerve agent. *Arch. Toxicol.*, 87: 421-437.

Chawanrat, S., Voravit, C., Chutarat, S., William, F., Beamish, H. (2007). Variability in acetylcholenesterase upon exposure to chlorpyrifos and carbaryl in hybrid cat fish. *Sci. Asia.*, 33: 301-305.

Cong, N.V., Phuong, N.T., Bayley, M. (2009). Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channa striata*). *Ecotoxicol. Environ. Saf.*, 72: 699-703.

Devi, Y. and Mishra, A. (2013). Study of behavioural and morphological anomalies of fry fish of freshwater teleost, *Channa punctatus* under chlorpyrifos intoxication. *Int. J. Pharma Bio Sci.*, 4(1B): 865-874.

Dube, P.N., Hosetti, B.B. (2010). Behaviour surveillance and oxygen consumption in the freshwater fish *Labeo rohita* (Hamilton) exposed to sodium cyanide. *Biotechnol.Ani. Husband.*, 26(1-2): 91-103.

Finney, D.T. (1971). Probit Analysis. Cambridge University press, Cambridge, 333p.

Fulton, M.H. and Key, P.B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environ. Toxicol. Chem.*, 20: 37-45.

Halappa, R. and David M. (2009). Behavioral responses of the fresh water fish, *Cyprinus carpio* (Linnaeus) following sub-lethal exposure to chlorpyrifos. *Turkish J. Fish. Aquat. Sci.*, 9: 233-238.

Hill, A.J., Teraoka, H., Heideman, W. and Peterson, R.E. (2005). Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicol. Sci.*, 86: 6-19.

Hulya, D., Sevgiler, Y., Uner, N. (2006). Tissue-specific antioxidative and neurotoxic responses to diazinon in *Oreochromis niloticus*. *Pest. Biochem. Physiol.*, 84: 215-226.

Ishi, S.S., Patil, Rd. (2017). Acute toxicity bioassay of new tech biopesticide on fresh water cyprinid *Danio aequipinnatus* (Ham Buch). *Intern. J. Fish. Aquat. Stud.*, 5(3): 584-586.

Ismail, M., Khan, Q.M., Ali, R., Ali, T., Mobeen, A. (2014). Genotoxicity of chlorpyrifos in freshwater fish *Labeo rohita* using Alkaline Single- Cell Gel Electrophoresis (Comet) assay. *Drug Chem. Toxicol.*, 37(4): 1-6.

Jeon, H.J., Lee, Y.H., Kim, M.J., Choi, S.D., Park, B.J., Lee, S.E. (2016). Integrated biomarkers induced by chlorpyrifos in two different life stages of (*Danio rerio)* for environmental risk assessment. *Environ. Toxicol. Pharmacol.*, 43: 166-74.

Jin, Y., Liu, Z., Peng, T., Fu, Z. (2015). The toxicity of chlorpyrifos on the early life stage of zebrafish: a survey on the endpoints at development, locomotor behavior, oxidative stress and immunotoxicity. *Fish Shellfish Immunol.*, 43(2): 405-14.

Kane, A.S., Salierno, J.D., Brewer, S.K. (2005). Fish models in behavioural toxicology: automated techniques, updates and perspectives. In: Ostrander GK (ed) Methods in aquatic toxicology, 2nd edn. Lewis Publishers, Boca Raton, 559-590.

Kristen, M.G., Roberts, A.P., Ellis, N., Swers, A.D., Klain, S.J. (2009). Biochemical and behavioral effects of diazinon exposure in hybrid striped bass. *Environ. Toxicol. Chem.,* 28: 105-112.

Khare, H.N. (2015). Determination of LC50 of an organophosphate pesticide in a fresh water catfish, *Mystus seenghala*. *Int. J. Appl. Univer. Res.,* 2(V): 9-12.

Majumder, R., Kaviraj, A. (2018). Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish *Oreochromis niloticus*. *Drug Chem Toxicol.,* 26: 1-9.

Miron, D., Crestani, M., Schetinger, M.R., Morsch, V.M., Baldisseroto, B., Tierno, M.A., Morales, G., Vieira, V.I.P. (2005). Effects of herbicides clomazone, quinclorac and metsulfuron methyl on acetylcholenesterase activity in the silver cat fish (*Rhamdia quelen*) (Heptapteridae). *Ecotoxicol. Environ. Saf.,* 61: 398-403.

Mishra, A. and Poddar, A.N. (2014). Acute toxicity and behavioral response of the food fish *Channa punctatus* (Bloch) to an insecticide dichlorvos. *Jr. of Indust. Pollu. Contr.,* 30(2): 219-222.

Mishra, A. and Verma, S. (2016). Acute toxicity bioassay of organophosphorus pesticide, chlorpyrifos on fresh water catfish, *Heteropneustes fossilis* (Bloch, 1794). *Int. J. Fish. Aquat. Stud.,* 4(6): 388-393.

Nwani, C.D., Ivoke, N., Ugwu, D.O., Atama, C., Onyishi, G.C., Echi, P.C., Ogbonna, S.A. (2013). Investigation on acute toxicity and behavioral changes in a fresh water African cat fish, *Clarias gariepinus* (Burchell, 1822), exposed to organophosphorus pesticide, Termifos. *Pakistan J. Zool.,* 45(4): 959-965.

Onyedineke, N.E., Odukoyaand, A.O., Ofoegbu, P.U. (2010). Acute toxicity tests of cassava and rubber effluents on the Ostracoda Strandesiaprava Kile, 1935 (Crustacea, Ostracoda), *Res. J. Environ. Sci.,* 4: 166-172.

Pandey, S., Kumar, R., Sharma, S., Nagpure, N.S., Srivastava, S.K., Verma, M.S. (2005). Acute toxicity bioassay of mercuric chloride and malathion on air breathing fish *Channa punctatus* (Bloch). *Ecotoxicol. Environ. Saf.,* 61: 114-120.

Parithabhanu, A. (2013). Pesticide toxicity and behavioural responses in the fish *Oreochromis mossambicus. Int. J. Fish. Aquacult. Sci.,* 3(2): 161-164.

Ramesh, H. and Munniswamy, D. (2009). Behavioral responses of the fresh water, *Cyprinus carpio* (Linnaeus) following sub-lethal exposure to chlorpyrifos. *Turkish J Fisheries Aquat Sci.,* 9: 233-238.

Ramesh, M. and Saravanan, M. (2008). Hematological and bio chemical responses in fresh water fish *Cyprinus carpio* exposed to chlorpyrifos. *Int. J. Integr. Biol.,* 3: 80-83.

Rao, J.V. (2006). Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. *Pest. Biochem. Physiol.,* 86: 78-84.

Reza, D., Glolamreza, A. (2012). Comparative study on the acute toxicity of synthetic pesticides, Permethrin 25% and Monocrotophos 36% and neem based pesticide, Neem gold EC 0.03% to juvenile *Cyprinus carpio Linn. J. Biol. Environ Sci*., 6(16): 105-108.

Richendrfer, H., Creton, R. (2015). Chlorpyrifos and malathion have opposite effects on behaviors and brain size that are not correlated to changes in AChE activity. *Neurotoxicol*., 49: 50-58.

Sharbidre, A.A., Metkari, V., Patode, P. (2011). Effects of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, *Poecilia reticulate. Pest. Biochem. Physiol.,* 101: 132-141.

Singh, S., Bahadur, M., Bhattacharjee, S., Pal, J. (2017). Acute toxicity of chlorpyrifos to zebrafish, *Danio rerio* (Cyprinidae). *NBU J. Anim. Sci.,* 11: 45-49.

Singh, A., Zahra, K. (2017). LC50 assessment of cypermethrin in *Heteropneustes fossilis*: Probit analysis. *Int. J. Fish. Aquat. Stud.,* 5(5): 126-130.

Sun, F., Chen, H.S. (2008). Monitoring of pesticide chlorpyrifos residue in farmed fish: investigation of possible sources. *Chemo.,* 71: 1866-1869.

Sunanda, M., Rao, J.C.S., Neelima, P., Rao, K.G., Simhachalam, G. (2016). Effect of Chlorpyrifos (an Organophosphate Pesticide) in fish. *Int. J. Pharm. Sci. Rev. Res.*, 39(1): 299-305.

Tanguay, R.L. (2018). The rise of Zebrafish as a Model for Toxicology. *Toxicol. Sci.,* 163(1): 3-4.

Timchalk, C., Nolan, R.J., Mendrala, A.L., Dittenber, D.A., Brzak, K.A., Mattsson, J.L. (2002). A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicol. Sci.*, 66: 34-53.

Tiwari, C., Ansari, B.A. (2014). Comparative study of acute toxicities of Endosulfan, Chlorpyriphos and Permethrin to Zebrafish, *Danio rerio* (Cyprinidae). *Scholars Acad. J. Biosci. (SAJB).*, 2: 404-409.

Topal, A., Atamanalp, M., Oruc, E., Demir, Y., Beydemir, S., Isik, A. (2014). In vivo changes in carbonic anhydrase activity and histopathology of gill and liver tissues after acute exposure to chlorpyrifos in rainbow trout. *Arh. Hig. Rada Toksikol.,* 65: 377-385.

Watson, F.L., Schmidt, H., Turman, Z.K., Hole, N., Garcia, H., Gregg, J., Tilghman, J., Fradinger, E.A. (2014). Organophosphate pesticides induce morphological abnormalities and decrease locomotor activity and heart rate in *Danio rerio* and *Xenopus laevis*. *Environ. Toxicol Chem.,* 33(6): 1337-45.

Yen, J., Donerly, S., Levin, E.D., Linney, E.A. (2011). Differential acetylcholinesterase inhibition of chlorpyrifos, diazinon and parathion in larval zebrafish. *Neurotoxicol.Teratol.,* 33(6): 735-741.