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Research Article

STUDIES ON CARBOSULFAN INDUCED OXYGEN DEPRIVATION AND BEHAVIORAL ANOMALIES IN FRESH WATER FISH *CYPRINUS CARPIO*

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ABSTRACT

A short-term toxicity study was conducted by a static renewal bioassay method to determine the acute toxicity (LC_{50}) of commercial-grade Carbosulfan (Marshal 25% EC) on freshwater fish *Cyprinus carpio*. The fingerlings were exposed to different concentrations of Carbosulfan for a 96-hour study. The acute toxicity (LC_{50}) of Carbosulfan for 96 hours was found to be 0.55mg/L. Behavioural patterns and oxygen consumption were performed in lethal (1, 2, 3, 4 days) and sublethal (1, 10, 20, 30days) exposures. The fingerlings of *Cyprinus carpio*in toxic media exhibited irregular, erratic and darting movements, hyper excitability, loss of equilibrium and sinking to the bottom, burst swimming etc. which might be due to the inactivation of AChE activity which results in excess accumulation of Acetylcholine in cholinergic synapses leading to hyperstimulation. Variation in Oxygen consumption was observed in both lethal and sublethal concentrations of Carbosulfan. This considerable variation in respiratory rates might be the consequence of altered Oxygen metabolism which leads to impairments in fish respiratory physiology and behavioural response. Even under recovery tenures respiratory stress was evident but not fatal, that might be due to the slow release of sequestered Carbosulfan from the storage tissue in sublethal exposure.

Keywords: *Cyprinus carpio*, Carbosulfan, Oxygen, Marshall, Behavior.

1. INTRODUCTION

India is one of the countries that most of the people practices agriculture as a main source of income for the family. Agricultural sector has made tremendous progress in crop production, integrated pest management and integrated fish farming in the past few decades [1]. Application of pesticide in agriculture became the most profitable, less expensive and convenient way of crop management method [2]. Integrated fish farming is also earning much attention because of the additional income along with main crop in the same agricultural field [3]. Pesticide runoff is one of the leading problems in integrated fish farming as this runoff pesticide residue ultimately reaches to the nearby waterbodies and harms the aquatic animals [4, 5].

Carbosulfan [2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl [(dibutylamino)thio] methyl carbamate] is a carbamate pesticide that is used to control insects in various crops including rice, citrus and potato [6]. Carbamates is well known for their inhibitory effects of AChE activity in fishes and mammals [7]. Acetylcholinesterase is a neurotransmitter that helps signal transduction in the nervous system. However, inhibition of AChE will cause reproductive defects, paralysis eventually leading to death in insects [8]. Cyprinus carp which was exposed to Indoxacrb exhibited liver damage, decreased protein synthesis, T_3 and T_4 production which is a serious threat to the fishes. Thyroid imbalance also observed due to the decreased levels of T_4 synthesis [9]. Low concentration treatment of carbosulfan to the fish rainbow trout affected the liver enzyme activities such as CAT, SOD and GST. However, these enzymes are considered to be biochemical markers that reflect oxidative stress in the animal [10]. Commercial formulations of carbosulfan induced mutagenic and genotoxic effects in *channa punctatus* by the formation of micronuclei in the blood [11]. Histological malformations of gill, kidney and liver are found in *L. rohita* exposed to different concentrations of carbosulfan indicated the potential toxic effects of carbamate pesticide on aquatic life [12]. The behavioral changes in fish considered as the serious stress and disturbance in the waterbodies. *Labeorohita* which was exposed to carbosulfan manifested the behavioral changes and pesticide induced oxidative stress in the animal [13].

The toxicity assessment in the rice field of integrated fish farming exhibited the persistence of small amount of carbamate pesticide in the water and this small amount pesticide was enough to bring down the AChE activity in the active fishes of the rice field. However, the fingerlings survived in the rice field but it was not recommended for the next stages of fish farming [14]. The combined treatment of chlorpyrifos and carbosulfan inhibited the Cholinesterase activity in the brain of Nile tilapia. Repeated dose of small amount of pesticide significantly affected the overall performance of the tilapia fish [15].

Apart from all these experimental results, data regarding effects of carbosulfan on *Cyprinus carpio* was meagre. The present experiment explains the behavioral anomalies and variation in oxygen consumption of the fish under the influence of lethal and sublethal concentrations of commercial formulation of carbo-sulfan pesticide.

2. MATERIALS AND METHODS

2.1. Animal Maintenance:

Healthy Fingerlings of *Cyprinus carpio* weighing 10±2g and length of 6±2cm were purchased from State fisheries department, Shivamogga, India. The fingerlings were acclimatized in laboratory condition for 15 days at 26°C. The fingerlings were further transferred to dechlorinated water filled large cement tanks that were previously treated with 1% $KMnO_4$ solution to avoid the microbial growth and Fungus attack to the fishes. The tanks were provided with sufficient aeration during the acclimatization period. Fishes were fed with finely powdered commercial food pellets (Optimum CP) regularly 2 times a day and allowed to 12-14 hours of photoperiod with daily renewal of water. Physico-chemical parameters were conducted daily according to the standard methods mentioned in APHA [16].

2.2. Test chemicals

Carbosulfan (25% EC) with Trade name Marshall was purchased from FMC India private Ltd., Maharashtra. For sublethal studies the $1/10^{th}$ dose of the pesticide was selected andthe required concentrations of the pesticide were drawn directly from the stock solution.

2.3. Acute toxicity test

Acute toxicity test was conducted according to OECD guidelines No. 203. Static renewal assay method was followed to test each concentration of pesticide. A range finding test was performed in order to find out the upper limit and lower limit of Carbosulfan toxicity, this test was employed in order to minimize the unnecessary killing of animals. Healthy fingerlings of *Cyprinus carpio* were selected for the test in which feeding was stopped 24hour before the commencement of assay. Carp fingerlings n=10 each transferred to separate clean glass aquaria each having 0.3mgL^{-1}, 0.35mgL^{-1}, 0.4mgL^{-1}, 0.45mgL^{-1}, 0.55mgL^{-1}, 0.65mgL^{-1}, 0.7mgL^{-1}, and 0.8 mgL^{-1} concentration of pesticide, respectively, another group (n=10) served as control which was filled with dechlorinated tap water without any pesticide residues. All the acute toxicity tests including control was conducted in triplicates simultaneously, the mean of triplicates was considered for the acute toxicity determination. The fishes were observed regularly for mortality during 96-hour test period, the dead fishes were removed as early as possible on sight and the mortality was recorded at every 24-hour interval up to 96 hours. The recorded mortality was used to calculate LC_{50} value using probit analysis [18].

2.4. Behavioral studies

The fingerlings were regularly monitored for better understanding of behavioral abnormalities. The fishes in each concentration were observed 1 hour continuously after introduction of toxicant into the test aquaria to assess immediate responses of fishes. The behavioral anomalies are noted down frequently for further data interpretation.

2.5. Oxygen consumption

Aquatic organisms rely on the dissolved oxygen in the water for the respiration process. Nowadays the waterbodies are polluted by various pollutants including pesticides. The respiratory distress in the fishes is one of the most important causes for loss of activeness and death. The percentage of dissolved oxygen in water-bodies decides the stress level of the fish. However, the pesticides are known to induce many physiological deformities in the fish. It is relevant to examine the oxygen consumption of the fish in pesticide treated medium. Oxygen consumption of the fish was measured from 24hr to 30 days at different intervals. The experiment was constructed to maintain above 70% oxygen levels in long term exposure period. Experimental setup was made as explained by Saroja [19]. The dissolved oxygen was measured by Winkler's iodometric method [20]. The oxygen consumption of the fish was expressed in O_2/mg/L/g/hr. No deaths were observed during the experimental period. The temperature and pH were maintained at 26±1°C and 7.2±0.3 respectively.

2.6. Statistical analysis

All the statistical values are expressed as means and Analyzed by ANOVA with Tuky'spost hoc test. The statistical analysis was performed using SPSS version 25 statistical package.

2.7. Ethical clearance

The present experimental procedure was accepted and carried out according to the Institutional Animal Ethics Committee guidelines (IAEC). The animals used in this study were handled according to the guidelines issued by Committee for the Purpose of Control and Super-vision of Experiments on Animals (CPCSEA), New Delhi, India.

3. RESULTS

3.1. Acute toxicity test

Physico-chemical parameters of test water used in the experiment were examined and the results were represented in Table 1. The toxicity of commercial formulation of carbosulfan for *Cyprinus carpio* for 96hr was observed with no mortality at 0.30mg/Ltr. The mortality rate was increased with increase in the concentration of carbosulfan and 100% mortality was found at 0.80mg/Ltr (Table 2). The 96hr LC_{50} values are obtained by the probit analysis method [18]. The 95% confidence limit were also calculated and presented in Table 3. Percent mortality was plotted against log concentration of carbosulfan. The graph was obtained with straight line representing the LC_{50} values of 0.55mg/Ltr (Fig.1 & 2).

Table 1: Physico-chemical parameters of water

Parameter	Obtained values
Temperature	26±1°C
pH	7.2±0.3
Dissolved oxygen	6.2±0.0.3 mg/L
Total Hardness	38.5±2.8 mg as $CaCO_3$/L
Salinity	Nil
Specific gravity	1.002
Calcium	22.02±0.4 mg/L
Phosphate	0.8±0.0 mg/L
Magnesium	0.78±0.2 mg/L

Table 2: Mortality of *Cyprinus carpio* exposed to different concentration of Carbosulfan

Concentration mg/Ltr	Log concentration of Carbosulfan	Number of fishes exposed	Number of fish alive	Number of fish dead	Percent mortality	Probit mortality
0.30	2.477	10	10	0	0	-
0.35	2.544	10	9	1	10	3.72
0.40	2.602	10	8	2	20	4.16
0.45	2.653	10	7	3	30	4.48
0.55	2.740	10	5	5	50	5.00
0.65	2.812	10	3	7	70	5.52
0.70	2.845	10	2	8	80	5.84
0.80	2.903	10	0	10	100	-

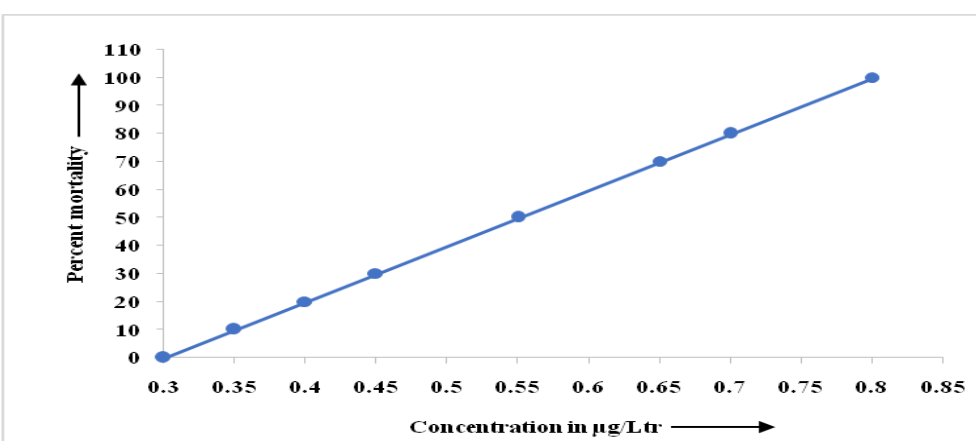

Fig. 1: Percent mortality of *Cyprinus carpio* exposed to different concentrations of carbosulfan.

A sigmoid curve was obtained by plotting a graph of Percent mortality against log concentration (Fig. 3). The upper and lower confidence limit were tabulated in Table 3. The 96hr LC_{50} of carbosulfan on *Cyprinus carpio* was found to be 0.55 mg/Ltr.

Table 3: Acute toxicity (96h LC_{50}) and 95% confidence limit of Carbosulfan to *Cyprinus Carpio*

Toxicant	96h LC50 (mgL^{-1})	95% Confidence limits	
		Upper limit	Lower limit
Carbosulfan	0.55 mgL^{-1}	300	800

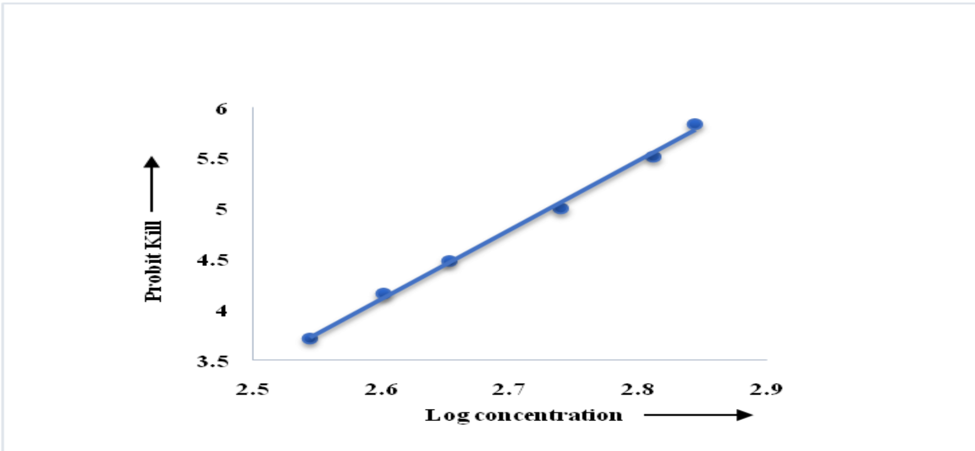

Fig. 2: Mortality of *Cyprinus carpio* exposed to different concentrations of carbosulfan

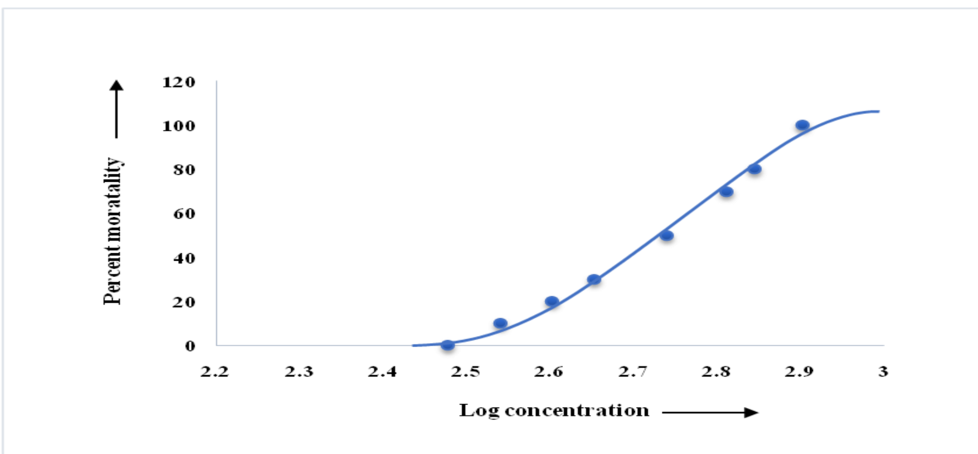

Fig. 3: Mortality of *Cyprinus carpio* exposed to different concentrations of carbosulfan.

3.2. Behavioral studies

The control fishes in the present study were very active and healthy throughout the experimental period. They were attentive for the slightest disturbances and manifested fine schooling behavior. The opercular beat, buccal movement, fin beat, pigmentation, swimming patterns all were normal and there were no behavioral differences between the control groups. The treated fishes with different experimental period exhibited different frequency of behavioral changes [Table 4]. On the first day of treatment with sublethal concentration the fishes displayed inverted swimming, delayed fright response and sinking phenomena. The fright response was gradually ceased at 30th day of exposure. Upward swimming was observed from 1st day to 20th day and was decreased up to 30th day. Buccal movement was high at 10th day and continued till 30th day. Dashing movement was slightly increased but the fin beat was moderately increased up to 30th day of exposure. Whirling cork movement and dyspigmentation was witnessed right from day 1st of exposure and the same increased until the last day of exposure period. Schooling behavior was disturbed during the experimental period. However, it was nil at 30th day of exposure.

3.3. Oxygen consumption

The mean values and percentage change of oxygen consumption of control group and treated groups are given in Table 5. The fishes exposed to lethal concentrations of carbosulfan for 24hr, 48hr, 72hr and 96hr expressed decrease in oxygen consumption with 0.1762, 0.1702, 0.1664, 0.1437 mg of oxygen/L/g/hr respectively. Initially at 24hrs of exposure the fishes exhibited increased oxygen consumption. However, the oxygen consumption decreased further withexperimental periods of 48hr, 72hr and 96hrs. The experimental results of treated fishes were significantly different from the control (0.2763 mg/L/g/hr). The percentage change in dissolved oxygen among the experimental fishes were shown in Figure 4. The fishes exposed to sublethal concentrations with different experimental period expressed increase in oxygen consumption. The mean values of 0.2112, 0.2213, 0.2242, 0.2348 oxygen in mg/L/g/hr were obtained for 1st day, 10th day, 20th day and 30th day exposure periods respectively. An increasing trend in oxygen consumption was observed in long term exposures. At first day of sublethal exposure the oxygen consumption of the fish gradually increased with increase in experimental days (Fig. 4).

Table 4: Behavioral changes in *Cyprinus carpio* exposed to different concentrations of Carbosulfan

Observed behavior	Control	Exposure days			
		1 Day	10 Days	20 Days	30 Days
Inverted swimming	-	+	+ +	+ +	+
Sinking phenomenon	-	+	+	-	+
Fright response	+ + +	++	+ +	+	-
Upward swim	-	++	+ +	+	-
Buccal movement	+	++	+ + +	+ +	+ +
Dashing movement	-	+	+	+ +	+
Fin beat	+	++	+ +	+ + +	+ + +
Whirling cork movement	-	+	+	+ + +	++
Schooling behavior	+ + +	++	+ +	+	-
Dyspigmentation	-	+	+	+ +	+ + +

Fishes exhibiting various behavior, indicates (+) as low, (++) as medium and (+++) as high intensity of behavior upon exposure to Carbosulfan.

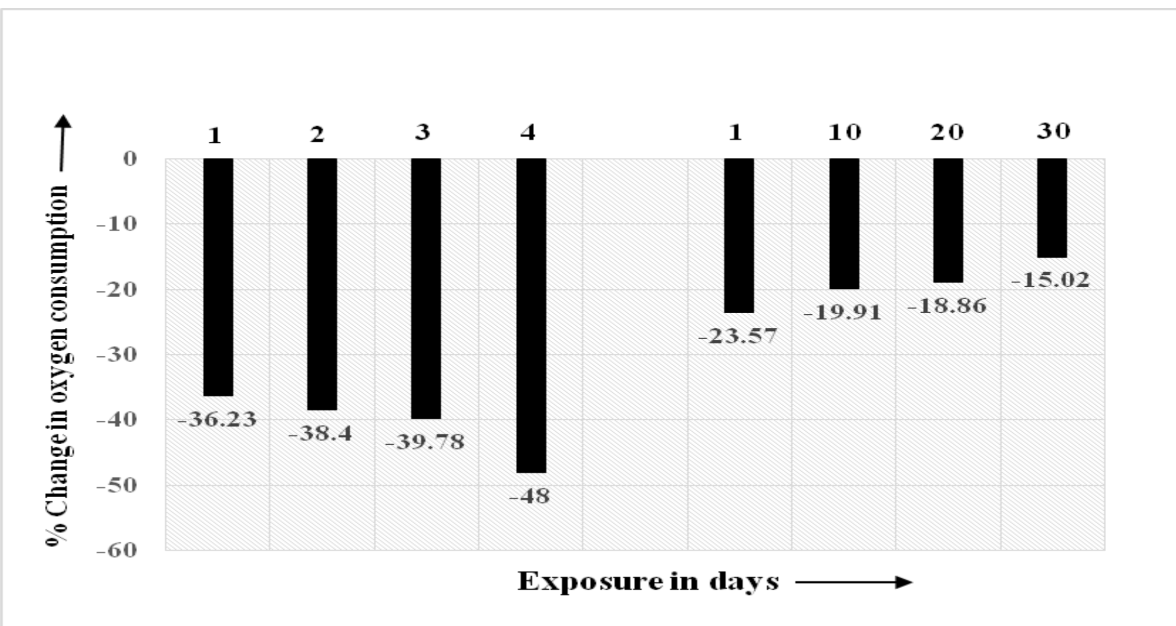

Fig. 4: Percentage change in dissolved oxygen of *Cyprinus carpio* exposed to Carbosulfan

Table 5: Oxygen consumption (O_2mg/gram wet wt. of the fish/hr) of the fish *Cyprinus carpio* following exposure to lethal and sublethal concentrations of Carbosulfan

		Lethal (In Days)				Sublethal (In Days)			
Oxygen consumption	**Control**	**1**	**2**	**3**	**4**	**1**	**10**	**20**	**30**
O_2mg/g/hr (Mean)	0.2763*	0.1762*	0.1702*	0.1664*	0.1437*	0.2112*	0.2213*	0.2242*	0.2348*
SD±	0.0508	0.0186	0.0191	0.0240	0.0134	0.0124	0.0066	0.0105	0.0147
% Change	--------	-36.23	-38.4	-39.78	-48	-23.57	-19.91	-18.86	-15.02

**The values are significantly different at* $p \leq 0.05$

4. DISCUSSION

The LC_{50} of *Cyprinus carpio* exposed to carbosulfan was found to be 0.55mg/L. The obtained values of LC_{50} suggest the extent of toxicity produced by the test chemical. Behavioral changes in the fishes are excellent tools to assess physiological and ecological interactions [21]. The control fishes expressed normal swimming movement, robust response for the slightest disturbances, well-coordinated movements, gills and buccal movements. However, the treated fishes were become very slow and unresponsive to any disturbances. Sinking phenomenon, upward swimming, whirling cork move-ments were attributed to loss of balance in the fish body. Initially these are very evident and varied throughout the experimental period. The loss of balance might be due to the adverse effect of carbosulfan on neurotransmitters and inhibition of AChE activity in the fish [22].

The initial increase of buccal movements could be attributed to the possible compensatory behavioral changes that were considered to be significant in stressful conditions [23]. The schooling behavior was disrupted right from the introduction of carbosulfan to the test media. The disruption in schooling continued and completely distorted at the end of experiment. Similar observations were made by Sobha *et al.,* Ural & Simsek. Dashing movement was observed that might be due to the loss of coordination in the fishes. Dyspigmentation was more evident in lethal doses and moderate in sublethal concentrations, this might be owing to the dysfunction of pituitary gland under high toxic stress [26]. The present experimental results were consistent with Marigoudar *et al.,* Nazeer Ahmed *et al.* and Zabin *et al.*

Stress is one of the most important factors that influence the metabolic and respiration rate. The oxygen consumption of the fish mainly depends upon the environment and the quality of water in which it is living. Slight disturbances in the water quality can affect the physiology and behavior of the fish [30, 31]. The behavioral changes and the change in levels of oxygen consumption could be considered as a bioindicators in assessing the industrial effluents before releasing into the environment. However, the variation in oxygen consumption might be attributed to the presence of contaminants in the water [32].

The present study revealed decreased oxygen consumption in lethal concentration and increased trend in sublethal concentration of carbosulfan (Fig. 4). High levelsof stress and first time contact of toxic chemical could be the reason for initial decrease in oxygen consumption that helps the animal to avoid absorption or to lower the intake of toxic materials. The gills also play an important role in the absorption of oxygen and other essential dissolved minerals through it. The oxygen consumption decreased from 24hr exposure period to 96hr that might be due to damaged gill components or accumulation of the toxic chemicals in the gill muscles [33]. On the other hand, the oxygen consumption was increased in sublethal exposure. The fish gradually increased the consumption of oxygen from day 1 to day 30exposure periods. The fish might have initiated specific protein synthesis to survive toxic environment or it might start to detoxify rapidly the chemical from the body [34]. The present obtained results were in accordance with observations made byDubey andHosetti 2010, Patil and David 2008, Shereena *et al.* 2009.

The toxic chemicals can be entering into the blood stream of the fish through gills. Upon entry of toxic substances, the physiological activities of the fish ceases and the metabolic rate also decrease [38]. Adaptive mechanism of the fish tries to accomplish the toxicity caused by the pesticide and starts to consume more oxygen in order to suffice metabolic need. Considering the results, it is evident that carbosulfan is highly toxic to fishes and it causes disturbances in oxygen consumption in the fish *Cyprinus carpio*.

5. CONCLUSION

Cyprinus carpio is one of the major carps that is consumed by most of the population as a proteinaceous food. The toxic chemicals such as heavy metals, pesticides and industrial pollutants in the water cause intoxication in aquatic organisms. The pesticide used to control target pest in the agricultural field is indirectly affecting the nontarget organisms. Carbosulfan is one of such chemicals that affect nontarget organisms in the environment. The effect of carbosulfan is highly evident in the present study with assessment of behavior and oxygen consumption in the fish. Carbosulfan is a carbamate chemical that mainly affects AChE activity in the organisms. However, the stress levels induced by the carbosulfan affected oxygen consumption in the fish *Cyprinus carpio*. Behavioral changes during the experimental period indicated the extent of toxic stress. Therefore, it is concluded that the pesticide is toxic to aquatic organisms and special precaution should be taken to avoid application near waterbodies with fisheries activities or in integrated fish farming as there might be chances of pesticide runoff and toxifying the waterbodies.

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Conflict of interest

Authors claim no conflict of interest.

7. REFERENCES

1. Sharma M, Patil C. *Jour of Pharma and Phytochem,* 2018; **7(2):**1906-1910.
2. Jingna Cui, Fang Wang, Jing Gao, Wangjing Zhai, Zhiqiang Zhou, Donghui Liu, Peng Wang. *Jou Agric Food Chem,* 2019; **67:**12348-12356.
3. Solomon MB, Natarajan P. *Intern Jou of Fish and Aqua Stud,* 2019; **7(4):**263-269.
4. Hakan Berg. *Crop Protect,* 2001; **20:**897-905.
5. Barbara C, Vania LL, Camila RMi, Tadeu LT, Bibiana M, Renato Z. *Scie of the Tot Envir* 2018; **626:**737-743.
6. Sahoo A, Sahu SK, Sharmila M, Sethunathan N. 1990; *Bull Environ ContamToxicol,* **44:**948-954.
7. Gomez-Gimenez B, Felipo V, Cabrera-Pastor A, Agusti A, Hernandez-Rabaza V, Llansola M. *Neurotox Res,* 2018; **33(2):**247-258.
8. Martin-Reina J, Duarte JA, Cerrillos L, Bautista JD, Moreno I. *J Toxins,* 2017; **4(1):**7.
9. Ghelichpour M, Taheri Mirghaed A, Mirzargar SS, JoshaghaniH, Ebrahimzadeh Mousavi H. *Aquacul Rese,* 2017; **48(11):**5666-5676.
10. Capkin E, Altinok I. *Enviro Toxic and Pharmac,* 2013; **36(1):**80-87.
11. Nwani CD, Lakra WS, Nagpure NS, Ravindra Kumar, Kushwaha B, Srivastava SK. *Foo and Chem Toxico,* 2010; **48:**202-208.
12. Dey C, Saha SK. *J Biol Tod World,* 2016; **5(7):**107-112.
13. Nagaraju B, Venkata Rathnamma V. *Jou of stre physio & bioche vol,* 2014;**10(3).**
14. De MelGWJLMVTM, Pathiratne A. *Jour of Appl Ichthy,* 2005; **21(2):**146-150.
15. Chandrasekera LKHU, Pathiratne A. *Bulle of EnvirConta and Toxi,* 2005; **75(6):**1228-1233.
16. APHA, AWWA, WPCP, Standard methods for the examination of water and waste-water. 21st ed. American Public Health Association, 2005; Washington, DC.
17. Test Guideline No. 203. OECD 18 June 2019. https://doi.org/10.1787/20745761.
18. FinneyDJ.1971; Probit Analysis, 3rd Edition, Cambridge University, Press, London, 333.
19. Saroja K. *Stu on oxy cons in trop poikil-II,* 1958; 183-193.
20. Moran JM, Morgan MD, Wiersma D, James H. Introduction to environmental science, 2nd edn. WH Freeman, New York, NY, 1980;.
21. Graham R Scott, Katherine A Sloman. *Aqu Toxico,* 2004; **68:**369-392.
22. Mushigeri SB, David M. *Environ Toxicol Pharmacol,* 2005; **20:**65-72.
23. Shivakumar R, David M. *Indi J Ecol,* 2004; **31:**27-29.
24. Sobha K, Poornima A, Harini P, Veeraiah K. *Jour of Engin Sciand Techn,* 2007; **3(2):**1-11.
25. Ural MS, Simsek S. *Bul Environ Contam Toxicol* 2006; **76:**871-876.
26. Pandey A, Kumar GK, Munshi JSD. *J Frewat Biol,* 1990; **2:**117-121.
27. Shambanagouda RM, Raichur NA, Muniswamy D. *Veterinars-kiarhiv,* 2009; **79(6):**583-590.
28. Marigoudar SR, Nazeer Ahmed R, David M. *WorJour of Zool,* 2009; **4(1):**19-23.
29. Zabin SB, Kartheek RM, David M. *Inter Jo of Fish and Aqu Stud,* 2018; **6(3):**221-226.

30. Tilak KS, Veeraiah K, Ramanakumari GV. *Rol Res,* 2001; **20(3):**438-445.
31. Alkhail ARAA, Askar AI, Younis LK, El-Gendy KS, Abbas MM, Marei ASM. *Pak J Biol Sci,* 2004; **7:**1117-1120.
32. Scherer E. *J Appl Ichthyol,* 1992; **8:**122-131.
33. Mushigeri SB. 2003; PhD. thesis, India, Karnataka, Dharwad: Karnataka University.
34. Connell D, Lam P, Richardson B, Wu R. Introduction to ecotoxicology. 1999; London, 170.
35. Dube PN, Hosetti BB. *Biotech in animhusba,* 2010; **26:**91-103.
36. Vineetkumar K Patil, David M. *Turki Jourof Fishe and Aqu Sci,* 2008; **8:**233-237.
37. Shereena KM, Logaswamy S, Sunitha P. *Rec Rese in Scie and Techn,* 2009; **1(1):**4-7.
38. Kalavathy K, Sivakumar AA, Chandran R. *J Ecol Res Bio,* 2000; **2:**27-32.