Journal of Stress Physiology & Biochemistry, Vol. 10 No. 3 2014, pp. 218-231 ISSN 1997-0838

ORIGINAL ARTICLE

Determination of Median Lethal Concentrations (LC_{50}) of Freshwater Fish *Labeo rohita* (Hamilton) for Carbosulfan and Its Behavioral Impacts

Nagaraju B.[1], Venkata Rathnamma V.[2]

[1] *Department of Biochemistry, Acharya Nagarjuna University, Guntur-522510, A.P, India.*
[2] *Department of Zoology, Acharya Nagarjuna University, Guntur-522510, A.P, India.*

*E-Mail: nagaraju.bantu301@gmail.com

Received April 19, 2014

In the present study, *Labeo rohita* were exposed to carbosulfan for 96 h. Experimental fishes which measured an average length of 6±7 cm and weighed 6.5±7.5g. Nine groups with three replicates of experimental fish were exposed to seven concentrations of carbosulfan. The containers used for the test media of 15 L capacity, filled with dechlorinated water. Where maximum 10 fishes were used in our experiments for each concentration, 10 fishes were also maintained in separate container along with experimental groups, served as control. The median lethal concentrations (LC_{50}) of carbosulfan for 96 h were 1.2mg/l, respectively. In addition, to dose and dose-time dependent increase in mortality rate, the behavioral changes of *L. rohita* at different concentrations of carbosulfan were determined. Physiological responses like rapid opercular movement, hyper secretion of mucus and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. The unusual behaviour of the fish *L. rohita* in stress condition may be due to obstructed functions of neurotransmitters. All these observations can be considered to monitor the quality of aquatic ecosystem and severity of pollution. In conclusion, carbosulfan was moderately toxic to freshwater fish *L. rohita*

Key words: carbosulfan, concentration, mortality rate, Labeo rohita

ORIGINAL ARTICLE

Determination of Median Lethal Concentrations (LC_{50}) of Freshwater Fish *Labeo rohita* (Hamilton) for Carbosulfan and Its Behavioral Impacts

Nagaraju B.[1], Venkata Rathnamma V.[2]

[1] *Department of Biochemistry, Acharya Nagarjuna University, Guntur-522510, A.P, India.*
[2] *Department of Zoology, Acharya Nagarjuna University, Guntur-522510, A.P, India.*

*E-Mail: ***nagaraju.bantu301@gmail.com***

Received April 19, 2014

In the present study, *Labeo rohita* were exposed to carbosulfan for 96 h. Experimental fishes which measured an average length of 6±7 cm and weighed 6.5±7.5g. Nine groups with three replicates of experimental fish were exposed to seven concentrations of carbosulfan. The containers used for the test media of 15 L capacity, filled with dechlorinated water. Where maximum 10 fishes were used in our experiments for each concentration, 10 fishes were also maintained in separate container along with experimental groups, served as control. The median lethal concentrations (LC_{50}) of carbosulfan for 96 h were 1.2mg/l, respectively. In addition, to dose and dose-time dependent increase in mortality rate, the behavioral changes of *L. rohita* at different concentrations of carbosulfan were determined. Physiological responses like rapid opercular movement, hyper secretion of mucus and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. The unusual behaviour of the fish *L. rohita* in stress condition may be due to obstructed functions of neurotransmitters. All these observations can be considered to monitor the quality of aquatic ecosystem and severity of pollution. In conclusion, carbosulfan was moderately toxic to freshwater fish *L. rohita*

Key words: carbosulfan, concentration, mortality rate, Labeo rohita

A major environmental impact has been the widespread mortality of fish and marine invertebrates due to the contamination of aquatic systems by pesticides. Industrial and agricultural endeavours are intimately associated with the extensive use of a wide array of chemicals. Historically, chemical wastes generated through industrial processes were disposed of through flagrant release into the environment. Pesticides and other agricultural chemicals revolutionized farm and forest productivity.

Potential adverse effects of the application of such chemicals to the environment (Hodgson, 2010). This has resulted from the agricultural contamination of waterways through fallout, drainage, or runoff erosion, and from the discharge of industrial effluents containing pesticides into waterways. However, fish are not usually target organisms for pesticides, and knowledge about effects of pesticides in the field is still sparse. Surprisingly, only a few studies have shown that fish, inhabiting natural freshwater ecosystems, may be affected by unintentional spreading of pesticides. The risk associated with contaminants was assessed using risk quotients based on the comparison of measured concentrations with original species sensitivity distribution-derived hazardous concentration values (Adedeji *et al*., 2012). Their worldwide annual use is estimated to range from 20,000 to 35,000 tones. When croplands are treated, some impacts of pesticides occur on non-target terrestrial and aquatic ecosystems, as well as on adjoining agro ecosystems (Caquet *et al*., 2013). Unfortunately, farmers may not be aware of the source of the pesticides sold to them and unwittingly introduce dangerous chemicals into the environment (Surendra, 2010). Accidental killing of fish due to the contamination of the aquatic environment remains among the most frequent poisoning cases. Every year, about 260 to 300 of such accidents are diagnosed (Weyman, 2007). Intensive use of pesticides particularly dithiocarbamates, in agriculture often leads to contamination of fresh water ecosystems through atmospheric fall out, agriculture runoff (Kreisler and Heiss, 2008). The toxicity of carbamate insecticides on mammals, birds and amphibians have been reviewed by (Vlcek and Pohanka, 2012). Acute toxicity is defined as toxicity elicited as a result of short - term exposure to a toxicant ,acute toxicity tests are designed to measure the effects of toxic agents on aquatic species during a short period of their life span and allow us to assess the effects of various pollutants on the biology of fish (Ebrahimpour *et al*., 2010; Javed, 2012a) to find out their abilities to adapt under certain toxicity levels and to forecast possible effects of toxicity on them (Javed, 2012b, Azmat *et al*., 2012).

Carbosulfan is a broad-spectrum carbamate pesticide used to control insects, mites and nematodes by soil, foliar and seed treatment applications, mainly on potatoes, sugar beet, rice, maize and citrus. Carbosulfan, a benzofuranyl methyl carbamate pesticide, has been widely used in agriculture and is reported to be very effective against pyrethroid resistant mosquitoes also; carbofuran is the main metabolite of carbosulfan in plants and is itself a pesticide (Guillet *et al*., 2001). On the other hand, it can pollute environment and become a problem when it reaches water supplies which, for example, was the case, reported by (Tarig *et al*, 2010) for carbosulfan.

Hence the number and quantities of carbamate pesticides used in agriculture continue to increase, replacing other type of pesticides such as organochlorine and organophosphorous pesticides. Carbosulfan is extremely toxic to mammals and including human being, its toxicity is mediated through inhibition of acetylcholine esterase (Renzi and Krieger, 1986; Sarbani Giri, 2003), the health problems in human individuals occupationally exposed to the pesticides in pesticide production industries and cotton picking women were observed (Rath *et al.*, 2011).

The aim of this current study was to estimate the acute toxicity of carbosulfan on fresh water edible fish *L. rohita,* it is abundantly used as food source in India. The fish was mainly grown in the rivers, canals, ponds connected to the fields. When it was sprayed it enters the water through runoff, drifts and leaching. So it is essential to determine how it affects non target aquatic organisms.

MATERIALS AND METHODS

The common edible fish *Labeo rohita* (Hamilton, 1822) is obtained from the local fish farm at Nandivelugu, Guntur District of Andhra Pradesh, India. The fish were brought to the laboratory for acclimatization. The length of the fish 6-7cm and body size 6.5 to 7.5g. The fish *L. rohita* were acclimatized to the laboratory conditions at 28±2°C. During the period of acclimatization, the fish were fed daily with commercial fish pellets and allowed to acclimate for 15 days. Feeding was stopped one day prior to the experimentation. The carbosulfan 25% EC, (Emulsifiable Concentrate), commercial grade formulations (Trade name as Marshal) was purchased from the local market in Guntur. Water was renewed every day to provide freshwater, rich in oxygen. If mortality exceeds more than 5% during the acclimatization, the entire batch of fish was discarded. The water used for acclimatization and conducting experiments was clear un-chlorinated ground water. The containers used for the test media of 15 L capacity, filled with dechlorinated water. Where maximum 10 fishes were used per each concentration of the carbosulfan pesticide, 10 fishes were also maintained in separate container along with experimental groups, served as control.

Acute toxicity Tests

After acclimation, the freshwater fish *L. rohita* were randomly transferred to 15 L capacity Glass chambers. Test solutions were prepared from commercial formulations the concentrations tested for carbosulfan; Fish were respectively exposed to a test solution of between 0.4-2.8 mg/l. Triplicate glass chambers were designated for each concentration. Carbosulfan were dissolved in acetone, and then added to the glass chambers. In control tanks, added with the quantity of acetone equal to the highest concentration used in the test. Precaution is taken to minimize the acetone as solvent. This study was conducted under acute toxicity test OECD Guidelines for the Testing of Chemical, Guideline No. 203 under static test conditions (OECD, 1992; Water quality, 1996).

During the test physiochemical parameters of water such as, pH, dissolved Oxygen, total hardness, temperature etc. were measured according to (APHA, 2005). During the experiment, dead fish were removed and the mortality was recorded after 24, 48, 72 and 96 h. the LC_{50} of carbosulfan and its 95% confidence limits for *L. rohita* were calculated using a basic program from the Probit analysis described by (Finney, 1971).

Physico-chemical analysis of water

Turbidity–8 Silica units, Electrical conductivity at

28^{o}C -816 micro ohms/cm, Alkalinity-1, Phenolphthalein-Nil, Methylorange-472, Total hardness (as $CaCO_3$) - 232, Non carbonate hardness (as $CaCO_3$) - Nil, Calcium hardness (As N) -Nil, Sulphate (as SO_4) -Trace, Chloride (as Cl)- 40, Fluoride (as Fl)- 1.8, Iron (as Fe) -Nil , Dissolved oxygen- 8-10 ppm, Temperature- 28±2^{o}C. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA, AWWA, WPCF, 1998) were followed. The dead fish were removed immediately; the incidence of fish mortality was recorded at 24, 48, 72 and 96 h after exposure to carbosulfan.

Statistical analysis

Percent Mortality data of fish at the end of 96 h exposure were analyzed using software- NCSS v.8; confidential limits were calculated by using with the help of probit analysis.

RESULTS

The results of the LC (median lethal concentrations) of the present study at 96 h were 1.2mg/l for carbosulfan, Results according to NCSS v.8 analysis showed the median lethal concentration LC_{50} of estimated carbosulfan to *L. rohita* for 96 h of exposure to be 1.2mg/l**,** the lower bound and upper bound 95% lethal confidence limits for carbosulfan indicated a wide range of values (0.916 to 1.329) respectively. The results showed that carbosulfan was toxic to the fish *L. rohita*, toxicity of the pesticide was both time and concentration dependent, thus accounting for differences in LC1-99 values obtained (Table 3) at different concentrations and times of exposure. The test result of the 96 h LC_{50} of *L. rohita* exposed to carbosulfan obtained in the present study was slightly higher than the 96 h LC value of 1.75mg/l for bluegill sunfish, the LC_{50} (96 h) is 0.042 mg/l for rainbow trout , 0.015 mg/l for bluegill sunfish, for fry of common carp is 0.60 mg/l, 0.231 and 0.122 mg/l estimated by Boran *et al.* (2007), for rainbow trout (*Oncorhincus mykiss*) and guppy (*Poecillia reticulata*), (Tomlin, 2003; Yi *et al.*, 2006) for the same pesticide. The results of the present work appear to agree generally with previous studies, where the main effective substance carbosulfan has been found to be moderately to highly toxic to fish, as LC_{50}-96 h values oscillate from 1.2mg/l, depending on the sensitivity of the species (Del, 2005; Howard, 1991). Carbosulfan decreased toxicity to mammals but increased toxicity to fish compared with carbofuran. It was not consistent with in vitro inhibiting brain AChE by carbofuran and carbosulfan. So, in vitro data do not explain the relatively acute toxicity of carbofuran and carbosulfan (Tomlin, 2003).

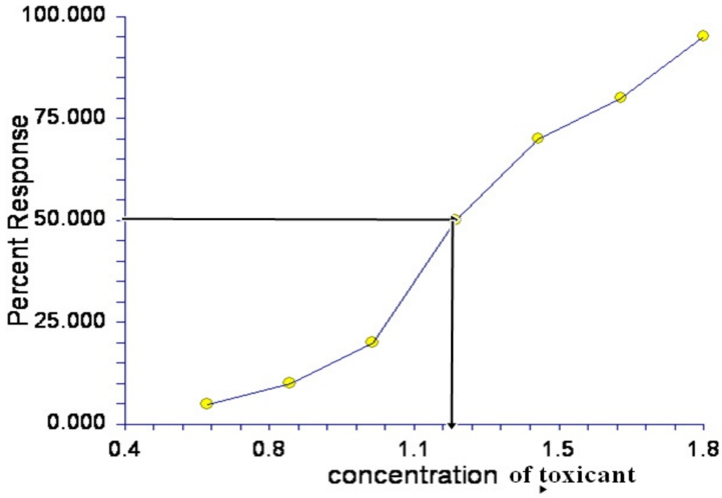

Figure 1. Percent response and concentration of pesticide carbosulfan

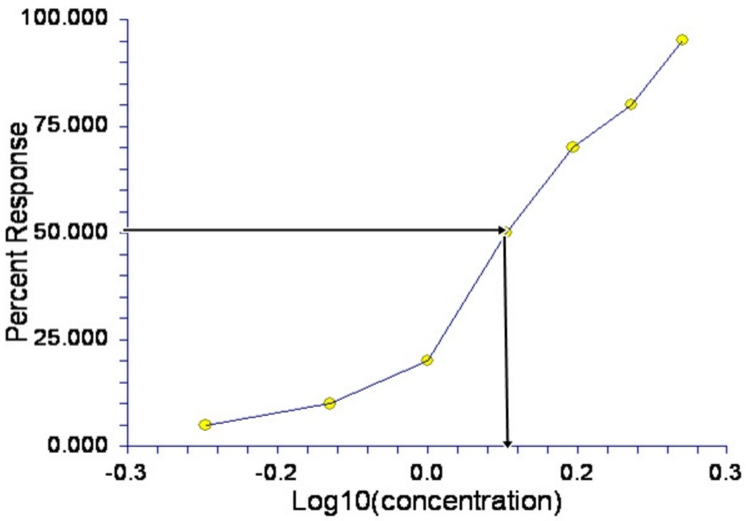

Figure 2. Percent response of fish *Labeo rohita* and log concentration of pesticide carbosulfan

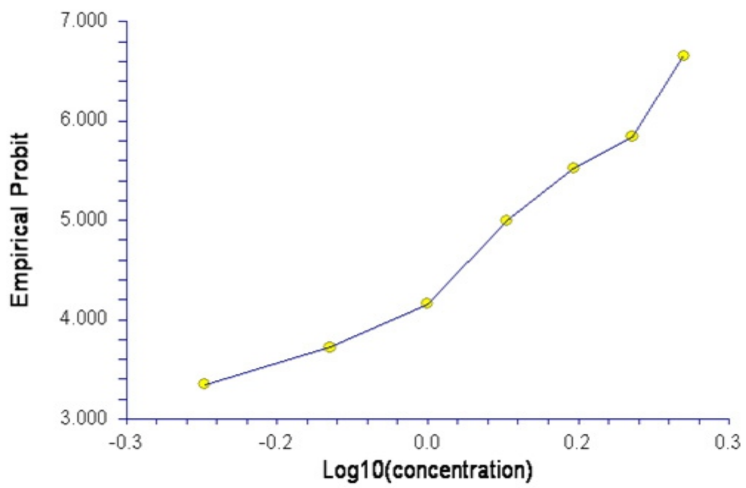

Figure 3. Probit line graph of Acute toxicity of carbosulfan on *Labeo rohita*

Table 1: Mortality of fish Labeo rohita in different concentrations of carbosulfan at 96h exposure period

Concentration mg/L	Actual percent	Probit percent	No.of Fish	Mortality	E(R)	Difference	χ^2
0.6	4.90	1.51	10	0.49	0.15	0.34	0.77
0.8	10.00	10.34	10	1.00	1.03	-0.03	0.00
1	20.00	28.77	10	2.00	2.88	-0.88	0.38
1.2	50.00	50.55	10	5.00	5.05	-0.05	0.00
1.4	70.00	69.10	10	7.00	6.91	0.09	0.00
1.6	80.00	82.09	10	8.00	8.21	-0.21	0.03
1.8	95.10	90.14	10	9.51	9.01	0.50	0.28

Total Chi-Square(χ^2) :1.46

D.F: 5

Prob Level: 0.92

Table 2: LC_{50} values with 95% confidence limits for carbosulfan based on dissolved concentrations estimated by Finney probit method

S.No	Concentration	exposed fish	%mortality	Log of toxicant concentration	Lower bound/upper bound
1	1.2mg/L	10	50	0.0773±0.0251	0.916-1.329

Table 3. Concentration of toxicant and Percentile responses of *Labeo rohita*

Percentile	Probit	Log(conc.)	Std. Error Log(conc.)	conc.	Std. Error Conc.
1	2.6737	-0.2438	0.0735	0.5705	0.0965
5	3.3551	-0.1497	0.0554	0.7084	0.0903
10	3.7184	-0.0996	0.0462	0.7951	0.0847
20	4.1584	-0.0389	0.0362	0.9144	0.0763
25	4.3255	-0.0158	0.0329	0.9643	0.0731
30	4.4756	0.0049	0.0303	1.0114	0.0705
40	4.7467	0.0423	0.0267	1.1024	0.0677
50	5.0000	0.0773	0.0251	1.1948	0.0691
60	5.2533	0.1123	0.0256	1.2950	0.0764
70	5.5244	0.1497	0.0284	1.4115	0.0922
75	5.6745	0.1704	0.0306	1.4804	0.1044
80	5.8416	0.1934	0.0336	1.5612	0.1209
90	6.2816	0.2542	0.0432	1.7954	0.1785
95	6.6449	0.3043	0.0521	2.0151	0.2417
99	7.3263	0.3984	0.0700	2.5024	0.4033

DISCUSSION

Carbosulfan, as with other carbamates, is extremely toxic to mammals and its toxicity is mediated through inhibition of acetylcholine esterase, a respiratory enzyme (Renzi and Krieger, 1986). A survey of literature revealed that very few studies have been carried out on the potential cytogenetic effect of carbosulfan. Studying on the genotoxicity of Marshal and its effective ingredient carbosulfan, Very few studies have been carried out on the potential cytogenetic effects of carbosulfan. Studying the genotoxicity of Marshal and its effective ingredient carbosulfan, reported that both test agents significantly induced the formation of chromosome aberrations in human peripheral lymphocytes *in vitro* (Topakata, 1993). Very few published reports could be found on the potential cytogenetic effects of carbosulfan (Topaktas, 1996). An increase in the number of the mortalities with increase in concentration of the toxicity of carbosulfan was observed and has been summarized in (Figure 1&2). There was no mortality at low concentration and as well as control group, the probit parametric estimates and the obtained results for acute toxic test 96 h estimated lethal concentration values and their confidence intervals were shown in (Table 2), respectively. It is clear from (Figure 1) that as the concentration of the pesticide chemical increased, fish mortality also increased, which indicates a direct proportional relationship between mortality and concentration of test chemical. (Table 2 and 3, Figure 2&3) depicts the relationship between concentration of test chemical and percentage fish mortality. It clearly showed that a higher concentration of carbosulfan is to kill number of fish. No mortality was observed during the experimental period in controls.

Probit analysis estimation of a common slope, common natural response rate and separate intercepts for each factor level shows (Table 3, Figure 1), Steep slope functions of the toxicity curve of 96 h mortality concentration data for carbosulfan indicate a large increase in the concentration of the insecticide. In the present study slope functions of the toxicity curve also clearly indicate the high acute toxicity of carbosulfan insecticide. Flat slope functions obtained for the toxicity curves of dimethoate and carbaryl indicate small increase in mortality associated with the relatively large increases in the insecticide concentrations. Steep slope functions of the toxicity curves are due to rapid absorption of the insecticides and rapid onset of effects. Flat slope functions indicate slow absorption, rapid excretion, detoxification or delayed toxicity of the toxicant (Caquet *et al.*, 2012). Comparing the other test organisms, *L. rohita* proved to be a particularly sensitive and reliable indicator as it appears from the high factor of determination LC_{50} for carbosulfan.

It is clear those earlier studies that LC_{50} of insecticides for a fresh water fish varies from species to species under influence of number of factors including size and time of exposure. Decreased swimming behaviour and increased respiration rate were other effects of pesticides in the present study, found that contaminants such as pesticides disturb normal fish behaviour after exposure (Scott and Sloman, 2004). Found that exposure of rainbow trout to carbaryl caused a decrease in brain ChE activity and this decrease was positively correlated with decreased swimming behaviour (Beauvais, 2001). Also found a positive correlation between changes in swimming speed and brain ChE activity following exposure to diazinon and malathion. Pesticides such as carbamates are known to affect fundamental physiological systems such that they affected salmonid olfactory- mediated behaviours in study (Brewer *et al.*, 2001). Carbamate insecticides are pseudo-irreversible inhibitors of AChE. They react with serine in estratic part of the enzyme active site in a process called carbamoylation (Darvesh *et al.*, 2008) Exposure to toxic substances may not result in immediate fish kills, but may affect fish populations by decreasing fecundity (number of eggs produced), reducing the viability of sperm, eggs and larvae, decreasing life expectancy, increasing the incidence of abnormalities and increasing natural mortality (Allan, 2004). Fish kills occur when pesticides are improperly applied to or otherwise end up in bodies of water through either misapplication or drift (Weyman, 2007).The response was initiated at the threshold dose when increased intensity of dose and exposure time is increased. This is reported in the basic concepts of the dose relationship. Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress at the toxicant, results increased swimming activity and entails increased expenditure of energy (Modra, Svobodova, 2009).The erratic swimming of the treated fish indicates loss of equilibrium. It is likely that the region in the brain which is associated with the maintenance of equilibrium should have been affected (Venkata Rathnamma *et al.*, 2008).

Alterations in the levels in tissues of fresh water *L. rohita* exposed to Fenvalerate, is observed by (David *et al.*, 2003). Carbamate did not cause cholinesterase depletion and cytotoxicity. However, genotoxic and pro-oxidant effects (increased levels of hemocytes with micronuclei and nuclear abnormalities, DNA-strand breaks and oxyradical in digestive gland), were common responses for both the exposed groups (Falfushynska, 2013).

Behavioral studies

The behavioural changes were also seen in the present investigation, over the duration of 96 h of exposure to carbosulfan, overall statistically significant changes in almost all behavioural patterns were observed in fish *L. rohita.* The surfacing phenomenon of fish observed under carbosulfan exposure. The control group shows the normal behaviour during the whole experiment and also normal responses was observed at the low concentration of the insecticide 0.1 mg/l was applied there is no variation takes place at control group and low insecticide concentration applied groups.

After 1day of exposure to carbosulfan significantly increased hyperactivity in terms of surfacing and scraping moments and schooling were observed in comparison to controls. At 2nd of exposure, the surfacing as well as scraping moments decreased in fish *L. rohita*, other behaviours increased significantly hyper secretion of mucus, opening mouth for gasping, losing scales, hyperactivity were observed. After 3rd day of exposure, the fish *L. rohita* showed decreased surfacing and jerky movements and increased grasping movements, sank to bottom of the test chamber and independency in swimming. Subsequently fish moved to the corners of the test chambers, which can be viewed as avoidance behaviour of the fish to the toxicant.

During the 96 h of exposure, all body activities were nearly ceased. Complete loss of body balance, exhibited irregular, erratic, darting swimming movements and loss of equilibrium followed by hanging vertically in water. Hyper excitation, loss of equilibrium, increased cough rate, flaring of gills, increase in production of mucus from the gills, darting movements and hitting against the walls of test tanks were noticed in all the species tested. A film of mucus was also observed all over the body and also on the gills. The increase ventilation rate by rapid, repeated opening closing mouth and opercula coverings accompanied by partially extended fins (coughing) was observed. This could be due to clearance of the accumulated mucus debris in the gill region for proper breathing. Nerve agents acting on the enzyme acetyl cholinesterase especially when dealing with neurotoxic compounds. Their toxicity is not limited to the acute phase, however, and chronic effects have long been noted. Neurotransmitters are acetyl choline profoundly important in the brain's development, in the absence of acetyl cholinesterase; acetylcholine level increased resulting in the failure of transmission of stimuli to the nerves or organs. Although carbamate pesticides tend to undergo fairly rapid degradation in the environment, repeat input into the aquatic environment may result in harmful exposures (Gracia de Liasera, 2001).The loss of balance in swimming and walking in both prawn species with increasing metal concentration may due to impairments either in nervous tissues or in muscle fibres (Shantha Kumar and Balaji, 2000; Verma *et al.*, 2005).

This leads to the abnormal functioning of the body including loss of balance, moving in circular form (convulsions) and at higher concentrations of insecticides resulting in death of the organism (Fukuto, 1990). Fish in the experimental group applied with highest concentration of the pesticide were lying laterally at bottom with loss of balance, swimming down in a spiral movement with jerks. Thus the behavioural changes of the fish under insecticidal stress may have deleterious effects of making the fish fall an easy prey in their natural habitat and may affect the stability of the population reported by (Jayantha Rao, 1984).

In the present study of test organism showed jerky movements, hyper secretion of mucus, opening mouth for gasping, losing scales, hyperactivity were observed in increase the insecticidal concentration. Behavioural characteristics are obviously sensitive indicators of toxicant effect. It is necessary, to select behavioural indices for monitoring that relates to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural systems. Insecticide toxicity is influenced by physical factors like temperature and biological factors like size, nutritional status and species specificity; for observing of experiments and all author report, the present study showed that carbosulfan is highly toxic to the common edible fish *L. rohita* as compared to the other fish species.

CONCLUSION

The result of the present study showed that carbosulfan insecticide was more toxic to *L. rohita* than other carbamate pesticides. All the pesticides induced oxidative stress inform of behavioural responses in the fish. Acute toxicity studies have been recognized as the very first step in determining the water quality management of fish and reveal toxicant concentrations that cause fish mortality even at short period of exposure. Additional research work is on the test species needed to arrive at more definite general conclusions.

ACKNOWLEDGEMENT

We are thankful to the Acharya Nagarjuna University, India, Guntur for providing financial support in the form of University Research Fellowship to carry out the research work.

REFERENCES

Adedeji, O.B. and R.O Okocha. (2012).Overview of pesticide Toxicity in fish. *Advances in Environmental Biology*, **6**,18-22

Ali, T., J.A. Bhalli, S.M. Rana and Q.M. Khan (2008). Cytogenetic damage in female Pakistan agricultural workers exposed to pesticides. *Environmen Mol Mutagen*, **49**, 374-380.

Allan. L. (2000). Fish kills in New South Wales. Department of Agriculture advisory. Note no 4/86.

APHA, AWWA, WPCF. (1998). Standard Methods for examination of water and Wastewater 20th Ed, American public Health Association, New York, USA.

APHA. (2005). Standard methods for the examination of water and waste water. 21st edn. APHA, AWWA, WPCF, Washington, USA.

Azmat H., Javed M., and Jabeen G. (2012). Acute toxicity of aluminium to the fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*). *Pak. Vet. J* **32**, 85–87

Beauvais, S.L. Jones S.B. J.T Parris S.K Brewer and E. Elittle. (2001). Cholinergic and behavioral neurotoxicity of carbaryl and cadmium to larval rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicol. Environ. Saf.* **49**: 84-90.

Boran, M. Altinok, I. Capkin. E. Karacam, H. Blcer V. (2007). Acute toxicity of carbaryl, methiocarb and carbosulfan to the rainbow trout (*Oncorhinchus myskiss*) and guppy (*Poecilia reticulata*). *Turk. J Vet Anim Sci*, **31**, 39-45

Brewer, S.K., Little, E.E., DeLonay, A.J., Beauvais, S.L., Jones, S.B. and Ellersieck, M.R, (2001). Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorhynchus mykiss*) exposed to cholinesterase-inhibiting chemicals. *Arch. Environ. Contam. Toxicol*. **40**, 70-76

Caquet, T., M. Roucaute, N. Mazzella, F. Delmas, C. Madigou, E. Farcy, T. Burgeot, J.P. Allenou and R. Gabellec (2013) Risk assessment of herbicides and booster biocides along estuarine continuums in the Bay of Vilaine area (Brittany, France). *Environ Sci Pollut Res Int*, **20**, 651-66

Darvesh, S. Darvesh, K.V., R.S. McDonald, D. Mataija, R. Walsh, S. Mothana, O. Lockridge and E. Martin (2008). Carbamates with differential mechanism of inhibition toward acetylcholinesterase and butyrylcholinesterase. *J. Med. Chem*, **51**, 4200-4212.

David, M. Mushier S.B. and Philip. G.H. (2003). Alterations in the levels in tissues of fresh water fish *Labeo rohitha* exposed to Fenvalerate. *Polin. Res*, **22**, 359-369.

De Mel GWJLMVTM and Pathiratne. A. (2005). Toxicity assessment of insecticides commonly used in rice pest management to the fry of common carp, *Cyprinus carpio*, a food fish culturable in rice fields. *Journal of Applied Ichthyology*, **21**, 146–150.

Drummond R.A, C.L. Russom, Diannel Geiger and David L Defoe.(1986). Behavioural and morphological changes in freshwater minnow *Pimephales promelas*. As diagnostic end point for screening chemicals according to mode of action. *Aqua. Toxicol*, **9**, 415-434.

Ebrahimpour, M M Alipur, and S. Rakhshah. (2010). Influence of water hardness on acute toxicity of copper and zinc on fish. *Toxicol. Ind. Health* **6**, 361–365.

Falfushynska H. (2013). In situ exposure history modulates the molecular responses to carbamate fungicide Tattoo in bivalve mollusk. *Ecotoxicology*. **22**, 433-445

Finney. D.J. (1971). Probit analysis 3ed, Cambridge University Press.

Fukuto. T.R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Environ Health Perspect.*, **87**, 245–254.

Gracia de Liasera M.P. and Bernal-Gonzalez. M. (2001). Presence of carbamate pesticides in environmental waters from the north west of Mexico: Determination by Liquid chromatography. *Water. Res* **35**, 1933-1940.

Guillet, P. Guessan, R N. Darriet F. Traore-Lamizana M. Chanre, F.P Carnevale. (2001). Combined pyrethroid and carbamate two-in-one treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*, *Med. Vet. Entomol*, **15**, 102–112

Hodgson. E. (2010). A textbook of modern toxicology, 4th ed. pp. 230-235.

Howard. P.H. (1991). Handbook of Environmental fate and exposure data for organic chemicals: pesticides. Lewis Publishers, Chelsea.

Jarrad, H.E. Delaney K.R. and C.J. Kennedy. (2004). Impact of carbamate pesticides on olfactory neurophysiology and cholinesterase activity in coho salmon (*Oncorhynchus kisutch*). *Aquatic Toxicol*, **69**, 133-148.

Javed M and M.A. Saeed, (2010). Growth and bioaccumulation of Iron in the body organs of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during chronic exposures. *Int. J. Agric. Biol.*, **12**, 881–886

Javed, M. (2012).. Effects of zinc and lead toxicity on the growth and their bioaccumulation in fish. *Pak. Vet. J*, **32**, 357–362

Javed, M., (2012). Tissue-specific bio-accumulation of metals in fish during chronic waterborne and dietary exposures. *Pak. Vet. J.*, **32**, 567–570

Jayantha Rao, K, Azhar Baig and K Ramamurthy (1984). Effects of a systemic pesticide phosphonidon some aspects of freshwater fish *Tilapia mossambica*. *Ind. J. Environ. Hlth.* **26**:

60-64.

Koesoemadinata. S. (1980). Pesticides as a major constraint to integrated agriculture-aquaculture systems. In: Integrated agriculture-aquaculture farming system. R.S.V. Pullin and Z. Shehadeh (Eds). ICLARM conf. Pro. 4, Languana, Philippines, p.167-174.

Kreisler E., and Heiss. R. (2008). Managing expired pesticides as hazardous waste across borders. The Eighth International Conference on Environmental Compliance and Enforcement, Washington, DC.

Modra H, Svobodova Z. (2009). Incidence of animal poisoning cases in the Czech Republic: current situation. *Interdisc Toxicol.*, **2**, 48–51.

OECD, (1992). (Organization for Economic Cooperation and Development): Guideline for the testing of chemicals: Fish, acute toxicity test, 203.

Prasanth, M.S., David. M. and Mathed S.G.(2005). Behavioral changes in freshwater fish *Ctenopharyngodon idellus* (Hamilton) exposed to cypermethrin. *J. Ecotoxicol. Environ. Monit.* **26**, 141-144

Rath N.C. Rasaputra K S. (2011). Dithiocarbamate toxicity-an appraisal In: Stoytcheva, M. (Ed), Pesticides in the modern world effects of pesticides exposures. In Tech Open Acess Publisher, pp-323-340.

Renzi B.E. and R.I. Krieger. (1986).Sublethal acute toxicity of carbosulfan [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-n-butylaminosulfenyl) (methyl) carbamate] in the rat after intravenous and oral exposure. *Fundam. Appl. Toxicol*, **6**, 7–15.

Sarbani Giri, Anirudha Giri, Gouri Dutt Sharma and Surya Bali Prasad. (2003). Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice in vivo *Mutagenesis*, **18**, 53–58.

Scott, G.R. and Sloman. K.A. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicol*, **68**, 369-392.

Shantha Kumar M and Balaji.M. (2000). Acute toxicity of an organophosphorus insecticide monocrotophos and its effects on behavior of an air breathing fish, Anabas testudineus (Bloch). *J. Envioron. Biol*, **21(2)**, 121-123.

Surendra, KY. (2010). Pesticide applications - threat to ecosystems. *J. Hum. Ecol.*, **32**, 37-45.

Tarig M.I., Afzal S., Shahzad. F. (2010). Fate of carbosulfan and monocrotophos in sandy loam soils of Pakistan under field conditions at different watertable depths. *J. Environ. Monit*, **12**, 1119-1125.

Tomlin. C.D.S .(2003).The pesticide manual. 13th ed., British Crop Protection Council, London,

Topakata S. and E. Rencüzo˘gulla. (1993). Chromosomal aberrations in human lymphocytes treated with Marshal and its active ingredient carbosulfan. *Mutat. Res.*, **319**, 103–111.

Topaktas, M. Rencu, E Zogullari and HB Ila. (1996). *In vivo* chromosomal aberrations in bone marrow cells of rats with Marshal. *Mutat. Res* ., **371**, 259–264.

Venkata Rathnamma, V. Vijay Kumar M. and Philip G.H. (2008). Acute toxicity and behavioral changes in freshwater fish *Labeo rohita* exposed to Deltamethrin. *J.Aqua Biol* **23**, 165-170.

Verma, R.S., Khan, M.A., R. Tripathi, S. Shukla, and Sharma. (2005). Heavy metal toxicity to fresh water prawn, *Macrobrachium dayanum* (Crustacea-Decapoda). *Aquaculture*. **6,** 57-62.

Vlcek V. and Pohanka. M. (2012). Carbamate insecticides in the Czech Republic: health and environmental impacts. *Mil. Med. Sci. Lett. (Voj. Zdrav. Listy)*, **81(1)**, 2-8.

Water quality, (1996). Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton – Buchana (Teleostei, Cyprinidae)] – Part 2: Semi-static method. International Standard ISO; 7346-2.

Weyman, M. (2007). How to prevent pesticide related fish kills. In. Bulletin 10. Clemson University, Department of Pesticide Regulation, Pendleton.

Yi, MQ . Liu, HX, Shi, XY. P Liang and XW Gao. (2006). Inhibitory effects of four carbamate insecticides on acetylcholinesterase of male and female *Carassius auratus* in vitro. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **143(1)**, 113-116.