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Research Article

STUDIES ON TOXICITY STRESS, BEHAVIOURAL ALTERATIONS AND BIOCHEMICAL CHANGES INDUCED BY GLYPHOSATE HERBICIDE ON THE FRESH WATER FISH *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT
Herbicide performs a vital role in the management of weeds. As the name indicates, herbicides are chemicals that kill or control vegetation. The indiscriminate use of herbicide i.e. "Glyphosate" careless handling accidental spillage or discharge of untreated effluents into aquatic environment have harmful impact on the fish population and other aquatic organisms and may contribute to long term eco toxicological effects to resident aquatic organisms. The aim of the present studies therefore, was to determine the acute toxicity of glyphosate (Excel Mera 71) herbicide and its impact on the toxicity stress, behavioural alterations and biochemical changes on fresh water fish *channa punctatus*. The bioassay studied after 24h or 96h exposure with 40% and 80% of LC_{50} of 24h. The freshwater fish *Channa punctatus* is an important fish of Indian capture fishery. The LC values (LC_{10}, LC_{50}, LC_{90}) was dose as well as time dependent. Alteration in biochemical profile (Total protein, amino acid, glycogen, and nucleic acid) of fish was also time & dose dependent. On the basis of present study we should avoid extensive use of this herbicide in near water bodies.

Keywords: *Channa Punctatus, Herbicides, Toxicity, Glyphosate,* LC_{50}*, Biochemical Parameters*

INTRODUCTION
Herbicides are important part of the agricultural field. It is specifically made for weed control, hence, it is also known as weed killers, and it is composed of a heterogonous family of chemical product. Herbicide can kill the plants, by affecting their biochemical mechanisms involved in photosynthesis, respiration, growth, cell and nuclear division or synthesis of protein carotenoides or lipids (Ecobichon, 1991). They can purposely direct or indirect reach aquatic ecosystem through soil surface runs off or from area where they are applied. Due to extensive usage of pesticide in agricultural field, the exposed non-target organisms are in a source of concern. Therefore, it seems essential to study the lethal toxicity and stress of such environmental pollutants on *Channa punctatus* so as to formulate the strategies for safe guarding aquatic organisms.

Fishes being the first level of consumers in the aquatic ecosystem, are widely accepted as bio indicator to study the health of an aquatic ecosystem as they are directly or indirectly exposed to chemicals resulting from agricultural production via surface run-off or through food chains of ecosystem. Accumulations of toxic chemical pollutant are known to adversely affect the liver, kidney, muscles and other tissues of fish. The various workers have reported on the effects of herbicides on aquatic organisms such as *Rainbow trout, Chinook and salmon* (Mitchell *et al.,* 1987), *Salmon, daphnia and trout* (Serivizi *et al.,* 1987), *Cyprinus carpio* (Neskovic *et al.,* 1996), and Nile tilapia *(Oreochromis niloticus)* (Jiraungkoorskul *et al.*, 2002).

Glyphosate (N-Phosphonomethyl glycine) is a broad spectrum, translocated herbicide, used primarily in agricultural field for the control of a great variety of annual, biennial, and perennial grasses, sedges, broad leaved weeds and woody shrubs. It is also used for aquatic weeds control in fish ponds, lakes, canals and slow running water (Tsui and Chu, 2008). Glyphosate act by the inhibiting the enzymes EPSPS, which block the aromatic amino acid biosynthesis via. Shikimate pathway (Steinruicken and Amrhein, 1980). In India there are some well known glyphosate formulations which are used widely and it is known as Glycel, Excel Mera 71, and Veggru glyphosate.

Bioaccumulations of these pesticides threat the long – term survival of fishes by disrupting the ecological relationships between organisms and loss of biodiversity. Long – term exposure of pesticides induces physiological disturbance, behavioural alteration, histological damages, haematological alteration, biochemical changes, immune-suppression, hormone disruption, diminished intelligence, reproductive abnormalities of aquatic organisms such as *Channa punctatus* (Bloch) (Pandey *et al.,* 2014), *Labeo rohita* (Mishra *et al.,* 2008), and *Tor putitora* (Ullah *et al.,* 2014).

Toxicity varies with species and age of the fish, their nutritional status (more toxic to hungry fish), and the temperature, pH, hardness of water (toxicity increases with temperature and pH), young fish are particularly vulnerable when water temperature increase in spring and summer and the glyphosate causes water temperature increases several years after herbicidal treatment hence could increase the toxicity of fish and aquatic invertebrates (Folmar *et al.,* 1979) and changes in the temperature regime of a valley (Holtby, 1989).

The sub-lethal doses also caused damage and change in the liver and kidney structure in Nile tilapia, *Oreochromis nitoticus* (Jiraungkoorskul *et al.,* 2003). The aim of this study was to evaluate the toxicity stress, behavioural alterations and biochemical effect of glyphosate on the level of total protein, total free amino acid, nucleic acid (RNA and DNA) and glycogen in the liver and muscle tissue of fresh water fish *Channa punctatus* (Bloch).

MATERIALS AND METHODS

Collection of Experimental Animals: The experimental species selected for present investigation is fresh water air breathing fish *Channa punctatus* (Bloch; Family: Channidae, Order: Perciformes) were collected from local water bodies of Gorakhpur, U.P. India. The specimens had an average weight and length of 160 to 200 gm and 10 to 15 cm, respectively.

Fish are belonging to both the sexes were used. Fish were stored in 45 litre capacity of glass aquaria containing de-chlorinated tap water for acclimatization under laboratory conditions for 3 weeks and fed with commercial fish food.

The dead animals (if any) were removed as soon as possible to avoid water fouling and contamination. The physico-chemical parameter of test water is (temperature = 22-25^0 C, pH= 7.2-7.4, alkalinity = 130-150 mg /L $CACO_3$, DO = 6.5-7.3 mg/L, free carbon dioxide = 4.4- 6.4 mg/L.) measured in the beginning of experiment by the standard method of (APHA/AWWA/WPCF, 2005).

Herbicide Used

Glyphosate is the isopropyl amine salt of N-(Phosphonomethyl) –glycine, glyphosate is a very effective non-selective herbicides and it is only herbicide that acts by blocking the Shikimate pathway through inhibition of S-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). Molecular Structure: (C3H8NO5P), Molecular weight = 169.09, Chemical group – Phosphinic acid, Chemical name – (N-Phosphonomethyl glycine).

Toxicity Test

Toxicity test had performed by the method of (Singh and Agarwal, 1988). Twenty experimental fish *Channa punctatus* were kept in glass aquaria containing 6 litre de-chlorinated tap water. Fish were exposed for 24h, 48h, 72h and 96h at four different concentrations of glyphosate "Excel Mera 71" herbicide in laboratory. Control fishes kept in similar conditions without any treatment. Each group of fish replicated six times. Mortality and Behaviour was recorded after every 24 hrs up to 96 hrs exposure periods.

Dead animals were removed to prevent the decomposition of body in experimental aquarium. The effective dose (LC value, upper and lower confidence limits, slope value and heterogeneity) calculated through POLO computer program of Robertson *et al.,* (2007). Product moment co-relation co-efficient was applied in between exposure time and lethal concentration (Sokal *et al.*, 1973).

Biochemical Analysis

Fishes exposed to 40% and 80% of LC_{50} of 24h. Experiment conducted from 24h or 96h. After completion of treatment the test fishes removed and washed with water and killed by severe blow on head and operated their liver and muscle quickly dissected out in ice tray and used for biochemical analysis. Control fishes kept in similar condition without any treatment. Each experiment replicated at least 6 times and values expressed as mean ± SE of six replicates. Following parameters estimated by different method.

Protein

Protein levels were estimated according to Lowry *et al.*, (1951) using bovine serum albumin as standard. Homogenates (50 mg/ml, w/v) were prepared in 10% TCA. Tissues homogenized for 5 minutes using an electric tissue homogenizer, centrifuged at 6000 g for 20 minutes. Values have been express as µg protein/mg of tissue.

Total Free Amino Acid

Estimation of total free amino acid on the basis of Spice, (1957) method. Homogenates (10 mg/ml, w/v) were prepared in 96% ethanol in an electric tissue homogenizer for 5 minutes and centrifuged at 8000 g for 20 minutes and supernatant was used for amino acid estimation. Free amino acids have been express as µg/mg of tissue.

Nucleic Acid

Estimation of nucleic acid (DNA and RNA) was performed, by the methods of Schneider, (1957) using diphenylamine and Orcinol reagents, respectively.

Homogenates (100 mg/ml, w/v) were preparing in 5% TCA at 90^{O} C by electric homogeniser for 5 minutes, and centrifuged at 5000 g for 20 minutes and supernatant was use for estimation. Both DNA and RNA have been express as µg/mg tissue.

Glycogen

Glycogen was estimated by the Anthrone method of (Van der Vies, 1954). Homogenates (100 mg/ml, w/v) were prepared in cold 5% TCA, and 1.0 ml of filtrate was use for assay. Result has been express as mg glycogen/g of tissue.

Statistical Method

Each experiment was replicate at least six times and data has expressed as mean ±SE. Student’s’-test was applied for locating significant differences (Sokal and Rohlf, 1973).

RESULTS AND DISCUSSION

Toxicity and Behavioural Response

Fish exposed to the different concentration of “Excel Mera 71” (Ammonium salt of Glyphosate 71% S.G., “Systemic Herbicide”) show uncoordinated behaviour. After fifteen minutes of treatment all the experimented fishes were alert, stopped swimming and remained static in position in response to the sudden changes in the surrounding environment. After some time they tried to avoid the toxic water with fast swimming and jumping.

And symptoms of toxicosis observed in fish behaviour with glyphosate include lack of balance; erratic swimming, air gulping, excessive secretion of mucus, rolling movement, and the treated fish became very weak, settled at the bottom and died and the skin colour was shining that’s why the behavioural changes or any mortality and colour of the control fishes was normal.

The toxicity of gyphosate was time & dose dependent. There was a significant negative correlation between LC values and exposure periods i.e. LC values of glyphosate is decreased from 47.05 mg/L (24h) > 39.23 mg/L (48h) > 34.35 mg/L (72h) > to 30.62 mg/L (96h) in case of *channa punctatus* (Table 1).

Biochemical Estimation

Exposure of sub-lethal doses (40% and 80 % of LC_{50} of 24h) of glyphosate for after 24h or 96h against the freshwater non-target fish *Channa punctatus* caused significant ($P<0.05$) alteration in biochemical parameters in liver and muscle tissues of this fishes (Figure 1 & Table 2). Total protein level was reduced to 85% and 88% of controls in liver and muscle tissues, respectively, after exposure to 40% of LC_{50} (24h) of glyphosate. The maximum decrease in protein level (80%) was observed in fish treated with 80% of LC_{50} (24h) of glyphosate. Same trend was observed in case of nucleic acid (RNA) also reduced of controls in liver and muscle tissues of the fish *C. punctatus* (Figure 1). Total free amino acid levels were induced to 120% and 115% of controls after treatment with 40% of LC_{50} (24h) of glyphosate in liver &

muscle tissues of fish respectively. The maximum increase in total free amino acid level (127% of control) was observed in fish treated with 80% of LC_{50} (24h) of glyphosate. Same trend was observed in case of nucleic acid (DNA) also induced of controls in liver and muscle tissues of fish after treated with (40% & 80% of 24h) glyphosate (Figure 1). Glycogen was also reduced to 87% and 82% of controls in liver and muscles tissues, respectively, after exposure to 40% of LC_{50} (24h) of glyphosate. The maximum decrease in glycogen level (78% of control) was observed in fish treated with 80% of LC_{50} (24h) of glyphosate. Likewise, the exposure of 40% of LC_{50} for 96h of 'glyphosate' herbicide, the level of total protein, total free amino acid, glycogen, nucleic acid (RNA & DNA) was observed at 40% and 80% of LC_{50} shows in (Table 2).

Table 1: Toxicity (LC Values) of Different Concentrations of Glyphosate "Excel Mera 71" Herbicide against Fresh Water Fish *Channa Punctatus* at 24h to 96h Exposure Period

Exposure Period (Hours)	Effective Dose (mg/L)	Limits (mg/L)		Slope Value	't' Ratio	Heterogeneity
		LCL	UCL			
24	LC_{10}=23.73	8.43	31.02			
	LC_{50}=47.05	39.82	59.85	4.312±1.357	3.178	0.126
	LC_{90}=93.29	68.47	316.98			
48	LC_{10}=20.73	7.62	27.70			
	LC_{50}=39.23	30.97	45.50	4.627±1.352	3.422	0.019
	LC_{90}=74.24	58.90	159.73			
72	LC_{10}=19.23	7.43	25.80			
	LC_{50}=34.35	25.41	39.51	5.089±1.421	3.581	0.065
	LC_{90}=61.34	51.25	102.61			
96	LC_{10}=18.03	6.70	24.38			
	LC_{50}=30.62	21.00	35.54	5.572±1.564	3.562	0.134
	LC_{90}=52.00	44.75	76.23			

- Batches of twenty fishes were exposed to four different concentrations of Glyphosate "Excel Mera 71" herbicide.
- Concentrations given are the final concentration (w/v) in glass aquarium containing de-chlorinated tap water. Each set of experiment was replicates six times.
- Mortality was recorded after every 24h.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL: Lower confidence limit; UCL: Upper confidence limit.
- There was no mortality recorded in the control group.

Glyphosate is highly toxic to various aquatic organisms. It is extensively used chemical in agricultural field. During the unsafe spraying and improper handling, accumulation in the water holding areas was mixed with river, pond, etc. and polluted the areas. Fish are particularly very sensitive to water contamination. Hence, pollutants such as insecticides, herbicides may significantly affect some physiological and biochemical processes when they enter into the organs of fishes *Oreochromis mossambicus* (Mathivanan, 2004), and *Hetropneustes fossilis* (Shrivastava and Singh, 2004). The result shows that the toxicity and behavioural alterations of glyphosate for *C. punctatus* is both time and concentration dependent, thus, accounting for differences in $LC_{10\text{-}90}$ value obtained at different concentration and time exposure and the significant behavioural changes is (Loss of equilibrium, erratic swimming, air gulping, excessive secretion of mucus, rolling movement, etc.). Bioaccumulations of atrazine in different organs of fish have been noted and thus we concluded that environmental pollutants can enter fish bodies through the gill and skin (Ortiz *et al.*, 2002). Behavioural changes are the most sensitive indicators of potential toxic effect in fishes and the changes like erratic swimming, interruption in schooling behaviour, restlessness and surfacing, as observed in present study may be an avoiding reaction to the herbicide present in medium. Loss of balance during swimming might be due to some neurological impairment in central nervous system as evident by inhibition of AchE by pollutants in fishes such as *Procambarus clarkii* affected by mercury, cadmium and lead (Devi and Fingerman, 1995), *Oreochromis mossambicu* (Patro, 2006) and *Clarias batrachus* (Srivastava and Singh, 2013d). The observed behavioural alterations in the studied formulation of glyphosate (Excel Mera 71) are consistent with previous reports on glyphosate – based herbicides on Nile tilapia (*Oreochromis niloticus*) (Ayoola, 2008), *Proechilodus lineatus* (Langiano and Martinez, 2008), Gold fish (Lushchak *et al.,* 2009), and Neotropical fish "*Piaractus mesopotamicus"* (Shiogiri *et al.,* 2012). Behaviour provides a unique perspective linking between physiological and ecological of an organism i.e., marine and Freshwater Teleost (Little and Brewer, 2001) and *Clarias batrachus* (Srivastava and Singh, 2013d).

Fish liver carries out essential body functions, including regulation of metabolism and detoxification of toxic compounds. Secondary metabolites of pesticides induces sever biochemical and enzymatic changes in aquatic organisms such as *Hetropneustes fossilis* (Rawat *et al.,* 2002) and *Colisa fasciatus* (Tiwari and Singh, 2009).

During stress, fish need more energy to detoxify toxicants and to overcome stress. Proteins are the building blocks of animal's body, and it is most fundamental biochemical substance to maintain the blood glucose and energy source during the stress period. Proteins play a major role in the interaction process of the cellular medium in the organism like *Channa punctatus* (Magar and Shaikh, 2012). The decrease in total protein level and increase in free amino acid level in both tissue and liver suggest the protein hydrolytic activity due to elevation of protease activity in fish *Labeo rohita* (Muley *et al.,* 2007). Hence, protein reduction might observe due to high – energy demand in TCA cycle. Decrease in protein content under toxicity stress has already been reported by several workers on the different fishes like Cyprinus carpio (Choudhary and Gaur, 2001), freshwater snail *Lymnaea acuminate* (Tripathi and Singh, 2003), *Anabas testudineus* (Kumar *et al.,* 2004), *Labeo rohita* (Muley *et al.,* 2007) and *Colisa fasciatus* (Tiwari and Singh, 2009).

Decrease in total protein content in the tissues of shrimp *Streptocephalus dichotomus* on exposure to sub-lethal concentration of Malathion and glyphosate (Arun Kumar and Jawahar, 2013).

Excel Mera 71 herbicide in tissue degrade into different types of amino acids based metabolites so it incorporate with several polypeptide chains and altered its fate. The quantity of protein may also be affected due to impaired incorporation of amino acid in the polypeptide chains (Singh *et al.,* 1996).

Total Free amino acid content is highest in the liver as it is the chief organ of free amino acid synthesis, least in the muscle as they are less involved in protein metabolism (Figure 1 & Table 2). This rapid increase in FAA levels is probably attributed to step up proteolysis or increased synthesis of free amino acid by transaminases reaction (James *et al.,* 1979), *Channa striatus* (Natarajan, 1983).

Glycogen is a sub divisional polysaccharide and major storage of Glucose that serve as a form of energy in animals. It plays an important role in the glucose cycle. The glycogen content in both tissues of *Channa punctatus* was decreased with increased toxicant concentration in the present experiment. Muscle and Liver tissues are a major glycogen parts in animals, which contains full of carbohydrates. It is found in the liver (10%) and skeletal muscles (1-2%).

Liver glycogen level was depleted during hypoxia or physical disturbances in fish (Heath and Fritechard, 1965).

Depletion of glycogen may be due to direct utilization of the compound for energy generation, a demand caused by pesticide-induced hypoxia.

Under hypoxia condition, the fish derives its energy from anaerobic breakdown of glucose which is available to the cells.

Under stress conditions the secretion of high amount of catecholamine depletes glycogen reserves (Pickering, 1981). Propiconazole treatment altered carbohydrate metabolism with the levels of a number of oligosaccharides reduced in a dose-response manner such as maltotriose and maltose level (Nesnow *et al.,* 2011).

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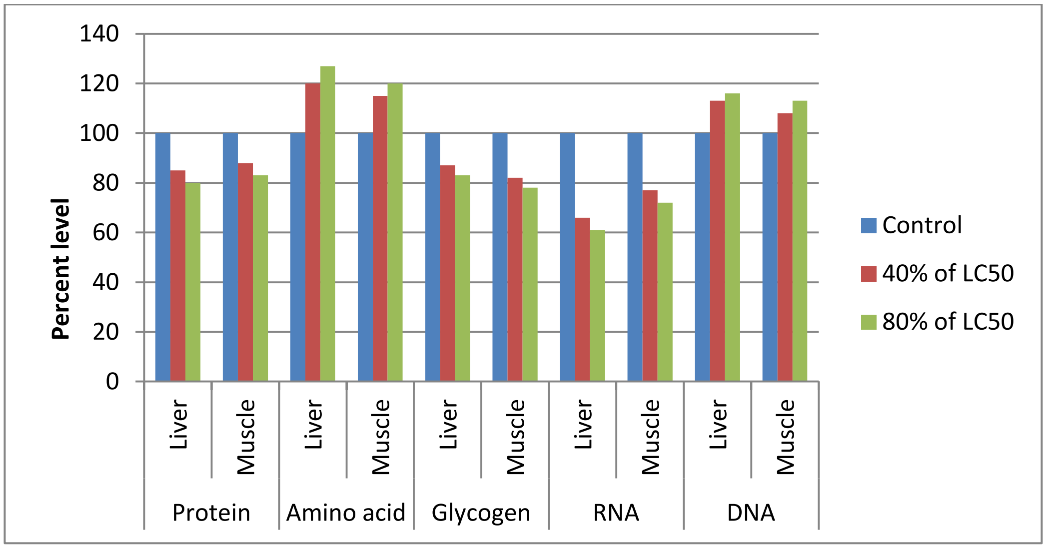

Figure 1: Total Protein (µg/mg), Total Free Amino Acid (µg/mg), Glycogen (mg/g) and Nucleic Acids (µg/mg) Level in Different Tissues of Fresh Water Fish *C. Punctatus* after Exposure to 40% and 80 % of LC_{50} 24h of Glyphosate

Table 2: Total Protein (µg/mg), Total Free Amino Acid (µg/mg), Glycogen (mg/g) and Nucleic Acids (µg/mg) Level in Different Tissues of Fresh Water Fish *C. Punctatus* after Exposure to 40% and 80 % of LC_{50} 24h of Glyphosate after 96h

Parameter	Tissue	Control	40% of LC_{50}	80% of LC_{50}
Protein (µg/mg)	Liver	0.760±0.0007 (100)	0.601±0.0004* (79)	0.581±0.0008* (76)
	Muscle	0.805±0.0007 (100)	0.676±0.0006* (84)	0.633±0.0004* (79)
Amino Acid (µg/mg)	Liver	0.183±0.0003 (100)	0.237±0.0004* (129)	0.243±0.0004* (133)
	Muscle	0.161±0.0004 (100)	0.199±0.0006* (124)	0.207±0.0003* (128)
Glycogen (mg/g)	Liver	0.402±0.0002 (100)	0.341±0.0004* (85)	0.321±0.0005* (80)
	Muscle	0.393±0.0003 (100)	0.306±0.0008* (78)	0.297±0.0003* (75)
RNA (µg/mg)	Liver	0.426±0.0003 (100)	0.254±0.0004* (60)	0.234±0.0004* (55)
	Muscle	0.385±0.0004 (100)	0.284±0.0003* (74)	0.266±0.0004* (69)
DNA (µg/mg)	Liver	0.304±0.0005 (100)	0.367±0.0004* (121)	0.379±0.0004* (125)
	Muscle	0.355±0.0005 (100)	0.416±0.0004* (117)	0.432±0.0007* (122)

- Values are mean ±SE of six replicates.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- *Significance (P<0.05), when student s' test were applied between control and treated groups.

Pesticide toxicity indicates alteration in nucleic acid synthesis. Impairment of nucleic acid metabolism the degradation of cells, resulting in the DNA content. Furthermore, inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery.

In present studies (Figure 1 & Table 2) show the result of glyphosate effect on nucleic acid i.e. reduced the RNA content but induced the DNA content in both tissues of fish. In present study, the maximum level of DNA was found in both tissues which is supported by earlier findings of Holbrooks, (1980) that, the thymine incorporation into hepatic DNA has markedly increased after 1 to 3 of days of administration of the various toxicants.

The increase of DNA contents in both liver and muscle tissues in the present study was due to hypertrophic nature of chloride cells, secreting cell leading to less transcription & enlargement of nuclei in *Channa punctatus* exposed to glyphosate. *Lepidocephalicthys thermalis* exposed to cypermethrin, endosulfan and pyrethroids also showed a decline in the RNA content of the liver and muscle tissues (Sheela and Muniandi, 1992) *Clarias batrachus* (Tripathi and Verma, 2004a). The synthesis of RNA plays an important role in protein synthesis.

The inhibition of RNA synthesis at transcription level may affect the protein contents (Singh *et al.*, 2010). The development and growth of the fishes upon the DNA & RNA which serve as biochemical indices (Nordenskjold *et al.*, 1979).

Conclusion

Our studies showed that the Glyphosate based herbicide (Excel Mera 71) which is widely used in agricultural fields have potential to damage aquatic organism. It is highly toxic for fish and it caused the severe toxicity stress, behavioural changes and different biochemical parameter of freshwater fish *Channa punctatus* (Bloch). Therefore, we should avoid extensive use of this herbicide in near water bodies.

REFERENCES

APHA/AWWA/WEF (1998). *Standard Methods for the Examination of Water and Waste Water*, 20th edition, (American Public Health Association, New York, USA).

Arun Kumar MS and Jawahar Ali A (2013). Toxic impact of two organophosphorus pesticides on acetyl cholinesterase activity and biochemical composition of freshwater fairy shrimp *Streptocephalusdichotomous. International Journal of Pharma and Bio Sciences* **2(4)** (B/P) 966-972.

Ayoola SO (2008). Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. *African Journal of Agricultural Research* **3**(12) 825-834.

Choudhary A and Gaur S (2001). Effect of sodium fluoride on the muscle and liver of a freshwater fish *Cyprinus carpio*. *Journal of Aquatic Biology* **16**(2) 67-68.

Devi M and Fingerman M (1995). Inhibition of acetyl cholinesterase activity in the central nervous system of the red swamp crayfish, *Procambarus clarkii*, by mercury, cadmium and lead. *Bulletin of Environmental Contamination and Toxicology* **55** 746-750.

Ecobichon DJ (1991). Toxic effects of Pesticides. In M.O. Amdur, et al., eds. *Casarett and Doull's Toxicology- The Basic Science of Poisons,* 4th edition (Mc Graw – Hill, Inc., New York, USA) PP. 565-622.

Folmar LC, Sanders HO and Julin AM (1979). Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology* **8** 269-78.

Heath AG and Fritechard AW (1965). Effect of severe hypoxia on carbohydrate energy. Stores and metabolism in two species of fresh water fish. *Physiological Zoology* **38** 325-334.

Holbrook Jr DJ (1980). Effect of toxicant on nucleic acid and protein metabolism. In: *Introduction to Biochemical Toxicology* edition: Hodgson, E and Guthrie, (F. E. Blackwell Scientific Publications, Oxford, UK) 261-284.

Holtby LB (1989). Changes in the temperature regime of a valley – bottom tributary of Carnation Greek, British – Columbia, Over - sprayed with the herbicide, Roundup (glyphosate). In Reynolds, P.E. (edition), *Proceedings of the Carnation Greek Herbicide Workshop*, (Sault Ste. Marie, Ontario, USA, Canada: Forest Pest Management Institute).

James JH, Ziparo V, Jeppsson B and Fischer JE (1979). Hyper ammonemia, plasma amino acid imbalance and blood brain amino acid transport: A unified theory of portal systemic encephalopathy. *Lancet* **2** 772-775.

Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri- Grams S and Pokethitiyook P (2002). Histopathological of Roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*). *Science Asia* **28** 121-127.

Jiraungkoorskul W, Upathan ES, Kruatrachue M, Sahaphong S, Vichasri - Grams S and Pokethitiyook P (2003). Biochemical and histopathocal effect of glyphosate herbicide on Nile Tilapia *Oreachronis niloticus*. *Environmental Toxicology* **18** 260-267.

Kumar K, Patri P and Pandey AK (2004). Haematological and biochemical responses in the freshwater air-breathing teleost, *Anabas testudineus* (Bloch) exposed to mercury. *Journal of Ecophysiology and Occupational Health* **4** 97-108.

Langiano VC and Martinez CBR (2008). Toxicity and effects of glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology* **147** 222-231.

Little E and Brewer SK (2001). Neurobehavioral toxicity in fish. In: *Target Organ Toxicity in Marine and Freshwater Teleosts New Perspective: Toxicology and the Environment*, **2** (edition, Schlenk D and Benson, W H), 139-174. (Taylor and Francis, London & New York, UK & USA).

Lowry OH, Rosenbrough NJ, Farr AL and Randell RJ (1951). Protein measurement with foline phenol reagent. *Journal of Biological Chemistry* **193** 265-275.

Lushchak O, Kubark O, Storey JM, Storey KB and Lushchak I (2009). Low toxic herbicide Roundup induces mild oxidative stress in gold fish tissue. *Chemosphere* **76** 932-937.

Magar RS and Shaikh A (2012). Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sub lethal treatment with the insecticide malathion. *Trends in Life Science* **1**(3) 2319–4731.

Mathivanan R (2004). Effects of sub lethal concentration of quinalphoson selected respiratory and biochemical parameters in the freshwater fish, *Oreochromis mossambicus*. *Journal of Ecotoxicology and Environmental Monitoring* **14** 57-64.

Mishra DK, Bohidar K and Pandey AK (2008). Effect of sub lethal exposure of cartap on hypothalamo-neurosecretory system of the freshwater teleost, *Channa punctatus* (Bloch). *Journal of Environmental Biology* **29** 917-922.

Mitchell DG, Chapman PM and Long TL (1987). Acute toxicity of Roundup and Rodeo herbicides to rainbow trout, Chinook and coho salmon. *Bulletin of Environmental Contamination and Toxicology* **39** 1028-1035.

Muley DV, Karanjkar DM and Maske SV (2007). Impact of industrial effluents on the biochemical composition of freshwater fish, *Labeo rohita*. *Journal of Environmental Biology* **28** 245-249.

Natarajan GM (1983). Metasystox toxicity: Effects of Lethal LC50 / 48 hrs concentration on free amino acids and glutamate dehydrogenase in some tissues in the air breathing fish, *Channa striatus* (Bleeker). *Journal of Comparative Physiology Ecological* **8** 254-256.

Neskovie NK, Poleksic V, Elezovic I, Karan V and Budimir M (1986). Biochemical and histopathological effects of glyphosate on Carp, *Cyprinus carpio* L. *Bulletin of Environmental Contamination and Toxicology* **56** 1028-1035.

Nesnow S, Padgett WT and Moore T (2011). Propiconazole induces alterations in the hepatic metabolism of mice: relevance to Propiconazole-Induced hepatocarcino-genesis. *Toxicology Science* **120** 297-309.

Nordenskjold M, Soderhall J and Moldeus P (1979). Studies on DNA strand break induced in human fibroblast by chemical mutagens and carcinogens. *Mutation Research* **63** 393-400.

Ortiz JB, Gonzalez ML and Sarasquete C (2002). Histological alterations in different tissues of fish under the impact of persistent chemical pollution. *Ecotoxicology and Environmental Restoration* **54** 45.

Pandey AK, Mishra DK and Bohidar K (2014). Histopathological changes in gonadotrophs of *Channa punctatus* (Bloch) exposed to sub lethal concentration of carbaryl and cartap. *Journal of Experimental Zoology* **17** 451-455.

Patro L (2006). Toxicological effects of cadmium chloride on Acetyl cholinesterase activity of fresh water fish *Oreochromis mossambicus* Peters. *Asian Journal of Experimental Sciences* **20** 171-180.

Pickering AD (1981). Stress and compensation in teleostean fishes: response to social and physical factor. In: *Stress and Fish* (edition Pickering A D), 295-322. (Academic Press, New York, USA).

Rawat DK, Bais VS and Agrawal NC (2002). A correlative study on liver glycogen and endosulfan toxicity in *Heteropneustes fossilis* (Bloch). *Journal of Environmental Biology* **23** 205-207.

Robertson JL, Russel RM, Preisler HK and Saven ME (2007). *Bioassay with Arthropods*. Polo: A new computer programme, (Germany, Berlin, CRC Francis and Taylor) 1-224.

Schneider WC (1957). *Determination of Nucleic Acids in Tissue by Pentose Analysis*, (Academic Press New York, USA) 680.

Serivizi JA, Gordon RW and Martens DW (1987). Acute toxicity of Garlon 4 and Roundup herbicides to salmon, daphnia and trout. *Bulletin of Environmental Contamination and Toxicology* **39** 15-22.

Sheela M and Muniandi S (1992). Impact of cypermethrin on protein conversion efficiency, RNA, protein, glycogen content and protease enzyme activity in different tissues of the fish, *Lepidocephalichthyes thermalis*. *Journal of Environment and Ecology* **10** 829-832.

Shiogiri NS, Paulino MG, Carraschi SP, Baraldi FG, Cruz C and Fermandes MN (2012). Acute exposure to glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus*. *Environmental Toxicology and Pharmacology* **34** 388-396.

Shrivastava S and Singh S (2004). Changes in protein content in the muscle of *Heteropneustes fossilis* exposed to carbaryl. *Journal of Ecotoxicology and Environmental Monitoring* **14** 119-122.

Singh A and Agarwal RA (1988). Possibility of using latex of euphorbiales for snail control. *Science of the Total Environment* **77** 231-236.

Singh A, Singh DK and Agrawal RA (1996). Molluscicides of plant origin. *Biological Agriculture and Horticulture* **13** 205-252.

Singh SK, Singh SK and Yadav RP (2010). Toxicological and biochemical alterations of Cypermethrin (Synthetic pyrethroids) against freshwater teleost fish *Colisa fasciatus* at different season. *World Journal of Zoology* **5**(1) 25-32.

Sokal RR and Rohlf FJ (1973). *Introduction to Biostatistics*, (USA, San Francisco, W.H. Freeman and Company) 368.

Spies JR (1957). Colorimetric procedures for amino acids. In: Calowick, S.P., Kaplon, N.O. (Eds.). *Methods of Enzymology*, (Academic Press, New York, USA) 468.

Srivastava P and Singh A (2013d). Study on some neural and behavioural changes induced by carbamate (Mancozeb) fungicide on fresh water fish, *Clarias batrachus*. *World Journal of Zoology*, **8** 376-380.

Steinrucken HC and Amrhein N (1980). The herbicide glyphosate is a potent inhibitor of 5-enplpyruvylshikimic acid-3-phosphate synthase. *Biochemical and Biophysical Research Communications* **94** 1207-1212.

Tiwari S and Singh A (2009). Changes in some biochemical parameters in the liver and muscle of *Colisa fasciatus* due to toxicity of ethanolic extract of *Narium indicum* (Lal Kaner) latex. *Journal of Natural Product Radiance* **8** 48-54.

Tripathi G and Verma P (2004a). Endosulfan mediated biochemical changes in the freshwater fish, *Clarias batrachus*. *Journal of Environmental Sciences* **17**(1) 47-56.

Tripathi PK and Singh A (2003). Toxic effects of dimethoate and carbaryl pesticides on protein metabolism of the freshwater snail *Lymnaea acuminate*. *Bulletin of Environmental Contamination and Toxicology* **70** 146-152.

Tsui MT and Chu LM (2008). Environmental fats and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland. *Chemosphere* **71** 439-446.

Ullah R, Zuberi A, Ullah S, Ullah I and Dawar FU (2014). Cypermethrin induced behavioural and biochemical changes in mahseer, *Tor putitora. Journal of Toxicolgical Sciences* **39** 829-836
Van der Vies J (1954). Two methods for determination of glycogen in liver. *Biochemical Journal* **57** 410-446.