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RESEARCH ARTICLE

TOXICITY EVALUATION AND PROTEIN LEVELS OF FISH *LABEO ROHITA* EXPOSED TO PYRACLOSTROBIN 20%WG (CARBAMATE)

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Abstract

Pollution the water by harmful chemicals can leads to entry of pollutants into food chain. Water bodies play a major role in assimilating or carrying of industrial and municipal waste water, runoff from agricultural fields, roadways and street which are responsible for water pollution. In the present study influenced the amount of carbamate Pyraclostrobin (20%) pesticide on freshwater fish *Labeo rohita*. The fish were acclimatized to laboratory conditions for 7-10 days and then exposed to different lethal concentrations of pyraclostrobin (1.0, 1.1, 1.2 and 1.3µg/L) for 96hr along with the controls. Water parameters were also taken during the experiment. The 96hr LC_{50} of pyraclostrobin was found in 1.4µg/l and the sublethal was taken as $1/10^{th}$ of the lethal concentration. The LC_{50} values were calculated in Finney Probit method. The 96hr value of pyraclostrobin lower bond was in (1.3503) and the upper bond was in (1.4497). The effect of carbamate, fish showed uncontrolled behaviour like mucus secretion, decreased agility and inability to maintain normal position and erradic swimming were seen in exposure time of the carbamate toxicant. The fish were exposed to lethal and sublethal concentrations for 24hr, 5^{th} and 10^{th} days. After each exposure period, different tissues were taken to estimate the protein levels. Consequently, the protein activity levels were decreased in all the tissues like liver, kidney, brain, gill and muscle exposed to lethal and sublethal concentrations of pyraclostrobin when compare with the controls.
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Introduction:-

Modernization and intensification of agricultural production is generally accompanied by a rapid increase in the use of chemical fertilizers and pesticides. Pesticides are useful tools in agriculture, but the gradual degrading of aquatic ecosystem. In addition to agriculture practices together with pest control programmes the surface runoff and aerial spraying forming the major source for translocation pesticides into aquatic ecosystems (Joseph and Raj *et al.*, 2011). The improper management of pesticides in agriculture crops could result in contamination of water bodies (Candida Toni *et al*., 2011; Capkin and Altinok, 2013). When pesticide reaches the aquatic environment, it may be present there for several days or weeks, depending upon its solubility, producing of mass mortality, morphological, physiological and behavioral changes in the organisms.

Material and methods:-

Experimental profile:-

Healthy freshwater fish, *Labeo rohita* (Hamilton) size [6±7 cm total length (TL) and 6.5±7.5 g body weight] were collected from the fish farm, Kuchipudi, Guntur District of A.P, India. Then the fish was acclimatized to the laboratory conditions with sufficient dechlorinated water for 10 to15 days at room temperature 28 ± 2^{0}C. The fish were fed with fish meal, rice and commercial fish pellets once in two days, at the same time water was renewed every day rich in oxygen (aeration) and feeding was stopped one day prior to the experimentation.

The test compound for the study belonged to Carbamate pesticide Pyraclostrobin (20%WG) widely used in agriculture field. Pyraclostrobin is a broad-spectrum pesticide used to control insects, pest, and mites in agricultural field. The stock solution of the toxicant was prepared in one liter of 100% pure acetone. Concentration of Pyraclostrobin was found in 96hr 1.4µg-l as a lethal and $1/10^{th}$ was taken as a sublethal concentration along with control group was maintained for each experiment. Where maximum 10 fish were used per each concentration of the test toxicant, 10 fish were also maintained in separate container along with experimental group, served as control. The protein activity levels were estimated in modified method Lowry *et al* (1951). The fish were exposed to lethal and sublethal concentrations in different tissues like liver, kidney, brain, gill and muscle for 24hr, 5 and 10days to determine the protein levels.

Result:-

The pesticide pollution is one of the major pollution in aquatic field which effect the aquatic animals. The carbamate pyraclostrobin (20% WG) pesticide exposed for 96hr the value was found in 1.4µg/l and the sublethal was taken where as in $1/10^{th}$ of the lethal. The values were calculated in Finney probit analysis test (1971). The 96hr LC_{50} values with 95% confidence limits for pyraclostrobin upper bond (1.4497) and the lower bond (1.3503) was shown in Table.1. The percent mortality of the freshwater fish *Labeo rohita* in different concentrations was shown in (Table.2. and Fig.1.) the probit mortality of the fish in different concentrations along with log concentration was shown in (Table.3.and Fig.2) under the toxicity evaluation. Decreased swimming behavior and increased respiration rate were other effects of pesticide in the present study, found that contaminates such as pesticides disturb normal fish behavior after exposure (Scott and Solman, 2004).

The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional changes of cellular proteins. The mean value obtained for total proteins in different tissues of fish and percent change over the control along with the standard deviation were shown in Table.4. and 3. graphically represented in Figure.5 and 4. In the present study under pyraclostrobin 24hrs sublethal exposure, maximum percentage of depletion was in liver (19.14) and minimum percentage was in muscle (9.12) decreased in the series of Liver>Kidney>Brain >Gill>Muscle. Under lethal exposure of pyraclostrobin for 24hr, maximum percentage of depletion was in liver (28.84) and minimum percentage was in Gill (11.96) decreased in the series of Liver>Kidney>Brain >Muscle>Gill. Under sublethal exposure for 5^{th} and 10^{th} days, maximum percentage of depletion was in liver (29.81) and (32.98) and minimum percentage of depletion was in gill (16.00) and (19.02) was more decreased in a series like Liver>Kidney>Brain >Muscle>Gill and Liver>Kidney >Muscle>Gill >Brain was observed in all the tissues of freshwater fish *Labeo rohita*.

Discussion:-

Pesticides can produce adverse effects in a biological system, seriously damaging its structure and function of living system finally leads to death of organism. Pesticides are entering into aquatic ecosystem by agriculture runoff from land, impairing the quality of the water and making it unfavorable for aquatic life (Tilak *et al*., 2009). The present investigation was revealed that LC_{50} value in 96hr lethal concentration found in static renewal method. The sublethal concentration was taken as $1/10^{th}$ of the lethal concentration. The pesticide effect influence the fish behaviour like jerking movements and mucus section were observed. When pesticide concentration was increase the mortality of the fish was increased. Nwani *et al.*, (2013) reported that median lethal concentration of chlorpyrifos based pesticide Termifos to African catfish *clarias gariepinus* were found to be 0.861 mg l^{-1}. The toxicity of a pesticide could vary from species and this variation is due to differential tolerance of animals to pesticide exposure (Prabhupatkar, 2004; Ram Nayan Singh, 2013).

Proteins are important organic constituents of the animal cells. They play a vital role in the process of interactions between intra and extra cellular media being a part of cell membrane and enzymes. Understanding of the protein components of cell becomes necessary in the light of the radical changes taking place in protein profiles during pesticide intoxication. Both the protein degradation and synthesis are sensitive over a wide range of conditions and show changes to a variety of physical and chemical modulators. The decrement in the proteins of freshwater fish *L.rohita* treated with sublethal concentrations of malathion suggests the existence of high proteolytic activity, and impairment of protein biosynthesis and degradation is due to oxidative stress, decreased protein levels may be attributed to the destruction or necrosis of cells and consequence impairment of protein synthesis (Patil and Devid, 2008; Mastan and Rammayya, 2010; Bakhshwan , 2009; Sathyavardhan, 2010).

Bijoy Nandan and Nimila (2011) observed significant biochemical changes in *Etroplus maculates* due to lindane toxicity. Proteins are primarily responsible for cellular responses to physical stimuli, as well as the phenotype of the organism (Benninghoff, 2007). It is presumed that reduction in protein content could be due to its utilization to mitigate energy demand when the fish is under stress as reported (Naveed *et al*., 2010; and Prashanth, 2008).

Under exposure to sublethal and lethal concentrations of pyraclostrobin, the decreased trend of the protein content in different tissues was observed; the percentage of depletion was more in liver followed by muscle. Naveed *et al*., 2010, reported that decreased protein content in different tissues of *channa puntatus* exposed to pesticide triazophos. The levels of protein decreased significantly in liver, brain, gill, kidney and muscle *Ctenopharygdon idella* treated with malathion (Satyavardhan, 2013). Exposure to carbryl for 4 and 24 days, decreased protein content in fish *Mugil cephalus,* reported by Shivanagouda *et al*., (2013).

Assessment of protein levels can be used as a diagnostic to determined the physiological status of the cells (Dogan and Can., 2011; Nwani *et al*., 2013). Detection of changes in activity levels of enzymes is widely used as quick method to deter mind the toxic effects of pesticides and other substances (Altinok *et al*., 2006). The decrease in the protein levels of pesticide or toxicant exposed fish suggests the disruption of carbohydrate metabolism, destruction or disturbance of protein synthesis machinery and inhibition of ATP synthesis (Ramalingam *et al.*, 2000). Veeraiah *et al*., (2013) observed that decreased levels of proteins in gill, liver, muscle and kidney of fresh water fish *L.rohita* exposed to sublethal concentrations of indoxacarb.

Table.1. LC_{50} values with 95% confidence limits for pyraclostrobin based on dissolved concentrations estimated according to (Finney Probit method,1971):

S.No	Concentration	Exposed fish	%mortality	Log of toxicant concentration	Lower bond/Upper bond
1	1.4µg/L	10	50	0.1461±0.098	1.3503-1.4497

Table:2. The 96 hr acute toxicity of Pyraclostrobin on freshwater fish, *Labeo rohita* percent mortality:

S. No	Conc. µg/L	Log Conc.	No. of fish exposed (N)	No.of fish alive	No. of fish died	Percent mortality
1	1.25	0.0969	10	10	0	0
2	1.30	0.1139	10	9	1	10
3	1.35	0.1303	10	7	3	30
4	1.40	0.1461	10	5	5	50
5	1.45	0.1631	10	2	7	70
6	1.50	0.1760	10	1	9	90
7	1.55	0.1903	10	0	10	100

Fig.1. The graph showing dose response curve between percent mortality against log concentration in freshwater fish, *Labeo rohita* exposed to Pyraclostrobin:

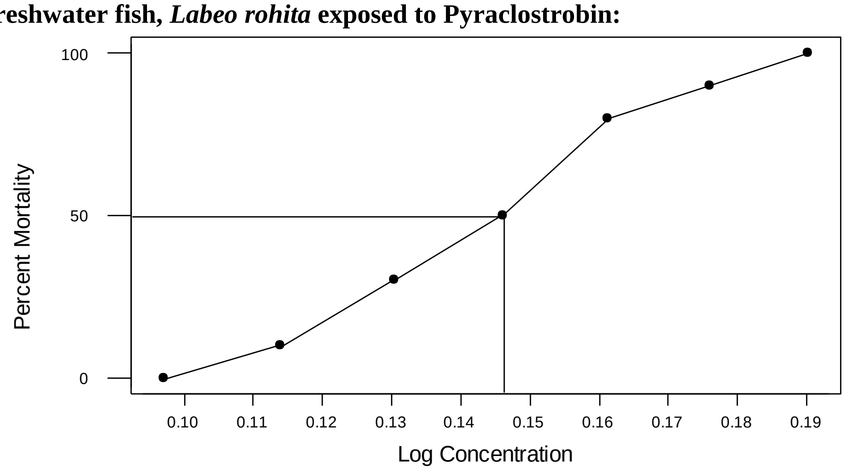


Table.3. The 96 hr acute toxicity of Pyraclostrobin on freshwater fish, *Labeo rohita* Probit mortality:

S. No	Conc. µg/L	Log Conc.	No. of fish exposed(N)	No. of fish alive	No. of fish died	Probit mortality
1	1.25	0.0969	10	10	0	-
2	1.30	0.1139	10	9	10	3.72
3	1.35	0.1303	10	7	30	4.48
4	1.40	0.1461	10	5	50	5.00
5	1.45	0.1631	10	2	70	5.84
6	1.50	0.1760	10	1	90	6.28
7	1.55	0.1903	10	0	100	8.09

Fig:2. The graph showing Probit mortality against log concentration in freshwater fish, *Labeo rohita* exposed to Pyraclostrobin 20%WG for 96hr:

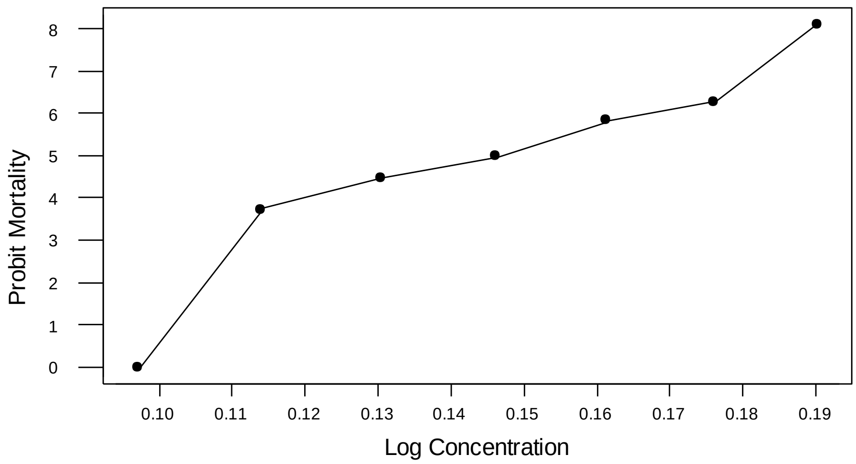


Table: 4. Changes in the **Protein content** (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of **Pyraclostrobin (20%WG) for 24hr**:

Tissues	Control (mg/g)	Sub-lethal (mg/g)	% Change	Lethal (mg/g)	% Change
Brain	81.57 ±0.017	70.12 ±0.04	14.03	67.34 ±0.03	17.44
Gill	78.94 ±0.04	71.49 ±0.02	9.43	69.50 ±0.04	11.96
Kidney	83.75 ±0.04	68.92 ±0.03	17.70	62.10 ±0.03	25.85
Liver	92.08 ±0.03	74.45 ±0.07	19.14	65.52 ±0.03	28.84
Muscle	72.65 ±0.03	66.02 ±0.13	9.12	61.90 ±0.04	14.96

Valuesare the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Figure: 3.

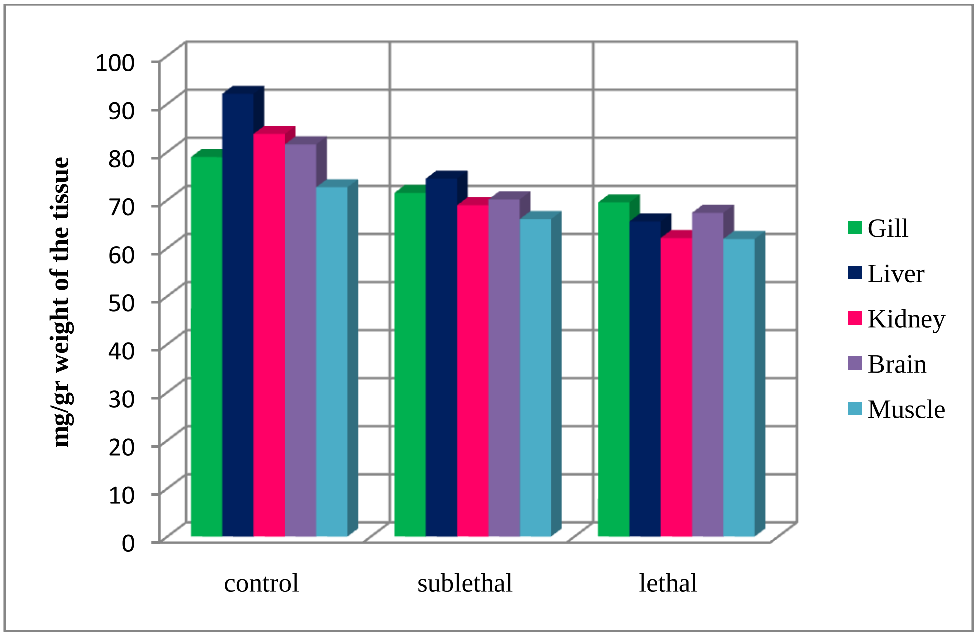
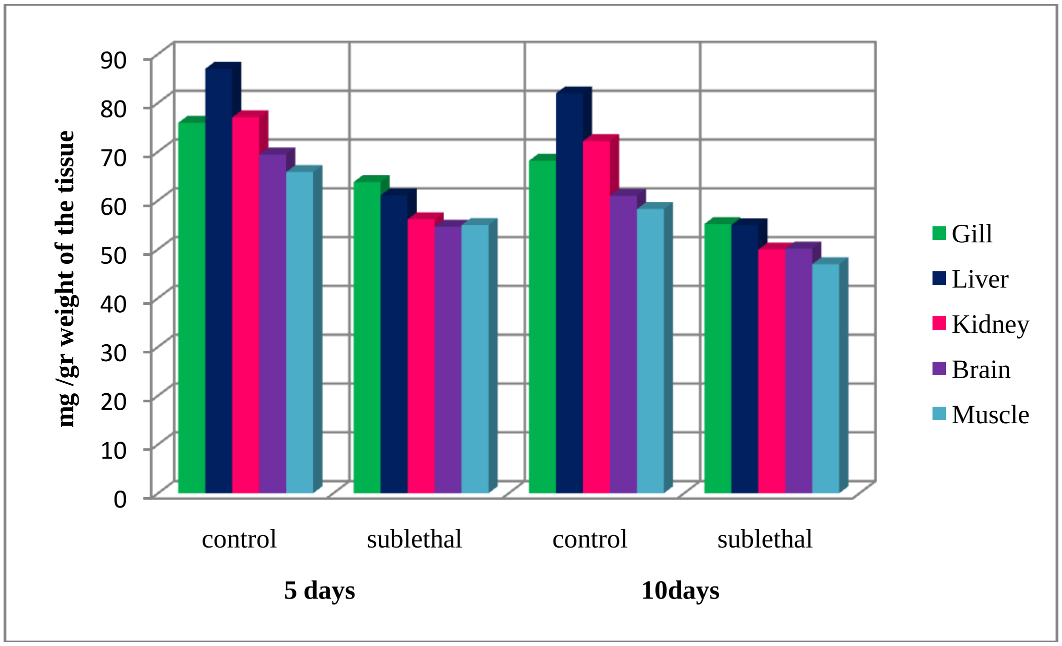


Table: 5. Changes in the **Protein content** (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Labeo rohita* exposed to sublethal concentrations of **Pyraclostrobin (20%WG) for 5 and 10 days**.

Tissues 24hr	5days			10days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change	Control (mg/g)	Sublethal (mg/g)	% Change
Gill	75.92 ±0.04	63.78 ±0.03	16.00	68.14 ±0.01	55.18 ±0.06	19.02
Brain	69.45 ±0.01	54.62 ±0.05	21.35	60.95 ±0.05	50.12 ±0.01	17.76
Kidney	77.05 ±0.12	56.14 ±0.05	27.13	72.16 ±0.10	49.94 ±0.08	30.74
Liver	86.98 ±0.03	61.05 ±0.03	29.81	81.95 ±0.07	54.92 ±0.03	32.98
Muscle	65.82 ±0.05	54.96 ±0.02	16.50	58.24 ±0.05	46.95 ±0.05	19.38

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Fig: 4.



Conclusion:-

When the effect of pesticide, fish show behavioral changes were observed. The pesticide concentration was increased and fish living survivability were gradually decreased. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways. The physiological and biochemical alterations observed in an animal under pesticidal stress can be correlated with the structural and functional changes of cellular proteins. The toxicity stress gradually decreases the protein activity levels compared with the controls.

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