IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

TOXICITY EVALUATION AND BEHAVIOURAL STUDIES OF FRESHWATER FISH *LABEO ROHITA* EXPOSED TO BIFETHRIN 10% EC (PYRETHROID)

V.Chaithanya kumari [1], Ch. Anithasmruthi [2], Dr.V.Venkata Rathnamma[3]

[1],[2]Department Of Zoology and Aquaculture, Research scholar

[3] Associate professor, Acharya Nagarjuna University, Guntur-522510. A.P, India

ABSTRACT:

Pesticides are the primary source for the achievement of green revolution. All over world pesticides are widely used to avoid the harmful pests from the crops. Bifethrin 10% EC is the synthetic pyrethroid insecticide which acts on nervous system of the target organisms but unfortunately it can show the effects on non target organisms like insects and other invertebrates including those that constitute the base of aquatic and terrestrial food webs. It enters the human systems through the food chain and shows its effects such as headache, nausea, breathing problems and other nervous disorders. The aim of the present studyis to determine the acute toxicity of the chemical Bifethrin 10% EC a synthetic pyrethroid on the fresh water carp *Labeo rohita* and also to notice behavioural alterations. In the experimental fish the LC_{50} values of **B**ifethrin 10%EC for 96hr was observed as 2.2µg/l respectively and the LC_{50} values were calculated in Finney Probit Method. The effect of Bifethrin on Fish had shown uncontrolled behaviour like schooling, hyper secretion of mucus, decreased agility, increased opercular movements, rapid jerking movements, discoloration of the body, erratic swimming, losing of scales, gasping, lose of equilibrium.

Key words:

Bifethrin, Pyrethroid, Insecticide, LC_{50}, *Labeo rohita* , Toxicity evaluation, hyper secretion of mucus and behavioural studies.

I.INTRODUCTION

Pesticides are the major hazardous chemicals to the environment; various types of pesticides are used to protect the crops and to achieve the yield. Due to the improper management of Pesticides while using leads to contamination of water bodies, because of this reason the non target organisms are also affected and from there it finally enters the humans through food chain. These toxicants affect fish first at molecular level, cellular level and finally shows physiological alterations. Fish serves as the bio indicator as it responds with great sensitivity for the changes in the aquatic environment, so it has an important role in monitoring of water pollination(1).The unrestricted use of synthetic pyrethroids causes lethal effect on various non-target organisms in the aquatic system and shows its effect directly or indirectly on users (2)(3)(4). Dimethoate and ʎ-cyhalothrin showed to be lethal for *Labeo rohita* (5).Acute toxicity is due to short-term exposure and happens within a relatively short period of time, whereas chronic exposure is due to repeated or long-term exposure and happens over a longer periods(6).Chronic low-level exposure to pesticides creates serious health problems as metabolism impairment, neurotoxicity, carcinogenicity, reproductive and endocrine disruption as well as immune dysfunctions (7)(8). Hence, in the present current study the effect of Bifethrin 10% EC a pyrethroid pesticide exposed to freshwater fish *Labeo rohita* to evaluate the toxicity effects and for the study of behavioural changes.

II. MATERIALS AND METHODS:

II.1. Test chemical:

The commercial grade formulations Bifethrin 10%EC a synthetic pyrethroid insecticide. Bifethrin comes into market as commercial product in many forms including sprays, granules and aerosols. These pyrethroid compounds are similar to the natural pyrethrins which are extracted from the flowers of pyrethrums. Bifethrin interferes with the nervous system of insects when they eat or touch it. It is more toxic to insects due to its low body temperature and small size. It is very effective against aphids, grasshoppers, fleas, flies, maggots, ticks, yellow jackets, spiders, beetles, mites, ants, termites etc. Now for the study I have chosen Bifethrin 10%EC which is available in the market as wilthrin. Unfortunately this chemical is highly toxic to the non-target organisms including fish and invertebrates and Bifenthrin acts on sodium channels at the nerve cell endings to depolarize the presynaptic terminals and It also affects cellular ATPase production (9).

II.2. Experimental organism:

The test fish *Labeo rohita* is one of the most important edible species of freshwater fish in India. This major carp is easily found in all over northern and central parts of India. It is also found in Godavari and Krishna rivers in south India. *Labeo rohita* belongs to the family cyprinidae, and is commonly known as ‘rohu’. It has fleshy protruding lips. It is rich in protein and is very suitable for human consumption. Hence, this carp has economic importance as edible fish and have a great commercial value.

II.3. Fish Collection and Acclimatization:

The freshwater fish *Labeo rohita* were collected from the fish hatcheries of Nandivelugu, Tenali mandal, Guntur (dt), A.P, India which is about 20km away from the university were brought to the laboratory and stored in large plastic containers filled with tap water. The average length and weight of the fish were about 5-6cm and 6.5 -7.5 gm respectively. At first fish were given prophylactic treatment by bathing them twice in 0.05% KMno4 solution for 4-5 minutes to avoid any dermal infections. The fish were acclimatized for the laboratory conditions at 28±2ºC for 10days before exposure to pesticide to observe the mortality. The fish which were unable to tolerate the climatic change had dead and they were removed from the tank immediately. During this period the fish were fed with groundnut oil cake and rice bran and the medium was replaced daily. The supply of oxygen (O_2) into the water of containers was done by electrical aerators and the water was renewed daily at regular intervals of time. Faecal matter and other materials were siphoned off regularly. All the precautions laid down by committee on toxicity tests to aquatic organisms APHA (1995)(10) were followed at the time of acclimatization.

II.4. Acute Toxicity Test:

To assess the susceptibility and the survival potentialities of the test organisms for 96hr LC_{50} tests had been conducted. Information generated from various toxicity tests can be of used in the management of pollution for different purpose like prediction of environmental damage due to wastage, comparison of various toxicants, animals or test conditions and regulation of waste discharge (11). The test solution was prepared by dissolving the commercial grade of Bifethrin 10% EC dissolved in 100% pure acetone and made into different concentrations. During acute toxicity trails, the fish were subjected to 12-hr photo period and were not fed for 24hrs. Experiment was conducted to select mortality of 96 hrs for the determination of LC_{50} values of Bifethrin in *Labeo rohita*. The fingerling stage of fish is more reliable to conduct toxicity test of various water borne toxicants (12)(13).The fish taken for the test were of equal size and weight approximately. Pilot experiments were conducted using 1L capacity bottles and a fish in each bottle was introduced to study the mortality concentration and at which concentration does the fish respond. For static renewal tests 10L capacity plastic containers were used. For each test seven different concentrations were chosen and 10 fish were introduced into each concentration. The dead fish were removed from test chambers immediately after death; for every 24hrs the water in the test containers were removed and again maintained the same Concentrations for 96hrs.The data regarding the fish mortality was observed and noted during the tests at the end of each specific time period. Static renewal tests were conducted to determine the LC_{50}values.The physic-chemical characteristics of water were determined by standard methods of APHA (1995). At lethal concentrations the death of the fish occurred even before noticing the behavioural abnormalities. In the present study of 96hrs LC_{50} values were selected for the study of the morphological, physiological and behavioural alterations.

III. RESULTS AND DISCUSSIONS:

III.1.Determination of LC_{50} values: To study the inherent capacity of the test chemical to affect any biological activity of an organism, the best method is to evaluate the toxicity of the toxicant by the determination of lethal concentration (LC), which represents the amount of chemical required for the death of 50% population among the experimental organisms(14).The values which I got in the study are calculated according to Probit Analysis Method (15) and the LC_{50} values were determined. In the present study Bifetherin caused 50% mortality in *Labeo rohita* for 96hrs at 2.2µg/l. The variation in the LC_{50} values is due to its dependence on various factors viz, sensitivity to the toxicants, its concentration and duration of exposure (16). Hence the value of LC_{50} help us to study acute toxicity effects of the test chemical on organism. Concentration of the toxicant is represented on x-axis and percentage mortality is represented on y-axis in the figure 1 graph. In figure 2 pesticide concentration is represented on x-axis and probit mortality is represented on y-axis. Results according to Finney probit analysis, the lower bound and the upper bound 95% lethal confidence limits for the test Bifethrin 10% EC is (2.08-2.29) respectively. The percentage mortality and probit mortality has been got increased with increasing the concentration of the toxicant. Tests were conducted to evaluate the mortality range from 10% to 96% for 96hrs (4days) in static renewal system. Finney Probit analysis as recorded by Roberts and Boyce (17) were followed to calculate the median lethal concentration (LC_{50}) values and Its 95% confidence limits. The mean values were derived by following the method of Finney Probit Kill theory. The data was subjected to the following statistical equations for LC_{50} values.

$$\text{Log } LC_{50} = \frac{Log\ A + 50 - a}{b - a \log 2}$$

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

S.no	Conc.	Exposed fish	Percent of morality	Log of toxicant concentration	Confidence limits
1	2.2 µg/l	10	50	0.342	2.08-2.29

Table II shows the 96 hr acute toxicity of Bifethrin on freshwater fish, *Labeo rohita* percent mortality and probit mortality

S.No	Cocµg/l	Log Conc	No of Fish Exposed	No of Fish Alive	No of fish Died	Percent mortality	Probity mortality
1	1.9	0.279	10	10	0	0	-
2	2.0	0.301	10	8	2	20	4.16
3	2.1	0.322	10	7	3	30	4.48
4	2.2	0.342	10	5	5	50	5.00
5	2.3	0.361	10	3	7	70	5.52
6	2.4	0.381	10	1	9	90	6.28
7	2.5	0.398	10	0	10	100	7.33

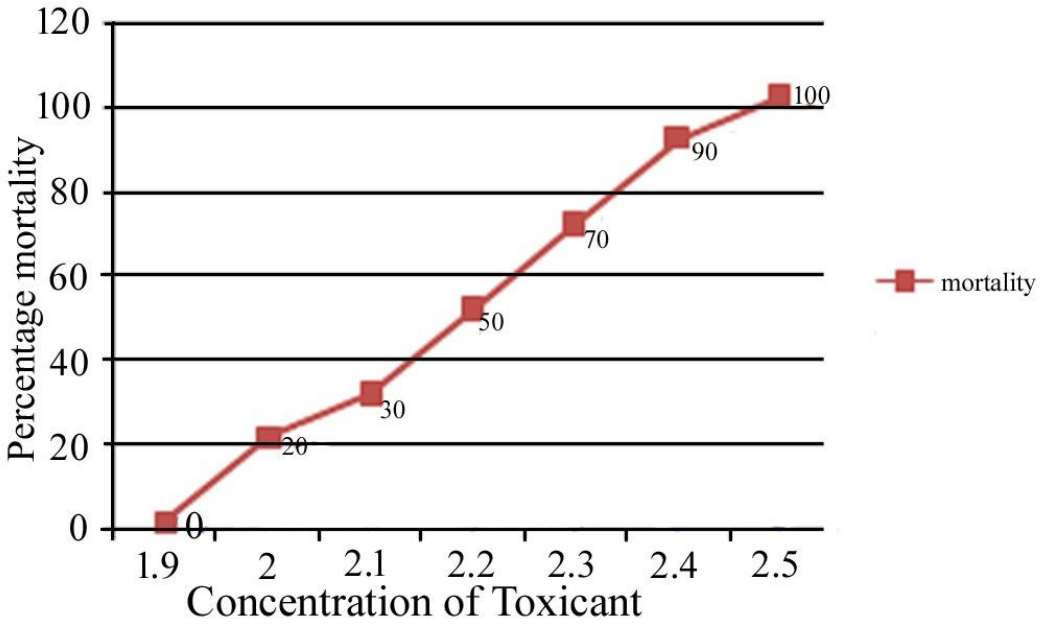

Figure 1 The graph showing dose response curve between percent mortality against concntration of toxicant in freshwater fish, *Labeo rohita* exposed to Bifethrin 10%EC

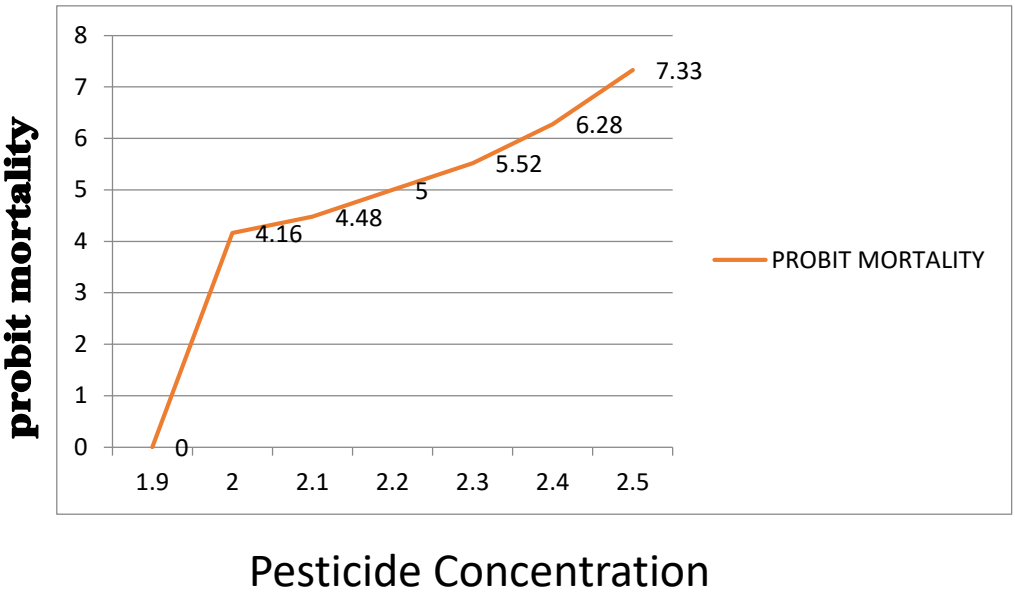

figure 2 The graph showing dose response curve between probit mortality against Pesticide concentration in freshwater fish, *Labeo rohita* exposed to Bifethrin 10%EC.

III.2. Discussion:

The present study is also supported by many authors and the variations in LC_{50} values depends on various parameters such as concentration of the toxicant, duration of exposure, sensitivity to the pesticide, dissolved oxygen level in water, size of the organism, age, quality of the water and temperature. The effect of the pyrethroid that is Bifenthrin, on rainbow trout (Oncorhynchusmykiss) was assessed based on biochemical, haematological and histopathological examination of fish exposed to Talstar 10% EC pesticide preparation (active substance 100 gm/l Bifenthrin) at a concentration of 14.7 µg/l (18). The effect of Fipronil pesticide on common carp (Catla catla) for the 96-hrs LC_{50} value was found at 0.23mg/l (10). The effect of Ethion 50% EC pesticide on common carp *Labeo rohita* for the 96 h LC50 value was found 1.20 µg/l (19). ʎ-Cyhalothrin of 5% EC formulation pyrethroid was used in static renewal bioassay to evaluate 96hrs LC_{50} in the freshwater fish *Ctenopharyngodon idella* and it was determined at 0.026 mg/l (20). In the present study Bifetherin 10% EC caused 50% mortality in *Labeo rohita* for 96hrs at 2.2µg/l and shows that it is harmful to the aquatic organisms as well as it gets transfer through the food chain to higher level organisms due to its inheritable capacities and shows it's impact.

III.3. Behavioural changes in fish due to acute toxicity:

Morphological and behavioural changes exhibited by the test fish can be taken as useful parameters in assessing the toxicity caused by the chemical up to some extent(21)(22). Thus, the studies on symptomology need much emphasis in understanding the changes in organisms which are exposed to toxicant (23). Aim of the present study is to evaluate the acute toxicity of Bifethrin 10%Ec and its toxicological effects on behavioural studies of Indian major carp *Labeo rohita*. During the experimental period, the behavioural

changes were observed and noted under the toxicant stress conditions and compared with the behaviour of control fish. The control group showed normal behaviour during the 96hrs duration where as the fish which were exposed to Bifethrin tried to jump out of water immediately to avoid its contact. Fish had shown random movements when they are exposed freshly later it had settled down at the bottom of the container and came to rest position. Fish which are exposed above the LC_{50} value (or) at higher concentration were dead by oozing out the blood due to the rupture of blood capillaries and the water in the container turned red color. Observations were also made on the changes in external morphology of the fish such as shedding of scales, secretion of thin layer of mucus around the body surface as well as gills. This might be the mechanism to avoid or to reduce irritating effect of the chemical. Fish in toxic media exhibited jerking movements, loss of equilibrium, schooling behaviour, moving in spiral fashion with jerky movements, convulsions and rapid flapping of the opercular movements with opened mouth, opening of mouth for gasping. When the fish was about to die at first the fish has lost its body balance and many times it turned its body upside down because of the lose of neuro muscular coordination. Hence Bifethrin is neurotoxin as it affects the central and peripheral nervous system by interfering with voltage gated sodium channels in neurons. The mechanism of action of pyrethroids, including Bifenthrin, is the same for mammals and invertebrates (24). Finally the fish settled at the bottom the container and tried to engulf lot of water to get oxygen supply to the gills (accelerated respiration) and there was continuous movement of operculum, There was no body movement, almost all the activities were ceased and finally it lead to death of the fish. It is observed that before few hours of death of the organism the colour of the body became pale (discoloration of the body). During the 96 hr of exposure, all body activities were nearly ceased. Complete loss of body balance exhibits irregular, erratic, darting swimming movements and loss of equilibrium followed by hanging vertically in the water (19). Bifethrin disturbs the normal fish behaviour after exposure and behavioural characteristics are obviously sensitive indicators of toxicant effects. After observing experiments and other authors reports had proved that Bifethrin 10% EC is highly toxic to common edible fish *Labeo rohita*.

IV. CONCLUSION:

Insecticides are the primary tool to maximize the yield but due to heavy and indiscriminate use of these chemicals results in negative consequences on non-target organisms which is leading to ecological imbalance. The LC_{50} value of *Labeo rohita* exposed to Bifethrin is 2.2µg/l for 96hrs. This shows that the more is the duration period the less is the concentration required. It is observed percentage mortality and probit mortality of *Labeo rohita* for Bifethrin in renewed static test and at different concentrations are shown in (Table No. II). Control group were normal where as experimental groups in response to insecticide Bifethrin has caused behavioral variations. In the present study exposure to Bifethrin a pyrethroid causes various effects on the survival and behavioural problems in *Labeo rohita* due to the action of the chemical on nervous system. The more duration period requires less concentrate

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