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TOXICITY OF MIXTURE INSECTICIDE CHANDIKA 505 (CHLORPYRIFOS 50% CYPERMETHRIN 5% EC) ON BIOCHEMICAL PARAMETERS OF ZEBRA FISH, DANIO RERIO

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Abstract:

The study was aimed to investigate the sub lethal effect of insecticide, Chandika 505 mixture of chlorpyrifos 50% and cypermethrin 5% EC to zebra fish (Danio rerio). The acute toxicity LC_{50} of insecticide was determined at 96 hrs and the value is 22.7 ppb and sub lethal concentration is 7.56 µg. Behavioral changes were also observed when fish were subjected to different concentrations of the insecticide. The results of the present study indicate increase in the total lipids, free fatty acids and cholesterol in gills, viscera and body of zebra fish. Hence it can be concluded from the results that measures should be taken for the usage of insecticides in order to protect the aquatic environment because combined toxic effects of pesticides are important in ecotoxicology since mixtures shave a greater effect than the individual pesticides.

Keywords: *Zebra Fish, LC_{50}, mixtures, Chlorpyrifos, Cypermethrin.*

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INTRODUCTION:

Pesticides are a group of compounds which are used to suppress and control harmful target organisms [1].They compromise inclusive compounds such as insecticides, fungicides, Herbicides etc. Pesticides are widely utilized in agriculture and there is need to monitor the impact of the chemicals on aquatic population. The indiscriminate utilization of pesticides causes toxicity, thereby polluting the environment and risks to non- targeted organisms. There are many classes of synthetic pesticides. The main class consists of Organochlorines, organophosphates, Carbamates and pyrethroid (Table 1) Some Pesticides are considered as biodegradable pesticides, which are separated by microorganisms and other living creatures or steady pesticides, which can take few months or years before they are separated.

CLASSIFICATION:

Table1 Type of Synthetic Pesticides

CLASS	EXAMPLES	AREA OF EFFECT
Organochlorines	DDT, Dieldrin, aldrin.	Nervous, endocrine, immune and reproductive system.
Organophosphates	Diazinoin, malathion, glypostae	Central nervous system
Carbamates	Aldicarb, Carbofuran, Carbaryl.	Central nervous system
Pyrethroid	Fenpropanthrin, deltamethrin, Cypermethrin	Poorly understood

Reponses of aquatic organisms are ranged broadly depending on the compounds toxicity, exposure time, species and water quality [2, 3]. Behavior and locomotion is commonly affected by the contaminants [4]. The swimming behavior of fishes is considered as toxic response of the fish which in turn indicated the effect on nervous system. Pesticides used in the pests control programs bring about many biochemical and physiological changes in freshwater creatures by affecting the activities of many enzymes. OP insecticides share a common mechanism, the inhibition of acetylcholinestersase (AChE), the enzyme which is responsible for the hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. Environmental pollutants toxicity results in negative effects on many organs and systems such as the detoxifying; central, nervous and hematological systems [5].

Interaction of Chlorpyrifos and Cypermethrin:

The interaction between OPs and pyrethroid is known well since formulations contain both the classes of insecticides. The non-reversible hindrance of the esterase by the organophosphates (OPs) leads to slow down of the activity of the enzymes which are responsible for the cleavage of the bonds of ester in pyrethroid molecule [6]. Blocking the pyrethroid hydrolysis lowers metabolic process hence a strong effect of the pyrethroid mixture is noted. From ecological point and economical view this combination mix reduces the usage of each pesticide, by keeping efficiency at the same level [7].

Ideally the pesticide mixture should be toxic to non-target organisms only and must be eco-friendly and biodegradable to certain extent. New formulations are being designed by researchers and manufacturers for mixture pesticides to meet the demand of the global and to reduce the environmental pollution.

Chandika 505 is a combination insecticide of the active ingredients of chlorpyrifos (CPF) and Cypermethrin (CYP). The former belongs to organophosphorous group and the other is a pyrethroid which is synthetic. It has a broad spectrum action and utilized against pod, fruit and stems borers, miners of leaf, for defoliating the caterpillars, termites and sucking pests etc which has contact and stomach action and this combination results in synergistic action and provides longer protection. It is tremendously in use in Telangana State to control various pests. Hence, Chandika 505 is selected for the present study to carry out sub lethal effects on zebra fish.

The effect of pesticides in aquatic environment

Pesticides are chosen depending upon their efficiency rather on their impact in an environment [8]. Many pesticides get into water bodies through different routes and are not easily degradable and persist in the aquatic biota and enter the aquatic organisms directly or indirectly [9] and may inhibit cell division, at cellular level, alter the growth, change the metabolic pathways and inhibits the action of enzymes [10]. The residue or the storage of the pesticide in bodies of the organisms will affect the development or growth stages of organisms.

Rationale for this study

Due to its small size, low cost of the feed, short tests cycle etc. zebra fish is used for acute and chronic toxicity studies and has become a choice for safety assessment and test pollution [11]. The combined or mixture toxicity of pests are important in ecotoxicology since mixtures have a greater effect than the individual pesticides [12].The present study deals with the determination of toxicity of mixture insecticide to aquatic organisms and to reduce the adverse effects on aquatic biota.

Fewer studies have been reported on physiological and biochemical effect of chlorpyrifos 50% cypermethrin 5% EC [13-15] on fishes. But no literature has been reported on Chandika 505(Chlorpyrifos 50% and cypermethrin 5% EC) on zebra fish, *Danio rerio*. Hence the study has been taken to study the sub lethal effects of insecticide, Chandika on lipid metabolites of zebra fish at different intervals of time.

MATERIAL AND METHODS:

Test compound:

The test compound, Chandika 505 is the ready mix of cypermethrin and chlorpyrifos purchased from local market and the purity is 92%.All chemicals and solvent were purchased from CSIR-IICT chemical supplier.

Experimental Animal

- The zebra fish, *Danio rerio*, the primary advantages of it are utilizing as a toxicological model over the other vertebrate species are:
- Unlike the other species of fish such as trout, adult zebra fish are approximately 1–1.5 inches long which greatly reduces living space and breeding costs.
- In differentiation to the larger species, the minute size of the larvae and adult zebra fish limits the costs by low quantities of dosing solutions.
- (experimental chemicals, drugs, pollutants) and thereby which reduces the waste and limits the amount of lab ware and chemicals, for both maintaining and treating live fishes, to perform various assays (reagents of low quantities) and for histological assessing (small amount of microscope slides and embedding materials).
- Their optical clarity permits easy developmental staging and phenotypic traits identification during mutagenesis screening and endpoint assessment during toxicity testing.
- A pair of adult fish lays 200–300 eggs in single morning, and when appropriately maintained, gives a yield for every 5–7 days.

Scientific classification		
Kingdom	:	**Animalia**
Phylum	:	**Chordata**
Class	:	**Actinopterygii**
Order	:	**Cypriniformes**
Family	:	**Cyprinidae**
Subfamily	:	**Danioninae**
Genus	:	***Danio***
Species	:	***rerio***

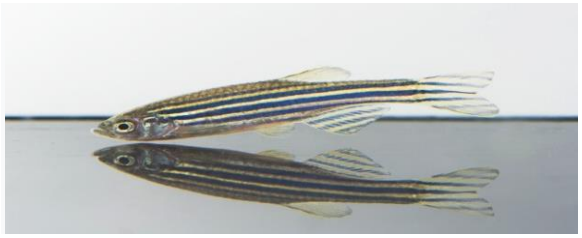

Figure 1 Danio rerio

The fish, *Danio rerio* (figure 1) were collected from Chennai fish farm, which is relatively free from pollutants and were brought in large aerated bags to the laboratory. Later, for 15 days they were acclimatized in glass aquaria (60 x 30 x 30 cm) and fed daily with (TAIYO). The average values for water quality data in exposure tanks is as follows:

PH : 7.10±0.05
Dissolved oxygen : 8.24 ±0.22 mgL^{-1}
Temperature : 24±2° C
Chlorides : 245.57±1.44 mgL^{-1}
Total hardness : 415±1.2 mgL^{-1} (as $CaCO_3$)
Alkalinity : 348±1.6mgL^{-1} (as CaCO3)

ACUTE TOXICITY:

DETERMINATION OF MEDIAN LETHAL CONCENTRATION (LC_{50}):

The acute LC_{50} value of Chandika 505 was determined in the laboratory using semi-static method (UNEP) [16]. The stock solution of the insecticide, Chandika 505 was prepared in ethanol and further dilution was carried out in water. The concentrations of test were chosen to determine the lethal concentrations (LC_{50}) based on the initial experiments for 96 hrs. The required concentrations 21, 22, 22.5, 23, 23.5 (µg L^{-1}) were maintained in five liters of water in plastic tubs by mixing the stock of Chandika into the water. Similar weight and a day starved fish (10 per aquarium) were released in to the selected concentrations with 2 replicates. Daily water was renewed and fresh toxicant was added to maintain the concentration constant. The control experiment without toxicant was also performed parallel. The mortality was recorded for every 24 hrs in each test concentration during 96 hrs of exposure. The test concentration, number of fish and percent mortality were used to estimate median lethal concentration (LC_{50}) by the method of Finney [17].

Sub acute toxicity:

Sub-acute study is the measure of the biological activity of the compound and provides a relative estimate of the lowest dosage required to produce the effect following repeated exposure. In this study the sub lethal concentration of the Chandika 505, 7.56 µg L^{-1} is selected to carry out biochemical parameter in tissues of zebra fish. Thirty healthy and one day starved fish were divided into two groups one is control and other is experimental. The experimental group was exposed to sub lethal concentration of toxicant 7.56 µg L^{-1} for 4 days. The water was renewed for every 24 hrs and fresh toxicant was added to maintain the concentration of toxicant constant. At the end of 1st, 2nd and 4th day five fish each from control and exposed groups were taken and sacrificed to isolate the gills, viscera and body. The biochemical parameters, total lipids, free fatty acids and cholesterol were estimated in these tissues.

Determination of total lipids:

Lipids were extracted as described by Folch et al [18] and estimated by Barnes and Blackstock [19]. Tissues (gills, viscera and body) were homogenized (5% w/v) in a waring blender in chloroform-methanol mixture (2:1).Through Whatman No. 1 filter paper the homogenates were filtered and residue was rehomogenised and filtered. The non-lipid matter from pooled filtrate was taken out by shaking vigorously with 0.88% KCI (added as 1/4th of the volume). In test tube 1 ml of filtrate was taken and evaporated under nitrogen, concentrated H_2SO_4 1ml was added and boiled for ten minutes. For total lipid estimation a solution of 0.2 ml was taken and 5ml of vanillin reagent was added. Colour developed in spectrocolorimeter was read at 520 nm against reagent blank.

Determination of free fatty acids:

Free fatty acid were estimated by method of Ragauw et al [20] 5% homogenate (w/v) of tissues (gills, viscera and body) was prepared in chloroform: heptane: methanol mixture (220:225:105), centrifuged for 5 mins at 4000 rpm. 5 ml of supernatant was taken in a centrifuge tube and 0.5 ml of copper triethanolamine reagent was added and shaken vigorously and horizontally. The mixture was centrifuged at 500 rpm for 5 min. To 1.5 ml of the supernatant, 3 ml of chloroform: heptane: methanol mixture and 0.75 ml of diethyl dithiocarbonate reagent was added and absorbency was read at 435 nm spectrophotometer using trichloromethane as blank.

Determination of cholesterol:

Cholesterol was estimated by the Zarrow et al [21] method. Homogenize the tissues (gills, viscera and body) in the 5% w/v of ethanol: ether (1: 1) solvent mixture and centrifuged for 10 minutes at 3000 rpm. 1 ml of supernatant is taken and (G.A.A) glacial acetic acid of 2ml and coloring reagent is added from sides of test tubes. The two layers are formed, a light brown color appear which changes to purple. Cool tubes to room temperature. Measure the OD at 560 nm along with reagent blank.

RESULTS AND DISCUSSION:

Toxicants alter the biochemical and physiological state of animals by inducing changes in the activities. In any individual lipids act as the source act as the source of energy to carryout different activities. Metabolic activity of an organism reflects the utilization of biochemical energy to counteract the toxic stress of the mixture chandika 505. In this present study sub lethal dose of the toxicant was taken to expose the fish for four days and total lipids, free fatty acids and cholesterol was determined in gills, viscera and body. Chlorpyrifos is poisonous to fresh water fish, oceanic invertebrates and marine organisms [22]. It is evident from the results that the insecticide Chandika 505 (Chlorpyrifos 50% and cypermethrin 5% EC) which is highly toxic to the zebra fish with an LC_{50} value of 22.7 µg L^{-1} and sub lethal concentration of 7.56 µg L^{-1}. It is observed in study that Chandika produced significant adverse effects on swimming behavior of zebra fish.

Total lipids content in gills increased at the end of 1, 2 and 4 day (Figure 2). End of the day 1 the percentage increase was 7.65 % followed by 26.47% and 54.15% on day 2 and day 4(Table 2). Similar trend in increase of total lipids was showed in viscera (Figure 3) and body (Figure 4) also showed increase and the increase was time dependent.

Cholesterol content of gills showed increase at 1, 2 and 4 day ending (Figure 5), after 24hrs the increase was 22.1%. At the end of day 2 and day 4, the % increase was 36.97%, followed by 95.46%. Increase in cholesterol content as noted in viscera (Figure 6) and body (Figure 7) of zebra fish. The increase was time dependent in gills, viscera and body (Table 3).

Increase in all tissues of free fatty acid content was observed (Table 4).The free fatty acid in gill tissue on day one increased (Figure 8) when compared with control. Till the end of the exposure period increase continued. Similar increase was shown in the viscera (Figure 9) and body (Figure 10).

Cypermethrin toxicity increased the lipids in all tissues, which possibly was due to enhanced rate of lipogenesis in tissues of C. batrachus when exposed to carbofuran [23]. Increase in the total lipids in gills, viscera and body is reported in present study. Total lipid content increased at low concentration when compared to control group. Free fatty acid (FFA) synthesis is dependent on the availability of excess carbohydrates, especially glucose and glycolytic activity of the cell [24]. This might have led to the free fatty acid content increase when exposed to mixture insecticide, Chandika 505 which contains cypermethrin.

In body metabolism cholesterol plays a vital role and being associated with every organ of body, is the foremost biochemical component of tissues. In the present study the insecticide Chandika caused alterations in cholesterol which suggested that the fish tried its best to accommodate with the situation and doing so body metabolism became rapid to fulfill energy requirement, utilizing organic reserves, yielding excess energy to compensate the toxic stress as suggested by Chandra [25].

The cholesterol in all tissues of fish increased in a time dependent manner with respect to controls. Lipids provide an essential source of energy for fish of which cholesterol is of major importance since its relationship to many physiologically active steroids and hormones [26]. Alterations in the cholesterol level on metabolic enzymes may be the indirect result of toxicant effect [27]. Chandika 505 had increased cholesterol levels in gills in this experiment. Gills being the primary sites of absorption but having poor metabolizing capacity indicated maximum rise (95.46%) in cholesterol level.

Table 2 Estimation of total lipids in gills, viscera and body of zebra fish for 4 days

TISSUE		EXPOSURE PERIOD (Days)		
		1	2	4
Gills	Control	70.30	70.32	70.32
	Exposed	75.705	88.94	108.4
	%variation	+7.65	+26.47	+54.15
Viscera	Control	316.18	316.15	316.18
	Exposed	338.04	248.53	404.59
	%variation	+ 6.91	+10.23	+27.96
Body	Control	201.75	201.70	201.75
	Exposed	216.53	243.19	310.49
	%variation	+ 7.32	+20.54	+58.7

Each value is the mean of five individual fish (mg/g wet weight of tissue)

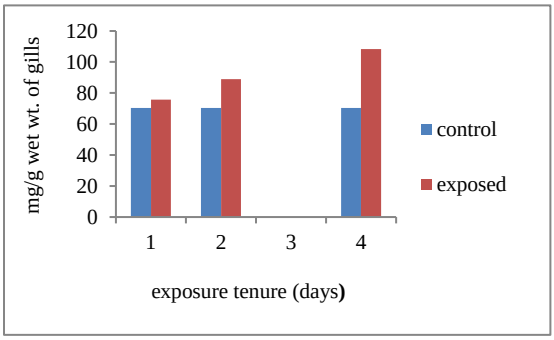

Figure 3 Total lipids estimation in viscera

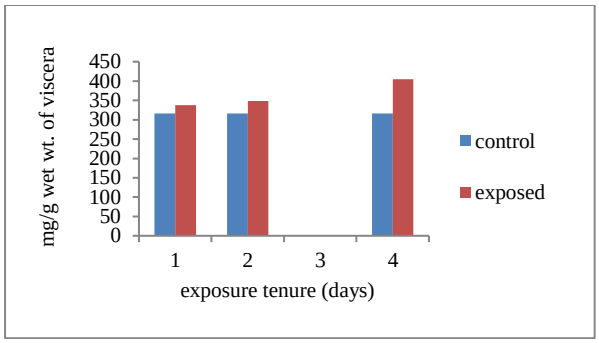

Figure 2 Estimation of total lipids in gills of zebra fish

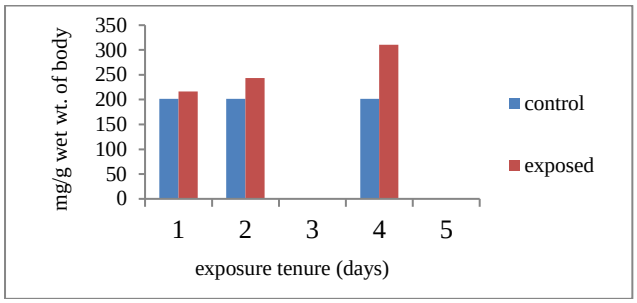

Figure 4 Estimation in the body of zebra fish of total lipids

Table 3 Estimation of cholesterol in gills, viscera and body of zebra fish for 4 days

TISSUES		EXPOSURE PERIOD (Days)		
		1	2	4
Gills	Control	3.09	3.11	3.11
	Exposed	3.80	4.26	6.08
	%variation	+22.1	+36.97	+95.46
Viscera	Control	4.50	4.50	4.48
	Exposed	5.56	6.39	7.57
	%variation	+23.55	+42.1	+68.2
Body	Control	3.31	3.30	3.31
	Exposed	4.50	4.91	5.91
	%variation	+35.95	+48.33	+78.24

Each value is the mean of five individual fish (mg/g wet weight of tissue).

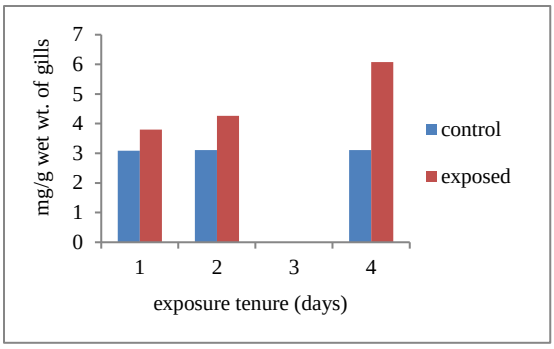

Figure 5 Estimation of Cholesterol of zebra fish in Gills

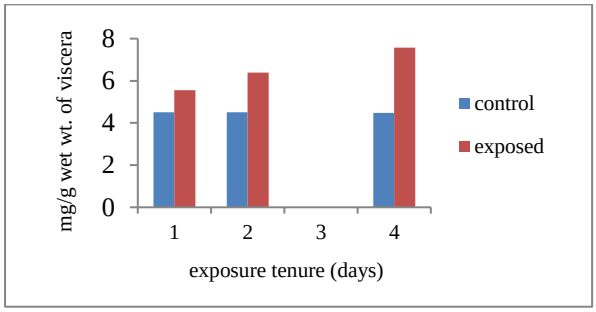

Figure 6 Estimation of cholesterol in Viscera.

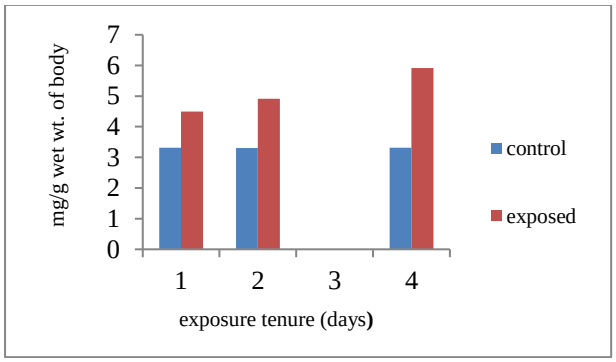

Figure 7 Estimation of Cholesterol in the body of zebra fish.

Table 4 Free fatty acid estimation in gills, viscera and body of zebra fish for 4 days

TISSUES		EXPOSURE PERIOD (Days)		
		1	2	4
Gills	Control Exposed %variation	1.20 1.625 +38.78 %	1.22 2.04 +67.2%	1.22 2.245 +84.01
Viscera	Control Exposed %variation	1.32 1.55 +15.6	1.34 1.88 +40.29	1.34 2.22 +65.67
Body	Control Exposed % variation	2.19 2.59 +18.26	2.18 2.90 +32.42	2.19 3.73 +70.3

Each value is the mean of five individual fish (mg/g wet weight of tissue)

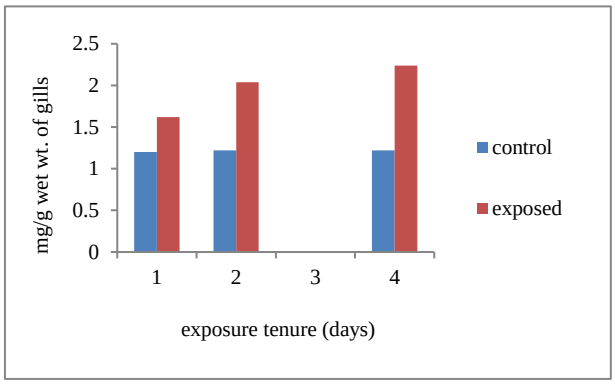

Figure 8 Estimation of free fatty acid in gills of zebra fish

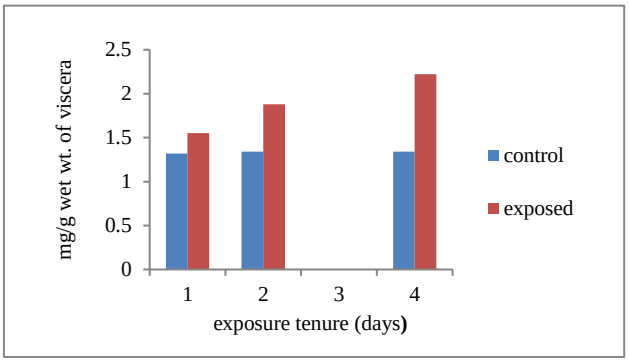

Figure 9 Estimation of free fatty acid in Viscera

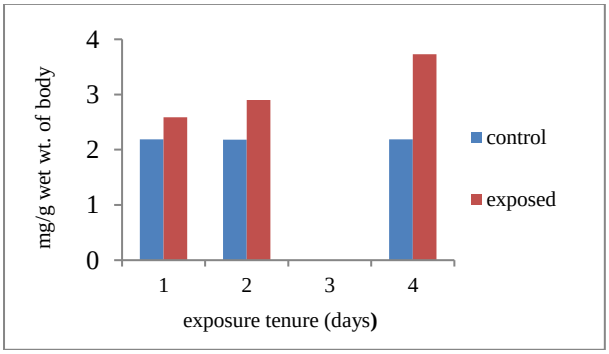

Figure 10 Free fatty acid estimation in the body of zebra fish

CONCLUSION:

This is a study of aquatic toxicological evaluation of mixture insecticide Chandika 505 on zebra fish, *Danio rerio*. The insecticide has shown toxicity of lethal concentration (LC_{50}) of 22.7 µg L^{-1} against the fish species, *Danio rerio*. They were exposed to the sub-lethal concentration (1/3rd of LC_{50} 7.56 µg L^{-1}) for acute study (4 days). To conclude from the results, that present study Chandika insecticide is very toxic and caused alteration in swimming movements, total lipids, free fatty acid and cholesterol in various tissues of exposed fish. The changes in various components of lipids indicate the impairment in vital physiological processes of exposed fish which in turn affects the health of fish. It can also be concluded from the results that measures should be taken for the usage of insecticides in order to protect the water resources, life of fish and other aquatic fauna.

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