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Comparative laboratory monitoring of organochlorine insecticide and synthetic pyrethroid on the fresh water fish, *Rasbora daniconius* (HAM.) in relation to LC_{50}, ethological and skin melanophore responses

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Abstract
The fresh water fish, *Rasbora daniconius* has great economic importance, in terms of nutritive values. The pesticides, Endosulfan (Organochlorine) and Synthetic pyrethroid (Cyhalothrin) from the agricultural runoff causing pollution of water bodies of Bhiwandi city wherein the fishes, the non-target organism get adversely affected. Therefore the efforts were made to study comparatively the effects of both the pesticides on percentage mortality (LC_{50}), behavioral and skin melanophore response (photochromatic effects) of fish *Rasbora daniconius.* The study shows that the used pesticides bring about various physiological disorders in the fish, the LC_{50} value is very low furthermore, there is marked behavioral and melanophore anomalies compare to control which proves that the selected pesticides have equal degree of toxicity on the test animal. Thus, the release of these pesticides in and around the water resources should be carefully monitored and controlled.

Keywords: organochlorine insecticide, synthetic pyrethroid, fish, LC_{50}, behavioral responses, skin pigmentation

Introduction

Nature took 600 million years to develop the environment as it exist today but man is barely one million years of his existence, has brought about changes which threaten the very existence of a healthy biosphere. On earth's surface, both in water as well as on land these changes result in impoverishment of biological system and degradation of biodiversity. In the atmosphere rising concentration of CO_2 causes global warming while pollution of stratosphere has been eating away the vital ozone - shield. These changes are expected to make human life more and more miserable in times to come.

Issues concerning environment now worry not only Scientist but statesman, rulers and general public as well. A pressing need has been felt to educate the common man about problems of environment.

Water is needed in almost every sphere of human activity. It is required for direct consumption or indirectly for washing, cleaning, cooling, transportation or even for waste disposal. Fresh water is a natural resource of fundamental importance. Without water life is not possible. In many respects the properties of water are unique. They seem to be especially designed for the living organisms. No other liquid can replace it.

Agrochemical (or agrichemical), a contraction of agricultural chemical, is a generic term for the various chemical products used in agriculture. In most cases, agrichemical refers to the broad range of pesticides, including insecticides, herbicides, fungicides and nematicides. It may also include synthetic fertilizers, hormones and other chemical growth agents, and concentrated stores of raw animal manure.

Many agrichemicals are toxic, and agrichemicals in bulk storage may pose significant environmental and /or health risk, particularly in the event of accidental spills. In many countries, use of agrichemicals is highly regulated. Government issued permits for purchase and use of approved agrichemical may be required. Significant penalties can result from misuse, including improper storage resulting in spillage. On farms, proper storage facilities and labeling, emergency clean-up equipment and procedures, and safety equipment and procedure for handling, application and disposal are often subject to mandatory standards and regulations. Usually, the regulations are carried out through the registration process.

There are various categories of agrochemicals. A pesticide is any substance used to kill, repel, or control certain forms of plant or animal life that are considered to be pest. Pesticides include herbicides for destroying weeds and other unwanted vegetation, insecticides for controlling a wide variety of insects, fungicides used to prevent the growth of molds and mildew, disinfectants for preventing the spread of bacteria, and compounds used to control mice and rats. Because of the widespread use of agricultural chemicals in food production, people are exposed to low level of pesticides residues through their diet. Scientist does not yet have a clear understanding of the health effects of these pesticide residues. Results from agricultural health study, an ongoing study of pesticide exposure in farm families, show that farmers who used agricultural insecticides experienced an increase in headaches, fatigue, insomnia, dizziness, hand tremors, and other neurological symptoms. Evidence suggest that children are particularly susceptible to adverse effects from exposure to pesticides used in a variety of setting including homes, schools, hospitals, and workplaces.

Since before 2000 B.C., human have utilized pesticides to protect their crops. The first known pesticide was elemental sulfur dusting used in ancient sumer about 4,500 years ago in ancient mesopotamia. The Rigveda, which is about 4000 years old, mentions the use of poisonous plants for pest control (Rao G. V. R. Rupela O.P., Rao V.R, Reddy Y.V.R, 2007) [49]. By the 15th century, toxic chemicals such as arsenic, mercury and lead were being applied to crop to kill pest. In the 17th century, nicotine sulfate was extracted from tobacco leaves for use as an insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum, which is derived from chrysanthemum, and rotenone, which is derived from the root of tropical vegetable (Miller, G.T. 2002) [35]. Until the 1950's, arsenic based pesticides were dominant (Ritter S.R. 2009) [51]. Paul Muller discovered that DDT was a very effective insecticide. Organochlorine such as DDT wasdominant, but they were replaced in the U.S. by organophosphates and carbamates by 1975. Since then, pyrithrin compound have become the dominant insecticide (Ritter S.R. 2009) [51]. Herbicides became common in the 1960s, led by "triazine and other nitrogen based compounds" carboxylic acid such as 2, 4-dichlorophenoxyacetic acid, and glyphosate (Ritter S.R. 2009) [51].

The first legislation providing authority for regulating pesticides was enacted in 1910 (Goldman L.R. 2007) [18]. However, decades later during the 1940's manufacturers began to produce large amount of synthetic pesticides and their use became widespread (Daly H. *et al*., 1998) [14]. Some sources consider the 1940's and 1950's to have been the start of the "pesticide era" (Graeme Murphy, 2005) [21]. Although the U.S. Environmental Protection Agency was established in 1970 and amendments to the pesticides Law in 1972 (Goldman L.R 2007) [18], pesticides use has increased 50 fold since 1950 and 2.3 million tonnes of industrial pesticides are now used each year (Miller, GT 2002) [35]. Seventy-five percent of all pesticides in the world are used in developed countries, but use in developing countries is increasing (Miller G.T 2004). A study of U.S.A pesticides use trends through 1997 was published in 2003 by the National science foundation's centre for integrated pest management (IPM) (Ritter S.R (2009) [51] and Arnold L. Aspelin (2003) [4]. In the 1960s, it was discovered that DDT was preventing many fish eating birds from reproducing, which was a serious threat to biodiversity. Rachel Carlson wrote the best selling book "silent spring" about biomagnifications. The agricultural use of DDT is now banned under the stockholm convention on persistent organic pollutants, but it is still used in some developing nations to prevent malaria and tropical diseases by spraying on interior walls to kill or repel mosquitoes (Lobe, J. 2006) [31].

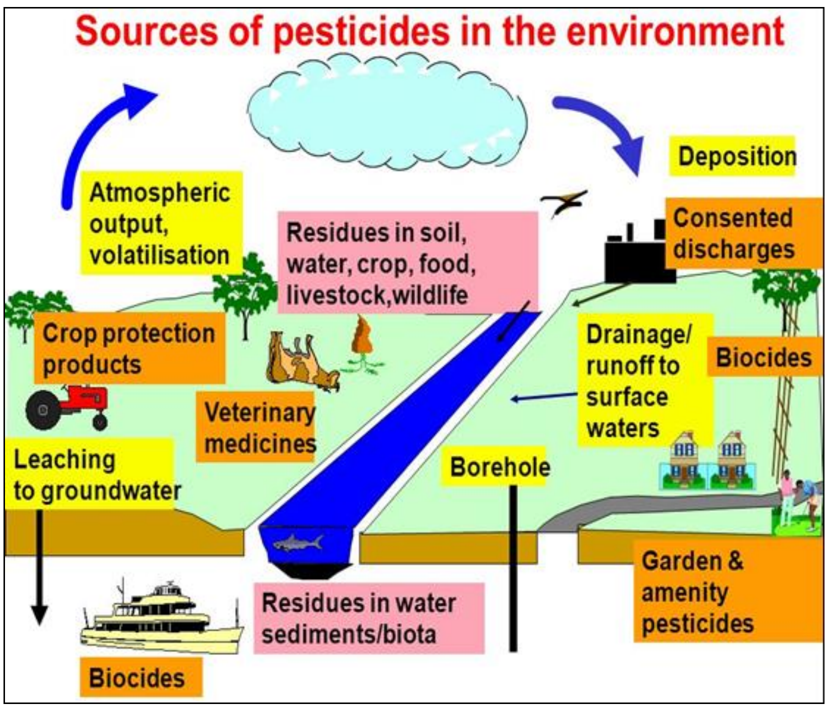

Fig 1: Exposure routes and environment fate of pesticides

When a pesticide is released into the environment, many things happen to it. Some of the pesticide reaches the target. The remainder will be broken down in the air, deposited on plants or soil in the target area and some will drift or run-off to non-target areas.

Many process affect what happens to pesticides in the environment. These processes include adsorption, transfer, breakdown and degradation. Transfer includes processes that move the pesticide away from the target site. These include volatilization, spray drift, run-off, leaching, and adsorption.

Experimental studies using live, intact creatures have played and continue to play, an essential role in developing new knowledge and better understanding of life processes, life forms, in the environment in which these forms and processes occur. The enormous evolutionary radiation of fishes comprises at least 25,000 species. Fishes exist in myriad forms and have developed many unique physiological, behavioral, and ecological specializations. Fishes occupy a variety of niches in virtually every kind of aquatic habitat. Understanding their biology simply cannot be accomplished in the absence of experimentation with live, intact animals.

Among the reasons for studying fishes are the following

Fishes are useful indicator of environmental quality and ecological integrity; fishes provide an important sources of food for many of the world's humans; catching and observing fishes are very popular and economically important recreational and commercial activities for millions of people; the unique adaptation and physiological specialization of fishes make them especially suitable for use as physiological and biomedical model; human existence is dependent on understanding our place and functions in the world's ecosystems, an understanding that cannot be accomplished without accurate and detail knowledge of fishes.

Since ages, fishes are being used as test animals for determining the toxic effect of pollutants. Sprague (1973) [58] and many others have even, standardized toxicity bioassay methods. Sonstegard (1977) [57] stressed the importance of fish as sentinel (an indicator of the presence of disease) for the early detection of water borne carcinogens. Certain species of fish can react in such a way, in vivo, that they produce active carcinogens from chemicals that are not themselves carcinogenic (Lech and Vodicnik, 1984) [29]. The basic assumption used in the present study, was if even a sturdy fish like *Rasbora daniconius*, affected by the cyhalothrin, then in the long run, other non target species would also be affected. With this view, endosulfan and cyhalothrin was selected to study its toxic effects, if any on the fresh water fish - *Rasbora daniconius*.

Fig 2: Test fish- *Rasbora daniconius*

The test fish *Rasbora daniconius* is of great economic importance, being one of the popular edible fresh water fish, as far as poor section of the society is concerned. It serves nutritive food for millions & consume whole (being smaller in size) without beheaded and gutted and therefore, it is a fish with considerable food value.

Massive research work has been carried out till date in the field of environmental ecotoxicology studies, using *Rasbora* as the test animal. T.S Pathan& *et al*. (2010) [60] examined histopathological changes such as swollen hepatocytes, nuclear hypertrophy, rupture sinusoids, haemorrhages, vacuolation in hepatic cells and broken central vein etc. in liver, kidney of*Rasbora daniconius* after 96 hrs. exposure to paper mill effluent. Pathan & *et al.*, (2009) determined the lethal concentration of 50 % mortality of paper mill effluents to fresh water fish *Rasbora daniconius* for 24 to 96 hrs. using bioassay method. The LC_{50} value of the prepared concentration for 24, 48, 72 & 96 hrs.were found at 11, 10.5, 10.1, & 9.5% respectively. Momin Shakir (2007) [38, 36] and Momin Heena (2008) [36] studied toxicological aspects of lead & zinc on histology of liver & gills of fresh water fish *Rasbora daniconius*. B.S. Khangarot V.K. Rasbanshi (1979) [5] [5] studied toxicity & behavioral response of fish *Rasbora daniconius* when exposed to zinc stress. Bhagadeadvaiti and *et al.*, (2013) [10] studied bioaccumulation kinetics and bioconcentration factors for Polycystic Aromatic Hydrocarbons in tissues of *Rasbora daniconius.* They found that PAH burdens differs from one tissue to another and that is possible to correlate the same with the lipid content of the tissue and exposure time in case of either PAH. Ghanbahadur Ashwini & associates, (2012) [12] evaluated effect of pH on endosulfan toxicity in *Rasbora daniconius*. They observed that the percentage of mortality increased with an increase in toxicant concentration, duration of exposure and also pH concentration. Similar observations were recorded by Marking *et al*, (1981) [34], Russo *et al*, 1981) [53]. R.M. Ganeshwade and coworkers (2009) estimated LC_{50} values for *Puntius ticto* and *Rasbora daniconius* with Dimethoate pesticide. They found that Dimethoate was found to be more toxic to *Rasbora* & *Puntius*, as the LC_{50} values were 3.936 ppm & 5.070 ppm respectively. Khanapure & Mudkhede (2013) [26] also evaluated toxicity of Dimethoate to fresh water fish *Rasbora daniconius*. The LC_{50} values of toxicant to the fresh water fish *Rasbora daniconius* at various exposure periods are 11.32, 10.69, 10.69 and 9.97 ppm for 24, 48, 72, & 96 hrs. respectively. They concluded that the fish *Rasbora daniconius* is more susceptible to Dimethoate toxicity as the LC_{50} values for this organophosphate is less than other reported fish species. K.B.Sangve (2015) [24] noticed histological alteration in the liver such as hypertrophy of hepatic cells, loss of polygonal shape, loss of cell boundaries, vacuolar degeneration etc. of fish *Rasbora daniconius*, exposed to sodium fluoride.

Thus it has been seen that considerable work has been done by using variety of toxicants on various physiological aspects of fish, *Rasbora daniconius*, but very meager data is available on the toxicity of organochlorine& synthetic pyrethroid insecticides on the fish *Rasbora daniconius*.

Bhiwandi is city, in the district of Thane, in the western state of Maharashtra, in India, located 20km to the north- east of Mumbai and 15 km to the north -east of Thane city. The exact location of Bhiwandi 19.296664ºN 73.063121°E. The Diwanshah pond is situated 2km away from Bhiwandi and it is second largest water body after Varhala Lake and is around 50 years old. It is located $19^{o}17^{l}41^{ll}$N $73^{o}2^{l}48^{ll}$E & around 1.03 hectare and 5.4 meters depth (www.bhiwandiinfo.com2011). It is being used for fish culture. It has been found to be mostly polluted because of washing, domestic waste, run - off from agricultural fields & dairy effluents.

Fig 3: Satellite image of Diwanshah Pond

Before starting the project, the water of Diwanshah pond was analysed for various pesticides and insecticides. It was observed that endosulfan, cypermethrin& cyhalothrin were present in the water. The present study has been confered to endosulfan and cyhalothrin only, as their amount were comparatively higher thencypermethrin.

Besides this the Fish *Rasbora daniconius*, was selected as model system because it is being cultured for commercial purpose in Diwanshah pond. It is sold in the nearly local market for human consumption.

Both the pesticides that have been selected for the present studies commonly used to control pest of vegetables, ornamental plants & fruits in Bhiwandi. Endosulfan was developed during early 1950s by Hoechst (now Bayer crop science) pharmaceutical. In 1954 Hoechst won USDA (United State Dept. of Agriculture) approval for the use of endosulfan in the United States (Agency to toxic substances and disease registry, 2000) [1]. Cyhalothrin was developed in 1997 by Cheminova, a company in Auriya industries A/S. (Denmark) & registered by the U.S. Environmental Protection Agency (EPA) (pesticide fact sheet number 171, 1988).

Endosulfan is an off- patent organochlorine insecticide and ascaricide that is being phased out globally. The two isomers, endo and exo, are known popularly as l & ll. Endosulfan sulfate is a product of oxidation containing one extra O atom attached to the S atom.

Endosulfan became a highly controversial agrichemical (Bayer to stop selling endosulfan-2009) due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor. Because of its threats to human health and the environment, a global ban on the manufacture & use of endosulfan was negotiated under the Stockholm convention in April 2011. More than 80 countries including the European union, Australia, Newzealand, several west African nations, the United States, Brazil and Canada had already banned it or announced phase- out by the time the Stockholm convention ban was agreed upon. It is used extensively in India, China and few other countries. It is produced by MakhteshinAgan and several manufacturers in India and China.

Endosulfan is one of the most toxic pesticides in the market today, responsible for many fatal pesticides poisoning around the world (Pesticide Action North America, 2006) [45]. Endosulfan is also a Xenoestrogen - a synthetic substance that imitates or enhances the effect of estrogen and it can act as an endocrine disruptor, causing reproductive and developmental damage in both animals and humans. Whether endosulfan can cause cancer is debated. With regard to consumersintake of endosulfan from residues on food, the Food & Agriculture Organization of United Nations has concluded that long term exposure from food is unlikely to present a public health concern, but short term exposure can exceed acute reference doses (pesticide residues in food, 2008).

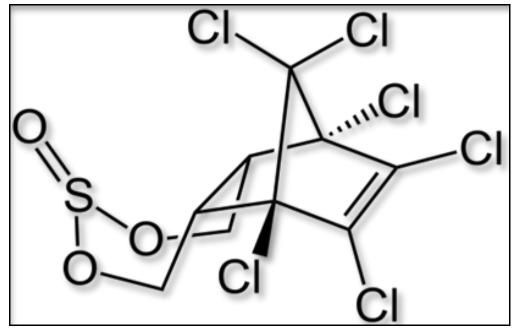

Fig 4: Structure of Endosulfan

Cyhalothrin an organic compound that is used as a pesticide. It is a pyrethroid, a class of man-made insecticide that mimics the structure and insecticidal properties of the naturally occurring insecticide pyrethrum - which come from the flowers of chrysanthemums. Synthetic pyrethroids, like Lamba - cyhalothrin, are often preferred as an active ingredient in insecticides because they remain effective for longer period of time. It is colorless, solid, although samples can appear beige, with a mild odor. It is non - volatile and used to control insects in cotton crops (Robert L. Metcalf, 2002) [52].

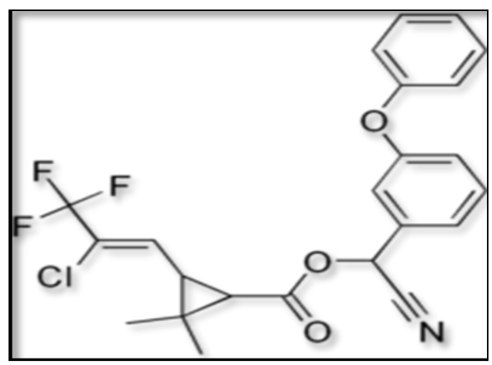

Fig 5: Structure of Cyhalothrin

Cyhalothrin is very toxic to aquatic organism. It is hazardous to the environment; special attention should be given to aquatic animals and mammals. The substances may cause long term effects in aquatic environment. Great care should be given to avoid its release, through inappropriate disposal (Momin Heena, 2011) [37].

Though both the pesticides used for the present studies are potentially toxic, hazardous and even banned by international bodies to manufacture, sale and use in the environment, but still find its use in different parts of world and even in different states of India because they are cheap, easily available and can kill broad range of pest.

Materials and Methods

An insecticide 'Endosulfan' and 'Cyhalothrin' that has been selected for the present studies are being commercially sold in all the agrochemicals stores and being commonly used to control the pest of fruits, vegetables and ornamental plants in Bhiwandi city.

Test fish selected for the present study is a common edible fresh water fish, *Rasbora daniconius* (HAM.) a species that not has been studied much especially in terms of pesticide toxicity in this country. It is easily available, hardy, adaptable in aquarium can be easily bred. Being smaller in size and rich in protein, mostly consume by poor section of society without beheaded & gutted (M.shakir, 2007 & M. Heena, 2008) [38]. It is also known as *Rasbora zanzibarensis*, *Leuciscus malabaricus, Oparius daniconius* etc.

The experiment was started in the month of June-2009. The test fish, *Rasbora daniconius* were regularly brought from Diwanshah Lake situated in Bhiwandi, Dist-thane with the help of local fisherman's. As a general rule of toxicology, these were acclimatized in the Laboratory for more than two weeks before actual use for bioassay. These fish were maintained in the large glass aquarium containing aged tap-water. This aged tap-water leads to stabilize the constituents of water and more important in the elimination of chlorine. The water in tank was about 2-litres per gram wet weight body of the test fish. The water in the tank was constantly aerated with the help of aerator to maintain the dissolved oxygen (DO) relatively constant. During this acclimatization period fishes were fed with dried tubifex worms and daphnia available in the market. But feeding was stopped 24 hrs. prior to the acute toxicity test to avoid focal matter and urine contamination. Care was taken to avoid overcrowding and over feeding or malnutrition of fish. Dead fish if any was immediately removed to avoid any type of infection. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. Every alternate day 90% of water was changed. During the Day time, fish were exposed to diffused light coming through the windows. Overcrowding was avoided and they were kept free from any disturbances and mechanical shock.

Glass aquaria measuring 30x 15 x20 cm were used as a test container. They were washed thoroughly by water and 1% potassium permagnate to avoid any type of bacterial and fungal infection before used for stocking the test fish.

As a general rule for an acute toxicity test, the minimal criterion for acceptable water is that the organism will live in it during acclimatization to the test condition. Ordinary tap-water was used for bio-assay tests and was stored in big reservoir (plastic trough) for more than 48hrs.before actual use. This is generally done to remove residual chlorine. Determination of physical and chemical characteristics of test water including P^H, dissolved oxygen, hardness, acidity, alkalinity ware carried out according to the standard methods of APHA (16th ed, 1985).The average values all the parameters studied are given below the each table.

Ten fish of approximately same size and weight were used for each concentration tested without sexual-discrimination. Ten fish of similar size and weight were also kept in toxicant free water under analogous conditions. The average size of the test fish, *Rasbora daniconius* was 5.3± 0.5 cm in length and the weight being 3.0 ± 0.5gms.

Before the beginning of the acute toxicity test it is necessary to calculate the upper and lower limit of the concentrations which give 100%and 0% mortality respectively. For this purpose some pilot experimental test were carried out using range of concentrations for 96hrs. The 1000 PPM stock solution of both the toxicants were prepared by dissolving 0.57ml of Endosulfan 35%EC and 0.75ml of Cyhalothrin 2.5% EC using water as a solvent. The desired concentrations were achieved by dissolving stock solution with water. The water was replaced after every 24hrs. to keep the desired concentration of the toxicant constant, during the 96hrs.experimental period. Control test with zero toxicant concentration were simultaneously performed exactly under similar condition.

The result of acute toxicity tests of both the toxicants were expressed as LC_{50}. It is an interpolation values based on the percentage of dead Fish at two or more concentration at which less than half or more than half of the fish died. With the help of mortality data straight line were drawn through the point expressing mortality at a particular concentration at 24hrs.interval during 96hrs. period. The concentration at which the line crossed the 50% survival line is respective LC_{50} values. Straight line graphical interpolation methods described by Litchfield & Wilcoxon, (1949) [30] were used.

During the toxicity test, the other important observations like fish behavior and skin melanophore responses under toxic environment were also critically observed, recorded and compared with fish under pesticide free water.

Fish behavior was studied by general observation and skin melanophore was studied by transferring the pesticides treated fish in enemal bowl and comparing with the control one.

Result and Discussion

The result of toxicity measurement and calculations (LC_{50}) by Litchfield & Wilcoxon (1949) [30] are summarized as follows.

Table 1: Percentage mortality of fish when exposed to different concentrations of Endosulfan

	Time of exposure in hrs.				
	Concentrations	**24**	**48**	**72**	**96**
Concentrations in mg/liter	.002	00%	00%	10%	20%
	.006	00%	10%	20%	30%
	.010	10%	20%	30%	40%
	.014	20%	20%	40%	50%
	.018	30%	30%	50%	60%
	.022	40%	50%	60%	60%
	.026	50%	60%	70%	70%

	.030	60%	80%	80%	90%
	.034	70%	80%	90%	100%
	.038	80%	90%	100%	-
	.042	100%	100%	-	-

Table 2: Percentage mortality of fish when exposed to different concentrations of cyhalothrin

	N				
	Concentrations	**24**	**48**	**72**	**96**
Concentrations in mg/liter	0.01	00%	10%	10%	20%
	0.05	00%	10%	20%	30%
	0.10	10%	20%	20%	40%
	0.15	20%	30%	40%	50%
	0.20	30%	40%	60%	70%
	0.25	40%	60%	70%	90%
	0.30	60%	70%	80%	100%
	0.35	60%	80%	90%	100%
	0.40	70%	80%	100%	-
	0.45	80%	90%	100%	-
	0.50	90%	100%	-	-

Table 3: Physico-chemical parameters of water used for toxicological study

Temperature	**28°C**
PH	7.5
DO	6mg|Litre
Free Chlorine	Nil
Total Acidity	3.2mg/Litre
Total Alkalinity	45 mg/Litre
Total Hardness as $CaCO_3$	32 mg/Litre
Length of fish	5.3 ± 0.5cm
Weight of Fish	3 ± 0.5gm

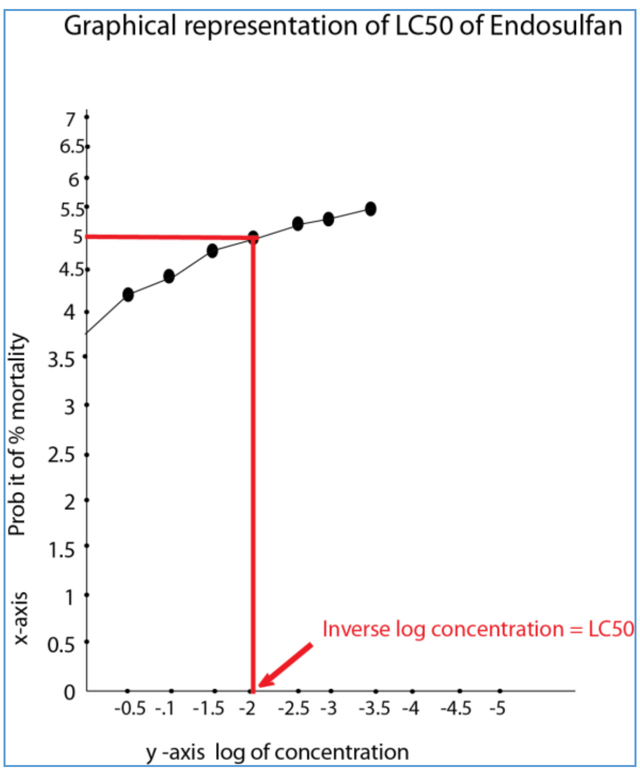

Fig 6: LC_{50} value of endosulfanby graphical interpolation method is found to be 0.01mg/litre.

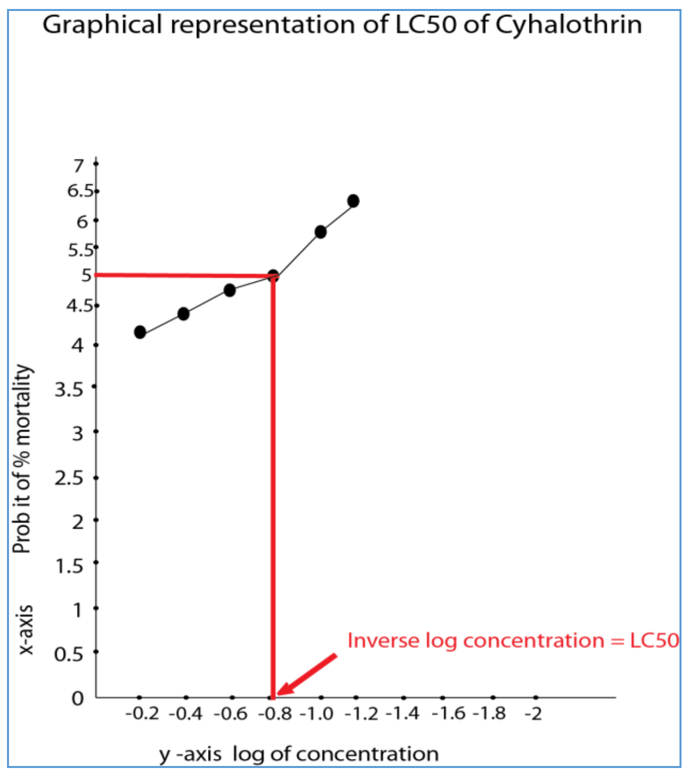

Table 7: LC_{50} value of Cyhalothrin by graphical interpolation method is found to be 0.15mg/litre

Fish behaviour exposed to Endosulfan

The fish, *Rasbora daniconius* when exposed to different concentrations of Endosulfan at different time interval showed marked behavioral changes compared to control as initial response to the toxicant, especially at higher concentration, a sudden hasty movement was noticed in the fish-which also involves erratic swimming, convulsion, jerky movement. The fish struggle hard for breathing, sometime engulfing atmospheric air as an adaptation to avoid toxic environment. The fishes tried to leap out the toxic medium and a thick mucus covering were seen over the whole body.

Fish behavior exposed to Cyhalothrin

The effect of Cyhalothrin on *Rasbora daniconius* is almost same as in case of fish exposed to Endosulfan. Thus behavioral change in feeding activity, jumpy and erratic swimming movements, and orientation of body part, opercular motion, and surfacing activity were observed when the fish were exposed to various concentrations of Cyhalothrin. These observations are well supported by earlier finding from other toxicity studies (Fingerman *et al*, 1980 and Tiwari *et al*, 2011) [15, 61].

The alteration in the general behavior is mainly due to its adaptive nature, by which it tries to adapt with the changed environment (Momin Heena, 2011) [37]. A number of other such investigations have been done so far by using variety of fish and various aquatic pollutants.

Soman (1987) [56] studied toxicity of fenthion, thiodan, DDT, malathion and nuvan to fish *Colisa fasicata*. He observed that with thiodan, fishes became excited; they showed loss of equilibrium, loss of skin pigmentation and grasping the atmospheric air. With DDT, fishes showed respiratory distress and a loss of equilibrium. Erratic swimming and convulsion after exposure to endosulfan have also been reported by Paul and Raut (1987) [44] in their study of toxicity of endosulfan to five different species of fishes including 3 carp's species and two Indian carps *Catla catla* and *Labeo rohita.* No correlation was found between the fish reaction and the insecticide nature by Basak and konar (1976) [7]. They observed that Singhi killed by organophosphorus insecticide lost skin pigmentation. Jacob *et al*, (1982) [23] noted undulation of the body, increased oscillation of fins, rapid and irregular movements of the opercular folds, loss of equilibrium and excitation in fishes A. *lineatus* and *M. cupanus* exposed to DDT, ekalux, malathion and sevin. Similar behavioral changes have also been reported by Lal and vohra (1976) [28] with thiodan exposed *Channa punctatus* and Sharma *et al*, (1984) [55] with *Lebeo rohita* exposed to sumithion 50% and kitazin 50%. Sambasiva Rao and Chandrashekhara Rao (1987) [54] studied combined and independent action of carbaryl and Phenthoate on snake head, *Channa punctatus.* They observed that fish exhibit erratic swimming which indicates loss of equilibrium which was more specific in carbaryl and phenthoate showing synergism. They have attributed this to the effect of insecticide on the region of brain which is associated with the maintenance of balance.

Webb and Brelt (1973) [62] studied the effects of sodium pentachlorophenate on growth rate and swimming performance on Sockeye salmon (*Oncorhychus nerka*). Opercular movement has been decreased with increase in toxicant concentration and accumulation of more fecal matter was observed in the container observed by Imtiyaz *et al*, (2012) [22]. Decrease opercular movement probably helps in reducing absorption of pesticide through gills. Behavioral changes of *Labeorohita* have been studied for various chemicals by Mariqouder *et al*., (2009) [33] and Pandey *et al*., (2009) [40]. The treated fishes also showed fading of their body colour before death, these changes can be considered as symptoms of stress on account of the toxicological nature of the environment. Sweilum (2006) [59] reported that the abnormal movements are noticed in the fish *Tilapia,* when applied to different concentrations of dimethoate pesticides. The surfacing phenomenon of fish might be due to hydrotoxic condition of the fish and these results are supported by AppaRao *et al*, (2009) [3] and Charjan *et al*., (2008) [12]. The decrease in body weight could be due to excessive expenditure of more energy on metabolism in fish growth and it was proportionate to the concentration of the pesticides. Similar results werereported by Balasubramani *et al*., (2008) [6] and Cook *et al*., (2005) [13].

Effects on skin melanophores

The body covering of multicellular animals is usually pigmented and it is a matter of general observation as well as careful experimentation that such coloration is frequently cryptic and often provides help in indicating the extent of pollution in the environment (Gopi, L.1994). The pollutants like pesticides are widely used in agriculture and are drained into the nearby rivers or ponds which are the natural habitat of fishes.

Fish skin, directly exposed to the ambient toxicants; is used extensively as a potential indicator of contaminated aquatic environment. Hence, it is important to study the insecticide effect on melanophore cells to evaluate the extent of stress.

In the present study, it is found that due to pesticide toxicity experimental fish were found to befall pale as compare to normal fish (control one) which readily adapt to the changed background, while treated fish showed delayed response. Due to continuous exposure of fish for 4 weeks at high sublethal doses of Endosulfan & Cyhalothrin, themelanophores granules may get aggregated and pigment cell got ruptured causing paleness of animal.

Several studies showed effects of hormones, chemicals & drugs on chromatophore have reviewed byFujii and Oshima 1986, Yamada et.al, 1984. Melanophores are effectively used as indicators of pesticides pollution.

However the effects of pesticide on fish chromatophore have focus little attempt taken by some investigators. Rao and Rao (1987) [54] have reported distinct change in skin colour, from normal black or dark grey to light grey under carbaryl and phenthoate influence in *Channa punctatus*. Gopi (1992) [19, 20] observed destructive changes in melanophore of *Cyprinus carpio* and has relate them to the concentration of fenthion used. Kaur and Toor (1978) [25] have reported the effects of different concentration of diazinon, fenitrothion, carbaryl, malathion and phosphamidon on the development of melanophores in young fry of *Cyprinus carpio*. Pandey *et al*., (1981) [41] has reported significant reduction in mean area of melanophores on exposure to malathion. Mankar and Kulkarni (2000) [32], focussed on endocoel impact on chromatophores population of fresh water fish *Channa orientalis*. Kulshrestha and Arora (1988) [27] observed that fish exposed to DDT showed dullness melanophores aggregation. The present study are apparent with the finding of other workers.

Conclusion

Acute toxicity studies are the first step in toxicological research. All the observations indicates the impact of Endosulfan and Cyhalothrin toxicity and caused behavioral alterations, such as those observed in this study may result in severe physiological problems, ultimately leading to the death of fish at relatively low concentration and even at short period of exposure. Therefore, the present investigation demonstrates a relation among pesticidal stress, behavioral disorder, and survival and mortality rates. Thus, this finding can be used as a tool for creating awareness among the local farmers so that the use of the highly toxic pesticides can be minimized. Morever paleness of skin of test fish, *Rasbora daniconius* caused by destructive changes in melanophores of treated fish are directly related to the concentration of pesticides(endosulfan& cyhalothrin) in the medium. This extensive damage noticed in melanophore could be due to the accumulation of pesticides i.e endosulfan& cyhalothrin in the nervous tissues and also may be due to the changes in electrolyte regulation. The photochromatic changes (changes in melanophore) seen in the present study is a significant abnormal response given by test fish, *Rasbora daniconius* under pesticides stress providing a symptomatic index of pesticidal toxicity. Further the effects of endosulfan & cyhalothrin on the melanophore of fish is found to be identical.

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