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Impact of Dicofol and Cypermethrin on protein metabolism of freshwater fish Catla Catla

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Abstract
The freshwater fish Catla Catla was exposed to Dicofol an Organochlorine pesticide and Cypermethrin a synthetic pyrethroid which is widely used in agriculture for several crops such as paddy, cotton and vegetables to control serious insects and mites in many areas of the Guntur district, Andhra Pradesh, India. The LC50 values determined for Dicofol 18.5%EC and Cypermethrin 10%EC at 24hrs were 1.82ppb and 1.68ppb respectively.1 /10th of lethal concentration for 96hrs LC50 value is considered as sub lethal concentration. The exposure of fish to a lethal concentration for 24hrs and sub lethal concentration for 8 days and investigated significant changes in the vital organs by following standard protocols. The percent decrease of total proteins was observed.

Keywords: Dicofol, Cypermehrin - Total proteins - Catla Catla – freshwater.

1. Introduction

Pesticides are extensively used worldwide in Agricultural Practices to control pests and increase crop yield. In recent years, their use has increased considerably. There are several definitions of pesticide; the Food and Agriculture Organization (FAO) defines a Pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest during the production, processing, storage or marketing of food in all agricultural commodities for controlling the pests (FAO 1986). For controlling the different types of pests a wide variety of chemical pesticides are used. These are primarily Organochlorines, Organophosphorous, Carbamates, Pyrethroids, and various inorganic compounds.

As an agricultural developing nation, India too relies predominantly on chemical pesticides to sustain a large population. Pesticides have the potential to kill a wide variety of insect pests and in doing so they harm the ecosystem in general and human health in particular. At present, India is the second largest producer of pesticides in Asia and also ranks twelfth in the consumption of world Pesticides (Eds, et al.,1972). The majority of the population in India (56.7%) is engaged in agriculture and are inevitable to exposure to the pesticides used in agriculture (Gupta, 2004).

Pesticides and related chemicals destroy the delicate balance between species that characterizes a functioning ecosystem. Pesticides produce many physiological and biochemical changes in freshwater organisms by influencing the activities of several enzymes. Alterations in the chemical composition of the natural aquatic environment usually affect behavioral and physiological systems of the inhabitants, particularly those of the fish [Radhaiah et al 1987].

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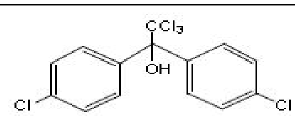
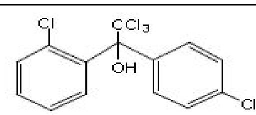
1.2. Dicofol

(IUPAC Name: 2, 2, 2-trichloro-1, 1-bis (4-chlorophenyl) ethanol) is an organochlorine miticide used on a wide variety of fruit, vegetable, ornamental and field crops [Binoy etal.,2004, Extoxnet,1992]. Dicofol is structurally similar to DDT. According to the World Health Organization [WHO,1996], Dicofol produces stimulation of axonal transmission of nerve signals, believed to be related to inhibition of ATPases in the central nervous system (CNS).

Table (1) Chemical identity of Dicofol

Common name IUPAC Chem. CAS chemical name	Dicofol 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol Benzenemethanol, 4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)-(CASRegistry)[1] 4-chloro-alpha-(4-chlorophenyl)-α-(trichloromethyl) benzene-methanol (WHO, 1996) 1,1-bis(4'-chlorophenyl)2,2,2-trichloroethanol (UNEP/POPS/POPRC.9/3)
Other names	1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol and 1-(2-chlorophenyl)-1- (4- chlorophenyl)-2,2,2-trichloroethanol ('p p'- and o, p'-isomer) (US EPA, 1998)
CAS registry number	115-32-2 (dicofol; p,p'-dicofol); 10606-46-9 (o,p'-dicofol
Trade name	1,1-bis(chlorophenyl)-2,2,2-trichloroethanol; 4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)-; Acarin; Benzenemethanol; Carbax; Cekudifol; CPCA; Decofol; Dicaron; Dichlorokelthane; Dicomite; Difol; DTMC; ENT 23648; FW293; Hilfol; Hilfol 18.5 EC; Kelthane; Kelthanethanol; Kelthane A; Kelthane (DOT); Kelthane Dust Base; Kelthane 35; Milbol; Mitigan; p, p-dicofol; NA2761 (DOT); NCI-C00486 (WHO, 1996).
Molecular formula	$C_{14}H_9Cl_5O$
Molecular weight	370.49
Structural formulas of the isomers	 p,p'-dicofol CAS No. 115-32-2  o,p-'dicofol CAS No. 10606-46-9

1.2.2. Cypermethrin

Chemical identity:

(RS)-alpha-cyano-3-phenoxybenzyl-(1RS,3RS,1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC name) (RS)-cyano(3-phenoxyphenyl) methyl(1RS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate (Chemical Abstracts name)

C.A.S. number: 52315-07-8

Cypermethrin is a mixture of all eight possible chiral isomers (see alphacypermethrin monograph) Structural formula:

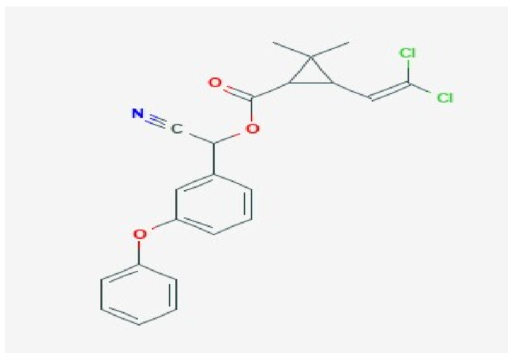


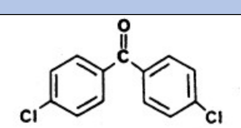
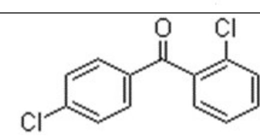
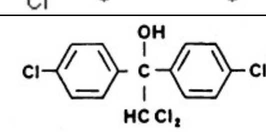
Figure (1) Cypermethrin

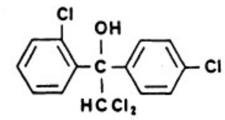
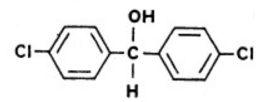
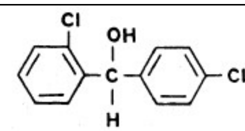
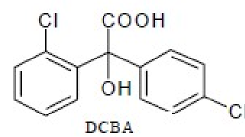
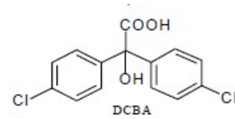
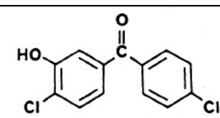
Molecular formula: C22H19Cl2NO3

Molecular weight: 416.3

Synthetic pyrethroids are one of the wide varieties of pesticides contributing to these situations. Cypermethrin is one of the newly synthesised insecticides. Hence the thought of using plant extracts, namely pyrethroids received much attention. But these insecticides also tend to affect the biology of non-target species along with pests (Reddy and Yellamm, 1991; Veeraiah and Durga Prasad, 1998).

Table (2) Chemical identity of environmental degradation products of dicofol (Source: US EPA 2009; Spain 2006; CAS REGISTRY 2015; Chemspider 2015)

Chemical (CAS Number)	Chemical Name	Molecular weight (g/mole)	Structure
p,p'-DCBP (90-98-2)	4,4'-dichlorobenzophenone	253	
o,p'-DCBP (85-29-0)	2,4'-dichlorobenzophenone	253	
p,p'-FW-152	1,1-bis(4-chlorophenyl)-2,2- dichloroethanol	336	

Chemical (CAS Number)	Chemical Name	Molecular weight (g/mole)	Structure
o,p'-FW-152	1-(2-chlorophenyl)-1-(4'-chlorophenyl)-2,2-dichloroethanol	336	
p,p'-DCBH (90-97-1)	4,4'-dichlorobenzhydrol	253	
o,p'-DCBH (43171-49-9)	2,4'-dichlorobenzhydrol	253	
o,p'-DCBA	2,4'-dichlorobenzilic acid	297	
p,p'-DCBA (23851-46-9)	Bis(4-chlorophenyl) (hydroxy) acetic acid, 4,4'-dichlorobenzilic acid	297	
3-OH-p,p'-DCBP	3-hydroxy-4,4'-dichlorobenzophenone	267	

1.2.3. ***Catla Catla***

Body short and deep, somewhat laterally compressed, its depth more than head length; head very large, its depth exceeding half the head length; body with conspicuously large cycloid scales, head devoid of scales; snout bluntly rounded; eyes large and visible from underside of the head; mouth wide and upturned with prominent protruding lower jaw; upper lip absent, lower lip very thick; no barbels; lower jaw with a movable articulation at symphysis, without a prominent process; gill rakers long and fine; pharyngeal teeth in three row, 5.3.2/2.3.5 pattern; dorsal fin inserted slightly in advance of pelvic fins, with 14 to 16 branched rays, the simple rays non-osseous; anal fin short; pectoral fins long extending to pelvic fins; caudal fin forked; lateral line with 40 to 43 scales. Greyish on back and flanks, silvery-white below; fins dusky.

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Figure (2) ***Catla Catla***

1. 3The Study Area

1.3.1Location

Guntur is located at 16.29°N 80.43°E. It has an average elevation of 33 m (108 ft) and is situated on the plains. There are few hills in the surrounding suburban areas and Perecherla Reserve Forest on the north west. The city is around 64 km (40 mi) to the west of the Bay of Bengal on the east coast of India. The Krishna delta lies partly in the Guntur district. There are other smaller rivers and channels in the region such as Guntur Channel, Chandravanka, Naagileru, Guntur Branch Canal.

Figure (3) The study area Location

3.1.1 Climate

As per Köppen-Geiger climate classification system the climate in Guntur is tropical (Aw). The average temperature is warm to hot year-round. The summer season (especially during May and June) has the highest temperatures, but these are usually followed by monsoon rains. The winter season (from November to February) is the most enjoyable with a pleasant climate. Winter months are usually dry, with little to no rainfall. The wettest month is July. The average annual temperature is 28.5 °C (83.3 °F) and annual rainfall is about 905 millimetres (36 in). Rain storms and cyclones are common in the region during the rainy season, which starts with the monsoons in early

June. Cyclones may occur any time of the year, but occur more commonly between May and Novembe.

2 Material and Methods

The freshwater fish *Catla Catla* of size 5-6 ±1cm and 6-7 g weight were brought from a local fish farm and acclimatized at 28 ± 2 0C in the laboratory for one week all fish bring it from Market of fish in Guntur India we made analysis in Lap of Acharya Nagarjouna University India. The stock solutions for Dicofol 18.5% Emulsifiable Concentrate (EC) and Cypermethrin 10% Emulsifiable Concentrate (EC) were prepared in 95% acetone to yield a concentration of 100mg100ml-1 which was further diluted with distilled water to get a working solution. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a capacity of ten liters. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from ten percent to ninety percent for 24hrs in static tests. The concentration that produced fifty percent mortality in test species noted. LC50 values were calculated by Finney's Probit analysis.

The total protein content of the pesticide exposed tissue samples was estimated according to the modified standard method (Lowry et al., 1951). The Quantity of 5% homogenate of brain, gill, kidney, liver and muscle were isolated, precipitated with 5% TCA then centrifuged at 3000 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 1N NaOH solution and 0.2 ml of extract taken into the test tube and mixed with 5 ml of alkaline copper solution. To this 0.5 ml of 50% Folin Phenol reagent was added after 30 minutes, the optical density was measured at 540 nm against the blank. The standard graph was plotted by using Lowry's method with bovine serum albumin (BSA) as the standard solution. The values are expressed as mg/g wet weight of the tissue.

3 . Results and Discussion

The mean values obtained for total proteins in different tissues of fish and percent change over control along with standard deviation are given in Tables 3 and 4 and are graphically represented in Figures 4 and 5.

In one day, control fish of Catla Catla, the total protein content is in the order of liver > muscle > kidney > brain > gill.

During the exposure of lethal concentration of test toxicants, percent change of total protein depletes in the order of

Dicofol - liver > muscle > kidney > brain> gill.

Cypermethrin-- liver > muscle > brain > kidney> gill

During the exposure of sub lethal concentration of test toxicants for 8days, percent change of total protein depletes in the order of

Dicofol - liver > muscle > brain > kidney> gill.

Cypermethrin-- liver > muscle > brain > gill> kidney

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Table (3) Changes in the total protein (mg/g wet weight of tissue) in different tissues of Catla Catla on exposure to lethal concentrations of Dicofoland Cypermethrin for 24hrs

		Dicofol		Cypermethrin	
Organs	**control**	**Lethal**	**% Change Control Vs lethal**	**Lethal**	**% Change Control Vs lethal**
Brain	115.37± 0.01	104.94± 0.01	8.9	102.80± 0.005	10.89
Gill	110.03± 0.005	101.01± 0.05	8.19	100.42± 0.05	8.73
Kidney	118.06± 0.005	107.50± 0.01	8.94	105.90± 0.01	10.29
Liver	131.09± 0.03	111.92± 0.05	14.62	109.68± 0.003	16.33
Muscle	122.02± 0.01	109.91± 0.01	9.92	107.21± 0.01	12.13

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

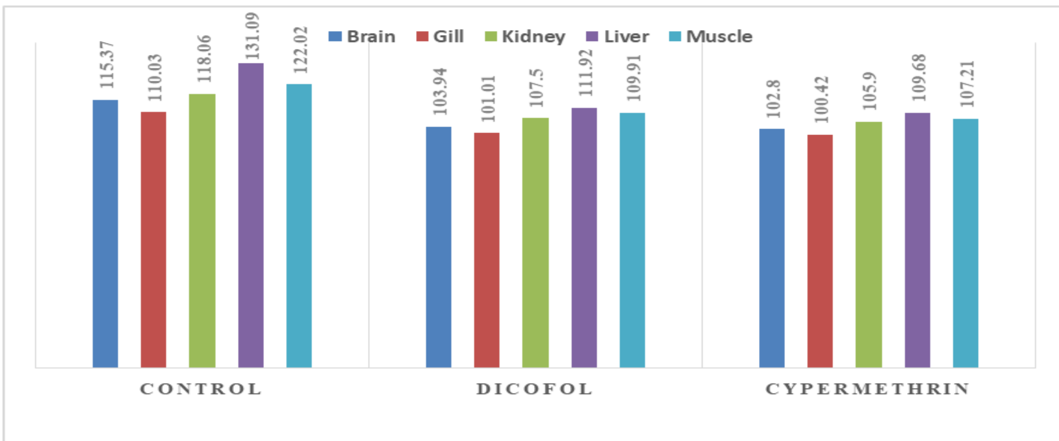

Figure (4) Changes in the total protein (mg/g wet weight of tissue) in different tissues of *Catla Catla* on exposure to lethal concentrations of Dicofol and Cypermethrin for 24hrs.

Table (4) Changes in the total protein (mg/g wet weight of tissue) in different tissues of *Catla Catla* on exposure to lethal concentrations of Dicofol and Cypermethrin for 8days.

		Dicofol (Control Vs sub lethal)		Cypermethrin (Control Vs sub lethal)	
Organs	**control**	**Sub lethal**	**% Change**	**Sub lethal**	**% Change**
Brain	87.78± 0.5	78.06± 0.005	11.07	74.02± 0.01	15.81
Gill	76.02± 0.03	67.99± 0.005	10.56	64.03± 0.005	15.77
Kidney	104.31± 0.005	93.20± 0.005	10.65	90.06± 0.005	13.66
Liver	122.57± 0.06	102.03± 0.005	16.75	98.04± 0.005	20.01
Muscle	115.20± 0.1	100.03± 0.005	13.16	96.02± 0.005	16.91

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

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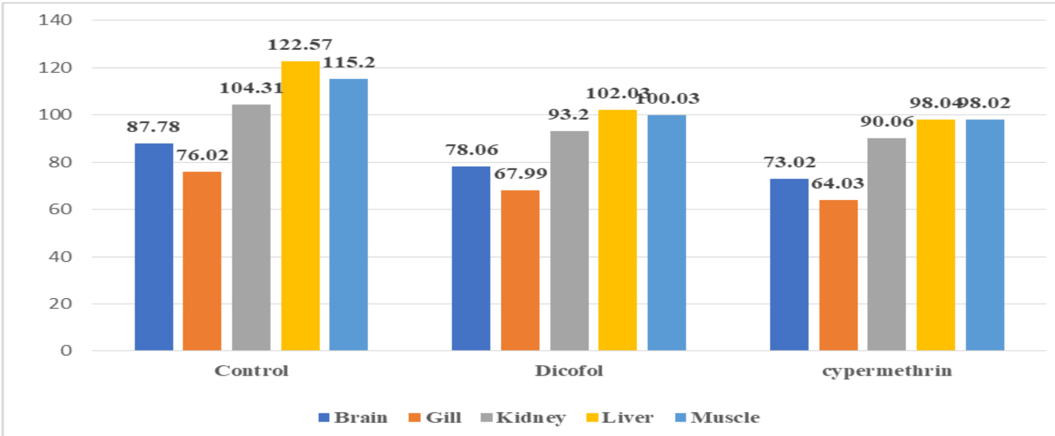

Figure (5) Changes in the total protein (mg/g wet weight of tissue) in different tissues of Catla Catla on exposure to Sub lethal concentrations of Dicofol and Cypermethrin for 8days.

Biochemical changes induced by pesticide stress led to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism [Tham et al., 2009]. The liver, kidney, brain and gills are the most vulnerable organs of a fish exposed to the medium containing any type of toxicant [Jana, S. and Bandyopadhyaya, S. 1987]. Proteins occupy a unique position in the metabolism of cells because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways [Harper 2006, Leninger,2008].

The decreased trend of protein content in various tissues of Catla Catla in the present study may be due to metabolic utilization of keto acids in the synthesis of glucose or for the osmatic and ionic regulation as mention by Mamata Kumari (2007), Chezhian et al., (2010).

The present study revealed the reduction in protein levels in the tissues of Catla Catla by following acute exposure of toxicants Dicofol and Cypermethrin. A similar change was observed in Labeo rohita exposed to Endosulfan and Fenvalerate by (Suneetha (2014) and Tilak et al., (2003) explained the reduction of protein content of liver, brain and ovary of C. punctatus exposed to fenvalerate.

4.Conclusion

Significant changes in the levels of proteins and transaminases were noticed under lethal and sublethal concentrations of Dicofol and Cypermethrin for 24 h and 8 days. The toxicant exposed tissue samples showed a significant decrease in total protein content and induction in enzymes were observed. The depletion of proteins under the stress of Dicofol and Cypermethrin toxicity observed in different tissues of *Catla Catla* indicates the proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The alterations in the levels of activity of aminotransferases induced by these pesticides clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems.

The use of pesticides has helped to enhance economic gains through crop protection yet they have had serious implications for human health and non-target plants and aquatic organisms by accumulating in food and water. Though one cannot avoid the use of pesticides, measures should be taken for the conservation of the water and also the aquatic resources, by following a number of least-toxic methods.

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التأثير السام للديكوفول والسيمبيرميثرين على استقلاب البروتينات السمكية

CATLA CATLA

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الملخص

تعرضت أسماك المياه العذبة *CATLA CATLA* لمبيد الديكوفول وهو عباره عن مبيد عضوي حيث يحتوي هذا المبيد على كليربيريثرويد وبيرويثرويد اصطناعي يستخدم على نطاق واسع في الزراعة ولعدة محاصيل منها الأرز والقطن والخضراوات للسيطرة على الحشرات والتي منها حشرة العث الخطيرة في منطقة كنتور ولاية أندرا براديش الهند، حيث كانت قيم LC50 المحددة بالنسبة للتوصيل الكهربائي ونسبة مبيد الديكوفول 18% وكانت نسبة السايبرو10% عند 24 ساعة الأولى عند تركيز 1.82 جزء من الألف و 1.68 جزء من الألف على التوالي حيث تعرضت الأسماك في هذه الدراسة إلي التركيز المميت للمبيد لمدة 24 ساعة متواصلة لمدة 8 أيام على التوالي حيث تم بحث التغيرات الهامه في زيادة النسبة المئوية لنشاط الأعضاء الحيوية بإتباع التحاليل والقياسات العلمية المتبعة.

الكلمات المفتاحية: ديكوفول – سايبر –البروتينات الكلية – *CATLA CATLA* – المياه عذبة.