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Toxic effects of the herbicide 2,4-Dichlorophenoxy acetic acid on fish Physiology and its Antioxidant

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Abstract :

The concept of modem herbicide technology began to develop in 1900, and accelerated rapidly with the discovery of Dichlorophenoxy acetic acid (2,4-D) as a growth regulator in 1944-1945, since the introduction of 2.4-D, a wide variety of organic herbicides have been developed and have received wide usage in agriculture, forestry and other industries. This study was designed in such a way to assess the toxicity effect of 2.4-D pesticide in fish physiology and its antitoxidant stress Channa Striatus fishes were administered with two different dose compensation of 2,4-D pesticide 100 and 200 mg/kg. After a matter of time interval the effect of 2,4-D on fish behaviour and its toxic level was determined by assessing its blood parameter and anti-oxidant enzyme level. Hemocyte count has cons derably reduced in 2,4-D treatment and its responsive antitoxidant enzyme level of SOD and catalase level was also drastically altered. Conclude the toxic effects of 2,4-D herbicide may cause physiologic and behavioural change in fish and also affect functions such as reproduction and metabolism.

Keywords : Herbicide, Fish, Metabolism

Chemical pollution in the environment by pesticide has been increasing due to their extreme usage in agriculture alternation in the chemical composition of natural aquatic environment can affect the fresh water fauna, particularly fish. Many of these compound of or their metabolites have show toxic effects related to oxidative stress (Anushiya Hemlata 2013) 2,4-D was one of the first herbicides to be commercially marketed.

In medical science and of other aspects of pest control the control of weeds by means of herbicides has benefitted practically every one. The value of agricultural crops has been increased. (Bramhi et. al. 2003) the herbicide is used primarily by cereal crop producers. The forestry industry uses 2,4-D to suppress the growth of hard wood and under growth in conifer plantations in recent years, a significant increase in the use of 2,4-

D against agricultural pests has been observed and the rest of the world one of the major reasons for the increase of using 2,4-D and ensuring an absolute result. 2,4-D is absorbed by human Skin by prolonged contact short-term contact leads to irritation of the skin. It is fact that 2,4-D is capable of entering the body via the food chain and are harmful to the ecosystem) The accumalation of these herbicides could be analyze in tissues, fluids and organs and there by increasing the risk of developing various disease. (O'Brien 1987, Randel and Rothmann et al. 1996) This study was designed in such a way to assess the toxicity effect of 2,4-D pesticide in fish physiology and its antioxidant stress. Channa striatus fishes were administered with two different dose compensation of 2,4-D pesticide. 2,4-D also bioaccumulates in fish, meaning that fish tissues will contain a higher concentration of 2,4-D than the surrounding water and puts them at even greater risk (Cox 1999

Material and Method :-

In the present investigation toxicity of 2.4-D (Dichlorophenoxy acetic acid) of fresh water fish Charma punctatus has been studies. The effect of pesticide in the form of Dichlorphenoxy acetic acid on channa punctatus were determined by APHA (1985) and EIFAC (1983). Fishes (weight 28 + 56g were collected fromn Sagar lake. The collected fish were maintained in glass aquaria containing 100L dichlorinated tap water for acclimatization to laboratory condition for one week.. This water in aquarea was aerated continuously than dead animals were removed to avoid any contamination. They were fed on alternate days with wheat flour mixed together with Soyabean flour and mustard cake, in ratio of 3:1:L Test Water qualities were as follows:-

Temperature	-	26.a C^0 1.5°C
PH	-	7.5 + 0.04
Dissolved O .	-	6.8 +0.2 mg/L
Alkalinity	-	2.4 +2.8 mg/L

Determination of LC50 values and sublethal values LC50 values of 2,4-D(Dichloro-Phenoxy acetic acic) in Channa punctatus during 24 hr, 48 hr, 72 hr and96 hr using the probit analysis method described by Finney 1971.

Biochemical Studies :-

To investigate the effect of 2,4-D healthy fishes and weighting between 28+56 gm were selected. Fishes were divided into four groups with 10 fishes in each group fish was exposed to various sublethal concentration 40, 45, 50, 55 and 60 ppm, of 2,4-D for a period of 7 days. After the doses of toxicant the fishes were watched carefully for any

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change in their behaviour. The various biochemical parameters were studied at intervals of 24 hr, 96 hr, 7 days and 15 days. Blood samples were collected from the fish and centrifuged in a capillary, II Sodium Citrate Solution was used as an anticoagulant. After centrifugation at 5000 rpm for 10 min. serum was collected marked and refrigerated determination of Antioxidant enzyme.

Method :-

Einzyme was estimated by UV Kinetic method using span Diagnostic Reagent Kit

αC-Oxoglutarate + L-aspartate = L-glutamine

NDH + NADH2 = L-malate + NAD

Sample material -

Blood Serum

Reagent

Table 1

Buffered Substrate	Concentration of Test
Trisbuffer (ph-7.5)	30 mmol/L
Asparate	90 mmol/L
OC-Oxoglutarate	6.0 mmol/L
MDH	0.12 ulml
NADH2	6.0 mmol/L

Procedure -

In Pipette -

Buffered Substrate	-	0.01 ml
NADH2	-	0.02 ml
MDH	-	0.01 ml
Mix all sample	-	0.02 ml
Oxoglutarate	-	0.04 ml

Mix and read initial absorbance. Reading noted after 1, 2, and 3 min

Calculation -

SGOT : u/L = 1051 X A/min

Procedure :-

Dry and clean test tubes, mixed with 'C' for control and 'T' for test, Iml of reagent was mixed in both the tubes, 0.1 ml of dilute Serum was added in tube 'T and 0.2 ml of distilled water was added to tube 'C' mixed well and incubated at 37°C for 5 minutes. 0.2 ml of solution 1(A) was mixed in tube 'T' incubated at 37°C for 15 minutes. 0.1 ml of reagent 3 was mixed in both the tubes and 0.1 ml diluted serum was added in tube 'C' and incubated at 37°C for 15 minutes. 10 ml of Solution I was added in both the tubes, mixed well by inversion, and allowel to stand at room temperature for 5 minutes, O.D. of test and standard was measured against distilled water on a colorimeter, using blue filter, reagent 6 working pyruvate standard was used for standard graph.

Calculation :-

The net O.D. of test (Tn) was calculated as follows:

In = 0.D Test - O.D. Control

Net O.D. of test (In) was marked on

Y - axis of the standard curve and extrapolated to the corresponding enzyme activity (In/Litre) on X - axis.

Result and Discussion :-

Effect of 24-D on the behavour of fish Channa punctutus - Daily observation were de after exposure of fishes to different concentration of 2,4-D and effect of toxicity. Such as changes inanmance and their behaviour were recorded Table 2 Toxicity of 2.4.D

Name of Herbicide	Grade with concentration	Concentration mg/L	Duration	Martality	LC Values
2,4-D	Technical 80%	350	96 hr	All died	LC 100
		300	96 hr	Half died	LC50
		250	96 hr	All alive	LC0
		200	15 day	All alive	Sub lethal concentration
		100	15 day	All alive	Sub lethal concentration

In response to 2,4-D changes in the locomotary activities were observed the spontaneous movement gradully ceased and Finally Fishes became still, sudden jerky

movements started sometimes quick start and sometimes delayed start were observed.

Table - 3: Enzyme status in Channa striatus fish after 2,4-D treatment

Group	SOD	Catalase	Glutathione Peroxidase	Glutathione transferase
Control	140.00+9.85	4.52+0.16	1.54+0.05	36.05+5.06
100 mg/L treatment	104.00+7.16	6.82+0.37	2.06+0.22	25.19+2.63
200 mg/L treatment	90.8 + 7.22	7.26+0.32	2.08+0.02	24.63+1.75

Data represent means + SD of these measurement Table 3 Shows the anti oxidant of status of fishes treated with 2,4-D herbicides! Super oxide dismutase (SOD) was relatively altered after 2,4-D treatment and at 200 mg the level of SOD was drastically altered.

Catalase and Glutathione peroxidase enzymes were also reflected

pronounced change in their level of 7.26+0.32 and 2.08 +0.2 which is affected in 200 mg/L of 2,4-D Glutathion transferase also incredibly reduced after 2,4-D treatment which tecs the fishes to get prone to ancillary stress. Herbicide may induce oxidative stress leading to the generation of free radicals and cause lipid per oxidation as molecular mechenisms involved in

pesticide - induced toxicity (Agrawal et. al 1991 and Khrer 1993) Increased lipid peroxidation and oxidative stress can affect the activities of protective enzymatic anticxidants that have been shown to be sensitive indicators of increased oxidative stress.

The 2,4-D function by maintaining high level of the plant hormone auxin, resulting in over stimulation of plant growth and death (Ateeg et. al. 2005) It is known that 2,4-D caused changes in the animal nervous system through complex formation with acetylcholine and inhibition of Ache (Sarikaya and Selvi 2005, Benli et. al. 2007)

This study shows that 2,4-D has drastic effect in lowering the antioxidant enzyme status of Channa Striatus fishes where by the fishes can prone to other diseases which tentatively cause fish mortality in aquaculture society. Hence the usage of 2,4-D on fish aquaculture should be very curious.

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