P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-4* ISSUE-1* (Part-2) April- 2019
E: ISSN NO.: 2455-0817 Remarking An Analisation

Impact of Chlorpyrifos on Molecular Parameters of *Channa Punctatus*

Abstract

Impact of chlorpyrifos (CPF) on DNA, RNA in blood of fresh water food fish *Channa punctatus* were investigated. It was found that there is significant alteration in DNA, RNA and DNA/RNA ratio in the blood of *Channa punctatus* exposed to chlorpyrifos at different concentrations and exposure periods.

Keywords: DNA, RNA, *Channa punctatus*, Chlorpyrifos.

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Introduction

A large number of pesticides are drained in water bodies where fish encounter with them and develop various metabolic abnormalities. They get accumulate in fish and affect human health too via ecological cycling and biological magnification. With the modernization of agricultural operations and rapid growth of industrial activities, there has been tremendous increase in the manufacture and use of pesticides. Incidents of fish mortality have been reported from different parts of world due to insecticidal treatment of the agricultural crops. Now days, due to advancement of technologies and requirement of huge amount of grains, the use of pesticides increased on large scale. (Chindah et *al* 2000)

For the control of pests, the pesticides are used since about 1850. Both types of pesticides are used by the farmers, natural as well as chemical. The natural pesticides are easy to use, safe and more biodegradable. But the synthetic pesticides like pyrethroids, polycyclic chlorinated hydrocarbon are less degradable and more dangerous to the environment. These chemicals when enters in food chain then they create most dangerous effects to human beings as well as other animals like fishes, reptiles and aves etc. Chlorpyrifos is also a dangerous pesticide which is used in the agricultural field for the control of pests. When the pesticides run off from the agricultural fields to the rivers many aquatic fauna have been effected and generally mammals and fishes show less susceptibility to Chlorpyrifos. (Chindah *et al* 2001)

Chlorpyrifosis moderately to highly toxic to fish under laboratory conditions. However, when products are used according to the label, chlorpyrifosis less likely to affect fish. This is because it is more likely to be bound to the sediment. Chlorpyrifosis practically non-toxic to birds when they eat it. Chlorpyrifosis highly toxic to honeybees under laboratory conditions. It did not harm bees in field studies, and formulated products actually had a repellant effect that lasted for 2-3 hours. Earthworms were not affected when soil was treated with Chlorpyrifos.

There are many uses for Chlorpyrifos, ranging from agricultural uses to home pest control. (Dieter *et al* 1995). Chlorpyrifoshas been instrumental in preventing the spread of diseases carried by tick-infested prairie dogs, rodents and other burrowing animals. It is helpful in eliminating and preventing a wide variety of household pests, especially spiders, fleas, ticks, carpenter ants, carpenter bees, cockroaches and bedbugs. Chlorpyrifosis also one of the primary ingredients in ant chalk. While chlorpyrifosis easy to use and very effective, it should always be treated with caution. It should be applied according to the instructions that come with the insecticide. When care is not taken, chlorpyrifospoisoning can occur. Since chlorpyrifosis a neurotoxin, it attacks the nervous system. Skin contact can lead to tingling or reddening of the skin local to the application. If taken in through the eyes or mouth, a common symptom is facial paraesthesia, which can feel like many different abnormal sensations, including burning, partial numbness, "pins and needles", skin crawling, etc. There are no reports indicating that chronic intoxication from pyrethroid insecticides causes motor neuron damage or motor neuron disease. However, in 2011, a case report was published demonstrating pathologically proven motor neuron death in a Japanese woman after

acute massive ingestion of pesticides containing pyrethroids and organochlorine (Omoregie 1995).

Aim of the Study

In the present investigation our main focus is to study the effect of chlorpyrifos on *Channa punctatus's* molecular level for that we observed the variations in the (DNA, RNA and the ratio of DNA and RNA) of *Channa punctatus*.

Materials and Methods

Channa punctatus, a catfish belongs to the family Channidae. It is commonly known as snakeheaded fish. It is found in India, Pakistan, Nepal, Srilanka, Thailand and Myanmar. Juvenile live fishes were purchased from the local fish market during September to April when the room temperature ranges from 25 to 36 ^{0}C and water temperature from 20 to 25 ^{0}C. The fish averaging 6-10cm standard length and average body weight of 60-70gm were used for the study. After examining carefully for any injury they were kept in one percent solution of potassium permagnate for few minutes to get rid of any dermal infection. After acclimatization for 15days they were reared in large glass aquaria measuring 75 cm X 37.5 X37.5 cm and fed on boiled egg yolk and fish food. Chlorpyrifos has been selected for present study. Chlorpyrifos products are among some of the most popular and widely used insecticides in the world and has become very popular with pest control operators and individuals in the United States in the past five years. This material is a member of one of the safest classes of pesticides: synthetic pyrethroids. The nucleic acid was isolated by the method adopted by Doyle (1990) with some modifications.

Methods for DNA isolation involve some basic steps which are as follows:

Lysing The Cell

One must first break open the cell to release the nucleic acids.

a. Detergents: - dissolve the lipid membrane of cells
b. Enzymatic digestion: - remove the cell wall of Gram +ve bacteria and plant cells. Examples- mutanolysin, lysostaphin, lysozymes, pectinases, cellulases, chitinases, etc.
c. Physical disruption: - grinding or homogenizing to remove cell wall

Remove Contaminating Material from Nucleic Acids

Major contaminants with the DNA are proteins.

a. Enzymatic digestion: - proteinases digest proteins.
b. Organic solvent extraction: - proteins, but not nucleic acids, dissolve in phenol and chloroform.
c. Chromatographic methods: - Anionic columns (recall that DNA is negatively charged; therefore, it binds to positively charged substances), glass or silica bases methods. DNA, but not other components, binds to the material of choice. Once the contaminating components are washed away, the DNA is eluted from the binding matrix.

Purification/concentration of Nucleic Acid

a. Precipitation with alcohol: - DNA is insoluble in either ethanol or isopropanol and thus is precipitated.
b. Centrifugation: - centrifugal force causes the precipitated DNA to form pellet in bottom of tube.
c. Dialysis: - concentrates and cleans DNA by removing salts and other impurities that are small enough to migrate through dialysis membrane.

Preparation

T. E. buffer (Tris-EDTA)
1 M Tris pH 8.0 20 ml
0.5 M EDTA 20 ml
Sterile water 100 ml
Proteinase K (10mg/ml)

Dissolve 100 mg Proteinase K in 10 ml TE for 30 min at room temperature (RT). Aliquot and store at –20°C

RNase A (20 mg/ml)

Dissolve 200 mg RNase A in 10 ml sterile water, boil for 15 min, and cool to RT. Aliquot and store at –20°C

Procedure

1. Put 60-80 mg of tissue in a petridish and wash it thoroughly with distilled water.
2. Grind the tissue finely in a chilled pestle mortar.
3. Transfer the homogenate in a sterilized centrifuge tube.
4. Add 500µl T. E. buffer.
5. Add 100 µl proteinase K (10 mg/ml) and 240 µl 10% SDS, shake gently, and incubate for 2hrs at 65°C in a waterbath. The tissue should be digested completely
6. If there are still some tissue pieces visible, add proteinase K again, shake gently, and incubate for another 1 hr at 65°C.
7. Add 500µl of phenol, shake by hand for 5-10 min, and centrifuge at 10, 000 rpm for 5 min at 10°C.
8. Pipette the supernatant into a new tube, add equal volume of chloroform/isoamyl alcohol (24:1); shake by hand for 5-10 min, and centrifuge at 10,000 rpm for 15 min.
9. Pipette the supernatant into a new tube, repeat extraction with chloroform/isoamyl alcohol (24:1) if necessary. Add equal volume of chloroform/isoamyl alcohol (24:1); shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min at 10°C.
10. Pipette the supernatant into a new tube, add 25 µl 3 M sodium acetate (pH 5.2) and 500µl isopropanol, shake gently until the DNA precipitates.
11. Pellet the DNA at 10,000rpm for 5 min. discard the supernatant carefully.
12. Wash the DNA in 70% ethanol and air dry it.
13. Dissolve the DNA in 50µl sterile water or T. E. Buffer and store at -20oC.

DNA Estimation

DNA is estimated by taking absorbance at 260nm as nucleic acids, calculating ratios of absorbance at A260/A280 gives an estimate of protein contamination, pure DNA free from protein contamination have a ratio of A260/A280 close to 1.8, if phenol or protein is present this ratio is less than 1.8, T.E. Buffer was taken as Reference. Elico spectrophotometer (model BL-192) was used for calculations.

A260 unit of Double stranded DNA = 50ug DNA/mL
Calculations were done as per following formula-
X ug DNA = 50 ug DNA/mL X Dilution Factor
OD Measured 1.0 OD

RNA Estimation

Pure RNA has absorbance ratio A260/A280 close to OD 2.0, relationship used for RNA quantization is A260 unit of RNA = 40ug/mL. T.E. Buffer was taken as Reference. Elico spectrophotometer (model BL-192) was used for calculations.
Calculations were done as per following formula-
X ug RNA/mL = 40 ug RNA/ml X Dilution Factor
OD measured 1 OD

DNA/RNA Ratio

The DNA/RNA ratio was calculated by the following formula-
DNA/RNA = Concentration of DNA/ Concentration of RNA

Results and Discussion

Values of DNA, RNA and DNA/RNA ratio in *Channa punctatus* After chlorpyrifos intoxication DNA(a) Control set ,The value of DNA ranges from 68.50-74.55 with mean 72.25±0.18µg/ml in control set. (b) Acute treatment (4days) In acute treatment (4days), the value of DNA ranges from 57.54-64.50 with mean 61.30±0.48µg/ml after chlorpyrifos treatment. The decrease was significant after treatment as compared to control set. (c) Sub-chronic treatment (20days) In sub-chronic treatment (20days), the value of DNA ranges from 42.66-48.20 with mean 45.34±0.67µg/ml after chlorpyrifos treatment. The decrease was FALSE after treatment as compared to control set. (d) Chronic treatment (45days) In chronic treatment (45days), the value of DNA ranges from 19.98-24.55 with mean 22.22±0.33µg/ml after chlorpyrifos treatment. The decrease was very highly significant after treatment as compared to control set. Lot of workers were also find such results such as Dambo (1993) RNA (a) Control set The value of RNA ranges from 52.44-57.50 with mean 55.33±0.15µg/ml in control set. (b) Acute treatment (4days) In acute treatment (4days), the value of RNA ranges from 42.56-48.20 with mean 45.50±0.33µg/ml after chlorpyrifos treatment. The decrease was significant after treatment as compared to control set. (c) Sub-chronic treatment (20days) In sub-chronic treatment (20days), the value of RNA ranges from 30.10-35.25 with mean 32.33±0.67µg/ml after chlorpyrifos treatment. The decrease was highly significant after treatment as compared to control set. (d) Chronic treatment (45days) In chronic treatment (45days), the value of RNA ranges from 16.45-21.57 with mean 18.44±0.50µg/ml after 50 chlorpyrifos treatment. The decrease was very highly significant after treatment as compared to control set. DNA/RNA RATIO (a) Control set The value of DNA/RNA ratio ranges from 1.25-1.35 with mean 1.32±0.07 in control set. (b) Acute treatment (4days) In acute treatment (4days), the value of DNA/RNA ratio ranges from 1.25-1.37 with mean 1.33±0.08 after chlorpyrifos treatment. The increase was non-significant after treatment as compared to control set. (c) Sub-chronic treatment (20days) In sub-chronic treatment (20days), the value of DNA/RNA ratio ranges from 1.10-1.17 with mean 1.15±0.04 after chlorpyrifos treatment. The decrease was non-significant after treatment as compared to control set. (d) Chronic treatment (45days) In chronic treatment (45days), the value of DNA/RNA ratio ranges from 1.00-1.05 with mean 1.02±0.01 after chlorpyrifos treatment. The decrease was significant after treatment as compared to control set. Our results were in close resemblance with Bostveld *et al* 1993.

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Table-1

DNA content (µg/ml) in blood of *Channa punctatus* after 4, 20 and 45 days treatment of chlorpyrifos

S.No	Experimental Set	Dose (µl/l)	No. Of Fishes	Range	Mean ± S.E	Significance level
1	Control	--------	5	68.50-74.55	72.25±0.18	-----------
2	Acute (4 days)	4.024	5	57.54-64.50	61.30±0.48	P≤0.05
3	Sub Chronic (20 days)	0.925	5	42.66-48.20	45.34±0.67	P≤0.02
4	Chronic (45 days)	0.452	5	19.98-24.55	22.22±0.33	P≤0.01

Table-2

RNA content (µg/ml) in blood of *Channa punctatus* after 4, 20 and 45 days treatment of chlorpyrifos

S.No	Experimental Set	Dose (µl/l)	No. Of Fishes	Range	Mean ± S.E	Significance level
1	Control	--------	5	52.44-57.50	55.33±0.15	-----------
2	Acute (4 days)	4.024	5	42.56-48.20	45.50±0.33	P≤0.05
3	Sub Chronic (20 days)	0.925	5	30.10-35.25	32.33±0.67	P≤0.02
4	Chronic (45 days)	0.452	5	16.45-21.57	18.44±0.50	P≤0.01

Table-3

DNA/RNA ratio in blood of *Channa punctatus* after 4, 20 and 45 days treatment of chlorpyrifos

S.No	Experimental Set	Dose (µl/l)	No. Of Fishes	Range	Mean ± S.E	Significance level
1	Control	--------	5	1.25-1.35	1.32±0.07	-----------
2	Acute (4 days)	4.024	5	1.25-1.37	1.33±0.08	P≤0.05
3	Sub Chronic (20 days)	0.925	5	1.10-1.17	1.15±0.04	P≤0.02
4	Chronic (45 days)	0.452	5	1.00-1.05	1.02±0.01	P≤0.01