

The Biochemical and Enzymes Effects of *Euphorbia Tirucalli* on Fresh Water Fish *Oreochromis Mossambicus* (Tilapia)

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Abstract

In the present study was conducted with latex powder of *Euphorbia tirucalli* and provide the various biochemicals parameters (e.g., lipids, protein, and carbohydrate) of *Oreochromis mossambicus*. At initial, fish was exposed to sublethal doses of 60 %, 80% of LC₅₀ values for 96 hours exposure time significant variation in the carbohydrate, total protein, and lipids. Further, various include aspartate aminotransferase, alkaline aminotransferase, acid phosphate, alanine phosphate, lactate dehydrogenase, and acetyl coelenterate were observed in the tissues of the fish, additionally overall biochemical parameters were significantly ($P < 0.05$) alteration in lipids, carbohydrates and total protein content in gill, liver, and kidney. Seven days of termination of treated shows limited recovery in those parameters in fish *Oreochromis mossambicus* with particularly supports the view of the plants used for aquatic environment areas.

Key words: *Oreochromis mossambicus*, *Euphorbia tirucalli*, enzymes, biochemical

Introduction

The pesticides are not every time useful be allowed to cause effects of toxic in different forms to non-target living organisms by modification of cell physiology, by the effects on all organisms (or) everybody changes of whole populations as well documented by Bernet *et al.* (1999) and Giari *et al.* (2008). Particularly, Fishes more important non-target resident of the aquatic ecosystem affected by environmental contamination both in direct contact with the environment contaminations through the delicate multi-functional structure, the gill which is responsible for the osmoregulation, respiration, nitrogenous waste excretion, and acid-base balance. The *Oreochromis mossambicus* (Mozambique tilapia) is tilapine cichlid. These fish originate from southern Africa. That is commercial fish for aquaculture. The tilapia often up to a decade. Due to the introduction of humans. That is now establishing in a lot of tropical and subtropical habitats all over the world where it can do something for invasive species because of robust nature. Those are matching features makes that a good species in freshwater from seeing that it readily adapts to the new situation. These fishes called as black tilapia in Colombia and such as blue Kuper in South Africa (Listado Oficial) (big bass)

Neuwinger (1994) reported that tropical Africa for 258 fish poisoning plants from 167 plant genera and 60 families. The wide choice of poisons noticed that the variety of wild and production of plants, fishermen to collect by the coast. These plants are used most from six sources. The main two plants were used for *Euphorbia nyikae* and *Euphorbia tirucalli*. The mature stems were cut and collected, tied into place in bags, and thrown into the poll or rivers where fish were sought.

Euphorbia tirucalli (Euphorbiaceae family) is a large unarmed a small pecil tree up to 5 meters tall with erect branches, that plants are generally known as bilk bush, is common medicinal plants of India. Stem and latex are used for traditional fishing purpose. Latex juice in small drops is purgative but large drops are purgative for emetic (Satyavati and Gupta, 1987). The reported this plant *Euphorbia tirucalli* ranks first among the plants which have been used for the control of permits. *Euphorbia tirucalli* have reported possessing antibacterial agent (Paresh and Chanda 2007). In the present study was absorbed to verify the enzymes and biochemical effects of *Euphorbia tirucalli* latex powder, their different concentration of action short time effects on freshwater fish *Oreochromis mossambicus* under exposure to sub lethal doses was investigation.

2. Materials and methods

2.01 Collections and maintenance of testing animals

Oreochromis mossambicus (Tilapia) 20±25g weights were collected from Govt, Fish Culture Center, KRP Dam Krishnagiri district, Tamil Nadu. Further, fishes are stored in water tanks, containing 1000 lit of dechlorinated tap water from our laboratory. Before the treatments fishes were adopt to laboratory conditions for 10 days. The electronic aeration was used continuously. The suitable artificial fish food supply has given the health maintenance of fishes. The death fishes were removed from the aquarium.

2.02 Collection of plant milky fluid

The stem apices plants *Euphorbia tirucalli* were collected from nedusalai village, Krishnagiri District, Tamil Nadu, India. The stem apices were cut to get the white milky latex product of the plant, the latex was drained into glass tubes. The latex was lyophilized at -40°C, given use the lyophilized latex in dried powder. The powder was saving in an airtight desecrator for further use. The wet weight of 1ml of the latex of *Euphorbia tirucalli* was 1.370g and the dry weight lyophilized at -40°C was 0.315g.

2.03 Toxicity experiment

The treatment was conducted in plastic tubs. About 10 fishes are stocked for each pond (20±25g weight). The treatment animals were exposed continuously from 24 hours up to 96 hours to three different concentrations. Control groups without any treatments every 24 hours of mortality were recorded for 96 hours. Death animals were suddenly removed from the aquarium.

2.04 Biochemical studies

The acclimation was treated with 60% and 80% of 96 hours LC₅₀ of *Euphorbia tirucalli* latex powder for 96 hours exposure periods. The six aquaria were set up for each dose and each aquarium contained 10 fishes in 20 lit dechlorinated tap water after the treatments, the test fishes was removed from aquaria and water wash and killed the animals the treated animals. Dissection of gill, liver and kidney tissues of fish were collected and used for biochemical's and enzymes analysis without the treatment control animals were held under similar condition

2.05 Estimation of total protein

The total protein ranges were getting the measurements of maintained to the methods of Lowry *et, al*, (1951). Make use of bovine serum albumin as standard homogenates (5mg/ml, w/v) were ready to 10% trichloroacetic acid (TCA).

2.06 Estimation of arbohydrate

The estimation of total Carbohydrates was estimated according to Nicholas *et al.*, (1956) method. Anthrone reagent was used as standard homogenates (5mg/ml, w/v) were prepared to 10% trichloroacetic acid (TCA).

2.07 Estimation of lipids

Predicted through the technique of Barnes and Black stock (1973).50 mg of tissues have been homogenized (5% w/v) in a waring blender withinside the chloroform-methanol mixture (2:1).

2.08 Aminotransferase activity

Alanine aminotransferase and aspartate aminotransferase was estimated according to Reitman and Frankel (1957) method. Homogenate (50 mg ml, W/V) was prepared in a 0.25 M cold sucrose solution. Oxaloacetic acid was used as a standard

2.09 Phosphatase activity

Acid phosphatase and alkaline phosphatase activities were estimated according to the method of Andersch and Szcypinski (1947), tissue homogenates (2% W/V) were prepared in ice-cold 0.9% sodium chloride n-Nitro phenol was used as standard.

2.1 Lactic Dehydrogenase activity

The LDH activity was estimated by the methods of Anonymous (1984). Homogenate (50mg/mL, W/V) was prepared in 1 ml of 0.01 M phosphate buffer PH 7.5, For 5 min in an ice bath. Sodium pyruvate was used as a standard.

2.2 Acetylcholinesterase

Acetylcholinesterase activity was estimated by the methods of Ellman *et al.*, (1961). Homogenate (50mg/ml) was prepared in 0.1 M-phosphate buffer; PH 8.0 for 5 min in an ice bath glutathione was used as standard.

3. Results

3.1 Toxicological studies

In the present study of the toxicity of *Euphorbia tirucalli* latex powder was treated against the freshwater fish *Oreochromis mossambicus*. The LC₅₀ values of *Euphorbia tirucalli* latex powder for test periods levels from 24 hours to 96 hours throughout are shown in (table-1). LC₅₀ values were noted for the treated time from 24 hours to 96 hours. The toxicity was noted the time, as well as various concentration and these, are a significant (P<0.05), negative correlation between LC values and treated time LC₅₀ values of *Euphorbia tirucalli* latex powder reduced from 0.08 mg/l (24H) >0.04mg/l (96H). There was no mortality in the controls group in each set of treatment

Table: 1. Toxicity (LC₅₀) of different concentration of *Euphorbia tirucalli* latex powder against freshwater fish *Oreochromis mossambicus* at different time intervals in laboratory conditions on treated ponds

Doses mg/l	24 hours (%)	48 hours (%)	72 hours (%)	96 hours (%)
control	0%	0%	0%	0%
0.02	0%	0%	16.6%	33.3%
0.04	0%	0%	33.3%	50%
0.06	0%	16.6%	33.3%	66.6%
0.08	16.6%	50%	83.3%	100%

3.2 Biochemical studies

Exposure of sub-lethal concentration (60% and 80% of LC₅₀) of *Euphorbia tirucalli* latex powder for 7 days against the freshwater fish *Oreochromis mossambicus* signification (P<0.05) alteration in carbohydrate, total protein and lipids parameters in different tissues (Table-2)

Table: 2 Changes in carbohydrate e, total protein and lipids levels in gill, liver and kidney tissues of fresh water fish *Oreochromis mossambicus* after exposure to sub- lethal doses of 60% and 80% of LC₅₀ (0.024mg/l and 0.032 mg/l)of *Euphorbia tirucalli* latex powder after 7days

Test	Organs	Control	60%of LC ₅₀ (7days) 0.024(mg/l)	80%of LC ₅₀ (7days) 0.032 (mg/l)	With drawal of after 7 days 0.032 (mg/l)
Carbohydrate (mg/dl)	Gill	45.23±	35.0±	30.12±	31.31±
		0.03	0.05	0.01	0.00
	Liver	67.1±	58.3±	51.21±	51.75±
		0.05	0.01	0.07	0.01
	Kidney	30.1±	21.5±	20.1±	21.21±
		0.08	0.00	0.01	0.07
Protein (mg/dl)	Gill	3.6±	2.8±	2.2±	2.76±
		0.02	0.05	0.01	0.00
	Liver	4.2±	3.7±	3.15±	3.62±
		0.01	0.00	0.01	0.04
	Kidney	5.3±	4.8±	4.0±	4.51±
		0.02	0.00	0.00	0.04
Lipid (mg/dl)	Gill	16.3±	10.20±	9.12±	9.72±
		0.08	0.00	0.01	0.04
	Liver	17.11±	14.9±	13.3±	14.0±
		0.00	0.07	0.04	0.06
	Kidney	9.12±	8.21±	7.98±	8.03±
		0.08	0.03	0.04	0.00

Mean values designated with different superscripts indicate that differences between treatments are significant according to the Tukey's honestly significantly different (HSD) multiple comparison tests ($p < 0.05$).

Carbohydrate level was reduced to 77% and 86%; 86% and 66%; 71% and 76% with compared to control. The protein levels were reduced to 77% and 61%; 88% and 75%; 90% and 75%. While reduced to 62% and 56%; 87% and 77%; 90% and 87% of control in gill, liver, and kidney tissues of fish *Oreochromis mossambicus* consecutively, after treatment with 60% and 80% of LC₅₀ of *Euphorbia tirucalli* latex powder (Table-2). The revelation of fish to the sub-lethal concentration of *Euphorbia tirucalli* affected signification enzymological changes in gill, liver, and kidney tissues of *Oreochromis mossambicus* (Table-3). Mean values designated with different showed that these alterations were significant (P < 0.05) time and concentration-dependent treatments groups was compared with 60% and 80%

Aspartate transaminase level was decreased to 69% and 62 %; 79% and 71%; 83% and 75 %; Alaine transaminase levels were reduced to 86 % and 75 %; 90% and 83%; 80 and 74%; Alkaline phosphatase level were reduced to 79% and 76%; 73% and 58%; 75% and 69%; Acid phosphatase level was reduced to 93% and 81 %; 87% and 79 %; 88% and 81%; Lactate Dehydrogenase levels were reduced to 75% and 68%; 92% and 90%; 93% and 84%; Acetylcolinitarese was level was reduced to 71% and 55%; 70% and 60 %; 65 % and 56% of controls in gill, liver and kidney tissues of fish *Oreochromis mossambicus* respectively, after treated with 60% and 80% of 96 hours of LC₅₀ of *Euphorbia tirucalli* latex powder (table-3)

Table: 3 Changes in Alaine and Aspartate transaminase; Alkaline and Acid phosphatase Lactate Dehydrogenase; Acetylcolinitarese levels in gill, liver and kidney tissues of fresh water fish *Oreochromis mossambicus* after exposure to sub- lethal doses of 60% and 80% of LC₅₀ (0.024mg/l and 0.032 mg/l) of *Euphorbia tirucalli* latex powder after 7 days

Parameters	Organs	Controls	60% of LC ₅₀ (7days) 0.024mg/l	80% of LC ₅₀ (7days) 0.032 mg/l	7 days after withdrawal 0.032mg/l
AST (u/l)	Gill	30.11±	20.8±	18.80±	19.11±
		0.04	0.00	0.05	0.05
	Liver	29.0±	23.15±	20.61±	21.71±
		0.00	0.04	0.03	0.03
	Kidney	18.7±	15.63±	14.14±	14.83±
		0.06	0.03	0.08	0.04
ALT(u/l)	Gill	19.02±	16.53±	14.36±	14.72±
		0.00	0.01	0.06	0.07
	Liver	20.17±	18.21±	16.83±	17.76±
		0.03	0.01	0.01	0.06
	Kidney	35.12±	28.12±	26.23±	27.38±
		0.05	0.00	0.04	0.03
ALP(lu/l)	Gill	21.78±	17.36±	16.73±	18.14±
		0.05	0.06	0.01	0.02
	Liver	27.42±	20.11±	16.14±	18.90±
		0.01	0.06	0.03	0.05
	Kidney	48.11±	36.12±	33.67±	35.79±
		0.00	0.07	0.09	0.08
ACP(lu/l)	Gill	20.12±	18.76±	16.34±	19.21±
		0.06	0.04	0.03	0.02
	Liver	22.12±	19.28±	17.69±	19.34±
		0.02	0.06	0.05	0.02
	Kidney	40.23±	35.67±	32.79±	33.10±
		0.06	0.07	0.01	0.04
LDH(lu/l)	Gill	305.2±	230.3±	210.13±	340.14±
		0.04	0.06	0.03	0.03
	Liver	280.4±	260.41±	252.48±	282.78±

		0.05	0.02	0.08	0.01
	Kidney	260.13±	242.17±	220.13±	257.18±
		0.06	0.01	0.01	0.04
AchE(u/l)	Gill	67.18±	48.20±	37.62±	38.02±
		0.06	0.04	0.04	0.01
	Liver	50.12±	35.17±	30.58±	31.34±
		0.03	0.05	0.07	0.01
	Kidney	60.90±	39.70±	34.37±	34.88±
		0.06	0.00	0.08	0.01

Mean values designated with different superscripts indicate that differences between treatments are significant according to the Tukey's honestly significantly different (HSD) multiple comparison tests ($p < 0.05$).

Seven days of withdrawal of 80% of the 96 hours LC_{50} of *Euphorbia tirucalli* latex powder (table-3). these are significant ($P < 0.05$) recovery followed in the carbohydrate, protein, lipids, and ALT, AST, ALP, ACP, LDH and AchE in gill, liver and kidney tissues of the freshwater fish *Oreochromis mossambicus*

4. Discussion

The beginning and immediate source of energy by carbohydrate (Lehninger, 1978). Below stress conditions of carbohydrates, relief is depleted to link up increased energy dictate (Arasta et al., 1996). consumption of glycogen as it may be due to immediate utilization for energy increased a low caused by latex persuade hypoxia, metabolism of carbohydrate are widely divided into glycolysis or anaerobic segment, in which disintegration of glycogen or glucose terminated the Embden Meyerhof through fare occurs of oxidation of pyruvate to acetyl Co-A whatever is utilized through the citric acid cycle (Lehninger 1978). Lactate dehydrogenates form the middle of an intricate balanced equilibrium between anabolism of catabolism of carbohydrate (Everse and Kaplan 1973). While the current study of LDH levels was decreased on *Oreochromis mossambicus* of *Euphorbia tirucalli*

The prompt of body tissues is significantly relevant to AchE activity. In control fishes, maximum AchE activity results in the brain through the brain is the seat neuronal tissue coordination and integration. The muscle tissue of highest in possessing the second activity of AchE in due the nerve muscular innervation brought about by nerve synopsis (Sambasiva Rao, 1999). While the present study was stress conditions of fish increased and decreased level of AchE after seven days of AchE similar levels of increased tissues of gill, liver, and kidney of *Oreochromis mossambicus* fish in *Euphorbia tirucalli* latex powder

Both aspartate and alanine- aminotransferase function as a connection between protein and carbohydrate metabolism by bringing about the interconvert of strategic compounds (Martin et al., 1983). While both the aminotransferase activity was higher in the liver, kidney than gill tissues of control fishes (table-3). Which kind of suggest that liver, kidney tissues is very worthwhile in utilizing amino acid for metabolic purpose. After treatment the activity of alanine and aspartate enzyme ALT, AST were highly exalted in both the tissue, which establishes the enhancement of stress as a sequel of *Euphorbia tirucalli* latex powder. Acid and alkaline phosphatase also after treatments were highly eleventh grades in both tissues.

5. Conclusion

According to our results, *Euphorbia tirucalli* latex powder can be used safely to use freshwater areas. The fisherman was to capture the fish. This plant was highly toxic affected to fish *Oreochromis mossambicus* with compared to other chemical pesticides. Sub-lethal doses of this latex powder substantially alter the treatments of *Oreochromis mossambicus* it becomes advantageous that poisonous impact of this plant latex powder has been reversible within 7 days after treatment.

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