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Biochemical and Hematological Effects of *Euphorbia Tirucalli* Latex Powder on *Oreochromis Mossambicus (Tilapia)*

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Abstract
The piscicidal activity of dry latex powder of Euphorbia *tirucalli* plant which is belonging to *Euphorbiacae* family and this latex powder against the fish tilapia *Oreochromis mossambicus* in laboratory condition was investigated. Toxicity of latex powder of this plant was timely as well as dose dependent against with fish *Oreochromis mossambicus.* This study reports that the decreasing nature of hematological parameters of freshwater fishes *Oreochromis mossanbicus* when treated with *Euphorbia tirucalli* latex powder at minimum concentration 0.04mg/l. The blood samples and tissue samples were analyzed according to the acquired result of the *Euphorbia tirucalli* latex powder is toxic to freshwater fish *O. mossambics*. This kind of physiological change may directly affect the survivability of these fish in their natural habitat. So, the use of this toxic plant should be a monitor in aquaculture areas. The present toxicity study, at the end of 28 days, there was considerable ($P<0.05$) alteration in different hematological parameters and biochemical parameters.

Keywords
Euphorbia *tirucalli,* latex powder, *Oreochromis mossambicus,* Heamatology, Biochemical.

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INTRODUCTION
Aquaculture, procurement has quality advantages such as high performance, high production, and high employment generation, etc., [1]. Fisheries sector contributes over 1.0% of the national GDP and 5.3% ofthe agricultural GDP in India [2]. The *Mozambique tilapia* is double colored fish of southern African native, line up a decade in its natural habits is the most popular fish for aquaculture. The *Oreochromis mossambicus* is the most extensively cultivated fish throughout India. It is one of the suitable fish models for toxicological studies due to its easy availability and adaptable to the laboratory condition.

Plants referred to as botanicals, which when turns poisonous to aquatic organisms are called piscicides [3]. Such piscicidal plants contain different active

ingredients of alkaloids such as resin, tannins, saponins, nicotine, and diosgenin [4]. However, these alkaloids were toxic to fish at high concentrations and wear off within a short time [5]. The different parts of plants such as latex, stem bark,root, stem, fruit,seeds, and leaves are poisonous[6,7,8]. Several plant products have shown to be toxic to Zooplanktons [9] and commercial fish species both in the laboratory and field studies [10]. *E. tirucalli* used as many countries such as Brazil, India, Indonesia, Malabar, and Malaysia. The juice of this plant is purgative, carminative, used in the treatment of gonorrhea, whoopingcough, asthma, dropsy, leprosy, jaundice, bladder stone and enlargement of the spleen [11].

Human beings have been using plants for catching fishes from time immemorial. Several plant parts such as seed, flower, leaf, stembark or latex are thrown by tribal people into the water to stupefy the fish [12]. *E. tirucalli* is also used to stupefy fish on the west coast of Maharashtra [13] and in Africa [3]. Mostly tribal use these poisonous plants for fishing [14]. Application of synthetic pesticides is one of the methods used to control predatory fish population [15]. Due to their long-term persistence, slow degradability in the water, toxicity to non-target organisms [16] and accumulation inside the fish body, synthetic piscicides adversely affect the quality and the status of fish [17] and contaminate the aquatic environment [18]. The possibility of using local plants as pesticides [19, 20] for solving this problem. Furthermore, the effect of these poisonous plants with humans is found safe and toxic to fishes [21].

The present study showed an insight into the adverse effect of latex powder *tirucalli* on fish organs (tissues) and blood, which required for swimming action and its coordination. The LC_{50} values, hematology, and biochemical analysis were investigated using standard techniques to observe the changes in the fish experimentally under laboratory conditions.

MATERIALS AND METHOD

Procurement of fish

Oreochromis mossambicus fish of average length 10–13cm and weight 15-20g procured from Tamilnadu Fisheries Development Corporation Limited, KRP Dam Fish Farm, Krishnagiri. Before the experiment, fish was allowed in a large tank for ten days with continuous aeration. In the present investigation dechlorinated tap water used with the following physicochemical parameters such as temperature (26.5°C), pH (7.27), dissolved oxygen (7.8 mgL/1 total alkalinity (180mgL/1), salinity (0.352 ppt), and total hardness (175 mgL/1).

Collection of the plants

The whole plants of *Euphorbia tirucalli* (Family-Euphorbiaceae) commonly known as Pencil tree (Barki-Thohar) collected from Nedusalai Village, Krishnagiri district, Tamilnadu.

The preparation of latex extracts

The sharp knife cut stem and branches of *Euphorbia tirucalli*, and the oozing white-milky latex collected in a glass tube. This latex was lyophilized at-40°C. This lyophilizedpowder was stored for further studies. The wet weight of 1ml latex *Euphorbia tirucalli* was 1.37 gram and dry weight (lyophilized) was 0.315g.

Acclimatization:

During acclimatization, fish fed with rice bran and groundnut oil cake. Water was replaced daily to minimize contamination from metabolic wastes and also to secure a healthy environment.

LC_{50} and Hematological Experimental setup

For 28 days of study, 60 healthy fishes were randomly collected from the stock pond and split into six groups and introduced into four separate plastic tubs. The LC_{50} value of latex powder of plant *E.tirucalli* concentration is 0.04 mg/l. From this value 0.04 mg/l was provided to ensure further experiment as $1/10^{th}$, the $1/20^{th}$ and $1/30^{th}$ of LC50 concentration for 96hr treatment. Three similar replicates are equally maintained. Experimental Control without latex also conducted simultaneously. Water was modified daily to prevent the dumping of fecal matter, excess feed and renewed with the toxic solution. The experiment performed periodically, and at the end of 28 days of exposure, fish were randomly taken from control and treated tanks, and its blood was collected by direct heart puncturing technique using sterile disposable plastic syringe with a 22-gauge needle [22].

Heparin sodium (1%) was used as an anticoagulant [23]. The collected blood samples immediately subjected to hematological analysis. The blood diluted with appropriated diluting fluids; RBC and WBC counts were determined using improved Neuberger counting chamber and calculated [24]. Replicated counts made for each blood samples. Sahli's haemoglobinometer was used to estimate the hemoglobin (HB) percentage (HB %). Hematocrit (HCT) was determined using microhematocrit capillaries filled with blood and centrifuged at 8,700 rpm for 5 min and calculated as a percentage of total blood volume [25]. Mean corpuscular volume (MCV), Mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC) calculated from the average values of HB% [26]. For determination of

erythrocyte sedimentation rate (ESR), the blood mixed well, and 200 mm drawn into a Westergren tube. The tubes were placed vertically and were left undisturbed for 60 min; after that, the level of the column of sediment noted as ESR.

Biochemical analysis

The samples were collected from the treated fish was analyzed by the under mentioned methods. The total carbohydrate was estimated,[27], Protein contents in the tissues determined [28], and the lipid was estimated by using the chloroform-methanol mixture as described [29].

Haematological analysis

The blood samples were centrifuged at 10,000 rpm for 6 minutes in microhematocrit glass capillaries to determine the haematocrit using the method of [24]. The hemoglobin (Hb) content was quantified by the cyanomethemoglobin method [30]. Total number of RBCs and WBCs were counted using Neubauer's improved haemocytometer using the method described [31]. The MCV (fL), MCH (pg) and MCHC (g/dl) levels were calculated using the equations given [32].

Statistical analyses

Statistical analyses were performed by using one-way analysis of variance (ANOVA) followed by me a value designated with different superscripts indicate the different (HSD) multiple comparison tests ($P<0.05$) against a control group.

RESULTS

The LC_{50} values of *Oreochromis mossambicus* were found to be 0.04 mg/l in 96hrs. The relation between the total mortality rate of *Euphorbia tirucalli* latex and concentration was noted. The various concentration of *Euphorbia tirucalli* latex powder 0.08, 0.06, 0.04, 0.02 mg/l andcontrol used for the analysis.The total mortality rate of the treated fishes was shown in Fig.I.

Results of fish treated in sub-lethal measurements of *Euphorbia tirucalli* latex from 24-96 hours revelation periods caused considerable ($p>0.05$) impediment in hematology and biochemical analysis in the gill, liver, kidney tissues of the experimental fish *Oreochromis mossambicus*. TheLc_{50}values for a long-time study observed for 28 days. At the end of the exposure period of *Euphorbia tirucalli*, hematology parameters analyzed. The results show that the treated groups showed an increased level of WBC compared with control and all other parameters (RBC, HGB, MCV, MCH, and MCHC) were decreased in compared with control as shown in (Table-I)

The data analysis of the biochemical parameters of gill, liver and kidney tissues of *Oreochromism ossambicus* after the exposure study period of *Euphorbiatirucalli* latex the carbohydrate level in Gill reduced from 47.0 to 45.3 when compared with control (48.8),carbohydrate level in the liver is reduced from 66.6 to 62.5 when compared with control (71.6) and carbohydrate level in the Kidney is reduced from 24.3 to 22.4 when compared with control (26.7)after 28 days exposure to the different concentration of *Euphorbia tirucalli* latex. (Table II)

The protein level in Gill reduced from 3.7 to 3.1 when compared with control (3.9), protein level in the liver is reduced from 2.8 to 2.3 when compared with control (4.5) and protein level in the Kidney is reduced from 3.4 to 3.1 when compared with control (4.6) after 28 days exposure to the different concentration of *Euphorbia tirucalli* latex. (Table III).

The lipid level in Gill reduced from 11.9 to 9.3 when compared with control (14.5), lipid level in the liver is reduced from 15.8to 14.1 when compared with control (18.5) and lipid level in the Kidney is reduced from 11.2 to 10.2 when compared with control (13.9) after 28 days exposure to the different concentration of *Euphorbia tirucalli* latex. (Table IV).

Fig–I: Mortality of *Oreochromis mossambicus* exposed to different concentrations of *Euphorbia tirucalli* (LC_{50} for 96 hours).

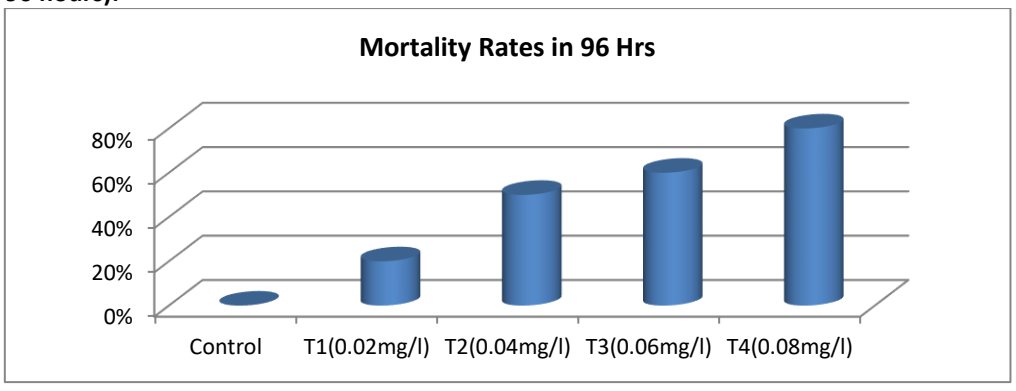


Table-I: Hematological analysis of *Oreochromis mossambicus* exposed to different concentrations of *Euphorbia tirucalli.*

PARAMETERS	CONTROL	TEST-1	TEST-2	TEST-3
WBC	38.95±0.05	45.45±0.24	43.34±0.43	42.1±0.76
RBC	2.46±0.10	1.17±0.56	1.07±0.45	1.4±0.63
HGB	4.78±0.11	1.67±0.34	2.9±0.45	3.7±0.43
MCV	152.7±0.13	138.98±0.98	135.1±0.13	122±0.34
MCH	28.30±0.34	19.98±0.43	31.6±0.87	27.3±0.87
MCHC	43.67±0.67	39.43±0.01	22.5±0.56	20.9±0.45

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).

Table-II & Fig.II: Carbohydrate level (mg/l) of fish (*Oreochromis mossambicus)* organs Gill, Liver, and Kidney exposed to different concentrations of *Euphorbia tirucalli* latex.

TEST	ORGAN	CONTROL	TEST-1	TEST-2	TEST-3
CARBOHYDRATE	Gill	48.8±0.35	47.0±0.45	46.0±0.00	45.3±0.09
	Liver	71.6±0.67	66.6±0.92	63.6±0.09	62.5±0.56
	Kidney	26.7±0.00	24.3±0.98	23.4±0.08	22.4±0.01

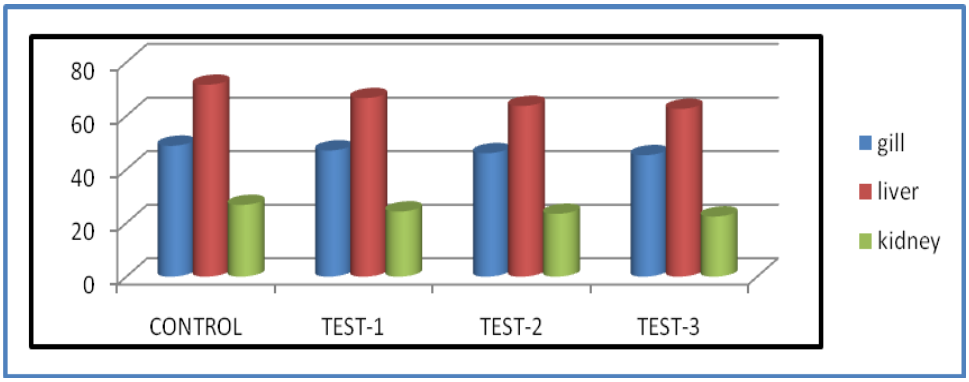


Table: III & Fig-III: Protein level (mg/l) of fish (*Oreochromis mossambicus)* organs Gill, Liver, and Kidney exposed to different concentrations of *Euphorbia tirucalli* latex.

TEST	ORGAN	CONTROL	TEST-1	TEST-2	TEST-3
PROTEIN	Gill	3.9±0.01	3.7±0.09	3.1±0.98	3.1±0.98
	Liver	4.5±0.05	2.8±0.00	2.4±0.98	2.3±0.67
	Kidney	4.6±0.08	3.4±0.03	3.2±0.45	3.1±0.54

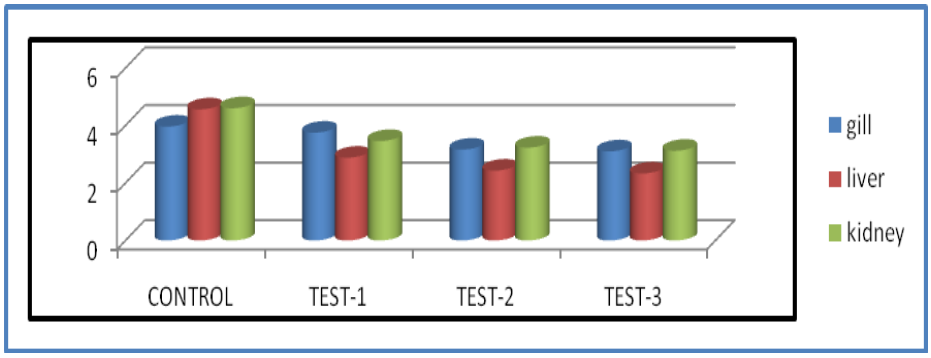
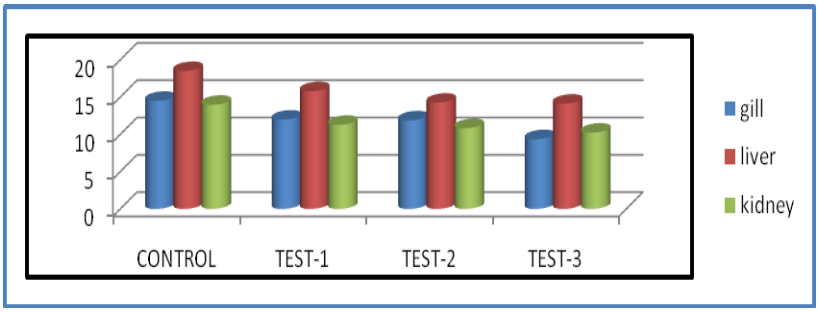


Table: IV & Fig IV: Lipid level (mg/l) of fish (*Oreochromis mossambicus)* organs Gill, Liver, and Kidney exposed to different concentrations of *Euphorbia tirucalli* latex.

TEST	ORGAN	CONTROL	TEST-1	TEST-2	TEST-3
LIPID	gill	14.5±0.48	11.9±0.01	11.8±0.62	9.3±0.43
	Liver	18.5±0.00	15.8±0.98	14.2±0.87	14.1±0.03
	Kidney	13.9±0.43	11.2±0.53	10.8±0.89	10.2±0.32



DISCUSSION

The reason of mortality by the extract shows the table signification increase correlation in the middle of does and mortality. It can be reason increased to various concentration in the water results in furthermore takes of their active moiety in fishes bodes. Date more showed the significations negative correlation in the middle of LC_{50} value and the period's time. It can be the reason for some factors. [33].

The different types of pressure in aquaculture ecosystem and aquaculture actions have been shown to induce more changes in the physicochemical nature of the variety of fishes [34]. The present study indicates that the *Euphorbia tirucalli* latex of lethal concentration (LC_{50} for 96 hrs.) in powder forms to the *Oreochromis mossambicus* at 0.04mg/lit is toxic. Carbohydrates are an immediate and significant source of energy [35]. In this study was performed the *Euphorbiatirucalli* stresses on fish. The stresses condition to fish is jumping in treated tubs. In the pressure, condition fishes need more energy like carbohydrates, proteins, and high dissolved oxygen. [36]. But the fishes treated with *E.tirucalli* powder shows adverse toxicity and high stress with reduced oxygen, protein and carbohydrates level proved biochemically and heamotologically.

The results of *Euphorbiatirucalli* latex powder on snails' shows many changes in their biochemical parameters. Glycogen, total protein level, and nucleic acid level also reduced [37]. The *Balanites aegyptica* on *Oreochromis niloticus* shows some difference in hematological parameters like HB, PCV, RBC, MCV, MCH, MCHC followed by different treatments. Some parameters such as MCV, MCH, MCHC found decreasing, and other values are equal [38]. This study reports the decreasing nature of hematological parameters of freshwater fish *Oreochromis mossambicus* when treated with *Euphorbia tirucalli* latex at minimum concentration 0.04mg/l. This study concludes *Euphorbia tirucalli* is toxic to freshwater fishes.). The Biochemical biomarkers such as glucose, protein and enzymes are used frequently as an indicator for the general state of health and also early warning of stress on fish under stressful conditions [39,40]. Many laboratorial studies have shown the toxicity of plant extracts to fish and changes in hematological and biochemical profiles leading to the death of fish [41, 42]

The generally reduce in the RBC, HGB, and HCT counts. In general, decreased level of RBC, HGB, and HCT counts attributed to the destruction of RBC and erythroblastosis leading to anemia [43]. In the results was reduced all the parameters. Euphorbia tirucalli latex was problems of environmental pressures on the fish.

Moringa oleifera is among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals that could help in stability and shelf life of poultry product [44]. The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in *Moringa* leaves [45].

CONCLUSION

In recent years, many of them for increasing the interest in environmental issues growing out of the realization that the state of the environment is fundamental importance to the quality of human existence. We depend on our physical and biological environment for ecosystem services. This study reports that the decreasing nature of hematological

parameters of freshwater fish *Oreochromis mossambicus* when treated with *Euphorbia tirucalli* latex powder at minimum concentration 0.04mg/l. At the end of 28 days, blood samples and tissue samples were analyzed according to the acquired result the *Euphorbia tirucalli* latex powder is toxic to freshwater fish *O. Mossambics*. The present toxicity study, it is concluded that exposure to sub lethal concentrations of *Euphorbia tirucalli*latex powder results in a significant alteration in different hematological parameters and biochemical parameters. This kind of physiological changes may directly affect the survivability of these fishes in their natural habitat. So, the usage of this toxic plant should be a monitor in aquaculture areas.

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