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Toxicological study on *Clarias batrachus* caused by Croton and Mahua extract

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Abstract
Mahua oil cake and croton are the major piscicide used by the fishermen in the Garhwal region. Croton contains two toxic proteins, those are essentially blood, poisons as well as mahua oil cake extract possess saponins, are poisonous towards the lower forms of life and are used for fish killing. *Clarias batrachus* was exposed to croton and mahua with concentration lethal 200 ppm and sub lethal 100 ppm and 4 ppm lethal and 2 ppm sublethal of croton 14 days duration. Protein changes in muscles, liver and intestine were analyzed after exposure period. Toxic effect of these two piscicides caused a remarkable protein fluctuation at lethal concentration as well as sub-lethal level of the toxicity.

Keywords: Mahua, Croton, Saponin, Garhwal, Piscicide

1. Introduction
Plant extracts are capable to alter the physico-chemical properties of the water bodies which ultimately causes the decline of small fishes in the streams and rivers. The toxicants released from the slurry and paste of these plants not only affect the fishes, but also damage the stream biota like Periphyton and Macrobenthos. Incalculable loss of Juvelines and small fishes occurs as a result of poisoning of the streams. Poison flow downstream over a long distance killing all fry and fingerlings of fish. Plant toxins have an impact on the target resource and may also affect non target species of streams and rivers [14].
Local people of Garhwal use various conventional methods to catch fishes for their domestic consumption and protein diet requirement. A large number of plant species growing in the wild in India are reported to be poisonous to fishes. Various types of plant products such as leaves of Khinna (*Sapium insigne*), Rambans (*Agave americana*), stem bark of Jamun (*Syzygium chumini*), latex of Surai (*Euphorbia royleana*), leaves and bark of Akhrot *(Juglans regia*), bark of Agali (*Acacia pennata*), fruits of Chilla (*Casearia elliptica*) and Maindul (*Catunaregam spinosa*), pounded seeds and stem bark of *Zanthoxylum armatum* and oil cake of Mahua (*Madhuca longifolia*) etc are used to catch various sizes of fishes from aquatic resources of Garhwal region. These plant products are first well crushed and grinded nearby the aquatic system and used in stagnant or semi stagnant pools, slow flowing streams and rivers for fish catching. These are mostly applied during morning or evening hours. Sometimes the streams are partly blocked to slow down the water flow to concentrate the power of poison without being washed away or diluted by a strong current. The active ingredients are released by mashing the appropriate plant parts [8].
Mahua oil cake and corton seeds are widely used as the fertilizer and manure by the farmers in the Garhwal region. Mahua oil cake is also used for piscicidal purposes by fishermen, is a product from the perennial Madhuka *(Bassia Koenig ex Linn*.) tree species, *Bassia longifolia var. latifolia Roxb* (Syn. *Madhuca latifolia*; Mindica J. F. Gmel) belonging to the family Sapotaceae [4, 9]. *Croton tiglium* is an evergreen shrub found throughout India. Croton seed is having piscicidal properties [7]. According to investigators [5] seeds of croton contain two toxic proteins Croton globulin and croton albumin and these are blood poisons.
The present investigation envisages the effect of Mahua Oil Cake and croton seed, piscicides of plant origin, on the protein profile of *Clarias batrachus.*

2. Material and Methods
Clarias batrachus, were collected and brought to the laboratory and kept in aquaria for a week using aged water for acclimatization with feeding on a daily basis. Fishes were exposed to mahua oil cake extract (200 ppm lethal, 100 ppm sub-lethal) and croton seed extract

(4 ppm lethal, 2 ppm sub-lethal) at 48 hrs intervals for 14 days. Simultaneously a control group of healthy fishes were maintained under identical conditions. The fishes were sacrificed immediately at the end of the exposure period. Liver, intestine and muscle were isolated and used to investigate biochemical contents under toxicant stress. Protein content was estimated by Folin phenol reagent method [10].

3. Results and Discussion

During present investigation mahua oil cake (saponin) caused significant protein alternation at a lethal concentration (+57.21% in muscles, +18.77% in liver and +19.63% in Intestinee) whereas at sub-lethal level protein level was found elevated (+48.30% in muscles, +36.29% in liver and +29.95% in Intestine). Croton seed extract exposure also caused disturbances in the protein profile as (-70.00% in muscles, -48.74% in liver and -26.41% in Intestine) at lethal concentration and (+4.80% in muscles, +28.13% in liver and +22.87% in Intestine) at sub-lethal level. The results obtained in the present investigation are summarized in Table 1 and Fig 1, 2.

Table 1: Change in Protein profile induced by Mahua oil extract (Saponin) and Croton (Globulin and albumin)

Toxicant	Control	Sub-lethal	Change in %	Lethal	Change in %
	Mahua				
Muscles	35.67**±3.51	83.33*±5.86	+57.21	69.00*±15.62	+48.30
Liver	62.00*±8.54	76.33*±10.69	+18.77	97.33**±12.34	+36.29
Intestine	87.33*±7.09	108.67*±14.98	+19.63	124.67**±20.31	+29.95
	Croton				
Muscles	111.33*±13.43	33.33**±5.51	-70.00	117.00**±4.36	+4.80
Liver	107.33**±14.19	55.00*±7.00	-48.74	149.33**±18.88	+28.13
Intestine	83.33*±10.26	61.33**±10.07	-26.41	108.00*±20.81	+22.87

The values are expressed in mg/100 mg dry weight (mean±SD) [*=P<0.05; **=P<0.01.]

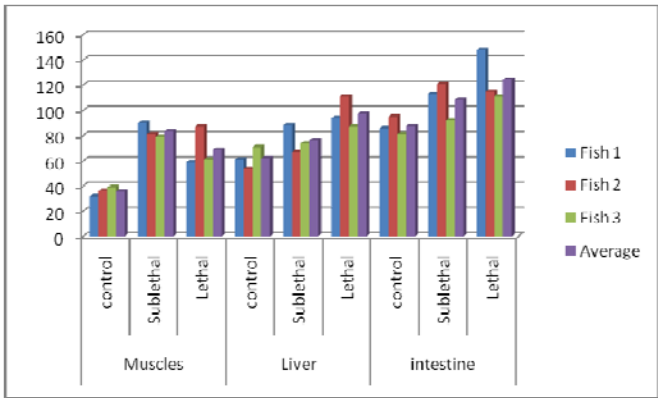

Fig 1: Effect of Mahua oil cake (saponin) toxicity on the fish protein

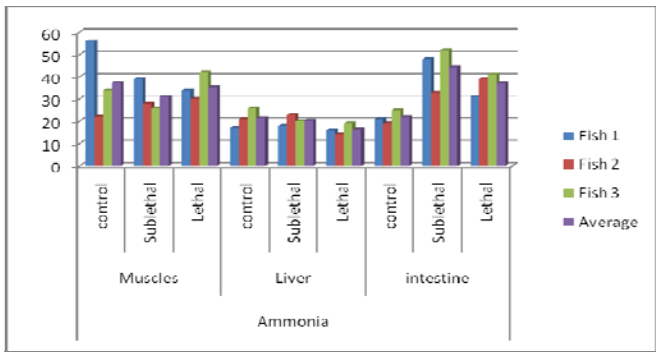

Fig 2: Effect of croton toxicity on the fish protein

The health of fish can be affected by environmental factors (stress), nutrition as well as by pathogens. Stress in fish may be induced by various abiotic environmental factors such as changes in water temperature, pH, oxygen concentration and water pollutants including pesticides, insecticides [3, 12], petroleum products and heavy metals [1, 2]. Biotic interactions such as predator pressure, parasitic invasions or strong competition with other organisms or among the fish in overcrowded areas and by human activities related to fish rearing and harvesting (manipulation, transport) can also be a source of stress to fish [1]. Stress reaction involves various physiological changes, including alteration in blood composition and immune mechanisms. Stress induces also changes in blood cell numbers and activities. An increase in hematocrit, red blood cell count and volume, and hemoglobin level usually has been reported to fish subjected to stress [13]. The increase in the number of circulating RBC is thought to be associated with the release from reservoirs (spleen contraction) and even division of circulating cells in fish subjected to low oxygen tension [15]. Haemoglobin and total plasma protein were found elevated due to biotic stress in *Clarias gariepinus* (catfish) by Jayaram 1981.

The protein concentration in *L. rohita* exposed to methyl parathion increased at different exposure periods. The increase in the protein content could be the result of an elevation in tissues metabolic activity induced by methyl parathion. The increases of the protein content in the fishes exposed to pesticides suggest that any possible protein loss is compensated by increasing the tissue protein synthesis. The loss of enzymes as a result of tissue necrosis or to meet increased demand to detoxify the pesticide necessitates enhanced synthesis of enzyme proteins [16]. Exposure to methyl parathion had significant effects on its protein metabolism, although the fish eventually developed tolerance during long-term exposure; but at lower sub lethal (0.25 and O.5 mg/L) concentration no significant changes were observed [16].

In the present study increase in the protein content was observed throughout the exposure period. The sublethal exposure results show the protein content increase is depend upon the concentration i.e. there is a progressive increase in the protein content with increase in concentration and time exposure. This finding follows the above mentioned findings, i.e. to combat the stress fishes produce various proteins and therefore to counter the stress induced by mahua oil cake (saponin) and Croton, an increase in the protein content has been observed.

4. Conclusion

Present investigation is capable to produce the conclusion that Saponin and Croton (albumin, globulin) are widely used by the local population, but these are reported toxic as both piscicides caused remarkable disturbance in protein profile of fishes. Saponin caused an elevation in protein content at lethal and sub-lethal concentration of toxicity, on other hand, in croton toxicity depletion of protein content was found at sub lethal level but increased protein content reported at lethal level. Hence, it may be concluded that the agricultural and piscicidal use of Mahua and croton must be avoided near the water bodies.

5. Acknowledgement

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