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IMPACT OF PISCICIDAL PLANTS ON PROTEIN PROFILE OF FISH

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Abstract: ***Cestrum nocturnum (CN) and Cestrum diurnum (CD) extracts are well-reported piscicides. Before its use in fisheries for removal of predatory and trash fishes, it's of most importance to study toxicological impacts on fishes. Both these plant extracts contain saponin as a poisonous constituent. Clarias batrachus was exposed to CN extract (LC10= 1.9 mg/lit, LC_{50} = 3.5 mg/lit) and CD extract (LC_{10}= 2.6mg/lit, LC_{50} = 4.4 mg/lit) for 48 hrs duration. Protein changes in muscles, liver and intestine were analyzed after exposure period. Toxic effect of these two piscicides caused a remarkable increase in protein at sub lethal concentration. The chronic application of lower dose of saponin containing extract may have initiated protein synthesis.***

Keywords: *Protein profile, Clarias batrachus, Cestrum nocturnum, Cestrum diurnum.*

I INTRODUCTION

In freshwater aquaculture, fish pond are periodically cleaned to remove unwanted and trash fishes. Screening and use of fish poison is standard method for this. [1]. Also where water is brought from the nearby water reservoir or rivers, entry of trash and predatory fish, eggs and juvenile is very common. Therefore the best way to ensuring total eradication of unwanted fishes is with fish toxicants (piscicide) in the pond water [2]. Ideally, ponds should be sundried and the pond bottom cracked dried to help get rid of fish predators. However, this practice is not always possible particularly during the rainy season. [3]. Presently, there is no legal registered safe fish poison is available in market except use of bleaching powder, tea leave cake, tobacco dust and rotenone. Because of unavailability and cost issue, farmers tends to use unregister fish toxicant such as agro-pesticides which are fast acting and readily available and low-priced. These organophosphate and other chemicals may produce negative and harmful effect on non-target animals, environment, and human health. Hence, there is an urgent need to search more specific, eco-friendly and safe fish toxicant of plant origin. These indigenous plant products are focus of attention as suitable alternative to synthetic pesticides due to their easy availability, inexhaustible resource, low cost, and biodegradability in nature [4]. Already a large number of biocide of plant origin is in use in aquaculture for control of aquatic pest and harmful snails [5, 6].

Diverse group of compounds like saponin, tannins, alkaloids, alkenyl phenols, esters, flavonoids, ichthyoethereol, triterpene and other ichthyotoxins [7, 8] have been found to be toxic to freshwater target and non-target organisms [9]. Thus, there is need for generation of more information on the piscicidally useful plants that have been reported biocidal property. In India there is a great biodiversity among fish poisonous plants in various ethno botanical reports [10,11,12]. Various authors have already demonstrated biocidal activities of plant *C. nocturnum* as mosquito larvicidal, insecticidal, molluscicidal, and antibacterial [6,10, 13,14,]. Therefore, present investigation was planned to assess impact of dried leaves aqueous extract of *C. nocturnum* and *C. diurnum* on the protein profile of *Clarias batrachus* juvenile, an important tropical catfish for aquaculture in India [15, 16].

II MATERIAL AND METHODS

Sample collection and preparation

As shown in Figure 1, the fresh green leaves of *Cestrum nocturnum* and *Cestrum diurnum* were collected from nearby garden at Nashik (M.S.) India. The plants were identified and authenticated by Department of Botany from same institute.

Preparation of aqueous extract

The samples were washed and shade-dried and then ground into fine powder and sifted using 0.25 mm sieve. The leaf powder thus obtained was soaked in 1lit of double distilled water for 48 h. The stored mixture was filtered through sterile gauze and the filtrate was collected. Further, it was subjected to vacuum evaporate in Rota-evaporator and stored in desiccators to ensure complete dehydration of aqueous extract. Such dried powder of aqueous extract was used for evaluating piscicidal activity of *C. nocturnm & C. diurnum.*

Figure 1. The piscicidal plant: Cestrum nocturnum.

Experimental design

The test fish, *C. batrachus* of average length (11.5 ±1.2 cm) and weight (16.0 ± 0.2g) were obtained from Government fishery farm at Nashik (M.S) India. The fishes were acclimatized to laboratory conditions (25°C) for 14 days before the exposure period using large glass aquaria. During the acclimation period, the fish were fed twice daily using standard commercial fish food. From earlier work in same laboratory, LC_{50} of these plant extracts were taken as reference for present investigation [17]. Fishes were exposed to CN extract (LC_{10}= 1.9mg/lit, LC_{50} = 3.5 mg/lit) and CD extract (LC_{10}= 2.6mg/lit, LC_{50} = 4.4 mg/lit) for 48 hrs. 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 [18].

III RESULT AND DISCUSSION

During present investigation *Cestrum* species caused significant protein alternation at a sub lethal concentration. At Sub lethal concentration LC_{10}, protein level in muscles was elevated in C.N. (74.21 ± 4.46 i.e. 41.87 %) and in C.D. (81.46 ± 5.51 i.e. 40.16 %). While at higher dose of sub lethal concentration protein level in muscles was elevated in C.N. (92.27 ± 23.51 i.e. 76.39 %) and in C.D. (96.38 ± 4.36 i.e. 65.83 %). (Table No.1) Chronic dose of toxicant lead into increased muscular activity, to cop up with the increased energy demand, new stress proteins are aggregated in muscles. Increased movement of fish during stress necessitate muscle synthesis that reflect into increased protein level in muscles at LC_{10} and LC_{50} dose.

In liver, at LC_{10}, dose, it increased by 27.84% with C.N. and 14.20 % with C.D. aqueous extract, and at LC50 dose, it increased by 35.95% with C.N. and 34.04 % with C.D. extract. (Table No.2) Due to prolonged exposure of toxicant, stress response initiate protein synthesis [19]. Many researchers reported increased opercula movement as indication of stress in fish when exposed to plant extract [20, 21]. Also observed increased mucus secretion in fish after treatment to reduce toxic effect. Such stress response may lead to increased protein synthesis in fish liver.

Whereas in intestine changes were noted as 17.87 % with C.N. and 14.16 % by C.D. During prolong toxicity at sub lethal dose at LC 10 and 7.59 % with C.N. and 5.53% by C.D. at LC 50. (Figure 2). Small quantity of extract may have been entered in the digestive system, which when entered in the intestine might have increased food absorption rate [22] as well as destruction of mucosal layer [23], that could have lead in to increased protein concentration in the intestine in response of enhanced protein synthesis.

Percent of protein in animal tissue indicates the physiological equilibrium between synthesis and degradation of proteins [24]. Enzymes, proteins, and cofactor involved in the binding, biotransformation, and excretion of foreign compounds have been proposed as specific biochemical indicator of xenobiotic exposure [25]. Protein serves as energy source to compete with stress conditions, which was exhibited by *Clarias batrachus*, when exposed to piscicidal extracts of CN and CD in present research. A significant gain of protein was seen in liver, muscle, and intestine. This indicates absence of proteolysis and initiation of protein synthesis, which in turn contributes to the increase of protein [26, 27, 28].

Saponin are generally known for their haemolytic effects and for being piscicidals property, where death of fish is due to suffocation and destruction of epithelial layer. [6]. CN and CD reported to have saponin as active toxicant. [21]Saponins have been reported to be highly toxic to fish.

Because of their damaging effect on the respiratory epithelia [29]. This effect lead to death of fish and not due to the damaging effect on liver, muscles and intestine.

TABLE 1: CHANGE IN PROTEIN CONTENT IN VARIOUS TISSUES OF FISH *CLARIAS BATRACHUS* INTOXICATED BY CN AND CD AT LC_{10}.

Toxicant	Control	LC 10 Sub lethal	Change In %
Cestrum nocturnum Leaves aqueous extract			
Muscles	52.31**±2.72	74.21*±4.46	41.87
Liver	71.51*±7.63	91.42±09.62	27.84
Intestine	83.45*±5.67	89.78*±10.23	7.59
Cestrum diurnum Leaves aqueous extract			
Muscles	58.12 *±13.43	81.46** ±5.51	40.16
Liver	76.67 **±14.19	87.56* ±7.00	14.20
Intestine	81.19 *±10.26	85.68 ** ±10.07	5.53

TABLE 1: CHANGE IN PROTEIN CONTENT IN VARIOUS TISSUES OF FISH *CLARIAS BATRACHUS* INTOXICATED BY CN AND CD AT LC_{50}.

Toxicant	Control	LC 50 Sub lethal	Change In %
Cestrum nocturnum Leaves aqueous extract			
Muscles	52.31**±2.72	92.27*±23.51	76.39
Liver	71.51*±7.63	97.22±11.42	35.95
Intestine	83.45*±5.67	98.36**±17.96	17.87
Cestrum diurnum Leaves aqueous extract			
Muscles	58.12 *±13.43	96.38 **±4.36	65.83
Liver	76.67 **±14.19	102.77 **±18.88	34.04
Intestine	81.19 *±10.26	92.69 *±20.81	14.16

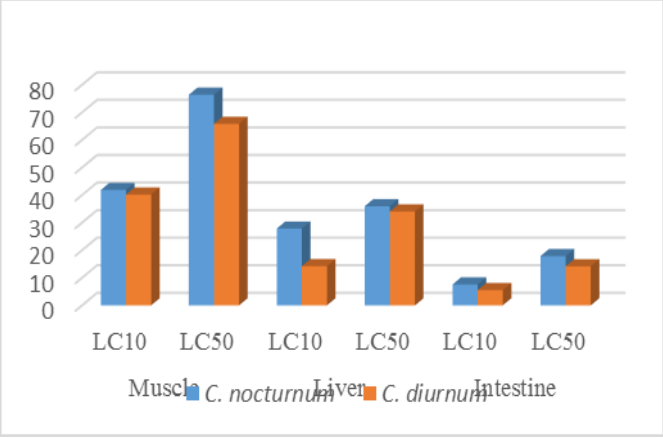

Figure 2: Effect of Cestrum species on Protein level various tissues at sub-lethal dose.

IV CONCLUSION

Protein changes in muscles, liver and intestine were analysed after exposure period. Toxic effect of these two piscicides caused a remarkable increase in protein at sub lethal concentration. The chronic application of lower dose of saponin containing extract may have initiated protein synthesis. Hence these plants stand beneficial for their use in aquaculture for removal of trash and wed fishes.

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