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Piscicidal Effect of Some Biologically Active Plant Compounds of *Alstonia scholaris* and *Codium variegatum* Commonly used in Fresh Water Bodies against Target Fish *Mystus mystus*

Singh Rakesh Kumar* and Singh Ajay

Natural Product Laboratory, Department of Zoology, DDU Gorakhpur University, Gorakhpur 273009, UP, India

**Corresponding Author*

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Abstract: Mortality caused by the aqueous extracts of leaf and stem bark of two plants *Alstonia scholaris* (family Apocynaceae) and *Codium variegatum* (family Euphorbiaceae) against freshwater fish *Mystus mystus* has been investigated in this study. It was observed that the fish may be killed by extracts of leaf and stem bark of these plants. The toxic effect of stem bark and leaf of two plants were time as well as dose dependent. The relationship between exposure durations and LC_{50} was significantly inverse. LC_{50} values of stem bark and leaf extracts of *Alstonia scholaris* and *Codium variegatum* were decreased from 60.84 mg/l (24 h) to 29.884 mg/l (96 h); 39.766 mg/l (24 h) to 7.93 mg/l (96 h) and 133.75 mg/l (24 h) to 64.79 mg/l (96 h); 115.10 mg/l (24 h) to 31.59 mg/l (96 h), respectively. It has been suggested that these plant products cannot be used directly in freshwater bodies, without their detailed studies on long-term effects on target animal as well their structure activity relationship.

Keywords: *Alstonia scholaris*, *Codium variegatum*, *Mystus mystus*, Piscicidal activity, LC_{50}

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Introduction

There are many different insecticides, pesticides, and herbicides on the market today for controlling pests in agriculture; however, these toxicants have an impact on our ecosystem. Our environment is a highly dynamic and interconnected system, in which every component is connected to other components or affected by changes in any one component. Living things are a component of the environment, and their interactions with the soil, plants, animals, and other biotic elements have an impact on growth and development.

Since the widespread and indiscriminate use of non-biodegradable synthetic pesticides and piscicides, the use of botanical extracts to control fish parasites, fish fry predators, and unwanted fish from aquaculture ponds has gained significant attention in recent decades. Chemicals have a negative effect on the aquatic environment and pose a serious risk to organisms that are not intended targets (Kumar *et al*. 2010; Das, 2013). Plant extracts are regarded as potential agents due to their low toxicity to non-targeted animals, high efficacy, ease of availability, rapid biodegrade-ability, and eco-friendliness (Yunis Aguinaga *et al*., 2014). Several plants have been examined to date world wide to determine their pesticidal (Rahuman *et al*., 2008; Dubey *et al*., 2010) and piscicidal (Murthy *et al*., 2010) properties. For the constantly expanding pisciculture industry, new sources of botanical pesticides and piscicides should be discovered as there are only few commercially available plant products (Ramanujam and Ratha, 2008). If plants extracts have toxicological effects on fish they are referred to as piscicides (Burkill, 1995).

In addition to their taxonomic differences, the plant parts used (leaves, barks, fruits, and seeds), the mode of use, the mode of extraction, and the species of target fishes can all vary significantly when it comes to the production of plant piscicides (Neuwinger, 2004). Numerous bioactive substances found in botanical materials can operate singly or in concert to act as piscicides (Obomanu *et al.*, 2007; Ramanujam and Ratha, 2008). Any plant extract's level of toxicity and piscicidal action can be evaluated by its exposure to fish and the median lethal concentration (LC_{50}) was then calculated (Ramanujam and Dominic, 2012). Fish hematological and biochemical parameters can change, which might have an impact on their biological and physiological processes (Winkaler *et al*., 2007, Kavitha *et al*., 2012). Mortality caused by the aqueous extracts of leaf and stem bark of two plants *Alstonia scholaris* (family Apocynaceae) and *Codium variegatum* (family Euphorbiaceae) against freshwater fish *Mystus mystus* has been investigated in this study.

Materials and Methods

Collection of plant materials:

The present study was carried out in DDU Gorakhpur University, Gorakhpur, India during the period of 2020–2022. The plant material used for the collection of stem bark as well as leaf of *Alstonia scholaris* and *Codium variegatum* belonging to family Apocynaceae and Euphorbiaceae, respectively were collected from DDU Gorakhpur University's botanical garden.

Preparation of plant extracts:

The fresh leaves and bark of *Alstonia scholaris* and *Codium variegatum* were dried at 35°C and then crushed using a mortar and pestle. Each plant portion was extracted for 70-75 h using 250 ml of methanol solvent in a Soxhlet device containing dried leaf and bark powder. The material that was dried using a vacuum pump.

Experimental animals:

Freshwater fish, *Mystus mystus* (Bleekeri) (13-13.2 cm) was taken from the banks of the Hetimpur along with Kushinagar Rivers in Uttar Pradesh, India. The 500 L glass aquarium holding the animals were filled with dechlorinated tap water. Animals were kept for one week for acclimatization to laboratory conditions before being used in the experiment. Animals that were sick, injured, or dead were removed from the glass aquarium as soon as possible to stop the body's natural decay. Fish were fed commercial food and the water is changed daily.

Toxicity Experiments:

Experiments on toxicity were conducted by using method of Singh and Agarwal (1988). For every group, there were six aquaria setup. Ten fish were placed in aquaria containing 3 L dechlorinated water. For the experiment, the appropriate concentration of various leaf and stem bark extracts from various plants was used (Table 1). Mortality was recorded at 24, 48, 72 and 96 h after exposure of the fish to various concentrations of plant extract (leaves and bark). If a fish did not react when prodded with a glass rod, it was deemed dead. To avoid polluting the water, any dead fish were taken immediately out of the aquarium. POLO computer program Russell *et al*. (1977) was utilized to calculate the LC values.

Table 1: Concentration used for determination of LC values

Plants	Source	Concentration used (mg/l)
A. scholaris	Leaf	20, 25, 30, 35, 40
	Bark	10, 12.3, 14.6, 17, 19.3
C. variegatum	Leaf	10, 16.6, 23.3, 30, 36.6
	Bark	3.3, 6.3, 9.3, 12.3, 15.3

Table 2: Physico-chemical properties of water

Temperature	25-30°C
pH	7.1-7.4
Dissolved oxygen	6.8-7.6 mg/l
Free carbon dioxide	4.3-6.5 mg/l
Alkalinity	9.7 mg/l

Results

Physico-chemical characteristics of Experimental water:

Under laboratory conditions, the physico-chemical characteristics of water were examined using the APHA/AWWA/WPCE (2005) method at the beginning of the experiment (Table 2).

Dose- mortality response:

Stem bark extract:

The piscicidal activity of stem bark extracts of *A. scholaris* and *C. variegatum* against fish *Mystus mystus* (Bleekeri) was shown to be dose and time dependent in a laboratory toxicity assessment. Table 3 displays a significant inverse relationship between the duration of exposure and the LC_{50} of the examined plants. Consequently, *A. scholaris* and *C. variegatum* stem bark extracts' LC_{50} values dropped from 60.84 mg/l (24 h) to 29.884 mg/l (96 h); and from 39.766 mg/l (24 h) to 7.938 mg/l (96 h). At every probability level, the heterogeneity factor was less than 1.0 and the "t" ratio was more than 1.96. Lower confidence limit (LCL) and upper confidence limit (UCL) are used.

Leaf extracts:

The piscicidal activity demonstrated by *A. scholaris* and *C. variegatum* leaf extracts was both dose- and time-dependent. Table 4 indicates a significant inverse relationship between LC_{50} values and exposure durations. The LC_{50} of *A. scholaris* and *C. variegatum* dropped with increasing exposure duration, from 133.75 mg/l (24 h) to 64.79 mg/l (96 h) and 115.10 mg/l (24 h) to 31.59 mg/l (96 h), respectively.

The results were found to be within the 95% confidence limits of the LC_{50} values, despite the high slope values shown in the Tables. At every probability level, the heterogeneity factor was less than 1.0 and the "t" ratio was more than 1.96.

Discussion

Fish *M. mystus* (Bleekeri) were killed by aqueous extracts of the stem bark and leaves of two plants, according to toxicity tests. The treated fish's

Table 3: Toxicity against the fish, *M. mystus*, caused by two plant stem bark at varied exposure time

Plant		Exposure period			
		24 h	48h	72h	96h
A. scholaris	LC_{50} (mg/l)	60.84	51.11	38.98	29.88
	LCL	55.31	46.97	35.78	25.90
	UCL	72.69	58.24	41.76	32.57
	Slope value	5.88 ± 1.10	4.65 ± 0.88	5.53 ± 0.86	6.69 ± 1.08
C. Variegata	LC_{50} (mg/l)	39.76	27.38	17.01	7.93
	LCL	35.11	24.06	13.55	4.47
	UCL	47.40	31.20	20.07	10.66
	Slope value	3.55 ± 0.54	3.08 ± 0.42	2.44 ± 0.37	2.37 ± 0.44

Mortality was determined every 24 h. Batches of 10 fishes were exposed to five different concentrations of above treatment. Concentrations given are the final concentration (w/v) in the aquarium water.

Table 4: Toxicity against the target fresh fish *M. mystus*, caused by two plant leaf extract at varied exposure time

Plant		Exposure period			
		24 h	48h	72h	96h
A. scholaris	LC_{50} (mg/l)	133.75	105.13	81.16	64.79
	LCL	119.06	95.61	72.10	56.44
	UCL	170.11	122.40	89.13	70.64
	Slope value	5.40± 1.10	4.09 ± 0.82	3.97 ± 0.79	5.59 ± 0.90
C. Variegata	LC_{50} (mg/l)	115.10	86.67	52.63	31.59
	LCL	100.48	75.72	42.63	21.23
	UCL	145.82	104.38	61.72	39.23
	Slope value	4.00 ± 0.71	2.92 ± 0.48	2.35 ± 0.42	2.44 ± 0.45

Mortality was determined every 24 h. Batches of 10 fishes were exposed to five different concentrations of above treatment. Concentrations given are the final concentration (w/v) in the aquarium water.

restlessness, difficulty in breathing, loss of equilibrium, and lethargic behavior were the most overt indicators of discomfort. Additional alterations in the behavior of fish exposed to plant extracts included a fast opercular beat, frequent surfacing, muscular titanic contraction, pectoral fin enlargement, and an out-of-water leap. The fish may have perished as a result of the poisonous effects that made it sink to the bottom of the aquarium.

Ologe and Sogbesan (1920) Wade *et al*. (2002) and Oti (2003) have reported some of these behavioral responses for *Brabus occidentalis* exposed to *Euphorbia heterophylla*, *O. niloticus* exposed to varying concentrations of cassava effluent in a laboratory setting, and *Heteroclarias* exposed to water extract of *Thevetia peruviana* bark, respectively. The kind and speed at which fish behavioral reactions begin to manifest suggest that these substances activate the fish *M. mystus* neuromuscular system. Neurotransmitter chemicals mediate the neuro tropically controlled phenomenon of animal behavior (Bullock *et al*., 1997). Fish that display stress-induced breathing may be suffering from respiratory impairment as a result of a toxin's influence on their gills.

The latex of *E. royleana* and *J. gossypifolia* has been reported by Singh and Agarwal (1984a, b,

1990) to inhibit acetyl cholinesterase as well as decrease endogenous levels of other known neurotransmitters, such as 5-hydroxytrytamine, epinephrine, and dopamine, in the nervous tissue of freshwater snails *Lymnaea acuminata* in a time- and dose-dependent manner.

There was a substantial positive connection between doses and mortality induced by the plant extracts. It might be because the concentration of extract in the water increased, causing the fish's body to absorb more of its active ingredients. Additionally, data demonstrated a strong inverse relationship between LC values and exposure times. There are multiple potential causes (Goodmann *et al.*, 1985) that may be operating independently or in combination. The link between exposure times and mortality is also affected by the stability (life span) of active molecules in the environment and the rate at which their bodies detoxify them (Mitra *et al.,* 1978; Matsumura, 1985).

In substitute of harmful chemical pesticides, newer biological insecticides are being created. Chemical pesticides are effective and target specific, yet they negatively affect the ecosystem. Plant-based insecticides have modest half-lives for their active ingredients, and therefore have negligible environmental consequences (Sharma *et al*., 1995). According to the current study, fish are less poisoned by plant-based pesticides than by synthetic ones. The fact that the 96-hour LC_{50} of pesticides derived from plants is significantly greater than that of synthetic pesticides suggests that plant-based pesticides are less harmful.

It is evident from the steep slope value that a small increase in the dose of plant extracts causes a large increase in fish mortality. A value of the 't' ratio greater than 1.96 indicates that the regression is significant. Values of the heterogeneity factor less than 1.0 denotes that in the replicate tests of random samples the concentration response lines would fall within 95% confidence limits and thus the model fits data adequately. The index of significance of potency estimation of 'g' indicates that the value of the mean is within the limits at all probability level (90, 95 and 99).

Conclusion

The present study indicates that the plants *Alstonia scholaris* and *Codium variegatum* had potent piscicidal effects at greater dosages. Therefore, without studying the long-term effects of plant extracts on target organisms and the functions of their structural connections, it is not possible to use them directly in water.

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