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Piscicidal Efficacy of Selected Plant Products against Murrel (*Channa* spp) in Tripura, Northeast India: A Bioassay Study

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Abstract

The objective of the study was to investigate the piscicidal efficacy of *Camellia sinensis* (Tea) leaves, *Jatropha curcas* (Bherenda) seeds, *Hevea brasiliensis* (Rubber) seeds, *Tephrosia purpurea* (Bannilgach) leaves and *Trichosanthes tricuspidata* (Makal) fruits against Murrel (*Channa* spp). Aqueous extracts were prepared (in a ratio of 1:5) using locally sourced materials from Tripura. These extracts were then subjected to a static bioassay technique, which was performed for 24 and 48 hours in glass aquaria. Test concentrations ranged from 5-100 ml/L. The behavioral responses of the fish were recorded upon exposure to the extracts, and the absence of reflexes was regarded as an indication of fish mortality. The median lethal concentrations (LC_{50}) of the extracts were determined by plotting fish mortality against the test concentrations. Among the tested substances, tea leaves exhibited the highest effectiveness, followed by Tephrosia leaves, Jatropha seeds, Trichosanthes fruits, and Rubber seeds. The 24 hour LC_{50} values varied from 11.0 to 52.5 ml/L, while the 48 hour LC_{50} values ranged from 9.4 to 29.5 ml/L. The results of this study suggest that tea leaves, bherenda and rubber seeds, bannilgach leaves, and makal fruits have the capacity to function as viable piscicides.

Keywords: Nursery fish rearing, predator fish, piscicides, acute toxicity

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Introduction

Aquaculture has gained considerable recognition in Northeast India as a strategy to enhance fisheries and safeguard the livelihoods dependent on them. It is important to note that currently, over 2.63 million people in the region earn a significant portion of their income from fisheries (Debnath et al., 2022). While there has been paradigm shift in aquaculture through the introduction of new fish species, feed-based aquaculture practices, integration of aquaculture with animal husbandry, advancements in fish prophylaxis and therapeutics, fish processing and value addition, entrepreneurship development through ornamental fish farming, and promotion of urban aquaculture through bioflocculation techniques, there are still several challenges that need to be addressed.

One of the long-standing issues in fisheries in Northeast India is the lack of suitable piscicides for managing unwanted fish in seed rearing systems. Fish species like murrels, gobies, featherbacks, mola, barbs, etc., are commonly referred to as "predators and weed fish" in fish seed rearing since they hinder the growth and survival of targeted fish species, mainly Indian major carps, Chinese carps, Bata, Gonia, etc. in Northeast India, through predation and competition for resources such as food, space, and dissolved oxygen. Studies have indicated that these unwanted fish can cause losses ranging from 30% to 40% in nursery rearing (Pillay & Kutty, 2005). The management of unwanted fish species remains a persistent challenge in nursery farming. Traditional methods using mahua oil cake (MOC) have proven to be effective, but mahua is not native to northeast India. Farmers in Northeast India face challenges in procuring MOC due to the high transportation costs involved in bringing it from the plains. While repeated netting is a cost-effective method, it is often insufficient in reducing the populations of predatory and weed fish. The use of synthetic chemicals is indeed very effective, but it has raised concerns due to residual effects, environmental sustainability, and the growing awareness of organic and natural farming in this part of India. Given this scenario, it becomes crucial to explore alternative piscicides for addressing the issue.

Locally available plant products could offer a potential solution in addressing the issue of managing predatory fish. The Northeast India is known for its extensive use of plant products in ethno-botanical remedies (Mao & Hynniewta, 2000), with more than 280 species utilized for various purposes (Chopra et al., 1958). Many of these plants contain natural compounds such as alkaloids, tannins, saponins, glycosides, etc., which are known to possess piscicidal properties (Dutta et al., 2019). However, their potential for removing predatory fish from nursery seed rearing systems remains largely unexplored. To generate valuable information in this aspect, a study was conducted to assess the piscicidal potential of selected plant parts in Tripura, Northeast India.

Camellia sinensis (Tea), *Jatropha curcas* (Bherenda), *Hevea brasiliensis* (Rubber), *Tephrosia purpurea* (Bannilgach), and *Trichosanthes tricuspidata* (Makal) are extensively naturalized as versatile crops in Tripura and other regions of Northeast India. While the tea seed cake is renowned as a commercially viable piscicide, the piscicidal potential of tea leaves remains largely unexplored. During the tea processing, substantial amount of leaves, approximately 10-15%, is discarded as "tea dust" or "tea fannings", presenting an opportunity for their recycling as piscicides. Furthermore, rubber cultivation is popular in Tripura, resulting in the generation of 150-200 kg/ha of seed waste, which can be repurposed as a piscicide. In addition, plants like *Moringa, Jatropha, Dioscorea* and others have become widely naturalized as flex crops in Tripura and neighboring areas of Northeast India. Leveraging these plant resources holds the promise of developing cost-effective biopiscicides. However, the piscicidal properties of these plants remain undisclosed, necessitating the present study to elucidate their potential in this regard.

Materials and Methods

The experiment was conducted in Tripura, Northeast India. Five plant products, namely tea leaves, bherenda seeds, rubber seeds, bannilgach leaves and makal fruits, were screened. The material was collected locally from Tripura. After collection, the materials were thoroughly cleaned with tap water and chopped into small pieces. Subsequently, the materials were lightly pounded using a mixer grinder to obtain macerated samples. Before pounding, the kernels were separated from the jatropha and rubber seeds. The macerated samples were then soaked overnight in distilled water, in a ratio of one part sample to five parts distilled water (Adelakun et al., 2017). After 18-24 hours, the aqueous solution was strained through a fine muslin cloth to remove solids, and various parameters were estimated. The pH was measured using a digital pH meter and ranged from 3.84 to 4.34. The color of concoction varied from tainted white to blackish green. The odor was evaluated through sensory analysis and ranged from sweet musky to waxy pungent. The acid-neutralizing capacity was quantified by determining the amount of sodium hydroxide solution (1 N) required to neutralize 100 ml of the extract, and it varied from 12.6 to 14.6 ml. To ensure the freshness and potency of the extracts, evaluations were performed immediately after extraction.

The products were tested on Murrel (*Channa* spp.), which was collected from the fish farm of ICAR, Tripura Centre. Approximately 1000 fish weighing 0.21 ± 0.03 g and measuring 3.5 ± 0.28 cm each were collected using netting techniques. Subsequently, they were acclimatized in FRP pools for a week at a stocking density of 2 fish per liter. During this period, they received regular feeding, aeration, and water exchange. The water quality parameters of the pools were closely monitored and maintained. The pH of the water was maintained between 7.5 and 8.2, the dissolved oxygen levels remained between 4.5 and 5.5 ppm, and the concentration of ammonia remained below 1 ppm (Debnath et al., 2015). Only the specimens that exhibited good health and condition were selected for the bioassay study.

The static bio-assay method described by APHA (1985) was followed using glass aquariums, each with a capacity of 20 liters. The fish were stocked at a rate of 6 fish per tank and were not fed for 24 hours prior to the study. They were then exposed to test concentrations that ranged between the safe limit (where no fish mortality occurs) and the lethal limit (where all fish die). These test concentrations were determined through a range-finding study conducted before the actual bioassay. The specific test concentrations used for each extract were as follows:

- Tea leaf extract: 0, 5, 7, 9, 11, 13, 15 ml/L
- Jatropha kernel extract: 0, 5, 10, 15, 20, 25, 30 ml/L
- Rubber kernel extract: 0, 5, 10, 50, 75, 100 ml/L
- Tephrosia leaf extract: 0, 5, 7.5, 10, 15, 20 ml/L
- Trichosanthes fruit extract: 0, 10, 20, 30, 40, 50 ml/L

After introducing the extracts into the water at the specified concentrations mentioned above, the behavioral responses of Channa fish were observed, including erratic swimming, skin discoloration, air engulfing, loss of reflex, and mucus secretion. These observations were made at specific time intervals: 1, 6, 12, 24 and 48 hours. Additionally, mortality was recorded, and fish that displayed an inability to respond to external stimuli or mechanical prodding were considered dead and marked as the endpoint of the assay. Each test was replicated three times. The deceased fish were removed and counted. Using Microsoft Excel, the fish mortality data was plotted against the corresponding test concentrations for each extract to determine the probit of kill and calculate the median lethal concentration (LC50) at 24 hours and 48 hours. The LC_{50} represents the concentration at which 50% of the fish die. The calculation of LC_{50} was performed following the method outlined by Finney (1971).

Results and Discussion

Predatory and weed fishes are of significant concern in fish farming, particularly in the rearing of fish seeds. These fishes can cause losses ranging from 30% to 40% in nursery farming (Pillay & Kutty, 2005). The results of this study demonstrate the effectiveness of tea leaves, bherenda seeds, rubber, bannilgach leaves and makal fruits as bio-piscicides. The extract from tea leaves exhibited the highest efficacy in killing Channa fish, followed by Tephrosia leaves, Jatropha seeds, Trichosanthes fruits and rubber seeds. The LC_{50} values of the extracts are presented in Table 1. The residues of tea leaves demonstrated the highest efficacy in killing the fish, with the lowest LC_{50} values of 11.0 ml/L (24 hours) and 9.4 ml/L (48 hours) at a significance level of $p \leq 0.05$. Tephrosia leaves also exhibited significant piscicidal activity, with LC_{50} values of 14.8 ml/L (24 hours) and 10.0 ml/L (48 hours) at a similar significance level. Jatropha seed kernel, fruits of Trichosanthes, and rubber seed kernel showed relatively higher LC_{50} values, indicating comparatively lower efficacy in killing the fish population. However, they still exhibited significant piscicidal activity at $p \leq 0.001$. Among these, the fruits of Trichosanthes had the highest LC_{50} values, suggesting a relatively lower potency compared to the other materials tested.

The present findings demonstrate similarities to previous studies conducted on other fish species, namely *Clarias gariepinus* (Adesina & Omitoyin, 2011), and *Puntius sophore* (Debnath & Sahoo, 2018; Debnath et al., 2022), albeit with some variation in the LC_{50} values. These variations can be attributed to a multitude of factors, including differences in fish groups, variations in experimental conditions such as water temperature, pH and oxygen levels, composition of bioactive compounds within the plant materials due to variations in plant varieties, growth conditions, and extraction techniques, as well as disparities in testing protocols (Maobe et al., 2013). Consequently, it is crucial to consider these factors

Table 1. Median lethal concentration (LC_{50}) of the extracts

Sl. No.	Materials	Median lethal conc. (LC_{50})	
		24 h	48 h
1.	Residues of tea leaves	11.0 ml/L (pd≤0.05)	9.4 ml/L (pd≤0.05)
2.	*Tephrosia* leaves	14.8 ml/L (pd≤0.05)	10.0 ml/L (pd≤0.05)
3.	*Jatropha* seed kernel	18.6 ml/L (pd≤0.001)	14.8 ml/L (pd≤0.001)
4.	Fruits of *Trichosanthes*	41.7 ml/L (pd≤0.05)	26.3 ml/L (pd≤0.001)
5.	Rubber seed kernel	52.5 ml/L (pd≤0.05)	29.5 ml/L (pd≤0.001)

when comparing results across different studies. Conducting standardized experiments would contribute to a more comprehensive understanding of the effectiveness of bio-piscicides.

Previous research provides insights into the potential compounds responsible for the piscicidal properties of these materials. For instance, saponin has been identified in tea leaves (Minsalan & Chiu, 1986), while prenylated flavonoid compounds have been found in Jatropha seeds and Tephrosia leaves (Subramanian et al., 1971; Chen et al., 1978). Rubber seeds have been reported to contain Linamarin, a cyanogenic glycoside compound (Lieberei, 1986) and Trichosanthes has been found to contain tannins, saponins, glycosides, and flavonoids (Kumar et al., 2012). Further research is recommended to identify the specific bioactive compounds present in the materials utilized in this study. This would aid in categorizing the materials based on their origin and facilitate their potential application as piscicides.

Channa sp. was chosen as the model predatory fish in this study due to its significant threat to fish during their nursery rearing. Although the precise number of Channa fish that can lead to the failure of fish seed rearing remains unknown, based on personal experience, it has been observed that 5 to 10 Channa fish weighing 20 to 30 g each can jeopardize the success of a fish seed rearing enterprise in a 150 to 200 m^2 pond. Fish farmers, particularly seed growers in Tripura, recognize the

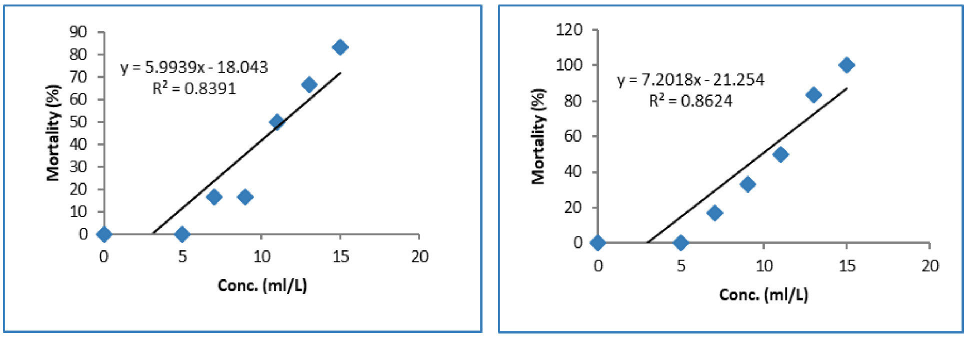

Fig. 1. Dose-response relationship with tea leaf extract in 24 h (Left) and 48 h (Right)

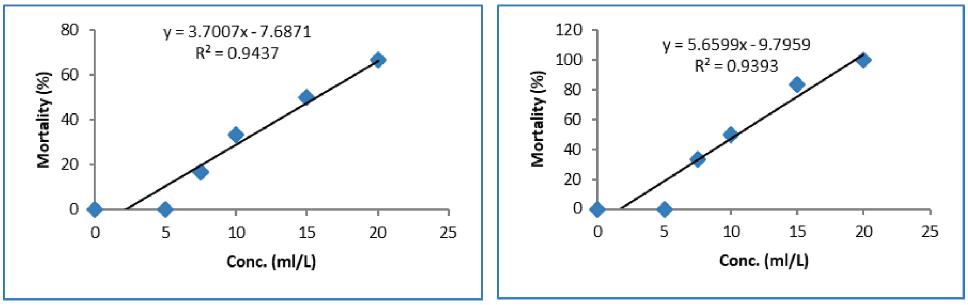

Fig. 2. Dose-response relationship using *Tephrosia* leaf in 24 h (Left) and 48 h (Right)

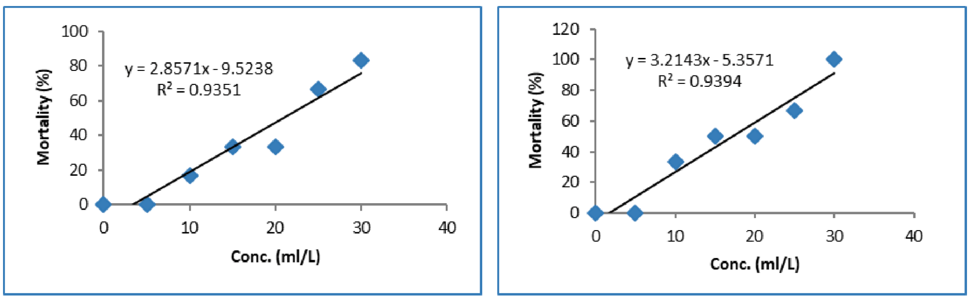

Fig. 3. Dose-response relationship using Jatropha seed kernel in 24 h (Left) and 48 h (Right)

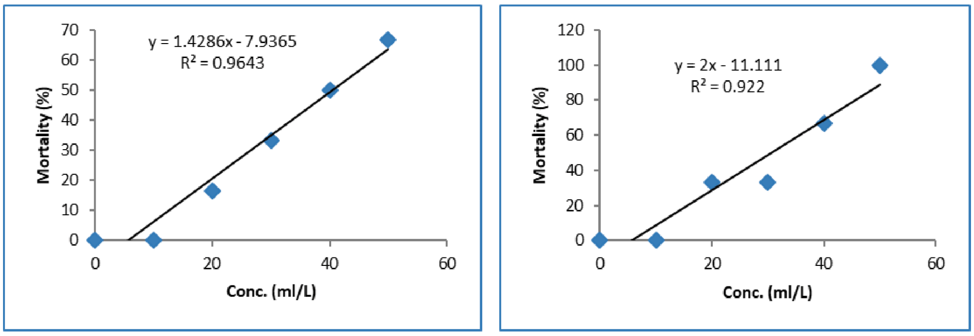

Fig. 4. Dose-response relationship using *Trichosanthes* fruits in 24 h (Left) and 48 h (Right)

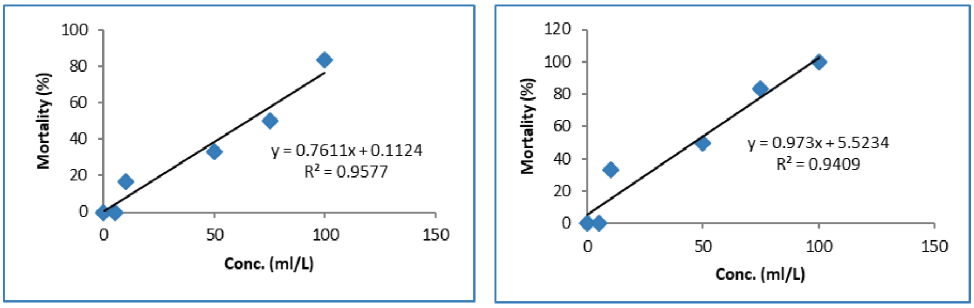

Fig. 5. Dose-response relationship with rubber seed extract in 24 h (Left) and 48 h (Right)

importance of maintaining a Channa-free pond to ensure safe nursery seed rearing. Therefore, effective strategies for Channa control are crucial to safeguard the success of fish seed rearing operations. Based on the present study, it can be concluded that plant-based solutions present a promising approach to address the challenge of Channa control during nursery fish rearing. This approach aligns with the principles of environmental friendliness and sustainability by utilizing biodegradable alternatives with reduced risks to non-target organisms and water quality (Das et al., 2013).

The dose-response relationship between the extracts and fish mortality is presented in Fig. 1-5, demonstrating the direct correlation between test concentrations and the observed effects. Lower concentrations caused minimal discomfort in the fish, while higher concentrations led to more pronounced symptoms such as skin discoloration, mucus secretion, and ataxia. This dose-dependent response confirms that the fish's reactions are directly influenced by the concentration of the extracts (Ayotunde et al., 2010).

Upon exposure to the extracts, the fish displayed specific behavioral responses including erratic swimming, air engulfing, skin discoloration, excessive mucus secretion and loss of reflex. These include impairment of gill function, which could affect respiration and overall health of the fish (Akinwande et al., 2007). Activation of melanocyte-stimulating factors and excess dispersion of melanin pigments in the skin may explain the observed skin discoloration (Oti, 2003). Disruption of metabolic and nervous functions could account for the erratic swimming and loss of reflex (Qadir & Muzaffer, 2014). Hemolysis of blood, formation of a cyanohemoglobin complex, inhibition of mitochondrial enzyme activity, triggering of fatal nervous failure and inhibition of cortisol metabolism are also potential mechanisms associated with the observed fish mortality (Reddy, 2015).

To develop more targeted and mechanism-based plant-based piscicides, a comprehensive understanding of these underlying mechanisms is crucial. Further studies are therefore recommended to explore and elucidate the specific mechanisms involved in fish mortality caused by these extracts.

Finally, it can be concluded that the leaves of Tea, seeds of Bherenda and Rubber, leaves of Bannilgach, and fruits of Makal possess piscicidal properties.

These plant products are readily available locally and require minimal processing before application, making them cost-effective options. Additionally, after their piscicidal action, these plant-based products have the potential to improve soil fertility, which is beneficial for fish culture (Unnithan, 1997). Therefore, these plant products can be recommended as alternatives to chemical piscicides in fish farming. Tea and rubber are widely cultivated in many Northeast Indian states. During tea processing, around 10-15% of the leaves go as waste, and during rubber cultivation, 150-200 kg/ha of seeds are generated as waste materials. Additionally, plants like *Moringa, Jatropha, Dioscorea* and others are widely naturalized as multipurpose crops in the region. By utilizing these plant resources, it is possible to develop low-cost biopiscicides and address the issue of the non-availability of effective piscicides for seed rearing in many places. Further studies are recommended to explore other plants products with similar properties, enabling the development of site-specific and cost-effective piscicides for sustainable fish seed production in Northeast India.

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