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***Research Article** (Open Access)*

THE JOINT ACTION EFFECTS OF THE BINARY MIXTURES OF SOME BOTANICAL PISCICIDES ON FRESHWATER FISH *MYSTUS MYSTUS*

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ABSTRACT
Piscicides activity of several plant moieties in different combinations (binary) were investigated against freshwater fish *Mystus mystus* in glass tank. Tengra is another name for these fish, which are members of the Bagridae family. In a glass aquarium, the piscicidal activity of binary (1:1) and 1:2 combinations of plant parts leaf, stem bark, and latex of Species *Codium variegatum* (Family: Euphorbiaceae) with plant parts leaf, stem bark, and latex of Species *Alstonia scholaris* (Family: Apocynaceae) was investigated. The fish target was *Mystus mystus*. It was shown that plant products in binary combinations have time- and dose-dependent piscicidal efficacy against predatory fish. There was a significant negative correlation between LC50 values and exposure periods thus increases in exposure time mixed in binary combination (1:1) of the *Codium variegatum* (Leaf, Stem bark and Latex) with *Alstonia scholaris* (Leaf, Stem bark and Latex) the LC50 values decreased from 95.70 mg/L (24h)> to 44.50 mg/L (96h); 43.02 mg/L (24h)> to 16.06 mg/L (96h) and 35.49 mg/l (24h)> to 6.24 mg/l (96h) respectively against *Mystus mystus* and binary combination (1:2) of the *Codium variegatum* (Leaf, Stem bark and Latex) with *Alstonia scholaris* (Leaf, Stem bark and Latex) the LC50 values decreased from 93.25 mg/l (24h)> to 53.69 (96h); 65.52 mg/l (24h)> to 30.66 mg/l (96h) and 60.68 mg/l (24h)> to 11.31 mg/l (96h) respectively against *Mystus mystus* . It has been stated that these plant products cannot be employed directly in freshwater environments without first undergoing thorough research on their structure-activity relationship and long-term impacts on the target animal.

Keywords: *Codium variegatum; Alstonia scholaris; Fish Mystus mystus; Binary; Piscicidal activity*

INTRODUCTION
Fishermen and fish cultivators utilize compounds derived from plants called botanical piscicides. They are used for fishing or when removing undesired, predatory and exotic fish species (Cagauan *et al.,* 2004). Piscicides derived from plants are increasingly used for fish removal and pond cleaning in sustainable aquatic environments. Botanical ichthyotoxins are less expensive, readily available, biodegradable, manageable, and safe for both humans and the environment (Singh *et al.,* 1996). Unlike chemicals, which kill the entire fish stock, fish used as pesticides are actually disoriented by plants (Kamalkishor and Kulkani, 2009). Fish exposed to piscicide plants recover quickly because the plants are biodegradable. When compared to the usage of prolonged chemicals, plant extracts utilized as piscicides in fisheries are thought to be beneficial (Gabriel *et al.,* 2009). Depending on the type of fish that is being attacked, different plant species used as piscicides have varying effects (Van Andel, 2000). Toxic plants are widely used around the world to catch fish.
The widespread use of artificial chemical pesticides has become a necessary component of modern farming methods. Non-target creatures are highly vulnerable to the indiscriminate application of synthetic pesticides by human activity (Burkill, 1986). Toxicants enter the environment as a result of excessive pesticide use, mostly in aquatic bodies (Minelli and Reberio, 1996; Dua *et al.,* 1996; Waliszewski *et al.,* 1999).

As a result of their widespread use and ongoing contamination by chemical pesticide waste (Bourgeois *et al.*, 1993; Nayak *et al.*, 1995; Nair and Sherief, 1998; Farag *et al.*, 1880), carbamates have drawn the most attention as pesticides. Additionally, they pose a direct threat to freshwater organisms, especially delicate species like fish and prawns (Sarvanan *et al.*, 2003; Selvarani and Rajamanickam, 2003; Park, 2004; Kumari and Paul, 2020).

Some of these plants are powerful weapons for catching or stupefying fish worldwide because of their ichthyotoxic properties. According to reports, some native Nigerian fisherman utilized particular plant-based biocides for fishing forty years ago (Sambasivam *et al.*, 2003). The twigs and roots of Derris elliptica (Family Papilionaceae) are often found in the tropics and have been utilized as natural piscicides in Nigerian aquaculture ponds and artisanal fisheries. Fish farmers often employ plant piscicides, such as *Tephrosia candida, Tephrosia purpurea, Mundulea sericea, Acacia pennata* (Weiss, 1973), *Adenia cissampeliodes* (Morah, 1985), *Tetra pleura tetraptera, Parkia filicoides,* and *Tephrosia vogelii,* to control pests and predators.Chemicals found in many plants have historically been employed in fish culture in practically every country on Earth (Jenness, 1967). The most well-known plants are Tephrosia species, which contain tephrosin, a natural biocide that is comparable to rotenone, and Derris species, which generate rotenone.

Some plant piscicides have been tested to phytochemical screens in petroleum ether, chloroform, and methanolic extracts. Flavonoids, alkaloids, steroids, glycosides, and anthraquinones were discovered to be present in the methanolic and chloroform extracts of flowers, barks, leaves, and seeds (Kathirvel and Sujatha, 2012). Rotenone and saponins are the two main categories of phytochemicals found in most plants utilized to make the beautiful fish. These families include almost all forms of fish poisons, while other ichthyotoxins, triterpene, and ichthyoethereol containing plants are also utilized (Bearez, 1998). Although a great deal of research has been done on botanical pesticides, nothing has been done to examine the synergistic impacts of their binary combination or their joint action.

As a result, the purpose of this study is to find out how specific botanical piscicide binary mixes affect the target fish, *M. mystus*.

The present study tries to determine the acute toxicity on *M. mystus,* a freshwater fish, by evaluating the LC50 values for various time intervals under binary (1:1) and (1:2) scenarios. This kind of research will support the baseline data required to analysed the relative sensitivity of plant pesticides and support in estimating the safe level dose.

MATERIALS AND METHODS

Experimental animal

The 13–13.2 cm freshwater fish, called *Mystus mystus* (bleekeri), was collected from the banks of the Kushinagar along with Chhoti Gandak Rivers in Uttar Pradesh, India, close the NH 27 of Hetimpur, a recently established Nagar panchayat. The creatures were kept in 500-liter glass aquariums with tap water that had been chemically dechlorinated. Before to the examination, the animals were allowed one week to acclimate to the lab environment. In order to prevent the body's natural deterioration, sick, injured, or deceased animals were taken out of the pond as quickly as possible. The water is replaced every day and the animals are fed standard food. The experiment was conducted with acclimated animals. The freshwater fish used in the experiment five distinct concentrations of the does in glass jars were frequently exposed to *Mystus mystus* during a period of 24 hours to 96 hours. The control group's experimental animals were housed in similar circumstances without any medical attention.

Collection of Plant:

The present research investigation was conducted from 2020 to 2022 at DDU Gorakhpur University in Gorakhpur. The botanical garden of DDU Gorakhpur University in Gorakhpur contributed the plant material that was utilized to harvest the leaves and stem bark of *Alstonia scholaris* and *Codium variegatum,* which are members of the Apocynaceae and Euphorbiaceae families, respectively.

Extraction of Compounds:
The leaf and stem barks have been crushed into a powder using a mortar and pestle after being dried at 35°C. The latex of the *Alstonia scholaris* and *Codium variegatum* was drained in glass tubes by cutting their stem apices, this latex was lyophilized at -40°C and lyophilized powder was stored for further use. A Soxhlet device containing powdered dried leaf and stem bark was used to extract each plant part for seventy to seventy-five hours using 250 mL of methanol solvent. A vacuum pump was used to dry the removed methanol solvent.

Toxicity Experiment:
Concentration utilized to assess the toxicity of a compound derived from *Alstonia scholaris* latex, stem bark and leaf (1:1) and binary (1:1) latex, stem bark and leaf of *Codium variagtum* plants against freshwater fish as the target of the experiment *Mystus mystus* (Table 1).

Using the technique of (Yadav and Singh, 2006), a mixed combination of binary (1:1) and (1:2) was established for the toxicity assay. Ten fish were placed in each of the six freshwater glass jars, each of which held three Liters of dechlorinated tap water, for the experiment. The suitable ratios of binary (1:1) and (1:2) leaf, stem bark and latex from different plants were used for the experiment. Mortality was observed at five alternate combinations of plant concentration every 24 to 96 hours. Fish were considered dead if they did not react when touched with a glass rod. Any dead fish removed from the test container will not pollute the water.

Table 1: Concentration of binary (1:1) and (1:2) combination of plant *Codium variegatum* and *Alstonia scholaris* are extract used for determination of LC values

Part combination	Ratio	Concentration (mg/l) (m/v)				
		1	**2**	**3**	**4**	**5**
Latex: Latex	1:1	2 (1+1)	4 (2+2)	6 (3+3)	8 (4+4)	10 (5+5)
Bark: Bark		4 (2+2)	6 (3+3)	8 (4+4)	10 (5+5)	12 (6+6)
Leaf: Leaf		12 (6+6)	16 (8+8)	20 (10+10)	24 (12+12)	28 (14+14)
Latex: Latex	1:2	3 (1+2)	6 (2+4)	9 (3+6)	12 (4+8)	15 (5+10)
Bark: Bark		9 (3+6)	12 (4+8)	15 (5+10)	18 (6+12)	21 (7+14)
Leaf: Leaf		18 (6+12)	21 (7+14)	24 (8+16)	27 (9+18)	30 (10+20)

Experimental Conditions
An assortment of physio-chemical characteristics, including temperature, pH, dissolved oxygen, and alkalinity, were measured in the water during the experiment. There were temperatures in a range of 26.5-28.5°C in the water's temperature. The remaining parameters dropped within the following ranges: pH 7.2–7.7, dissolved oxygen 7.2–8.3 mg/L, and total alkalinity 9.5 mg/l.

Up to a period of 96 hours, mortality was measured at 24-hour intervals. A computer application called POLO was used to analysed the bioassay data and determine the doses that are fatal (LC50 values), as well as the upper and lower confidence limits (UCL, LCL), and slope values (Jacqueline *et al.*, 2007). According

to Sokal and Rohlf (1973), the regression coefficient was calculated between exposure time and various LC50 values.

RESULTS

Effect on Behavioural changes and Poisoning Symptoms:

After chemical compounds were exposed for just a few minutes, changes in both their behaviour and their appearance were observed. The fish's skin turns a pale grey colour, and exposure causes the black dots on their fins to become less intense. Fishes may often come to the top of the water to gasp for air and will begin to scratch their noses at the bottom of glass jars. Fish attempt to break free from the test jar after 15 to 30 minutes. They slowed down after thirty minutes, but they still glided close to the surface. After that, the fish proceeded to move more irregularly, quickly, and frequently as the exposure duration expanded. After 10–12 hours, at greater doses, the fish eventually fell due to loss of bodily homeostasis and haemorrhage, which showed up as a reddish colour in the head region. Fish under control were not showing any behavioural abnormalities.

Determination of LC value against different concentrate combination:

Table 2: Toxicity (LC_{50}) of methanolic extraction combination of Binary (1:1) Compound of leaf, Stem bark and Latex of *Codium variegatum* and *Alstonia scholaris* against fish *Mystus mystus.*

Hours	Combination	Effective Does (mg/l)	Limits (mg/l)		Slop value	't' ratio	Heterogeneity
			LCL	UCL			
24	Leaf: Leaf	LC_{50}=95.70	83.07	127.43	4.32±0.86	4.99	0.34
	Bark: Bark	LC_{50}=43.02	35.88	62.31	3.38±0.68	4.93	0.36
	Latex: Latex	LC_{50}=35.49	28.58	54.39	2.63±0.52	5.01	0.21
48	Leaf: Leaf	LC_{50}=74.12	66.33	88.62	3.64±0.69	5.26	0.31
	Bark: Bark	LC_{50}=31.12	26.95	39.27	2.81±0.53	5.22	0.33
	Latex: Latex	LC_{50}=22.22	18.20	29.71	1.90±0.36	5.18	0.19
72	Leaf: Leaf	LC_{50}=56.64	51.39	62.15	4.19±0.67	6.21	0.55
	Bark: Bark	LC_{50}=21.98	19.37	24.78	3.24±0.52	6.20	0.54
	Latex: Latex	LC_{50}=12.47	10.06	14.79	2.23±0.35	6.33	0.33
96	Laef: Leaf	LC_{50}=44.50	39.80	48.29	5.36±0.74	7.21	1.00
	Bark: Bark	LC_{50}=16.06	13.88	17.87	4.11±0.56	7.25	0.99
	Latex: Latex	LC_{50}=6.24	4.30	7.83	2.56±0.40	6.32	0.53

Batches of 10 fishes were exposed to Six different concentrations combination of plat *Codium variegatum* and *Alstonia scholaris*.

The concentrations indicated were the final concentrations (w/v) in laboratory circumstances.

Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.

LCL=Lower confidence limit; UCL=Upper confidence limit.

Table 3: Toxicity (LC_{50}) of methanolic extraction combination of Binary (1:2) Compound of leaf, Stem bark and Latex of *Codium variegatum* and *Alstonia scholaris* against fish *Mystus mystus*.

Hours	Combination	Effective Does (mg/l)	Limits (mg/l)		Slop value	't' ratio LCL	Heterogeneity
			LCL	UCL			
24	Leaf: Leaf	LC_{50}=93.25	86.51	107.31	7.47±1.40	0.28	0.28
	Bark: Bark	LC_{50}=65.52	58.88	79.27	5.11±0.92	0.21	0.21
	Latex: Latex	LC_{50}=60.68	46.44	108.48	2.33±0.50	0.25	0.25
48	Leaf: Leaf	LC_{50}=81.26	76.07	90.02	5.96±1.13	0.29	0.29
	Bark: Bark	LC_{50}=46.06	42.21	50.67	4.41±0.69	0.29	0.29
	Latex: Latex	LC_{50}=34.30	27.79	47.40	1.80±0.36	0.25	0.25
72	Leaf: Leaf	LC_{50}=65.82	61.60	69.43	7.14±1.11	0.30	0.30
	Bark: Bark	LC_{50}=36.65	32.44	40.18	4.21±0.67	0.26	0.26
	Latex: Latex	LC_{50}=19.82	15.03	24.63	1.71±0.34	0.29	0.29
96	Leaf: Leaf	LC_{50}=53.69	48.12	57.35	8.74±1.41	0.53	0.53
	Bark: Bark	LC_{50}=30.66	26.24	33.91	4.65±0.73	0.45	0.45
	Latex: Latex	LC_{50}=11.31	7.90	14.08	2.23±0.37	0.57	0.57

Batches of 10 fishes were exposed to Six different concentrations combination of plat *Codium variegatum* and *Alstonia scholaris*.
The concentrations indicated were the final concentrations (w/v) in laboratory circumstances.
Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
LCL=Lower confidence limit; UCL=Upper confidence limit

Toxicity against fish *Mystus mystus* was time as well as dose dependent. There was a significant correlation between LC50 values of extract compound of leaf of *Codium variegatum* in binary (1:1) combinations with extract compound of leaf of *Alstonia scholaris* is decreases from 95.70 mg/L (24h);> 74.12 mg/L (48h);> 56.64 mg/L (72h);>44.50 mg/L (96h) , bark+ bark is decreases 43.02 mg/L (24h);> 31.12 mg/L (48h);> 21.98 mg/L (72h);> to 16.06 mg/L (96h) and latex+ latex is decreases from 35.49 mg/L (24h);> 22.22 mg/L (48h);> 12.47 mg/L (72h);> to 6.24 mg/L (96h) respectively against *Mystus mystus* (Table 2). Same trend of toxicity was observed in the binary (1:2) combinations of leaf + leaf is decreases from 93.25 mg/L (24h);> 81.26 mg/L (48h);> 65.82 mg/L (72h);>53.69 mg/L (96h) , bark+ bark is decreases 65.52 mg/L (24h);> 46.06 mg/L (48h);> 36.65 mg/L (72h);> to 30.66 mg/L (96h) and latex+ latex is decreases from 60.68 mg/L (24h);> 34.30 mg/L (48h);> 19.82 mg/L (72h);> to11.31 mg/L (96h) respectively against *Mystus mystus* (Table 3).

DISCUSSION

As can be observed from the results, both the freshwater fish *Mystus mystus* in the glass jar and the mixed binary (1:1) and (1:2) combinations of *Alstonia scholaris* leaf, stem bark, and latex are poisonous to Codium variegatum leaf, stem bark, and latex. Fish attempt to withstand environmental changes in the aquatic environment and minimize the adverse effects of integrating various compounds by modulating a broad spectrum of emotional and behavioural responses. These include a change in colour of the skin, the beginning of nose-picking at the bottom of the aquarium, and frequent rises to the water's surface to breathe. A number of factors may be contributing to the higher mortality observed with longer exposure periods, either independently or in combination. For instance, the time-dependent uptake of the active moiety causes the drug's entrance and effects in the snail body to gradually increase (Singh and Agarwal, 1993a; Singh and Agarwal, 1993b). According to Singh and Singh (2005), there is a significant molluscicidal activity in

the aqueous latex extracts of *Thevetia peruviana* and *Alstonia scholaris*. More precisely, the LC50 of the extracts decreases from 4.76 mg/L (24 hours) to 1.76 mg/L (96 hours) and from 0.43 mg/L (24 hours) to 0.17 mg/L (96 hours) when applied to the freshwater snail *Lymnaea acuminata*.

It is evident that the naturally occurring toxicity of the plants under evaluation was decreased. According to Dawson *et al.*, (1991), the cause of the decreased toxicity may be the adsorption of soil particles or the temperature-induced acceleration of the toxicant breakdown process. (Chiayvareesajja *et al.,* 1997) discovered a similar tendency, where fish could be stocked in ponds four days after the plant pesticides were applied due to the short toxicity persistence of *Masea ramentacea* and tea seed cake plants. It has been demonstrated that the plant *Masea ramentacea* can be used in place of tea seed cake to kill predatory fish in freshwater. However, the effective concentration against fish that breathe air, such as *Clarias species*, *Ophicephalus striatus*, and *Anabas testudineus*, which are typically more tolerant than other fish species, needs to be determined (Chiayvareesajja *et al.,* 1997).

The LC50 values of the studied plant compound against freshwater fish in a lab setting were found to be binary (1:1) and (1:2) combinations of *Codium variegatum* leaf, stem bark, and latex with *Alstonia scholaris* leaf, stem bark, and latex. *Mystus mystus* was found in methanol extracts at 95.70 mg/L (24 hours), 43.02 mg/L (24 hours), 35.49 mg/l (24 hours), and 93.25 mg/l (24 hours), 65.52 mg/l (24 hours), and 60.68 mg/l (24 hours), respectively.

There was a substantial positive connection between dose and mortality across every one of the compounds that resulted in death. It might be due 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 exposure duration and LC value. According to Goodmann *et al.,* (1985), there could be several of factors at play, both independently and to one another. The link between exposure times and mortality is additionally impacted by the stability (life span) of active moieties in the environment and the frequency at which they are detoxified in the body of an animal (Mitra *et al.*, 1978; Matsumura, 1985).

In substitute of harmful chemical pesticides, newer insecticides that are biological in origin are being created. Chemical pesticides are effective and target specific, yet they negatively affect the ecosystem. Pesticides derived from plants have low half-life periods for their active ingredients, and their impacts on the environment are extremely harmful (Sharma *et al.*, 1995).

It is recommended that the usage of plant-based pesticides be promoted in order to minimize the amount of chemicals that are released into the environment (Schmutterer, 1990). Even so, consideration should be used while using plant-based pesticides, only using small amounts. Additionally, in many environmental locations, plant-based insecticides dissolve readily into constituent elements without leaving a permanent mark on the environment (Khan and Ahmed, 2000).

CONCLUSION

In summary, plant *Codium variegatum's* leaf, stem bark, and latex extract compound, as well as plant *Alstonia variegatum's* leaf, stem bark, and latex extract compound, can be combined in binary (1:1) and (1:2) combinations with other plant origin piscicides in a glass jar to control the population of predatory fish in aquatic mediums. These binary (1:1) and (1:2) combinations can increase the effectiveness and lower the dosages of pesticides originating from plants, ensuring that the treated water regions are safe for the environment.

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