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Antigonon leptopus Hook. & Arn.- a new plant origin icthyotoxicant on *Channa punctata* (Bloch, 1793) for aquaculture management

Dulal Kumar De[1], Sujit Kumar De[2], Rama Prasad Bhattacharya[3], Bratati Bhanja[4], Dipankar De[4]

[1]Department of Botany, Midnapore College (Autonomous), Midnapore, West Bengal, India
[2]Department of Mathematics, Midnapore College (Autonomous), Midnapore, West Bengal, India
[3]Bikash Bhavan Kolkata, West Bengal, India
[4]Research Scholars, Midnapore College (Autonomous), Midnapore, West Bengal, India

ABSTRACT

The present study was conducted to develop an ecofriendly and effective organo-piscicide. Here piscicidal effect of locally available plant *Antigonon leptopus* (Hook. and Arn.) Against common freshwater air breathing predatory fish *Chana punctatus* (Bloch,1793)and their behavioral changes were investigated. The test fish was exposed to five different concentrations of fresh leaves aqueous extract (10,20, …50ppm) for toxicity test remaining one a control. Attempts had been made to access the impact of 24h $LC_{100}$of the aqueous extract of *A. leptopus* leaves on the test fish *Chana punctatus*. To determine the LC_{100} of the leaf extracts, relationship between observed and expected rate of mortalities of the test fish and dose-mortality relationships, Chi-square and ANOVA tests were done. This study indicates that the leaf extract of the plant species may be used as potential organo-piscicide to remove the unwanted weed fish species from the fish culture ponds as an environmentally safe and effective phyto-piscicide necessary for aquaculture management.

KEYWORDS: *Antigonon leptopus, Chana punctatus, ANOVA, Chi-square test, Ichthyotoxicants*

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1. INTRODUCTION

Since prehistoric times piscicidal plants have been used throughout the world for fishing. Plants are valuable and inexhaustible sources for biologically active substances. Insecticidal, piscicidal, molluscicidal, medicinal properties of plants have also been used widely[1].

Besides, the plants are used as traditional piscicidal agents for stupefying or killing wild fishes. Plant derived fish toxicants are also used in aquaculture management for controlling the predatory and weed fishes. The eradication of these predatory or weed fishes from the culture ponds is an important step in pond management before the stocking of desired species.

In this respect, the air-breathing predatory fish species are of particular importance as they are highly resistant to toxicants[2] and may survive in moist borrows and mud even when ponds are drained. The use of plant origin ichthyotoxicant as a fisheries management tool has been practiced in at least 30 countries[3-5] due to its low cost, easily availability and eco-friendliness.

The control and eradication of unwanted fishes in the pond require effective piscicides which are usually not easily accessible. But due to lack of excessive plant based piscicides farmers are using synthetic compounds including malachite green, sodium cyanide, antimycin etc. and even pesticides[6-9].

These synthetic piscicides have long-term persistence in the ecosystem as well as in the cultured species. Therefore, alternative piscicides such as botanicals, which are biologically degradable, and having

piscicidal activities with shorter residual effects are being appreciated.

Again due to lack of modern technique for preparation of plant materials as a fish poison and also their limited availability in the market the fish farmers of this district use insecticides and piscicides viz. DDT, Aldrin, Dieldrin etc. for cleaning the nursery and stocking ponds[9]. The increasing use and the toxicity of these synthetic insecticides and pesticides have a lot of adverse effects on the environment viz. (i) residual toxicity which may cause the biomagnification in the aquatic food chain (ii) most of them are highly toxic to mammals, and also require precaution in handling and technical skill in operation [7].However, the most effective and safe method is the use of plant origin fix toxicants as these satisfy all the requirements of ideal fish poison[9].

Keeping this view in mind, the present research work has been undertaken to prepare ichthyotoxicant from plant parts. Findings of this research may be useful to develop some low-cost plant origin herbal piscicide which may help to protect the fish from predators and thus to increase the fish production in commercial fish farms and aquatic habitats without creating environmental hazards.

2. Study area

Paschim Medinipur district is situated between $25^{0}06^{/}$ N-$27^{0}04^{/}$N latitude and $93^{0}20^{/}$- $95^{0}15^{/}$E longitude and covers an area of Paschim Medinipur district is one of the districts of the State of West Bengal, India**.** On 1st, January, 2002 it was formed after the partition of earlier Midnapore District into Purba Medinipur and Paschim Medinipur district. The District of Paschim Medinipur is located in the South- Western part of West Bengal. It is at 23 meters above Sea level. The climate of this district follows a hot tropical monsoon weather pattern. Soil types near Kangsabati River are alluvial, whereas towards Rangamati are lateritic. Vegetation includes Species of *Eucalyptus* and Sal (*Shorea robusta* L). Forest is located on the North West side of the Midnapore Town. The average annual precipitation of this district is 2,111mm. According to the 2011 Census Paschim Medinipur district has a population of 5,943,300 (54-56). Schedule Caste (SC) constitutes 19.1 % while Schedule Tribes (ST) are upto 14.9% of total population of the district. 12.2% people live in urban areas while 87.8% live in the rural areas (Census 2011).

3. Experimental design

A research design has been developed by the following ways.

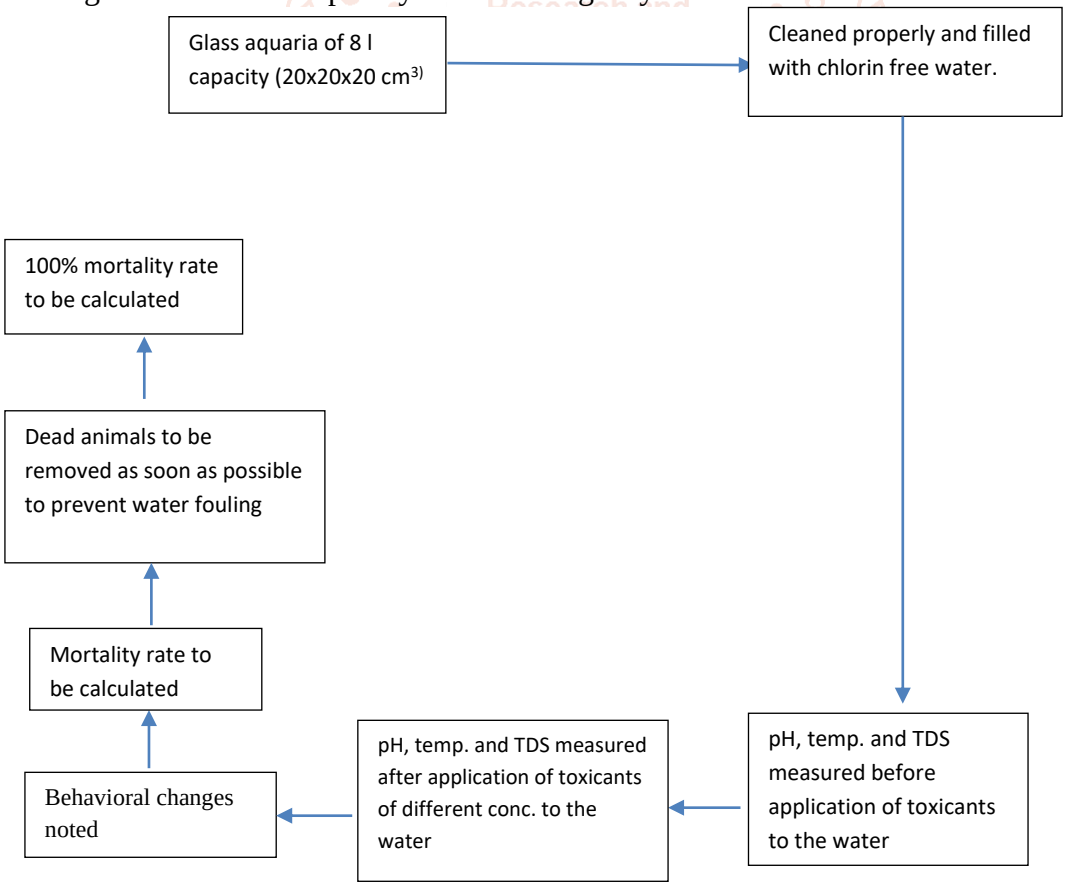


Fig.1:Schematic diagram of present Research design

4. Materials and Methods

4.1. Materials Used

4.1.1. Experimental plant material

A. Diagnostic character of *A. leptopus*

Coral vine or *A. leptopus* (Hook. and Arn.) is a native of Mexico. It is a fast growing, evergreen vine. Here inflorescence axis has been modified into tendrils and can climb about 40 ft. Leaves are dark green, heart shaped to arrowhead-shaped and about 5 inches long. Due to the presence of heart shaped leaves and the delicate pink flowers, it is called "Chain of love" in Mexico. The actual flowers are tiny but the sepals are longer and provide the brilliant colour that range from white to rose pink to deep coral flowered varieties[10, 10.a].The plant coral vine is listed as category II invasive, exotic by Florida's Pest Plant Council[11].

Fig-2 (A): A flowering twig of *A. leptopus*

B. Phytochemical

Phytochemical screening of methanolic leaf extract of *A. leptopus* revealed presence of saponin, phenolic compounds, tannins, flavonoids, alkaloids, fixed oils and amino acids.

Fish pathogens, *Providencia vermicola* and *Aeromonas hydrophila* were sensitive to the methanolic leaf extract[14].

C. Medicinal importance

Consumption of *A. leptopus* as a tea is beneficial for hydration, but preliminary evidence regarding its anti-inflammatory, analgesic properties will deserve further confirmation[15].

Different parts of the plant are investigated for pharmacological properties. The plant is reported to possess various bioactivities such as antimicrobial, antioxidant, hepatoprotective, analgesic, anti-inflammatory, cytotoxic and antidiabetic activities. Besides the plant can be utilized as a remedy for various ailments and to develop drugs with potent pharmacological activities that can benefit human being[12].

Other uses- Seeds are edible in Spain

4.2. Experimental animal

A. Habit and habitat of Lathya

Channa punctatus (lathya fish) is a species of snake headed, inhabits fresh water ponds and ditches. It is a common edible, wild fish. It is usually carnivorous and each other small fishes and small aquatic animals. Lathya fish is notable for migration overland from one pond to another during rain. The ability to breath in air by its accessory respiratory organ helps to fish to thrive well out of water for a considerable period of time.

Its natural habitats are swamps, ponds and brackish water systems[16]. It is a fish of high food value and has little values as aquarium fish.

It is listed as least concern in IUCN, due to lack of major threats to this species populations [17]
Journal of Entomology and Zoology Studies.

B. Geographical distribution

Channa punctatus is found in the Indian Subcontinent and hereby area ranging across -Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar and Tibet[16].

C. External Structure

The body of *Channa punctata* (Bloch,1793) (Lathya/Takifish) is elongated and covered with ctenoid scales. It has been recorded that; cycloid scales are also present. Both the scales are systematically arranged cycloid and prectenoid. The body of Channa punctatus is elongated and covered with ctenoid scales. It has been recorded that; cycloid scales are also present. Both the scales are systematically arranged cycloid and prectenoid. The body is divided into head, trunk and tail regions. The head is depressed and covered by large plate like scales resembling the "head -shields" of snakes. The eyes are placed on the lateral sides of the head. The dorsalfin is single. The anal fin extends from the posterior end of the anus. Both the dorsal and anal fins are undivided and are supported by spinous bony fin rays. The caudal fin is unilobed[13].

Fig.-2 (B): External morphology of *Chana punctatus*

4.3. Methodology Implementation

Step -1: Collection and preparation of leave extracts

Fresh leaves of *A. leptopus* were collected from different places of Paschim Medinipur district. Leaves were chopped and then homogenized using electric grinder to get the pure leaf extract.

Step- 2: Collection and acclimatization of experimental fishes

Healthy and live fishes of *C. punctatus* (average weight of 77 gm) were collected from the nearby farm.

Collected fishes were acclimatized for seven days in the laboratory condition before using for experiment. During that period, the aquarium water was aerated continuously and fishes were daily fed an artificial feed like fish meal. Water was changed at every 24 h. The water temperature in aquaria is $20^0C \pm 1^0C$ and pH is 7.2 ± 0.2

Step-3: Analysis of water quality parameter

Some important physio-chemical parameter of water solution- TDS, DO, total alkalinity was studied. Water quality parameter during median lethal test for 24hour experiment was analyzed at the beginning and end of the experiment by using the methods described in .

Bioassays:

Experiments were conducted in glass aquaria each measuring ($20x20x20\ cm^3$) and containing 8 l of water in the month of January. At first the aquaria were cleaned and filled with water. The pH, temperature and TDS values of experimental as well as the control aquaria were recorded before the application to the toxicants into the water and at several time interval of the experiment and at the time when detoxication occurred completely.

Five concentrations (0,10, …50g/l) of all the different fresh leaf extracts were used. Each treatment had three replicates. These concentrations were made on the basis of the results of initial experiments with arbitrary concentrations. (Four) fishes were kept in each aquarium. Room temperature was maintained thorough at the study period. Different specific quantity of leaf extracts was added in the test aquaria and gently mixed with glass rod.

Step-4: Behavioural responses of fishes to test concentrations of ichthyotoxicants

When fishes exposed to the different concentration of leaf extracts exhibited various abnormal behavioural patterns before death occurred. Erratic and agitated movement, trying to jump out of the test media, gulping for air and loss of equilibrium, restlessness, were frequently observed. Their rate of operculum movement also increased. Control group of fishes are devoid of such behavioural changes.

The fishes which became affected by the toxic action of leaf extract first expressed distressed symptoms. Gradually, they became inactive and started losing their balance. For this action, they went to the bottomof the aquaria; lie down on their sides till death. Here a fish was considered dead when its respiratory movement

checked and became non- sensitive to mechanical stimuli. Experiments were also conducted to determine the detoxification time of the toxicants. The dead animals were removed as soon as possible from test container to prevent water fouling.

Fig-3: Behavioral response of test fishes to ichthyotoxicant

5. Data Collection and Data Analysis

Here, we put our observed collected data according to the time required to clean [Table 1 (a)] under L_{25}, L_{50} , L_{75} and L_{100}respectively and that with the average weights of fishes used are given in Table 1 (b) alone.

Table 1 (a): Record of average time (hours) under room temperature 22^0 C.

Methods	Dose		Observation			
	TDS	ppm	L_{25}	L_{50}	L_{75}	L_{100}
	10	210	2.167	3	4.75	6.75
	20	309	1.5	2.25	3.83	5.75
	30	346	1.083	1.583	3.05	4.467
	40	417	1	1.367	2.667	3.933
	50	481	0.917	1.15	1.917	3.417

Table 1 (b): Record of average weight (grams) under room temperature 22^0 C.

Methods	Dose		Observation			
	TDS	ppm	L_{25}	L_{50}	L_{75}	L_{100}
	10	210	17.6	56	74.4	105
	20	309	17.2	57.7	76	104
	30	346	4.3	66.2	77.8	107.5
	40	417	3.9	54	76.2	84.6
	50	481	4.9	24.5	68.3	104

5.1. Statistical Analysis

The design of experiment was sent in such a way to make it possible to do analysis of variance in completely randomized design (ORD). LSD analysis was done to test mean difference. The linear correlation of various doses of piscicide with body weight of fishes and time killing of fishes were analyzed.

Now to calculate the ANOVA table we compute the following

1. $CF = \frac{T^2}{N} = \frac{56.548^2}{20} = 159.88$,
2. ${}_1x_{ij}^2 = (4.6959 + 2.25 + 1.1729 + 1 + 0.8409) + (9 + 5.0625 + 2.5059 + 1.8687 + 1.3225) + (22.5625 + 14.6689 + 9.3025 + 7.1129 + 3.6749) + (45.5625 + 33.0625 + 19.9541 + 15.4685 + 11.6759) = 212.7645$
3. $TSS = \sum_{i=1}^{5}\sum_{j=1}^{4} x_{ij}^2 - CF = 212.7645 - 159.88 = 52.88$

4. Let us compute, $) = \frac{694.353}{4} =$ 173.59

Let us compute, $\sum_{i=1}^{5}\left(\frac{T_i^2}{4}\right) = \frac{694.353}{4} = 173.59$

5. $$Therefore, SS\ due\ to\ Observation(SSV) = \sum_{i=1}^{5}\left(\frac{T_i^2}{4}\right) - CF = 13.71$$

6. $$Therefore, SS\ due\ to\ Observation\ (SSV) = \sum_{i=1}^{5}\left(\frac{T_i^2}{4}\right) - CF = 13.71$$

7. $$SS\ due\ to\ Methods(SSB) = \sum_{j=1}^{4}\left(\frac{T_j^2}{5}\right) - CF = \frac{986.0813}{5} - 159.88 = 37.336$$

8. $SS - SSB - SSV = 52.88 - 37.336 - 13.71 =$ 1.834

Table 2: Computation of ANOVA for time of five methods and observation

Sources of Variation	SS	*df*	MSS	*F*	Tabulated *F*
Observation	13.71	3	4.57	29.908	$F_{0.01}(3,12) = 5.95$ $F_{0.05}(3,12) = 3.49$
Methods	37.336	4	9.334	61.086	$F_{0.01}(4,12) = 5.51$ $F_{0.05}(4,12) = 3.26$
Error	1.834	12	0.1528		
Total	52.88	19			

Here the null hypothesis to be tested are i) H_{01} :Observation means do not differ significantly and ii)H_{02} :Method means do not differ significantly

1. Since the observed *F* value is greater than tabulated *F* value so, H_{01} cannot be accepted for both 1% and 5% level of significance, that is the observations are significantly different.
2. Since observed *F* value is greater than tabulated *F* value so, H_{02} cannot be accepted for both 1% and 5% level of significance, that is the method means are also differ significantly.

Thus, we may conclude that, the observation means as well as the method means are significantly different. Here, the error mean square $(\sigma^2) = 0.1528$. Therefore, standard error between any two observations means $= \sqrt{0.1528\left(\frac{1}{4}+\frac{1}{4}\right)} = 0.076$. Similarly, standard error between any two methods means= $\sqrt{0.1528\left(\frac{1}{5}+\frac{1}{5}\right)} = 0.061$.

Since these errors are very small and almost equal, so the *S*tatistic represents a stronger test regarding the conclusion of the experiment.

We further consider the data set of Table 1 (b), representing individual weights of test fishes and compute the ANOVA Table 3.

Table 3: One way ANOVA for weight of five methods and observation

Sources of Variation	SS	*df*	MSS	*F*	Tabulated *F*
Observation	627.667	3	209.22	2.546	$F_{0.01}(3,12) = 5.95$ $F_{0.05}(3,12) = 3.49$
Methods	22514.6135	4	5628.65	68.48	$F_{0.01}(4,12) = 5.51$ $F_{0.05}(4,12) = 3.26$
Error	986.309	12	82.19		
Total	24128.59	19			

Here the null hypothesis to be tested are i) H_{01} :Observation means do not differ significantly and ii)H_{02} :Method means do not differ significantly

1. Since observed *F* value is less than tabulated *F* value so, H_{01} is accepted for both 1% and 5% level of significance, that is the method means do not differ significantly.
2. Since *F* value for methods used is greater than tabulated *F* value so, H_{02} cannot be accepted for both 1% and 5% level of significance, that is the observations are significantly different.

Thus, we may conclude that, the method means are significantly different while the observation means have less significant difference. Here, the error mean square $(\sigma^2) = 82.19$. Therefore, standard error between any two observations means $= \sqrt{82.19\left(\frac{1}{4}+\frac{1}{4}\right)}$=41.095 Similarly, standard error between any two methods means= $\sqrt{82.19\left(\frac{1}{5}+\frac{1}{5}\right)}$=32.876. We indeed compute χ^2 test to validate the research study for the expected cleaning time under five different methods. Considering the data set of Table 1 (a), we perform the following χ^2 –Table 4as follows:

Table 4: Computation of χ^2 value for the observed value of Table 1 (a)

f_0	f_e	$f_0 - f_e$	$(f_0 - f_e)^2$	$(f_0 - f_e)^2/f_e$	$\chi^2 - value$
2.167	1.965	0.202	0.0408	0.0208	
1.5	1.572	-0.072	0.0052	0.0033	
1.083	1.200	-0.117	0.0137	0.0114	
1	1.057	-0.057	0.0032	0.00302	
0.917	0.873	0.044	0.0019	0.0021	Tabulated value:
3	2.756	0.244	0.0595	0.0215	$\chi^2_{0.01}(19) = 7.633$
2.25	2.204	0.046	0.0021	0.00095	And
1.583	1.684	-0.101	0.0102	0.00606	$\chi^2_{0.05}(19) = 10.117$
1.367	1.483	-0.116	0.0135	0.0091	
1.15	1.224	-0.074	0.0055	0.0045	
4.75	4.779	-0.029	0.0008	0.0016	
3.83	3.822	0.008	0.000064	0.000017	Observed value:
3.05	2.920	0.13	0.0169	0.0058	$\chi^2 = \Sigma(f_0 - f_e)^2/f_e$ = 0.158837
2.667	2.571	0.096	0.0092	0.0036	For
1.917	2.122	-0.205	0.0420	0.0198	degrees of freedom
6.75	7.167	-0.417	0.1739	0.0242	= 20-1=19.
5.75	5.732	0.018	0.0003	0.00052	
4.467	4.379	0.088	0.0077	0.00176	
3.933	3.856	0.077	0.0059	0.00153	
3.417	3.183	0.234	0.055	0.01728	

Since, the computed value of Chi square is much lower than the critical values in 1% and 5% level of significance, so the null hypothesis is accepted. Hence, we can say that there is no difference between expected average cleaning time and the observed cleaning time. Moreover, referring to Table 1 (a) and Table 1 (b), considering the average weight (gram) of each fish as *X* variable and that for maximum cleaning time as *Y* variable, we obtain the correlation coefficient between X and Y as $\rho_{XY} = 0.953$which conclude that cleaning time is highly related to the average weight of the fishes. However, if we wish to have a toxicity relationship between TDS and average death time with full clean (L_{100}). The correlation indicates a highly negative value $\rho_{XY} = -0.973$ so it is expected that, increase of TDS might minimize the full cleaning (L_{100})time.

Table 5: Computation of confidence intervals for TDS and average death time and weight

Variate	Mean	Standard deviation	Z score $z = \frac{Q - \bar{Q}}{\sigma}$	99% confidence interval	95% confidence interval
TDS (ppm):*X*	352.6	92.64	$z = \frac{X - 352.6}{92.64}$	$\left\|\frac{X - 352.6}{92.64}\right\| \leq 2.58$	$\left\|\frac{X - 352.6}{92.64}\right\| \leq 1.96$
Clean time (hours) Y	4.86	1.22	$z = \frac{Y - 4.86}{1.22}$	$\left\|\frac{Y - 4.86}{1.22}\right\| \leq 2.58$	$\left\|\frac{Y - 4.86}{1.22}\right\| \leq 1.96$
Weight (grams) W	59.2	33.55	$z = \frac{W - 59.2}{33.55}$	$\left\|\frac{W - 59.2}{33.55}\right\| \leq 2.58$	$\left\|\frac{W - 59.2}{33.55}\right\| \leq 1.96$

To get the more precise form of the confidence intervals we have,

95% confidence interval: $352.6 - 1.96 \times 92.64 \leq X \leq 352.6 + 1.96 \times 92.64 \Rightarrow 171.03 \leq X \leq 534.17$

$4.86 - 1.96 \times 1.22 \leq Y \leq 4.86 + 1.96 \times 1.22 \Rightarrow 2.47 \leq Y \leq 7.25$

$59.2 - 1.96 \times 33.55 \leq W \leq 59.2 + 1.96 \times 33.55 \Rightarrow 6.56 \leq W \leq 124.96$

Similarly, for 99% confidence interval:

$352.6 - 2.58 \times 92.64 \leq X \leq 352.6 + 2.58 \times 92.64 \Rightarrow 113.59 \leq X \leq 591.61$

$4.86 - 2.58 \times 1.22 \leq Y \leq 4.86 + 2.58 \times 1.22 \Rightarrow 1.71 \leq Y \leq 8.0$

$59.2 - 2.58 \times 33.55 \leq W \leq 59.2 + 2.58 \times 33.55 \Rightarrow 27.36 \leq W \leq 145.76$

We further investigate the p-values related to observation (based on percentiles) and the methods (based on doses) and they are put in Table 6 and Table 7 respectively.

Table 6: Observation- based *p*-values and their test of significance

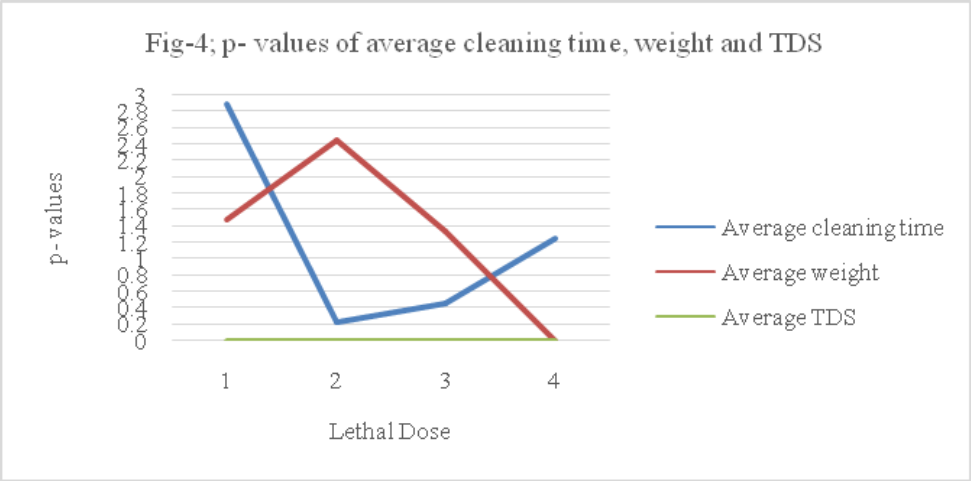
observations	parameters	Values	p-values	decision	Remarks
L_{25}	Average time	1.33	2.893*	Rejected under 5% level of significance, but accepted as 1% level of significance	Significantly different
	Average weight	9.58	1.478	Null hypothesis is accepted for 1% and 5% level of significance in all the cases	There is no difference between observed data and the expected data
	Average TDS	352.6	0		
L_{50}	Average weight	51.68	0.224		
	Average time	1.87	2.45*	Rejected under 5% level of significance, but accepted as 1% level of significance	Significantly different
	Average TDS	352.6	0	Null hypothesis is accepted for 1% and 5% level of significance in all the cases	There is no difference between observed data and the expected data
L_{75}	Average weight	74.54	0.457		
	Average time	3.24	1.327		
	Average TDS	352.6	0		
L_{100}	Average weight	101.02	1.246		
	Average time	4.86	0		
	Average TDS	352.6	0		

Table 7: Method based *p*-values and their test of significance

Methods (ppm)	parameters	Values	p-values	decision	Remarks
$10g$ /L	Average weight	63.25	0.12	Null hypothesis is accepted for 1% and 5% level of significance in all the cases	There is no difference between observed data and the expected data
	Average time	4.17	0.565		
	Average TDS	210	1.539		
$20g/L$	Average weight	63.725	0.134		
	Average time	3.33	1.254		
	Average TDS	309	0.47		
$30g/L$	Average weight	63.95	0.141		
	Average time	2.55	1.893		
	Average TDS	346	0.071		
$40g/L$	Average weight	54.675	0.134		
	Average time	2.24	2.147*	Rejected under 5% level of significance, but accepted as 1% level of significance	Significantly different

	Average TDS	417	0.695	Null hypothesis is accepted for 1% and 5% level of insignificance in all the cases	There is no difference between observed data and the expected data
50 g/ L	Average weight	50.425	0.261		
	Average time	1.85	2.467*	Rejected under 5% level of significance, but accepted as 1% level of significance	Significantly different
	Average TDS	481	1.386	Null hypothesis is accepted for 1% and 5% level of significance in all the cases	There is no difference between observed data and the expected data

6. Graphical illustrations



Note: 1=L_{25}, 2=L_{50}, 3=L_{75},4=L_{100}

Fig-4 reveals the p-values under various parametric components like average cleaning time, weights and TDS with respect to the various lethal doses L_{25}, L_{50}, L_{75} and L_{100} respectively. It is seen that the average weight's p-value under L_{25} lies within 1.4 and 1.6 which is less than the table values for 95% and 99% confidence intervals but that values of the average cleaning time it becomes beyond 2.8. For the study in L_{50} the average weight's p-value is less than that of table values under 99% confidence interval and that for average cleaning time lies within 95% confidence interval. Again, if we wish to study L_{75} and L_{100} then we see that it is accepted for 1% and 5% level of significance. Thus, from the figure we may conclude that there is no significant difference between our experimental design and the corresponding expected values. However, we see that the p-values of the average TDS get negligible value.

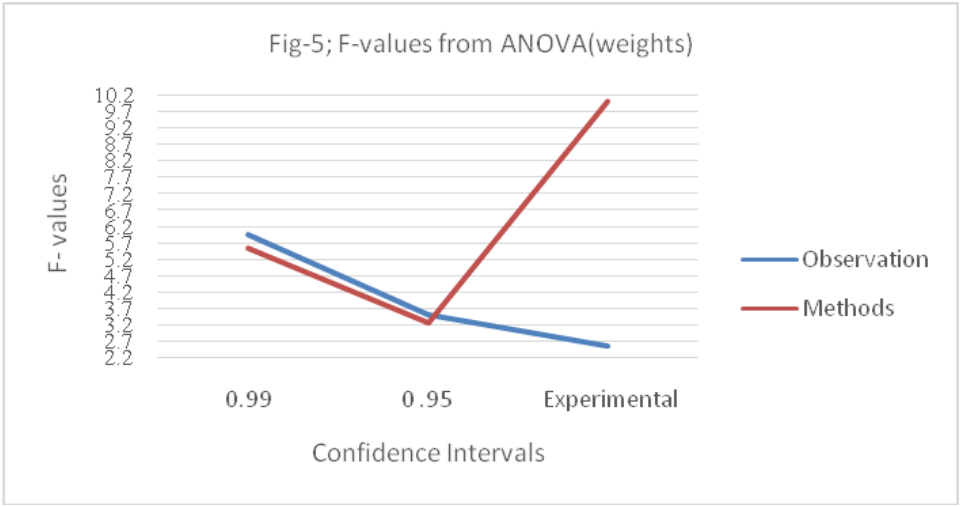


Fig-5 shows the f-values under ANOVA test for the observation and methods used in different confidence intervals like 99% and 95% respectively. It is seen that the experimental values (observation) are smaller than the table values throughout the studies but the methods values in the experiment are higher than the table values for standard 95% and 99% confidence intervals.

7. Conclusion

Aquaculture is the fastest growing food producing sector in the world. For successful or commercial aquaculture, control and eradication of unwanted fishes from the water bodies in prerequisite. Synthetic chemicals are very effective in killing unwanted fishes in a shorter period of time, but not environmental safely to use. In other cases, plant piscicides are chosen because of their eco-friendliness and have dual effects, killing fishes as well as act as manure after certain period of time interval. To sum up, it can be concluded that locally available plant material, *Antigonon leptopus* is a potential ichthyotoxic plant to eradicate unwanted fishes and can be used as piscicide in large scale which is biodegradable and environmentally safe. Moreover, the statistical analysis implies that, studying with *p*-values, the average time of cleaning under L50 might significantly different between observed and expected values; but the average weight of each fish and the average TDS employed must not differ significantly. Again, if we wish to study the p- values under L75 and L100 , in all the cases the average weight of each fish, the average cleaning time and the average TDSimposed have no significant difference between observed and expected values for 99% and 95% confidence level of null-hypothesis testing. Hence our experimental study might carry a high scientific value to the present research in the related field. From the experimental result it can be concluded that leaves of *A. leptopus* would be a potential source of ecofriendly plant based piscicide for aquaculture pond management though further studies are needed to investigate the effect of such organic piscicide to actual pond environment and in futureto produce the product for commercialization.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

Credit Author Statements

Dulal Kumar De: Conceptualization, funding acquisition, project administration, resources, original draft writing and methodology development

Sujit Kumar De: Data curation, Software use, statistical analysis, validation, review and editing.

Rama Prasad Bhattacharya: Visualization, supervision and revision.

Dipankar De: Experiment and observation and Data collection.

Bratati Bhanja: Measurement and recording/ reporting of data.

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