Leaf extract of *Antigonon leptopus* (Hook and Arn.)- An Effective Ichthyotoxicant of plant origin on predatory fish, *Oreochromis niloticus* (Linnaeus,1758)

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

[1]*Dept. of Botany, Midnapore College (Autonomous), Midnapore-721101, W.B., India, E-mail: ddtiger@rediffmail.com*
[2]*Dept. of Mathematics, Midnapore College (Autonomous), Midnapore-721101, W.B., India, E-mail: skdemamo2008.com@gmail.com*
[3]*Bikash Bhavan Kolkata-700001, W.B., India, *E-mail: raprbht@gmail.com*

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ABSTRACT

In newly stocked fishponds, the presence of unwanted predatory fish creates a great nuisance as they compete with desired fishes for food in culture ponds. In this perspective attempt has been made to prepare eco-friendly organic piscicide, for aquaculture management and in future to produce the product for commercialization instead of non-biodegradable chemical pesticides. The acute toxicity study of an ichthyotoxin of plant origin, methanolic extract of *Antigonon leptopus* (Hook and Arn.) on nile Tilapia, *Oreochromis niloticus* (Linnaeus,1758) was carried out for evaluating its potential use in the eradication of predatory fishes from culture system. The experiment was designed by using static renewal bio-assay techniques in transparent glass aquaria in triplicates with concentration of 0 (control), 0.2,.03,0.4,0.5,0.6 mg/5l water for 24 hours. Data on temperature, pH, TDS (Total Dissolve Solutes), DO (Dissolve Oxygen), salinity, alkalinity of the experimental water was collected before and after exposure of plant extract analyzed. The strong statistical tools like interpolating polynomial function, ogives, correlation coefficients, confidence intervals, p-values, F- values of ANOVA test could confirm our findings and claims. Thus, phyto-piscicide or ichthyotoxicants can be the alternative source of synthetic chemicals. Mortality is believed to be direct toxic effects of the plant extracts on the fish tissues due to the present phytotoxic substance, saponin. This toxicant can also be used for stupefactant and easy harvesting of fishes.

1. Introduction

For scientific management of inland aquaculture systems like ponds, tanks etc. there are some problems arises like invasion of unwanted organism including nuisance fishes, insects etc. For eradication of predatory and weed fishes some traditional methods like dewatering and de-silting of ponds, repeated hitting operation, hooks and lines with baits are used but complete eradication is not possible and also it is uneconomical [Rath RK 1993]. For this reason, limited poisoning of the pond with selective toxicant is done. [Sarkhel C 2002]

For scientific fishery management piscicides have long been used as the best rehabilitation tool available.[Prevost G 1960. Klar GT, Schleen LP 2000].Synthetic piscicides are not eco –friendly as it creates environmental hazards and also have detrimental effects on non-target organism.[Fafioye OO, Adedisi AA, Fagade SO 2004]

On the other hand, organic plant piscicides extracted from different plant parts like flower, leaf, bark, stem, fruit, seeds, roots and in some cases the whole plants are used which do not create any environmental hazards and low cost.[Sirivam V et al 2004].So, uses of herbal piscicides are always advantageous over synthetic ones for their effectiveness in killing targeted organisms, eco-friendliness, rapid degradation and non-residual effect.[Das SK, Sarkhel C, Mandal A, Dinda R 2017]. According to Olufayo 2009, usage of such

2. Experimental design

A completely randomized design was used in the experiment.

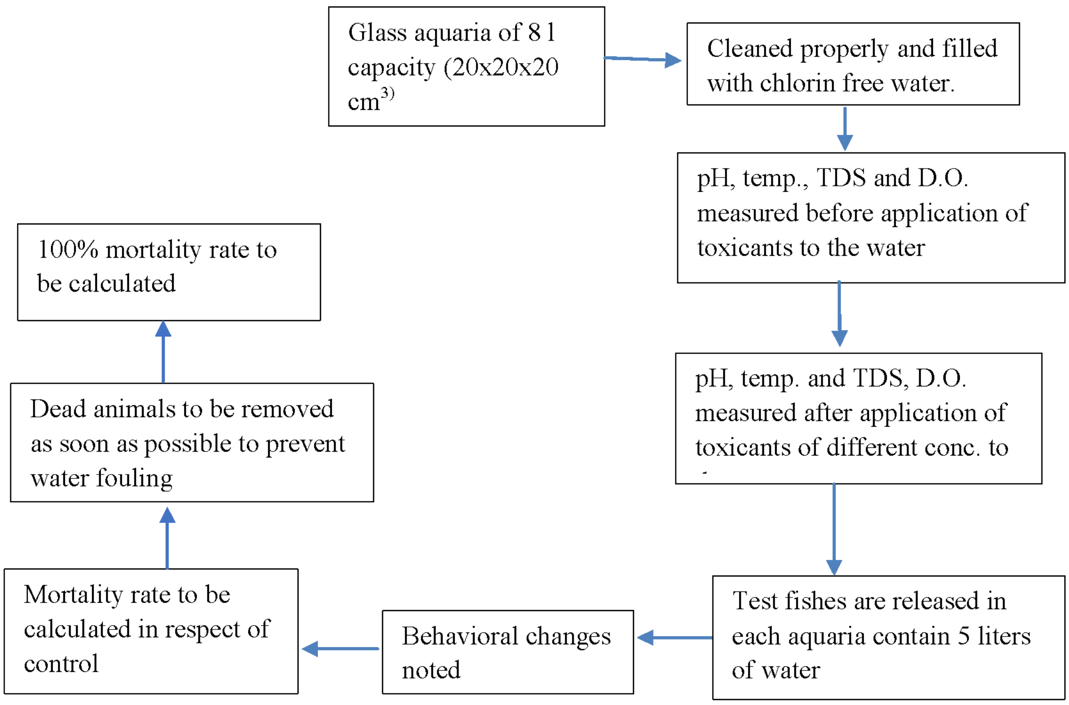


Figure 1. Schematic diagram of present Research

piscicides is encouraged due to their minor toxicity to aquatic organism and non-hazardousness to the environment . The piscicidal potential and phytotoxic properties of plant extracts on *Oreochromis niloticus* have been reported by workers like Nasiruddin et al 2009,2014; Ibrahim 2000; Agbon et al 2002; Ayotunde and Benedict 2008; Mousa et al 2008; Ayoola 2011; Akinduyite and Oyedapo 2011; Fafioye 2012; Okey et al 2013; Adesina et al 2013; Kishore et al 2016.The plant species *Antigonon leptopus* (Hook and Arn.) is a new potential piscicide on *Oreochromis niloticus*. No such piscicidal effect of methanolic extract of *A. leptopus* has yet been done earlier on *Oreochromis niloticus*. Only fresh aqueous leaves extract of this plant has been tested on *Chana punctatus* (Bloch, 1793) as organic piscicide [De et al. 2022]. In context, the study was undertaken to determine the piscicidal effects of leaves of *Antigonon leptopus* on predatory fish, the nile tilapia (*Oreochromis niloticus*). Behavioural responses and mortality rates were observed during the bioassay, to assess the extent of toxicity of the extracts of the plant species (*A. leptopus*).

3. Materials and Methods

3.1.1 Experimental plant material

Coral vine or *A. leptopus* (Hook. and Arn.) (Fig-2) 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.by tendrils, that is the modification of inflorescence axis. Leaves are dark green, heart shaped to arrowhead-shaped and about 5 inches long. For 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.

The plant coral vine is listed as category II invasive, exotic by Florida's Pest Plant Council [Florida Exotic Pest Plant Council.2019.]

3.1.2 Phytochemicals present in *A. leptopus*(Hook. and Arn.)

The qualitative phytochemical analysis of the aqueous extract of the leaf of *A. leptopus* was done using standard procedures to identify the various constituents described by Sofowara A, 1990., Trease GE, Evans WC, 1989. and Harborne JB, 1973.Study of phytochemical screening of methanolic leaf extract of *A. leptopus* revealed presence of saponin, Cardiac glycosides, tannins, flavonoids, alkaloids.

Table I:

Plant	Parts used	Flavonoids	Alkaloids	Terpenoids	Cardiac glycoside	Tannins	Saponin
A. leptopus	Leaves	+	+	+	+	+	+

3.2 Experimental fish

Tilapia, *Oreochromis niloticus* (Linnaeus,1758) belonging to the family cichlidae is a popular and easily cultivated and edible fish. It is an invasive species and fond to cultivate in different corners of our planet. Tilapia are shaped like sunfish or crappie and are easily identifiable by the interrupted lateral line characteristics of the chiclid family of fish. They are laterally compressed with deep bodies and have long dorsal fin. The faremost portion of the dorsal fin is heavily spined. Spines are also found in the pelvic and anal fins. There are usually wide vertical bars of dark coloration found along the sides of fry, fingerlings and sometimes adults.

Fig-2. A flowering twig of *A. leptopus*

Conservation Status – Least concern(IUCN 3.1)[Diallo, I. et all.,2020].

4. Results and calculation

4.1. Behavioural responses by toxic effect-

In the present study, different behavioural activities were observed in *Oreochromis niloticus* (Fig-3) when exposed to the dried methanolic leaf extracts of *Antigonon leptopus*. The fishes of the control solution exhibited regular swimming movements all through the experiments without any mortality. The treated fishes showed their aggressive behaviour within thirty minutes after being exposed to the toxic media. The fishes expressed initial distress via swimming pattern, loss of balance and restless gulping of air. Excessive mucus secretion and unusual lethargy were also observed during this period. In some cases, erratic swimming before settling at the bottom

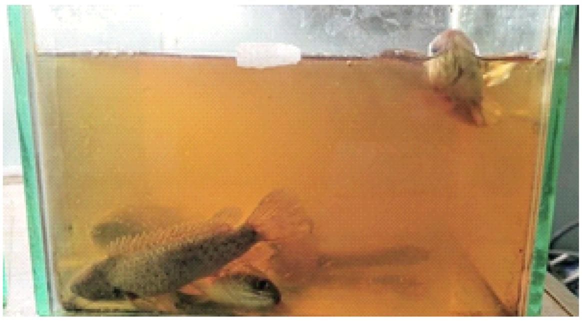


Fig 3. Behavioral response after exposure of toxicant

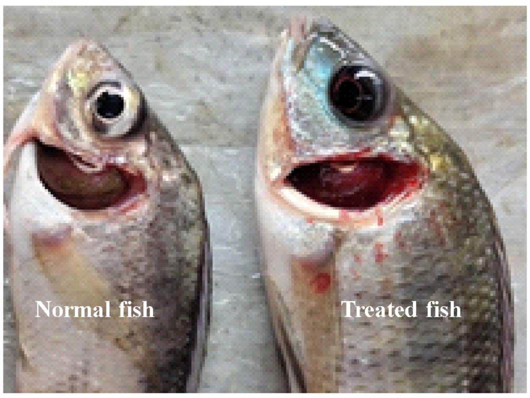


Fig 4. Changes in gill and eyeball after treatment

of the tank with slow operculum movement can be found prior to death. Discolouration of eye and changes of colour of gills (reddish to blackish) has been observed.

4.2. Cytological observation by toxic effect

Blood smear with Leishman stain of non-treated and treated fish was prepared and observed under binocular microscope at 40x.

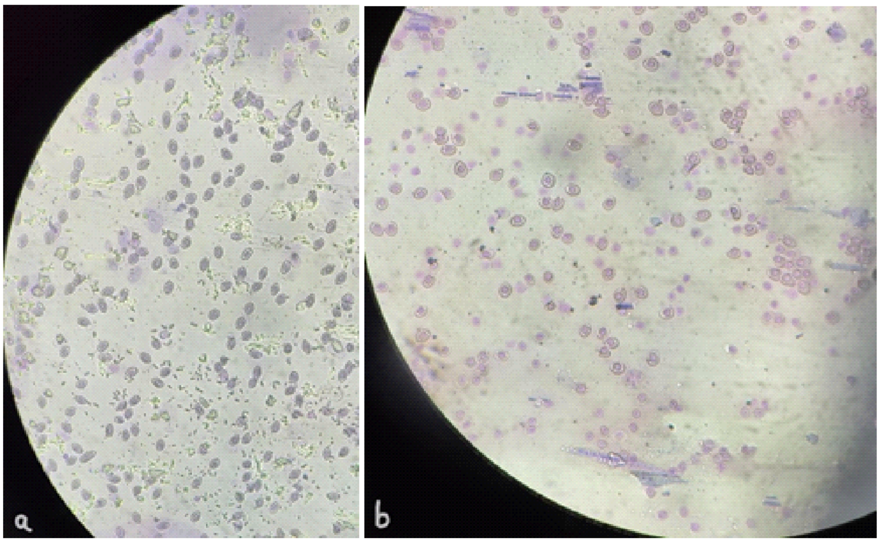


Fig 5. Microscopic view of RBC non-treated (a) and treated fish (b)

Some changes in the structure of RBC observed between pre and post treatment with plant toxicant. In case of non-treated fish RBC with entire cell wall observed (Fig 5.a) while in treated fish, ruptured RBC are found (Fig 5.b).

4.3. Statistical analysis

Here, we put our observed collected data according to the time required to clean [Table II(a)] under L_{20}, L_{40} , L_{60} , L_{80} *and* L_{100} respectively and that for the average weights of fishes used are given in Table II (b) alone. The ANOVA table for the above data Table II(a)) indicates that the F value becomes 12.325 and 3.87 for the observation and method employed in the test which are higher than the tabulated [4.77,] value under 99% and 95 % confidence intervals respectively. We may assume that the null hypothesis, to be tested are i) Observation means do not differ significantly and ii) Method means do not differ significantly. The decision for the above hypothesis reaches to:

i) Since observed F value is greater than tabulated F value so, cannot be accepted for both 1% and 5% level of significance, that is the observations are significantly different.

ii) Since F value for methods used is less than

Table II(a): Record of average time (hours) under room temperature 31^0 C

	Dose		**Observation**				
	TDS	ppm	??20	??40	??60	??80	L_{100}
Methods	0.2g/5l	196	1.75	3.35	5.58	7.33	8.58
	0.3g/5l	202	1.58	1.66	1.86	2.05	4.25
	0.4g/5l	206	0.83	1.42	1.75	1.783	2
	0.5g/5l	210	0.66	1	1.33	1.5	1.83
	0.6g/5l	216	0.5	0.75	1.83	1.083	1.25

tabulated F value for 1% but greater than tabulated F value for 5% so, accepted for 1% level of significance but cannot be accepted for 5% level of significance, that is the method means do not differ significantly.

Thus, we may conclude that, the observation means as well as the method means do not differ significantly. Here, the error mean square (σ^2)=1.239. Therefore, standard error between any two observations means $\sqrt{1.239\left(\frac{1}{5}+\frac{1}{5}\right)}$ =0.4956. Similarly, standard error between any two methods means $\sqrt{1.239\left(\frac{1}{5}+\frac{1}{5}\right)}$ =0.4956. Since these errors are very small and almost equal, so the Statistic represents a stronger test regarding the conclusion of the experiment.

Moreover, if we wish to Compute the ANOVA table (based on Table II(b) values) regarding the average weights of each fish then we see that, the F values become 2.6128 and 26.146 respectively for the observation means as well as the method means. We conclude that,

Table II(b): Record of average weight (grams) under room temperature 31^0 C

	Dose		**Observation**				
	TDS	ppm	??20	??40	??60	??80	L_{100}
Methods	0.2g/5l	196	3.2	5.3	3.5	9.4	12.3
	0.3g/5l	202	4.6	4.7	6.4	7	15
	0.4g/5l	206	3.5	4.2	9	12.3	15.3
	0.5g/5l	210	5.2	6	6.7	8.9	11.2
	0.6g/5l	216	1.7	5	5.3	6.4	10.4

iii) Since observed F value is less than tabulated F value so, is accepted for both 1% and 5% level of significance, that is the observations do not differ significantly.

iv) Since observed F value is greater than tabulated F value so, can't be accepted for both 1% and 5% level of significance, that is the method means differ significantly.

Thus, it is expected that, the method means are significantly different while the observation means have no significant difference. Here, the error mean square (σ^2)=2.5195 Therefore, standard error between any two observations means $\sqrt{2.5195\left(\frac{1}{5}+\frac{1}{5}\right)}=1.008$. Similarly, standard error between any two methods means $\sqrt{2.5195\left(\frac{1}{5}+\frac{1}{5}\right)}=1.008$.

Moreover, 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 $\rho_{XY}=0.942$ which concludes 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 $\rho_{XY}=0.872$ so it is expected that, increase of TDS might minimize the full cleaning (L_{100}) time.

The following **Figure 6** shows that, the less than type and the greater than type ogives have intersected in a single time point (hours) for each lethal dose. We see that, for L20, L40, L60, L80 and L100 the respective times are at most 15 hours, 5 hours, 3.5 hours, 3 hours and 2.8 hours respectively.

Indeed, if we wish to consider the TDS (ppm) as X variable, the average clean time (hours) as Y variable and the average Weight (grams) of each fish as W variable then their confidence intervals can be obtained as:

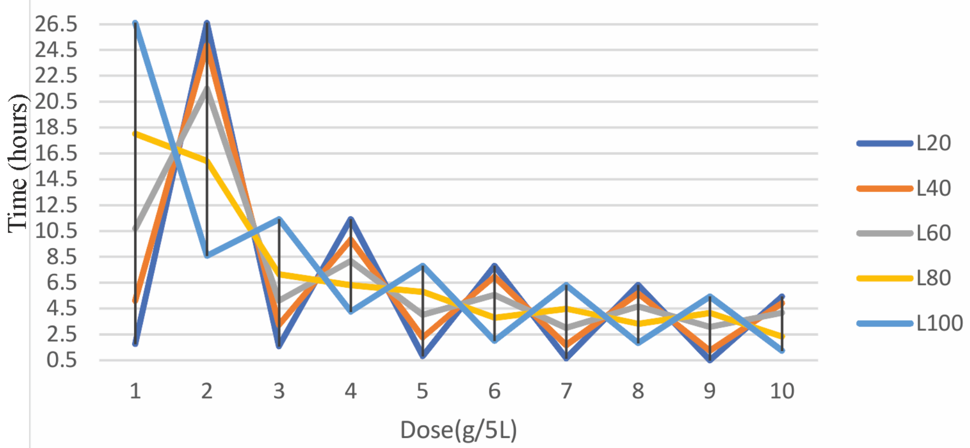
95% confidence interval:

$192.65 \le X \le 219.35$, $0 \le Y \le 8.87$ and $0.936 \le W \le 13.664$ respectively and that for

99% confidence interval:

$188.43 \le X \le 223.57$, $0 \le Y \le 10.545$ and $0 \le W \le 15.68$ respectively.

Figure 6. Ogive drawn from less than and greater than cumulative time hours



Again, analyzing the p-values related to observation (based on percentiles) and the methods (based on doses) we may reach to the decision that: the null hypothesis is accepted for 1% and 5% level of significance in all the cases and hence there is no difference between observed data and the expected data. Although the mmethod based (0.2g/5L to 0.6g/5L) *p*-values and their test of significance gives null hypothesis is accepted for 1% and 5% level of significance in all the cases and hence there is no difference between observed data and the expected data.

Constructing the interpolating Polynomial function based on most likely observed cleaning time (Y hours) based on L_{100} with respect to the X ppm used we get

Now applying the notion of differential calculus, we have the minimum expected time 1.158 hours with respect to the ppm used 0.538 g/5L. Moreover, the maximum expected time gives 1.541 hours using ppm 0.733 g/5L which differs from the minimum expected time by 23 minutes only.

4.4. Relationship between Probit and log concentration (1 hours)-

The relationship between Probit and log concentration based on mortality against log concentration Fig 7. Regression equation that showed the relationship between the mortality and the concentration used. The correlation coefficient (R) value between concentration of the extract and the fish mortality was 0.9971 and the LC50 value of methanolic leaf extract of *A. leptopus* in case of *Oreochromis niloticus* is 107mg/l for 1 hour exposure period. No mortality was recorded in control.

Fig 7. Percentage mortality of *O. niloticus* against log conc. of *A. leptopus* methanolic leaf extract at 1 hour exposure period

5. Discussion

The result obtained in this study revealed that the plant species *A. leptopus* could be a potential source of organic piscicide. The result of the study showed that *A. leptopus* collected from local floras is a potential organic piscicide

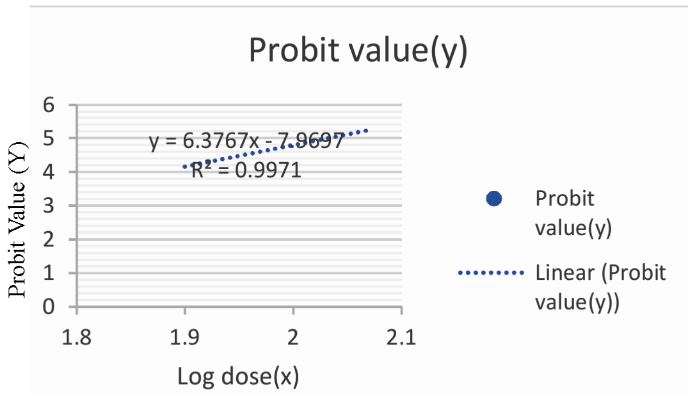


Fig 7. Percentage mortality of *O. niloticus* against log conc. of *A. leptopus* methanolic leaf extract at 1 hour exposure period

and can be a good alternative to harmful commercial piscicides. The plant extract could be used as organic piscicide in aquaculture pond management to eradicate predatory fishes to stocking for successful aquaculture management.

The statistical analysis reveals that, the proposed method and the product can be highly recommended for quicker cleaning mechanism because it requires only 0.538 g/5L lethal dose to clean within 1.158 hours only where the average weight of each Tilapia fish would be 15.68 g only. Based on this research, further studies are recommended such as the effect of organic piscicide to actual pond environment and later mass produce the product for commercialization. Not only can that it be used for easy harvesting by stupefying the fishes. Here piscicidal effect is found due to the presence of saponin in methanolic leaf extract of *Antigonon leptopus.* It has been reported that saponin is highly toxic to fish due to their damaging effect on respiratory epithelia [Roy PK, Munshi JD, Dutta HM,1990]. Saponin can cause saponification and the destruction of epithelial layer show haemolytic effect which ultimately leads to death of fish [Jawale CS and Dama LB, 2010]. Further work against the piscicidal effect of this experimental plant in molecular level and its quantitative study of chemical constituent of the toxicant is needed, which would be helpful for making future industrial product.

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