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Piscicidal Effect of the Aquous Leaf Extract of *Antigonon leptopus* Hook. & Arn. on Invasive Predatory Fish *Stramoteus sinensis* Forst

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Abstract:- For aquaculture management, the presence of unwanted predatory or trash fishes in the newly stocked fish ponds create a great nuisance because of their competition with desired fishes for food and shelter.

In this perspective for eradication of such predatory fishes by using phytopiscicides the present study was conducted to determine the median lethal concentration (LC 50) value of *Antigonon leptopus* aqueous fresh leaf extract on Rupchand fish (*Stramoteus sinensis)* in laboratory condition. Piscicidal effect had been studied by using five different concentration of aqueous leaf extract (40mg/L, 80mg/L, 120mg/L, 160mg/L and 200mg/L) on fingerlings of Rupchand fish. The phytopiscicidal effect was tested for 24 hours of exposure and the LC50 value 57.54mg/L was estimated by probit analysis. LC50 values were found to be inversely proportional to exposure duration. Moreover, the acute (LC 100) effect that killed all the test fishes within 24-hrs was determined to be the highest concentration of 235.59mg/L. where as in control no such effect had been found. The phytochemicals present in tested plant material and also the water parameters (TDS, DO, pH etc.).

The dose response mortality and behavioural changes were studied against the aquous leaf extract of *Antigonon leptopus* Hook. The result indicated the potential piscicidal property of this botanical. Being a potent biodegradable, easily available, eco-friendly, icthyotoxicant against environmentally hazardous synthetic chemicals, aqueous fresh crude leaf extract of this plant species may be used for eradicating unwanted predatory or trash fishes for pre stocking management of pond-based aquaculture which would be helpful for making future ecofriendly industrial product.

Keywords:- *Antigonon leptopus, Stramoteus sinensis, Phytotoxicity, LC50.*

I. INTRODUCTION

Eradication of unwanted predatory fishes is essential for increasing the productivity in aquaculture ponds. Generally farmers are using synthetic pesticides for to kill predatory fishes, but these have long term residual effects because of their less biodegradability and harmful effect on other organisms and also the unscientific and indiscriminate use of chemical pesticides create threat to aquatic ecosystem and biodiversity label. [(1,2,3)] In this perspective attempt has been made to find out the plant based piscicide a botanical because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability[(4)] and reduce toxicity to non-targeted animals and. During the study of ecthyotoxicants of plant origin it has been found that aqueous leaf extract of *A. leptopus* can be used as potent piscicide.[(5)] The piscicidal effect of this plant leaf extract on Rupchanda fish has yet not been studied earlier. In this experiment LC50, LC90 values of different concentration during different exposure periods have been studied.

II. MATERIALS AND METHODS

A. Experimental Design :

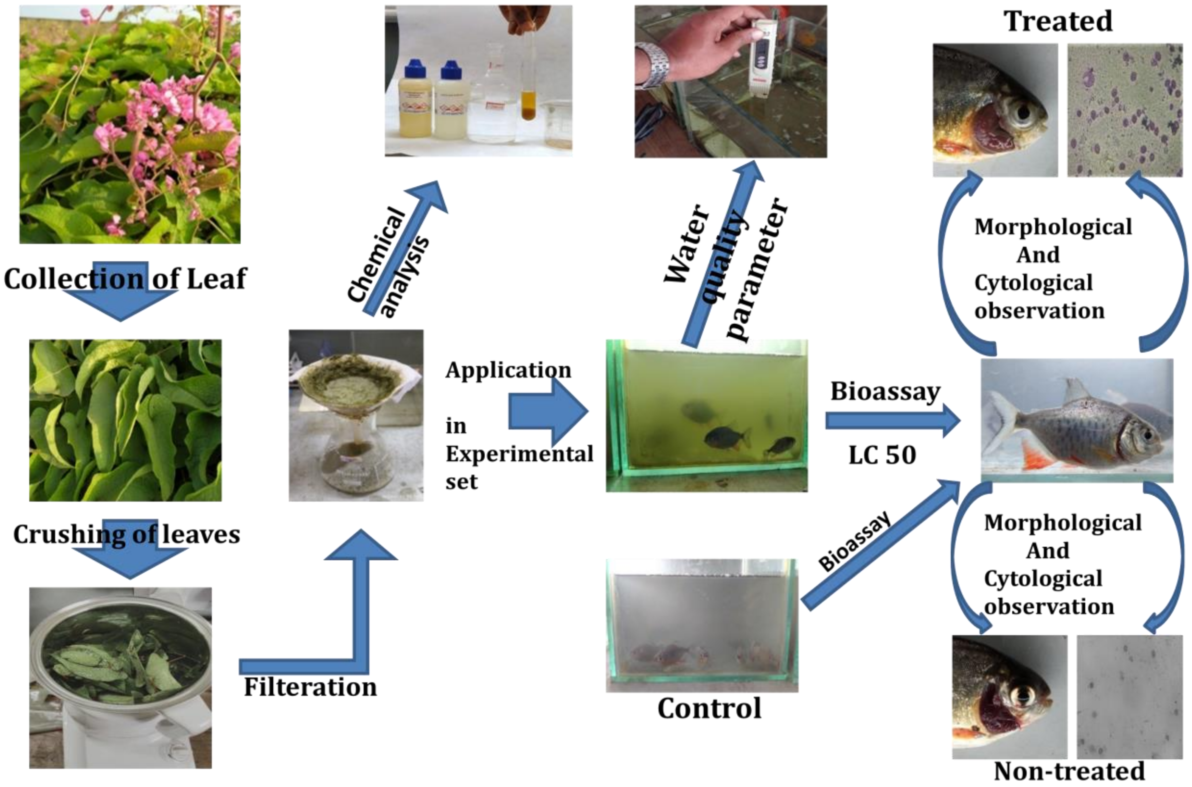

Fig. 1: Experimental Design

B. Experimental Plants:

Antigonon leptopus (belonging to the family – Polygonaceae) is an invasive, fast-growing climbing vine where inflorescence axis has been modified into tendrils. It has cordate (heart-shaped), sometimes triangular leaves. The flowers are borne in panicles, clustered along the rachis producing pink or white flowers from spring to autumn.

- **Distribution:** It is found to grow in wild condition along the road sides and near the rail lines, also it can climb on trees, shrubs covering them.

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

Fig. 2: Flowering twig of *Antigonon leptopus* Hook. & Arn.

C. Preparation of Aqueous extract:

Fresh leaves were collected from local flora of Paschim medinipur district of West Bengal washed and grinded (by using Bajaj electrical grinder). The grinded matters are properly mixed with one litre of distilled water for 2hr. Then the extracts were filtered by using whatman filter paper to get leaf extract.

D. Phytochemical Analysis:

The qualitative phytochemical analysis of the aqueous extract of the leaf of *A.leptopus* was done using standard procedures to identify the various constituents.[6,7]

The aqueous extract of *A. Leptopus* leaves have the following biochemicals, which are shown in Table -1.

Table 1: The aqueous extract of *A. Leptopus* leaves

Plant	*Antigonon leptopus*
Part tested	Leaves (Aqueous extract)
Flavonoids	-
Alkaloids	+
Terpenoids	++
Cardiac glycoside	+
Tannins	+
Saponin	++

E. Experimental fishes:

Rupchand (*Stramoteus sinensis Forst.*), a native fish of the Amazon river of South America , recently introduced into India through Bangladesh as alien introductions . *Stramoteus sinensis* is a robust fish, with ovoid shape, flattened laterally. Its colour is dark grey to silver, with a white belly and a yellow breast, mostly carnivorous in nature. [8]

F. Collection and acclimatization of experimental fishes:

Healthy fingerlings of *Stramoteus sinensis* with average length of 5 ± 2 cm and average weight of 9 ± 7 gm were collected from the local fish farmer and maintained in an aquarium (60 cm × 30 cm × 30 cm) in laboratory for 7 days before the experiment. Fishes were fed pelleted feed and maintained optimum level of water quality.[9] Fish aquaria were well aerated and the water was exchanged when it is required.

Fig. 3: Experimental fish *Stramoteus sinensis* Forst.

G. Analysis of water quality parameters:

Some important physico-chemical parameter of water such as pH, TDS, Dissolved Oxygen (DO) etc. were studied 2hr interval during the experiment .[10]

The recorded water quality parameters during the experiment are mentioned in table -2.

Table 2: Water Quality Parameters

Total Dissolve Solute (TDS)						
	0 hr	2 hr	4hr	6 hr	8 hr	10 hr
Control	157 ppm	159 ppm	162 ppm	167 ppm	170 ppm	177 ppm
40 mg/L	170 ppm	173 ppm	177 ppm	182 ppm	187 ppm	190 ppm
80 mg/L	171 ppm	176 ppm	181 ppm	186 ppm	191 ppm	196 ppm
120 mg/L	175 ppm	181 ppm	185 ppm	189 ppm	192 ppm	197 ppm
160 mg/L	181 ppm	188 ppm	191 ppm	194 ppm	198 ppm	205 ppm
200 mg/L	192 ppm	199 ppm	202 ppm	206 ppm	212 ppm	219 ppm
Dissolve Oxygen (DO)						
Control	1.8	1.6	1.5	1.3	1.2	1.0
40 mg/L	1.76	1.5	1.3	1.0	0.8	0.6
80 mg/L	1.7	1.3	1.2	1.0	0.8	0.5
120 mg/L	1.7	1.3	1.1	0.9	0.7	0.4
160 mg/L	1.7	1.2	1.0	0.9	0.7	0.3
200 mg/L	1.7	1.2	1.0	0.8	0.5	0.3
pH						
Control	6.8	6.8	6.8	6.9	6.9	6.9
40 mg/L	6.7	6.8	6.8	6.8	7.1	7.1
80 mg/L	6.7	6.8	6.8	6.9	6.9	7.1
120 mg/L	6.5	6.6	6.7	6.7	6.8	6.9
160 mg/L	6.5	6.6	6.7	6.7	6.8	6.8
200 mg/L	6.4	6.6	6.7	6.7	6.8	6.8
Temperature (^{0}C)						
	26.5	26.9	28	27.7	27.2	26.6

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H. Statistical analysis:

The LC50 value of *A. Leptopus* for *Stramoteus sinensis* was calculated using Probit analysis method.[11,12] The regression analysis were done by Microsoft excel to assess the relation between mortality and exposure period in different concentration of aqueous extract of *A. Leptopus* leaves.

III. RESULT AND DISCUSSION

A. Probit analysis for the mortality percentage of exposed to different concentrations of aqueous extract of A. leptopus leaves :

The lethal concentration (LC50) of the test plant was determined by plotting logarithm concentration of the plant against fish mortality within 24 hours. The median lethal concentration (LC50) is the concentration in which 50% of the test fish survived and 50% died while LC100 is the concentration in which 100% of the fish died.

The data were collected for probit analysis. The logarithm of concentration of *A. leptopus* was determined. The percentage of the mortality response was found for each treatment and the relationship between the probit values and the logarithm concentration was establishing using regression analysis.

Table 3: Mortality observed in *S. sinensis* exposed to different concentrations of aqueous leaf extract of *A. leptopus* .

Concn.	Fish taken	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h	11h	12h
Control	10	0	0	0	0	0	0	0	0	0	0	0	0
40 mg/L	10	0	0	0	0	0	1	4	0	2	1	0	2
80 mg/L	10	0	0	0	0	0	2	1	1	2	0	3	1
120 mg/L	10	0	0	0	0	1	3	2	1	3	-	-	-
160 mg/L	10	0	0	0	1	3	4	2	-	-	-	-	-
200 mg/L	10	0	0	0	1	3	5	1	-	-	-	-	-

Table 4: Probit values obtained for different concentrations of piscides (7 hrs.)

concentration	Mortality	% mortality	Probit	Log- concentration
40 mg/L	1	10%	3.72	1.6
80 mg/L	2	20%	4.16	1.9
120 mg/L	4	40%	4.75	2.07
160 mg/L	8	80%	5.84	2.2
200mg/L	9	90%	6.28	2.3

Table 5: Relationship between probit and log concentration (7 hours).

Log – dose (x)	Probit value (y)
1.6	3.72
1.9	4.16
2.07	4.75
2.2	5.84
2.3	6.28

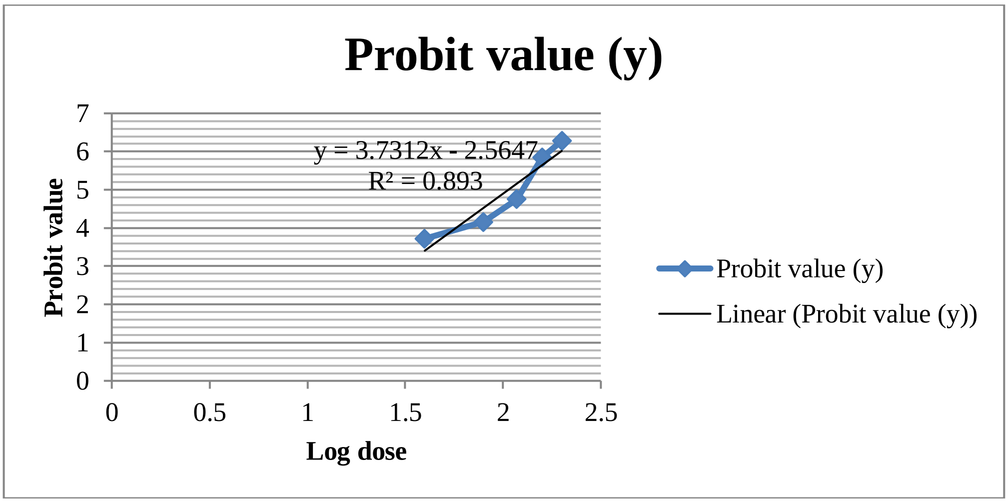

Fig. 4: Probit value

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y = 3.731x – 2.564
X=(5+2.564)/3.731 [as Lc50=5]
X= 2.02 (log dose)

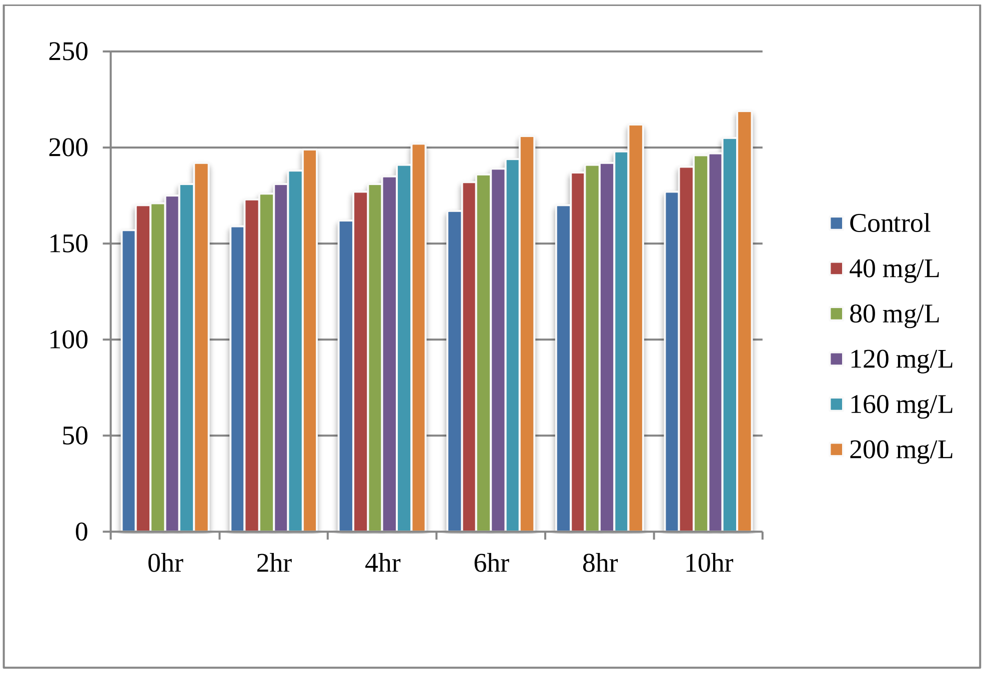
Lc50= Antilog of (2.02) =104.71 mg/L
Lc16, y=4.01
X= (4.01+2.56)/3.731
=1.76 (log dose)
Lc16= Antilog of (1.76) =57.54 mg/L
Lc 84, y=5.99
X= (5.99+2.56)/3.731
=2.29 (log dose)
Lc84= Antilog of (2.29)=194.98mg/l
Slope=(Lc84/Lc50+Lc50/Lc16)/2
S= (2.29/2.02 + 1.76/2.02)/2
= 1
Log S=0.26
Log f95= (2.77/N) *(1/logS)
= (2.77/30) *(1/0.26)
= 0.353
f95= 2.25

- **Upper limit** = (Lc50*f95)
  =**235.59 mg/L**
- **Lower limit** = Lc50/f95
  =**46.53mg/L**

The antilogarithm of the value of X is found to be 104.71mg/l. This value is taken as the LC50 of *A. leptopus* at 7 hours. Rupchanda (*S. sinensis*) juveniles exposed to aqueous extract of the leaves of *A. leptopus* was recorded two mortality at the initial 4 hours in last two experimental set (160mg/L and 200mg/L) but no mortality in other experimental set except some sign of stress. The mortality of fish also increased as the concentration and time of exposure are increased. The result of LC16, LC50 and LC84 of *A. leptopus* leaves are respectively 57.54mg/L, 104.71mg/L and 194.98mg/L at 24 hour exposure into the experimental sets. Through the probit analysis of the experimental data the lower limit is 46.53mg/L and the upper limit is 235.59mg/L. Hence, this present result shows that *A. leptopus* leaf is a potent piscicide.

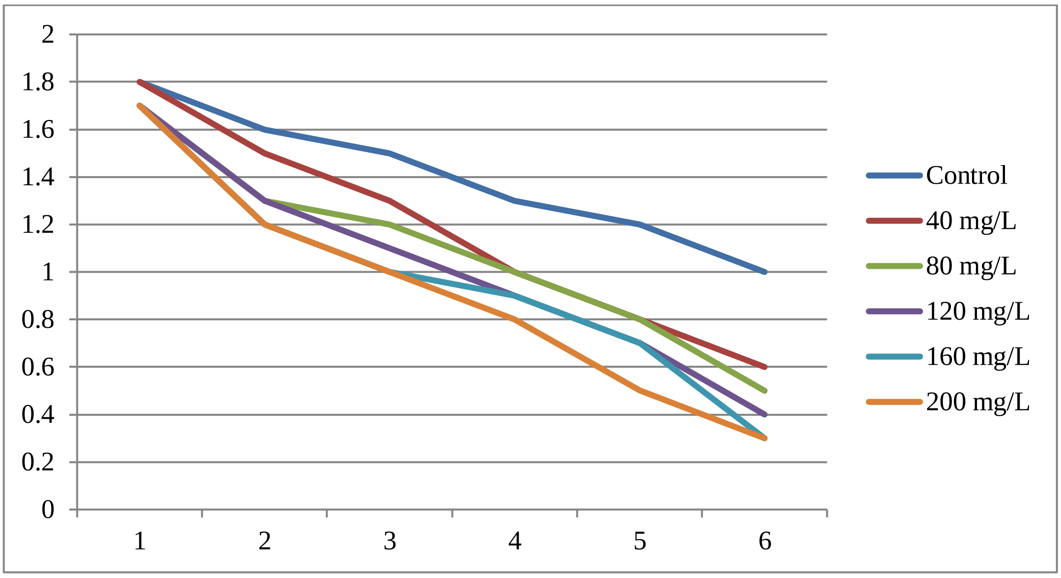
The statistical analysis reveals that, the proposed method and the product can be highly recommended for quicker cleaning mechanism because it requires only 235.59 mg/L lethal dose to clean within 7 hours only where the average weight of experimental fish would be 9 ± 7 g only. Based on this research, 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 that it can also be used for easy harvesting by stupefying the fishes. Here piscicidal effect is found due to the presence of saponin, alkaloids, tannin in fish poison plant has been reported. This phytochemicals are known to sapocate the fish by destroying the respiratory organs of interfering with the bio-chemical respiratory pathway thereby forcing the fishes to gulp on surface for air and leads to death.[13,14,15]

GRAPHICAL ANALYSIS



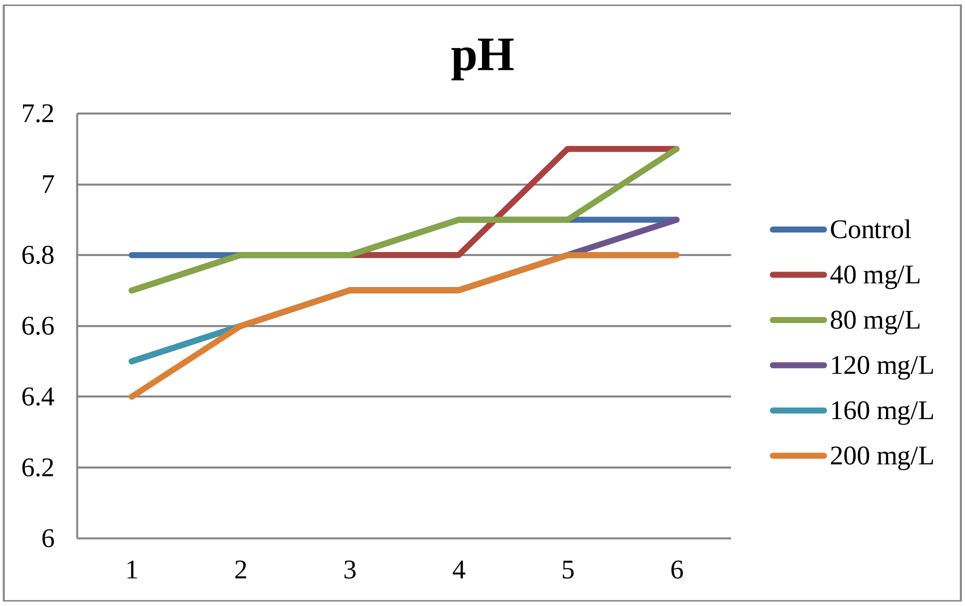
Graph 1: Total Dissolve Solute (TDS)

The graph (Graph -1) shows the amount of total dissolve solute (TDS) in different experimental set. During this experiment the result reveals that the TDS value of different experimental set is increased in respect to time and also respect to the concentration. The experimental set with highest concentration shows the rapid changes in TDS value with respect to the control.



Graph 2: Dissolve Oxygen (DO)

The graph (Graph - 2) shows the amount of dissolve oxygen (DO) in different experimental set. During this experiment the result shows that the DO of different experimental set is decreased gradually in respect of time and concentration of plant material. The last two experimental set with highest concentrations shows the rapid decreasing of DO whereas the control set shows slight changes.



Graph 3: The changes of pH in different experimental set

The graph (Graph - 3) shows the changes of pH in different experimental set. During this experiment the result reveals that the pH of different experimental set is decrease gradually in respect of time and concentration. The experimental set with highest concentration shows the rapid decrease of pH in comperison to the control.

By analysing the result of the water parameters it shows a clear relation among the values of different parameters. With the increasing concentration of plant material, the TDS value is increased and with respect to the TDS value the DO and pH values are decreased.

IV. BEHAVIOURAL CHANGES OF EXPERIMENTAL FISHES

The present study showed that *A. leptopus* has great affects on the experimental fishes *S. sinensis*. It was observed that the sign of discomfort increases with the increasing concentrations. In first few hour erratic swimming was observed in experimental fishes and also tried to escape from experiment tank by jumping. In next few hour Fishes were shown to move to the surface of water of the aquarium and started air gulping. In last few hour eexcessive mucus secretion, unusual lethargy, slow operculum movement was observed and finally settling down at the bottom of the tank and sacrificed. These

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behavioral changes indicate the stress effect of experimental fishes imposed by the leaf extract, but no such behavioral changes observed in control.

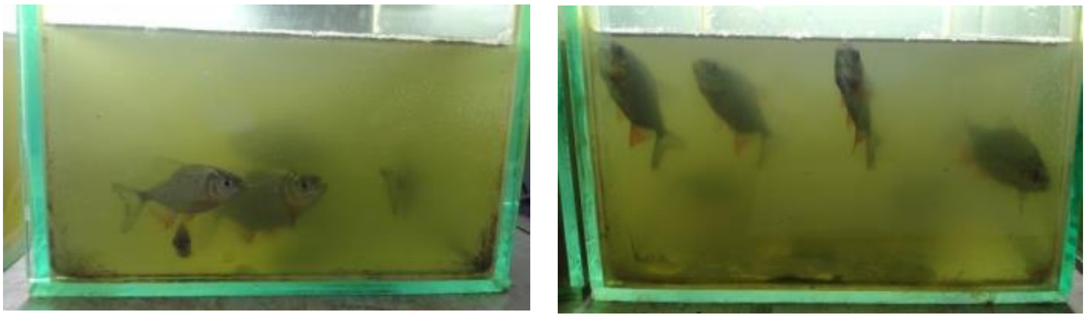
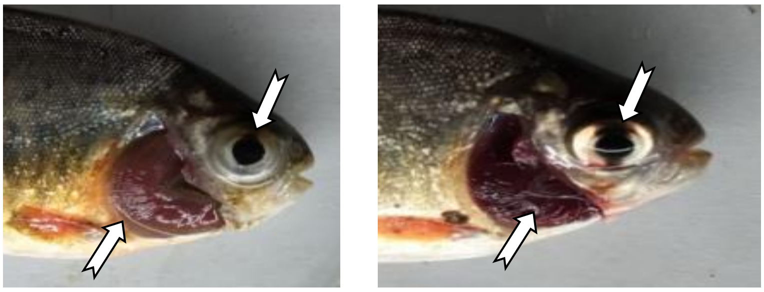


Fig. 5: Behavioural changes observed during experiment

V. PHYSIOLOGICAL CHANGES AND CYTOLOGICAL OBSERVATION OF THE EXPERIMENTAL FISHES

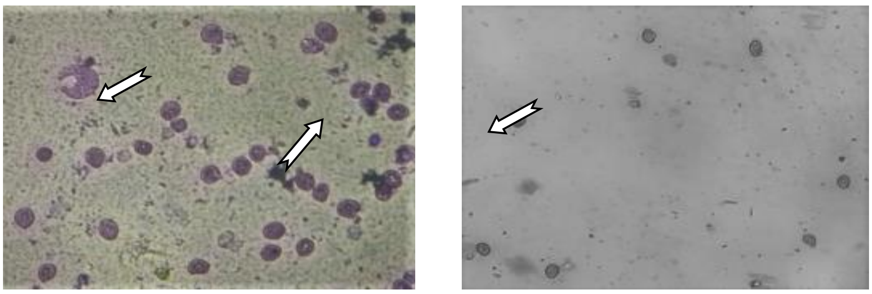
During the experiment it was observed some physiological changes on test fishes. The gills become whitish-red in case of treated fish but in non-treated fish the gills were dark red in colour. The eyeball becomes oval shaped in case of treated fish but in non-treated fish it was round.



(a) (b)

Fig. 6: Treated (a) and Non-treated (b) fish showing gills & eye ball.

Haemolytic test has been done to test the toxic effect on test fishes.[16] The prepared blood smear with Leishman stain of non-treated and treated fish was observed under binocular microscope at 40X and it was observed that there were some changes in the structure of RBCs. In case of non-treated fish the RBCs remain free with unchanged shape while in treated fish, RBCs were slightly larger, accumulated and some also ruptured.



(a) (b)

Fig. 7: Microscopic view of RBCs of (a) treated and (b) non-treated fish.

VI. CONCLUSION

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

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REFFERENCES

[1.] Ahmed S, Grainge M. Potential of Neem tree (*Azadirachta indica*) for pest control and rural Development., Economic Botany, (1986) 40;201-209.

[2.] Rath, R.K. In. Freshwater Aquaculture. Scientific Publishers. Jodhpur, India, (1993)pp 493.

[3.] Sarkhel,C.Comparative Evaluation of some selected piscicides on certain environmental health parameters with special reference to nutrient cycling microbes. M.F.Sc. thesis. West Bengal University of Animal and Fishery Sciences. Nadia, West Bengal,India.(2002).

[4.] Kamalkishor HN, Kulkarni KM,; Fish stupefying plants used by the Gond Tribal of Mendha village of Central India,(2009) Ind J Trad Knowl8(4):531-534.

[5.] Dey D.K., De S.K., Bhattyacharya R.P., Dey D., Bhanja B. *Antigonon leptopus* (Hook & Arn.) – A new Potential Plant Origin Ichthyotoxicant on *Chana punctatus* (Bloch,1793) for Aquaculture Management. International Journal of Trend in Scientific Research and Development.(2022) Vol-6, Issue-3, 1658-1668.

[6.] Kakate KC.,Practical pharmacognosy.Edn 4, Ballav Prakashan,Delhi,1997,218.

[7.] Harbone JB., Phytochemical methods: A guide to modern techniques of plant analysis.Edn 2,Chapman and Hall,London,1998,54-84.

[8.] Froese, Rainer, Pauly, Daniel (eds.) (2017). “Piaractus mesopotamicus” in fish Base. March 2017 version.

[9.] APHA, AWWA,WWF.Standard Methods for examination of water and wastewater.Edn 22. American Public Health Association,Washington.2012,1360.

[10.] APHA,AWWA,WPCF – American Public Health Association(APHA), American Water Works Association(AWWA) and Water Pollution Control Federation(WPCF).1971. Standard Methods for examination of water and wastewater. Edn 13. American Public Health Association,Washington DC.USA.

[11.] Finney D.; Probit Analysis. Edn 3.; New York Cambridge University Press.1971.

[12.] Weber C.; Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Cincinnati. Environmental Monitoring System Laboratory, Office of Research and Development, US Environmental Protection Agency;1991.

[13.] Roy, P.K., Munshi, J.D. and Dutta, H.M. Effect of saponin extracts on morpho-history and respiratory physiology of an air-breathing fish, *Heteropneustes fossilis* (Bloch). Journal of Freshwater Biology,(1990)Vol.2,135-145.

[14.] Andel V.T. The diverse uses of fish-poison plants in Northwest Guyana. Economic Botany. 2000,5:39-50.

[15.] Dey D.K., De S.K., Bhattyacharya R.P., Bhanja B., Dey D., Leaf extract of Antigonon leptopus (Hook. And Arn.) – An Effective Ichthyotoxicant of plant Origin on Predatory fish, Oreochromis niloticus (Linn. 1758).International Research Journal of Basic and Applied Sciences. (2022) Vol-7.98-109.

[16.] Jawale, C.S. and Dama, L.B. Haematological changes in the freshwater fish, *Cyprinus carpio* exposed to sub-lethal concentration of piscicidal compounds from *Cestrum* species (Family: Solanaceae). Nat. J. Life Sci. (2010) Vol. 7 No.1:81-84.