Acute fish toxicity study of *Antigonon leptopus* Hook and Arn. to eradicate unwanted fish fauna from nursery pond enhancing the Aquaculture development

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ABSTRACT

Eradication of undesired fish fauna from the aquaculture ponds is the prime needs for the development of aquaculture farming. Due to this the present investigation envisages the acute fish toxicity study of leaf extract of Antigonon leptopus, an ecofriendly icthyotoxicant of plant origin which would be an alternative of environmentally hazardous synthetic piscicides.

During this study a comparative acute lethal toxicity was studied for 24 hours exposure of six predatory fishes (Koi, Lyatha, Tilapia, Tyangra, Magur, Singi) against different concentrations (2,4,6,8,10 g/l) of aqueous leaf extract of experimental plant species. The LC50 of different (six) predatory fishes against different conc. of dry leaf aqueous extract of *Antigonon leptopus* was noted. Behavioral responses of these experimental fishes also recorded against this botanical or phyto-piscicide.

Gradual bio-degradation of the potency of the plant extract after the experiment also studied for seven days. Again, efficacy of toxic study of aqueous extract of plant materials in dried and fresh condition in different water resources (Pond, tap, distilled) was also studied and recorded.

Such type of comparative study of this plant species against different fish species in different water resources and also the bio-degradability of the phytotoxicant were not studied earlier and also this study would help the fish farmer to use this plant product in the aquaculture management tool as an alternative of environmentally harmful synthetic piscicides. This toxicity may be due to the presence of botanicals like flavonoids, alkaloids, terpenoids, cardiac glycosides, tannins, saponins in the leaf extract of the plant species.

1. Introduction:

Presence of predatory and weed fishes in the Nursery- pond create a great nuisance for scientific management of carp culture as these unwanted fishes compete for food, dissolved oxygen and also space with desired

cultured varieties. (Chatterjee and Ganguli, 1993; Chakroff,1993; Rath, 1993).In comparison with the other cultivable varieties of fishes the weed or trash fishes have high fecundity and very fast sexual maturity. (Jhingram,1991).Therefore, eradication or complete removal of such predatory or trash fishes from the fresh water ponds is most vital for the development of fish farming as well as economic development.

Though dewatering and desilting of ponds, hooks and lines with baits, netting operation repeatedly are the traditional practices for mitigating this problem but could not give complete success in one hand and also uneconomical on the other. Now-a-days fish farmers are more interested in limited poisoning of the pond with selective toxicant. Generally, two types of piscicides can be used for this purpose; plant derived piscicides or botanicals like saponin, rotenone and chemical compounds like organo- phosphate compound, chlorinated hydrocarbons. (Jhingram,1991; Rath, 1993; Mahapatra and Thosar,1999). Plant derived piscicides are more suitable rather than the chemical compounds as these have target specificity at very low doses, not harmful to human-beings and cattle when treated water is used, easily biodegradable, affected fishes are not unsuitable for human consumption, do not show permanent resistance, economical and less side effects (Jhingram, 1991). For these reasons, phyto-piscicides are eco-friendly in comparison with chemical toxicity and would be the alternative one against harmful synthetic pesticides.

In this perspective, the attempts have been made to search the plant derived piscicides which could be effective on target organism without creating environmental hazards. Here phytotoxicity study of the leaves of the plant species both in fresh and dried form has been done on different predatory or unwanted fish species using different water resources like pond, tap and distilled water. Potency of ichthyo-toxicity of Antigonon leptopus leaf extract has already been established by earlier workers (De et al, 2022; De et al,. 2023) but a comparative acute lethal toxicity study for 24 hours exposure of the on six predatory fishes (Koi, Lathya, Tilapia, Tyangra, Magur, Singi)against different concentration (2,4,6,8,10 g/l) of aqueous leaf extract of experimental plant species was not done earlier. Again Gradual degradation of the potency of the plant extract after the experiment also studied during 7 days and efficacy of toxicity study of aqueous extract of plant materials in dried and fresh condition in different water resources (Pond, Tap and Distilled) also studied and recorded.

In this regard this work claims its importance to help the fish farmers to use this plant product in the aquaculture management tool as an alternative of environmentally harmful synthetic piscicides.

2. Material & Methodology:

2.1. Test plant:

A. leptopus Hook. and Arn. is the fast growing, perennial, coral climbing vine belonging to Polygonaceae family . It is a native plant of Mexico. It is an invasive species. Flowers are borne in panicles, clustered along the rachis. Flower colour is pink or white and produced from spring to autumn Inflorescence axis of A. leptopus has been modified into tendrils. It is generally found to grow along road side, railway tracks and in forest areas. Some cases it is also cultivated due to its beautiful inflorescence *A. leptopus* is listed as category II invasive, exotic by Florida's Pest Plant Council (Florida Exotic Pest Plant Council.2019)

Fig-1: Antigonon leptopus Hook and Arn.

2.2. Test animals:

Six different types of predatory healthy and adult fishes of *Clarias batrachus* Linn.1758 (Magur), *Mystus tengara* Hamilton,1822 (Tyangra), *Oreochromis niloticus* Linn.,1758 (Tilapia), *Anabus testudineus* Bloch,1792 (Koi), *Chana punctatus* Bloch,1793 (Lytha), *Heteropneustes fossilis* Bloch,1794 (Singi) were collected from the nearby farm and 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 artificial feed like fish meal. Water was changed at every 24 h. Test fishes are categorized as least concern worldwide (IUCN,2017). The selection of experimental fish based on three reasons viz. (i) all these three fish species are distributed throughout the Indian subcontinent as wild and cultivated condition.(ii) aggressive behaviour of these wild predatory fishes can cause severe economic loss to fish farmer especially in carp culture system: and (iii)presence of bimodal respiration in case of Magur, Singi, Koi and Lytha is facilitated by accessory respiratory organ as well as gills.

2.3. Methodology:

2.3.1. Test plant collection and preparation of plant extract :

Mature green leaves of *A. leptopus* were collected from different places of Rangamati, Paschim Medinipur located nearby Midnapore town, Paschim Medinipur, West Bengal, India. This plant has been identified with the help of exhisting literature. Voucher specimen was kept in the herbarium of Dept. of Botany, Midnapore College,

Paschim Medinipur, W.B. with specimen no SCANT-10. Fresh leaves were used to prepare aqueous extract of fresh leaves of *A. leptopus* were left to dry and processed into powder to prepare aqueous extract of air- dried leaves of *A. leptopus*.

For fresh leaves:

Collected fresh leaves were washed and grinded → Filtration using absorbent cotton and cloth

↓

Again, filtration using Whatman filter-paper

↓

Prepared aqueous extract of fresh leaves of different concentrations

For dried leaves:

Collected fresh leaves were washed → leaves get air- dried, powdered and preserved air tightly

↓

Dried leaf powder absorbed in water for 24 h

↓

Filtration using absorbent cotton and cloth

↓

Filtration using filter-paper

↓

Prepared the aqueous extract of dried leaves of different concentrations

2.3.2. Experimental design:

Phytochemical content of the plant was analyzed using standard screening ,methods. Standard static bioassay procedures were employed (APHA, AWWA and WPCF) (1971;1980).

For experimental set up rectangular shaped glass container, measuring 20cmx 20cmx10cm were used. Each container was filled with 5-liter non-chlorinated tap water. Before adding different conc. of the plant extract, water was pre-aerated for 20 min for full oxygen saturation. Previously prepared crude extract from fresh leaves of test plant i.e., *A. leptopus* five different concentrations i.e., 2,4,6,8,10g/l were prepared. A total of six different types of adults, healthy test-fishes were exposed to each aquaria containing different concentration of leaf extract of *A. leptopus* for 24 hours. A control set was maintained, where no plant extract was used and contained same number of fishes. The same procedure was followed for the experiment where aqueous extract of dried leaves were used. Behavioral changes and mortality data was noted through-out the experiment. Obtained mortality data were noted and plotted against different concentration of the test plant extract on different experimental fishes (graph 1). All the experiment were conducted in the Research Centre, Midnapore College (Autonomous) at room temperature and under diffused light.

For observing the mortality percentage of fresh leaves extract in different water resources (Pond, Tap and Distilled) three

glass aquaria contain water from the above mentioned three different sources. Then same methodology was applied as described earlier in fresh leaves condition. Here Tilapia was used as test fish and the potency of phytotoxicity of the test plan was studied and recorded against a single concentration i.e. 10g/l of the test plant(Graph - 3).

3. Result and discussion:

Phytochemical analysis:

The phytochemical analysis of the aqueous leaf extract was done according to the methodology of Augusthy KT,1979 and Burkill HM,1985.

behavioural activities which is different from control. In the treated tank fishes showed aggressive behaviour after being exposed to the plant-toxicant. These fishes showed erratic swimming, hyperactivity, gulping for air at the surface. After that fishes became lethargic, imbalanced and their fins get stiffened and barbels were straightened. Finally, mortality occurred and some of the dead fishes floated parallel or vertical or at an angle with the surface water or remained flat at the aquarium bottom. Behavioural activities increased with the advancement of time of exposure and concentration.

Table-I. Preliminary Phytochemical Screening analysis of *A. leptopus* (leaves) extract.

No. Tested *A. leptopus* (leaves)	
1. Unsaturated Sterol And/or tri-terpenes	+
2. Alkaloids	+
3. Flavonoids	+
4. Tannins	+
5. Saponins	+
6. Anthraquinone glycoside	+
7. Coumarins	+
8. Cardiac glycoside	+

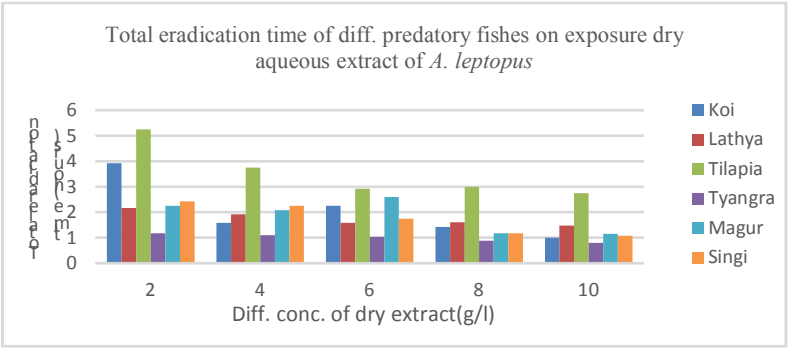
(+) = Present ; (-) = Absent

Behavioural response:

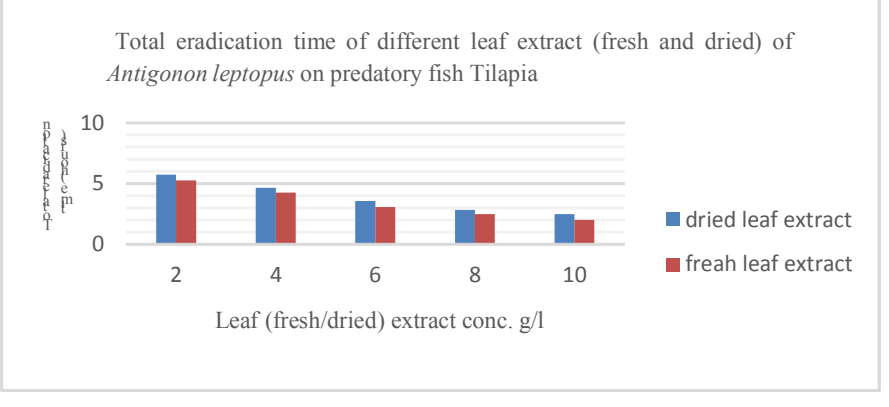
Upon exposure to the plant toxicant (aqueous fresh and dried leaf extract) of different concentrations test fishes exhibit several Discolouration with reddish tinge in the body, bulged eyes, with few scales being shed off and damaged fins and tails were also observed.

Fish samples	Behavior changes during exposure peroid
Heteropneustes fossilis	• erratic swimming • loss of equilibrium • bulging eyes • hanging vertically with head above in water medium
Clarias batrachus	• initially resting at bottom • air gulping at surface of exposure medium • barbel deformation • erratic swimming • hanging vertically in water column • skin discoloration
Oreochromis niloticus	• loss of balance and restless gulping of air. • excessive mucus secretion and unusual lethargy • erratic swimming before settling at the bottom of the tank with slow operculum movement can be found prior to death. • discolouration of eye • changes of colour of gills (reddish to blackish)

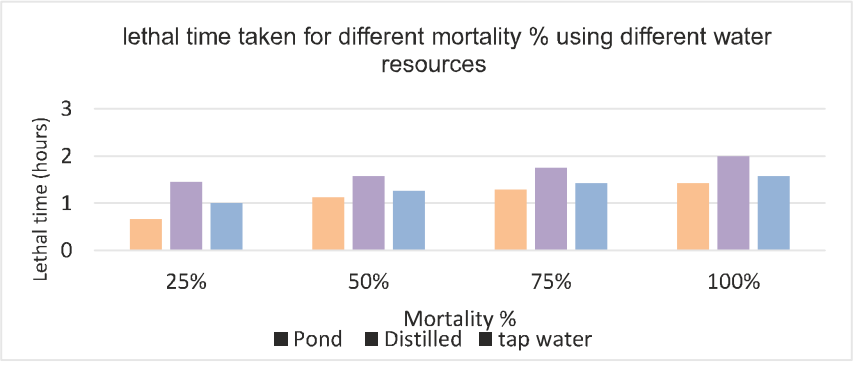
Channa punctataus	• initially expressed distressed movement • they became inactive gradually and started losing their balance • erratic and agitated movement • gulping for air • restlessness • went to the bottom of the aquaria; lie down on their sides till death.
Anabus testudineus	• immediately after exposure to the toxicants, the fishes jumped upwards • then they started moves up and down rapidly • subsequently, their movement became slow and likely stopped • gradually they relaxed to the bottom and ultimately died
Mystus tengara	• erratic swimming initially • restlessness • gulfing for air, hanging vertically with head above in water column • finally settled down



Graph-1: The graph represents LC100 of diff (six) predatory fishes against different conc. of dry leaf aqueous extract of Antigonon leptopus. The graph also indicates that in the same concentration the mortality rates of different species are different, where the mortality time is greater in Tilapia and then followed by *Channa punctatus*>*Clarius batrachus*>*Heteropneustes fossilis*>*Anabus testudineus*>*Mystus tengara*. The graph also indicates that for all type of fish the mortality time is inversely proportional with the concentration of products.



Graph-2: The graph represents the lethal time against different leaf extract (fresh and dried) of *Antigonon leptopus* on predatory fish Tilapia. The graph indicates that mortality percentage in dried leaf extract of the experimented plant material is slightly higher than the fresh leaf extract.



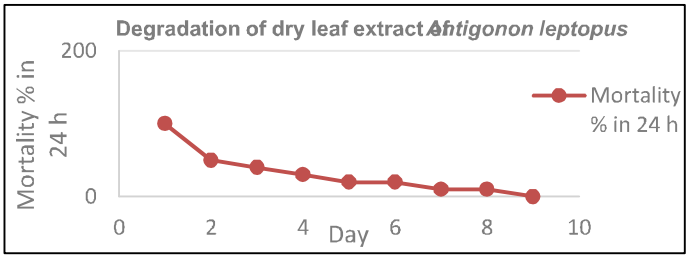
Graph-3: The graph represents the effect on lethal time of different of water extract of A. leptopus on test fish using different water resources i.e., pond water, distilled water and tap water. The graphical result shows that the mortality percentage in pond water is higher than other two water resources.

Biodegradability test of the plant product :

In Lab condition (Glass Aquarium):

In this experiment the mortality % of test fishes against 24 hrs for a specific conc. i.e., 10g/ l of leaf extract(dry) of *Antigonon leptopus* was observed.

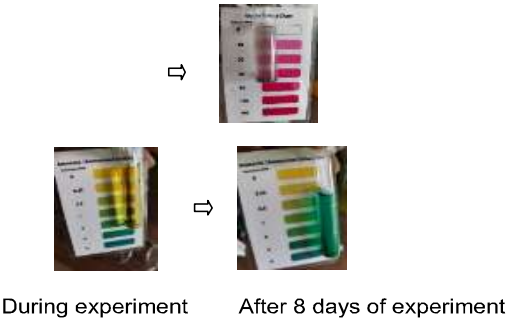
The result represented in the graph clearly shows that the potency of the toxic effect of the leaf extract is degraded day by day. So, mortality% in 24 h of observation period gradually decreased. The toxic effect has been found up to 8 days of experiment. After that no toxic effect has been found.



Graph- 4: Degradation of dry leaf extract of Antigonon leptopus

Table II: Changes of ammonia and nitrate in experimented glass- aquarium water after using the plant extract and after 8 days of experiment.

Chemical component	During experiment	After 8 days of experiment
Nitrate	0 ppm	0<10 ppm
Ammonia	0ppm	4 ppm



During experiment After 8 days of experiment

Fig-2: Changes of ammonia and nitrate in experimented glass- aquarium water

Table II indicates ammonia and nitrate component of aquaria-water increases due to degradation. Hence, the plant product of *A. leptopus* considered as environment-friendly as they are bio-degraded within a short period of time into nitrate and ammonia which can be used as fertilizer.

Table-III: Chemical parameters of water used for toxicity test in the glass-aquaria:

Chemical parameter	
Arsenic(As)	0 ppm
Iron(Fe)	0.3 ppm
Free Cl	0 ppm
Fluoride(F)	0.5 ppm
Alkalinity	300 ppm
Cupper(Cu)	0 ppm
Calcium hardness	80 ppm
Total hardness	110 ppm

Various physico-chemical parameters of tap water used during experiment were also estimated using Aqua-Check test kit to ensure the quality of water used during experiment.

4. Conclusion:

From these experiments it is clear that aqueous leaf extract (fresh and dry) has the

Fig-3: Fluoride and free chlorin test of water

potency of phytotoxicity on different predatory fishes though slightly higher toxic potency can be found in dry condition. This plant product could be used for complete eradication of unwanted fish species in aquaculture treatment which would be helpful for the economic development of fish farming. Again, the biodegradability of plant materials after the experiment proofs the eco-friendliness of this ichthyotoxicant. Not only that after degradation it can be used as fertilizer.

For these reasons it can be concluded that *Antigonon leptopus*, would be a potent botanical and could be used industrially for economic development as an alternative of synthetic pesticides. Further detail study of active phytocomponent of the test plant and its biochemical stress response on different predatory fishes are needed

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