ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 08, pp.35787-35793, August, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

A PRELIMINARY STUDY ON A NEW PHYTO-FISH TOXICANT

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ARTICLE INFO

Article History:
Received 21st May, 2016
Received in revised form
30th June, 2016
Accepted 15th July, 2016
Published online 20th August, 2016

Key words:

New, Plant toxicant, *Butea monosperma, Channa gachua*, LC_{50} value.

ABSTRACT

The idea of the present research work was originated from a fish poaching event in a remote tribal dominated village of Paschim Medinipur located in Block, Gopiballavpur – I, where the fish fauna of a pond was totally destroyed due to application of a plant twig locally known as Palash. The name of the plant was collected by the second author from a tribal community of the village Singdhuee of the said block. The Plant part for experiment was collected from forest and identified by plant taxonomist as *Butea monosperma*. During the present study experiment was designed to isolate the toxic part of the plant materials and the toxicity has been measured for both the crude material as well as the supernatant extract of the plant materials. The entire experiments have been performed on *Channa gachua* as test organism. LC_{50} value of the plant toxicant on *Channa gachua* has been assayed.

Citation: Pranita Mishra and Angsuman Chanda, 2016. "A preliminary study on a new Phyto-fish toxicant", *International Journal of Current Research*, 8, (08), 35787-35793.

INTRODUCTION

It is known that Soil on earth provides the scope of generation, growth and survival of the flora and fauna in certain favorable environments since their origin. Again flora and fauna are interdependent as they supply each other the rich resources for their living and nourishment through food, nutrition and curative ingredients for survival and betterment of living. At the same time the natural resources essentially provides some ingredients and products which harms and even causes fatal extinction of certain population even community of an ecosystem. The events may occur due to toxic effect of toxicants. Toxicants are such ingredients which are harmful to living beings. The study of role of the toxicant is known by the term "Toxicology". Toxicology is a branch of biology, chemistry, medicine concerned with the study of the adverse effects of chemicals on living organisms. It also studies the harmful effects of chemical, biological and physical agents in biological systems that establish the extent of damage in living organisms. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Factors that influence chemical toxicity includes the dosage of the toxicant (and whether it is acute or chronic); the route of exposure, the species, age, sex and environment of the system (Schrager, 2006). The goal of toxicity assessment is to identify adverse effects of a substance (Committee on Risk Assessment of Hazardous Air Pollutants, 1994). Adverse effects depend on two main factors: i) routes of exposure (oral, inhalation, or dermal) and ii) dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models. Generally, different sets of experiments are conducted to determine whether a substance causes cancer and/or to examine other forms of toxicity (Human Health Toxicity Assessment, 2012).

Aquatic toxicology is one of the parts of toxicology that refer to the effect of the toxicants to 'organism' living in marine water or fresh water and the 'organism' represents Vertebrate (fish), Invertebrate (crustacean) and Plant (algae). It is a multidisciplinary field which ingrate toxicology, aquatic ecology and aquatic chemistry (Rand *et al*., 1985). The field of study include standardized acute and chronic toxicity tests lasting 24 - 96 hours (acute test), up to 7 days or more (chronic test). These tests measure end point such as survival, growth, reproduction. Tests are done by several concentrations of toxic ingredients. Same also measured with each concentration of an ingredient along with a control test ("Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, 2001). The dose of the substance is an important factor in toxicology, as it has a significant relationship with the effect experienced by the individual. It is the primary means of classifying the toxicity of

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the chemical or exposure to the substance. All substance has the potential to be toxic if given to living organisms in the right conditions and dose (Ottoboni 1991).

Acute tests are short-term exposure tests (hours or days) and generally use lethality as an endpoint. In acute exposures, organisms come into contact with higher doses of the toxicant in a single event or in multiple events over a short period of time and usually produce immediate effects, depending on absorption time of the toxicant. These tests are generally conducted on organisms during a specific time period of the organism's life cycle, and are considered partial life cycle tests. Acute tests are not valid if mortality in the control sample is greater than 10%. Results are reported in EC50, or concentration that will affect fifty percent of the sample size (Rand *et al*., 1985). The toxicity tests are used to provide qualitative and quantitative data on adverse effects on aquatic organisms from a toxicant. *LC50* are common term used in toxicology which refers to the dose of a substance that displays toxicity in that *it kill 50% of a test population.* In scientific research, in aquatic medium fisher are usually used to determine toxicity. ("Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, 2002) *Butea monosperma (Lam.)* is commonly known as Flame of forest, belongs to the family Fabaceae (Patil *et al*., 2006). It is locally called as red-palas, Generally it grows gregariously on open grasslands and scattered in mixed forest (Flame of forest). *Butea monosperma* holds an important place because of its medicinal and other miscellaneous uses of economic value. The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhea, dysentery, ulcer, sore throat and snake bite. Besides medicinal uses it is also having the economic use such as leaves are used for making plates, cups and bowls. Bark fibers are obtained from stem for making cordage (Kirtikar and Basu, 1935). The present project is a primary toxicological study of plant Red Palash (*Butea monosperma*) on fresh water fish *Channa gachua*. Toxic part of the plant has been determined by application of leaf, bark, root and stem in separate experiment. It has been determined by experiment that the Woody Part of this Plant contains the Toxic materials.

MATERIALS AND METHODS

A. Preparation of test Sample, *Channa gacghua* :

1.Collection of aquarium :

One 100 lit water containing aquarium for controlling of 25 living sample fishes and two small 30 lit water containing aquarium for testing purpose were collected. They were properly cleaned and made ready for use.

2.Collection and preparation of experimental specimen (*Channa gachua*):

- *Channa gachua* were collected from nearby village ponds and market of Medinipur town, during 10.09.2015 to 25.02.2016 for several time.
- The size and weight of fishes were almost same weight ranging from 40-50 gm (mature).
- For survival adjustment of fishes in the aquarium, two weeks were spread for their feeding, nourishment etc.
- They were fed twice daily. The fish food was '*Annelids*'. The study room was maintained at 25 ±2◦C; the photoperiod was 12 hrs light followed by 12 hrs darkness. Cleaning, changes of water aquarium were made the regular interval. After 2 weeks, two fishes were dead and others were alive.

Fig.1. Preparation of *Channa gachua*

3. Collection of plant materials of *Butea monosperma*:
Plant twigs has been collected from nearby forest of our college campus.

B. Isolation of the toxic part of the plant:

B.1 Preparation of different parts of *Butea monosperma* :

For identification and confirmation of the presence of toxic part in the *B. monosperma* the different parts like bark, wood, leave, root were collected from the surrounding of college campus of Raja N.L. Khan Women's College, Paschim Medinipur and separated them and then cutting into small pieces. Flowers were not available in time of experiment process (10.09.2015 to 25.02.2016).

The steps taken for testing of toxic effect on the fish were observed and recorded as follows-

S.No.	Experiment	observations
1	*Treatment with bark of the plant*	The bark was applied to the water of aquarium containing five *C. gachua*. Stirred the water well. The movement of fishes as observed up to 24 hours were lessened.
2	*Treatment with woody part of the plant*	The small pieces of wood were applied to the water of aquarium containing five *C. gachua*. Stirred the water well. Observation of behavioral changes in fish exposed. The opercula beat and swimming were slower than normal condition. After 18 hrs the 3 fishes swam near the surface. After 20 hrs they were lay at the bottom and finally *dead* after 24 hrs.
3	*Treatment with leaves of the plant*	The leaves were applied to the water of aquarium containing five *C. gachua*. Stirred the water well. But no reaction was observed up to 24 hrs. The fishes were normally moved and swam.
4	*Treatment with root of the plant*	The small pieces of root was applied to the water of aquarium containing five *C. gachua* Stirred the water well. Up to 24 hrs, no effect was seen on the fishes. Still they were normally moved and swam.

Inference

From the above experiments and observation it has been found that only the woody part of the plant *(B.monosperma)* made the toxic effect on fishes. Hence, it has been decided to conduct experiment with the woody part of the plant to determine the toxic effect along with determination of LC50 value of the phytotoxicant.

Determination of LC50 value of the phytotoxicant

B.2. Preparation of different parts of *Butea monosperma*

For the preparation of plant material, steps were followed as per the system adapted by M.S.A.Mamun; M.Shahajahan; M.Ahamed, 2009 in their project 'Indigenous plant extracts as toxicant against red flour beetle' (Mamun *et al*., 2009).

Fig. 2. Small pieces of dried woody part of *B. monosperma*

a) Crude material of wood part of plant

Fresh stem of *B.monosperma* were collected. Afterward these were washed in running water. Then peeled the bark from the stem and collect the wood part. The materials were kept in shade for air drying and cut into small pieces. Then it grinds in grinder machine. Then ground samples were passed through a 25-mesh-sieve to obtain fine and uniform dust. The dust was preserved in air tight condition in polythene bags till their use in extract preparation and experiment.

b) Soluble extract of woody part of the plant

The dust ready for extraction 10 gm of sample was taken in a 500ml beaker and mixed with methanol (100ml). The mixture was stirred for 30 min by centrifuge machine and left to stand for next 24 hrs. Then mixture was filtered through filter paper (Watt man no. 1). The filtrate were taken in round bottom flak and condensed to 15 ml by evaporation of solvent in water both maintained at 55 ^{o}C. After evaporation of solvent the condensed extracts were preserved in a tight corked labeled bottle and stored in a refrigerator.

Fig. 3. Mixed with methanol, Centrifuge extract sol. Extracted solution

Experiment

A laboratory test for direct toxicity by topical application method was conducted according to the method of Talukdar and Howse (1993) which has been slightly modified. Eight fishes were taken from stock aquarium and putting these in each 20 lit aquarium, where amount of water is 6 liters. The fishes were not fed during the 24 hrs acclimation to aquarium before the test or during the test period.

For Control Treatment

The 8 specimen of *Channa* fishes were placed into 6 lit water of the control aquarium. Normally they were fed twice daily. The fish food was 'Annelids'. It was observed several times during 8 weeks. After completion of every experiment for a particular dose, the aquarium has been cleaned and again prepared for next experiment.

Fig. 4. Normal condition of control aquarium

Experiment with Crude Plant materials

The 5 different amounts of materials were taken into the experimental aquarium i.e., 6gm, 12gm, 18gm, 30gm, and 32gm. Now I observed for 24 hrs to 48 hrs.

Fig. 5. Application of crude materials into experimental aquarium in several time against *Channa gachua*

Experiment with soluble extract of wood

Same process and procedure were maintained in the present case. Here quantity of plant extract solution were 0.75ml, 1.2ml, 1.8ml, 3ml, and 6ml. These amount of solution were applied on 6 lit water and 8 fishes in each aquarium respectively. The different amount of materials has been taken for determination of the low toxic level to high or maximum toxic level.

Fig. 6. After application of extract solution of toxic plant materials against *Channa gachua*

Observation

The activities and effect of fishes were observed. Fish responses to *B.monosperma* were recorded for several hours and daily thereafter throughout the 48-hour bioassay. The observation of behavioral changes in fish exposed to *B.monosperma* indicated that at high concentration there was an increase in opercula beat but slower swimming activity than the control treatment. Dead fish were recorded and removed. Fish were regarded as dead when all opercula movements were completely ceased. Fish which survived after the 48-hour bioassay has been counted.

Growth Study: A feeding experiment was conducted to study growth rate of *Channa gachua* continuously exposed to therapeutic levels of toxic substance concentration. The experiment was conducted in 6-liter aquaria for an 8-week feeding period. Tap water has been used during the experimental period. *C. gachua* averaging 1.2 g were acclimated 2 weeks in the laboratory before stocking in the experimental aquaria. They were fed a nutritionally complete practical diet (Annelids) during this holding period. Water was changed every two days and new toxic materials and extract solutions were added into aquaria. Aeration was supplied through air stone during the 8-week experimental period. The fish were fed with Annelid diet twice daily at a rate of 3% (dry matter) their body weight per day. Fish were weighed after every two weeks and the feed allowance adjusted accordingly.

Activity of fishes after application of materials: Fish responded to the woody materials of *B.monosperma*. The activities were recorded for several hours and daily thereafter throughout the 48 hour bioassay. The dead fish number were recorded and removed. Fish were regarded as dead when all opercula movement ceased. When the fishes survived the 48

hours bioassay, the experiment was stop. The water of aquarium becomes polluted and foaming. Numbers of dead fishes were increased after 24 hrs. In different concentration the numbers of dead fishes were different. It was observed that soluble plant extract materials more toxic than the crude materials.

Fig. 7. Weighting of fishes after 2 weeks

Fig. 8. Effected fishes after application of different concentration of toxic materials

RESULTS

Growth study: C. gachua averaging 1.2 g were acclimated 2 weeks in the laboratory before stocking in the experimental aquaria. They were fed a nutritionally complete practical diet (Annelids) during this holding period and do not show any significant weight change.

Acute Toxicity: Percentage mortalities of fish during the 24 hrs and 48 hrs exposure to crude material of *B. monosperma* have been recorded. The affected number of fishes against the various concentrations of materials are given below

Table 1. List of number of dead fishes (*Channa gachua*) after control treatment and after applying Crude material woody part of *Butea monosperma* at 24 hrs & 48 hrs

A.Results of after control treatment

Volume of water in aquarium (lit)	Total no. of live control fishes before treatment	No. of dead fishes at 24 hrs control after treatment	No. of dead fishes at 48 hrs control treatment
6	8	0	0

All fishes remain alive in control system after treatment period of 8 weeks.

B.Result after applying Crude material (woody part) of *Beutea monosperma*

S.No.	Volume of water in aquarium (lit)	Total no. of fishes for treatment	Percentage of Dose of Crude materials (mg/lit)	No. of dead fishes after 24 hr	No. of dead fishes after 48 hr
1	6	8	0.1	No die	No die
2	6	8	0.2	2	4
3	6	8	0.3	3	5
4	6	8	0.5	4	7
5	6	8	0.6	8	8

Diagram of mortality of fishes after 24 hrs. and 48 hrs for crude material

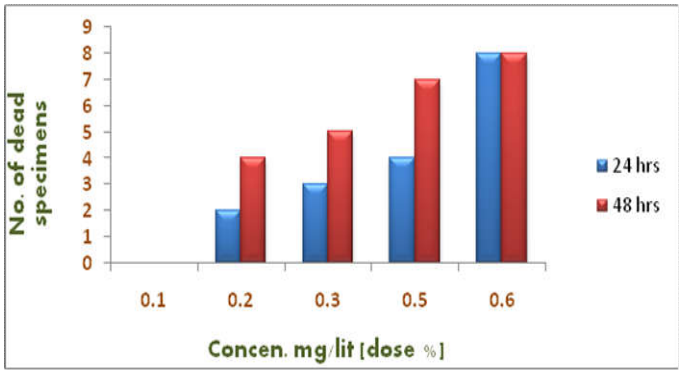


Table No. -2: List of number of dead fishes (*Channa gachua*) after control treatment and after applying Extract solution (of woody part) of *Butea monosperma* at 24 hr & 48 hr.

A. Results of control treatment

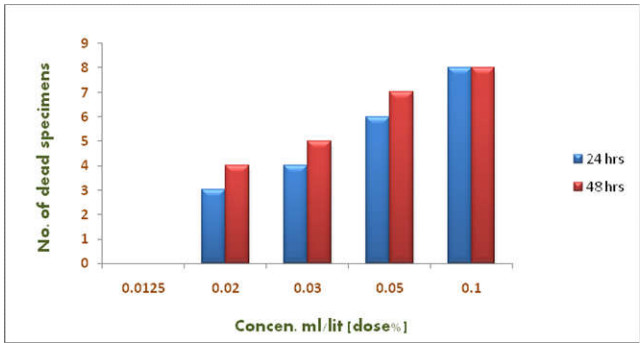
Volume of water in aquarium (lit)	Total no. of live control fishes before treatment	No. of dead fishes at 24 hrs control after treatment	No. of dead fishes at 48 hrs control treatment
6	8	0	0

All fishes remain alive in control system after treatment period period of 8 weeks.

B. Result after applying extract solution (of woody part) of *Beutea monosperma*

S. No.	Volume of water in aquarium (lit)	Total no. of fish for treatment	Percentage of dose of Extract sol. (ml/lit)	No. of dead fishes after 24 hrs	No. of dead fishes after 48 hrs
1	6	8	0.0125	No die	No die
2	6	8	0.02	3	4
3	6	8	0.03	4	5
4	6	8	0.05	6	7
5	6	8	0.1	8	8

Diagram of mortality of fishes after 24 hrs. and 48 hrs for extract sol.



The calculation of LC_{50} was using Probit analysis (Finney 1971). The experimental data were statistically analyzed by USEPA Probit Analysis Program V 1.5 software in computer.

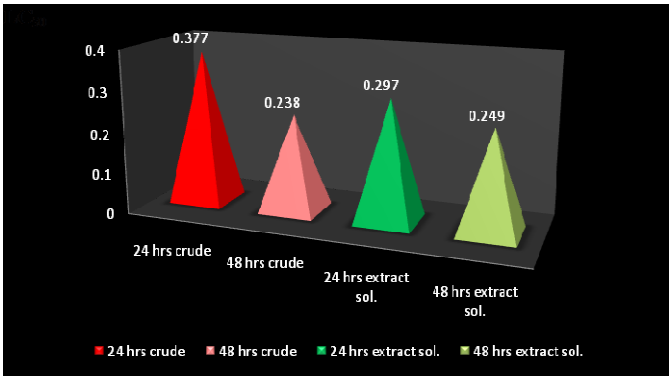
Table 3. LC_{50} of Crude Material (woody part) of *B. monosperma* against *Channa gachua* at 24 hrs & 48 hrs

S. No.	Experimental time duration (hours)	The concentration of toxic in water cause the death of 50% of fishes (LC 50 Value)
1	24	**0.377**
2	48	**0.238**

Table 4. LC_{50} of Extract Solution (woody part) of *B. monosperma* against *Channa gachua* at 24 hrs & 48 hrs

S.No.	Experimental time duration (hours)	The concentration of toxic in water cause the death of 50% of fishes (LC 50 Value)
1	24	**0.297**
2	48	**0.249**

LC_{50} value of crude and extract sol. Of toxic materials in column



DISCUSSION

Behavioral changes: Behavioral changes in fish exposed to toxic *Butea monosperma* as observed was at high concentration with both crude and extract solution there was an increase in opercula beat but slower swimming with passing of time than the fishes at controls. *Channa* fish swam up and down rapidly and frequently gulping at the surface. After 18 hours of exposure fish swam near the surface. Fish showed uncoordinated movements. Finally they lay down on the bottom within 12–18 hours. It was due to less oxygen availability in water. In case of extract treated group the rate of stupefaction was higher than the woody powder *(B.monosperma*) treated group.

Effect on growth: In the woody powder (*B.monosperma)* treated group, gain in body weight was gain was observed like that of the control group, but the magnitude of body weight comparatively lesser. This indicates that there might have been non-drug influence on the body weight changes. Since none of the observed changes reached statistically significant level, it can be suggested that the woody powder at the dose level studied have no significant influence on the body weight, even on long duration of administration. In case of extract treated group the result was more or less same.

Mortality & LC_{50}: The present investigation revealed that the indigenous fresh water fish species (*C. gachua*) those were maintained under control treatment survived successfully for 8 week. But among the tested sample fishes of the same species treated with Butea monosperma showed slope of regression line as recorded after 24 and 48 hours. The rate of mortality is presented in Table No.1 in case of crude (woody) treatment and in Table No. 2 in case of extract solution treatment. The result indicates the rate of mortality is almost directly proportional to the increase of dose applied. The trend is found to be the same as at the end of periods of 24hrs and 48hrs.The study on values of LC_{50} shown in the Table No. 3 & 4 indicate the distinct and discrete difference in mortality in case of crude (woody) and extract sol. treatment. Higher mortality rate noticed in case of extract treatment because of the concentration of toxicant in its dose is comparatively higher than that in the crude dose.

Conclusion

It may be concluded from the investigation and result of toxic effect of the plant extract used in present experiment shows direct toxic effect on fish under study. Controlled and specified application of the plant product may be used for multiple purposes, e.g., fish harvesting in large-size aquatic body by stupefying fish fauna. It may be applied for predator eradication before commencement of pond culture, of course a study on residual effect of the toxin is needed before application of the toxin as eradicator in pond culture system. Further research on this subject is needed for identification of the Chemical Constituents of woody part, responsible for toxic effect. Further, It may be investigated that weather the plant extract can be used as herbal insecticide in pest management or not.

Acknowledgements

Authors are very much grateful to Dr. Jayasree Laha, Principal and Dr. Partha Pratim Chakravorty, HOD of Zoology, of Raja N. L. Khan Women's College for their permission to use infrastructural fascilities. Authors are also indebted to DBT, Government of India for financial support by approving our college under DBT Star College Scheme.

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