

Original Research Article

doi: <http://dx.doi.org/10.20546/ijcmas.2016.502.xxx>

## Effect of *Azadirachta indica* Leaf extract on protein content in muscles and gills of semi fingerling of *Catla catla*

Rukhsar Shaikh\*, M.A.Majid and KalimShaikh

Department of Zoology, AKI'S Pooa College of Arts, Commerce and Science Camp,  
Pune-411001, India

\*Corresponding author

### ABSTRACT

*Azadirachta indica* is an important medicinal plant having diverse chemical compounds with various biological properties. The aim of this study was to assess the effect of leaf extract of neem plant on protein content of muscles and gills in semi fingerling of *Catla catla* a common fresh water fish. The fingerlings were exposed to sub-lethal concentration of neem extract (g/lit) for 24 and 96 hours as time intervals. The total protein level was changed in muscles and gills of semi fingerling. Semi fingerling of first group were exposed to  $\frac{1}{2}$  24 hrs Lc50 and  $\frac{1}{2}$  96 hrs Lc100 (4.25gm/lit and 3.25gm/lit) for 2 and 7days and second group were exposed to  $1/10^{\text{th}}$  24hrs Lc50 and  $1/10^{\text{th}}$  96 hrs Lc100 (0.85g/lit and 0.65g/lit) for 2 and 7days. The result revealed that total protein (mg/dl) in muscles and gills decrease from 0.793-0.520, 0.776-0.512 and 0.793-0.636, 0.776-0.586 for 2days. When it was exposed with concentration 4.25g/lit and 0.85g/lit for  $\frac{1}{2}$ ,  $1/10^{\text{th}}$  24hrs Lc50. and 0.793-0.504, 0.776-0.495 and 0.793-0.628, 0.776-0.578 for 7 days when it was exposed with concentration 4.25g/lit and 0.85g/lit for  $\frac{1}{2}$ ,  $1/10^{\text{th}}$  24hrs Lc50. as well as total protein (mg/dl) in muscles and gills decrease from 0.793-0.495, 0.776-0.479 and 0.793-0.628, 0.776-0.603 for 2 days, when it was exposed with concentration 3.25g/lit and 0.65g/lit  $\frac{1}{2}$ ,  $1/10^{\text{th}}$  96hrs Lc100 and 0.793-0.471, 0.776-0.462 and 0.793-0.603, 0.776-0.576 for 7 days, when it was exposed with concentration 3.25g/lit and 0.65g/lit  $\frac{1}{2}$ ,  $1/10^{\text{th}}$  96hrs Lc100, respective different exposure period compare to control.

### Keywords

*Azadirachta indica*,  
Semi fingerling,  
*Catla-catla*,  
Muscles,  
Gills,  
Protein

### Article Info

Accepted:  
28 January 2015  
Available Online:  
10, February 2016

### Introduction

*Azadirachta indica* is one of the most promising medicinal plant having a wide spectrum of biological activity and well known for its insecticidal properties (ICAR, 1993). Every part of neem tree have been known to possess a wide range of pharmacological properties, especially as antibacterial, antifungal, antiulcer, antifeedant, repellent, pesticidal, mollus-

cidal, ecdysome inhibitor and sterilant is commercially exploitable (Biswas *et al.*, 2002; Das *et al.*, 2002) and hence traditionally used to treat large number of disease (Van Der Nat *et al.*, 1991).

Both fish parasites and fish predators which cause great economic losses in productivity are mainly controlled with toxic chemicals,

mostly applied indiscriminately and without adequate training (Senhorini, 1991). Thus use of pesticides in aquaculture system to control fish disease, parasites and other pests not only leads to high level of residues in animals but also may interfere with the maintenance of their homeostasis and thus affect their performance (Barton and Iwama, 1991; Wendelaar Bonga, 1997). In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative method of pest control that minimize this damage, there has been extensive research on pest control by substance from plant (Wan *et al.*, 1996). One of the most promising compound is Azadirachtin (AZA) an active compound extracted from the *Azadirachta indica*, its antiviral, antibacterial and antifungal properties have been known for several year (Harikrishnan, *et al.*, 2003). The chemistry and biological activity of both neem extract and purified AZA have been investigated in various countries (Biswas, *et al.*, 2002).

*Azadirachta indica* has been used successfully in aquaculture system to control fish predators (Dunkel and Ricilards, 1998); Martinez 2002) stated that aqueous extract of *Azadirachta indica* leaves and other *Azadirachta indica* based products have been extensively used in fish farms as alternative for the control of fish parasites and fish fry predators such as dragon fly larvae.

Although *Azadirachta indica* extract is considered of low toxicity toward non target aquatic life water extract of the bark of the neem plant caused respiratory problems in *Tilapia zilli* (Osmoregie and Okapanhchi, 1997) while long exposure to low concentration of the crude extract of *Azadirachta indica* delayed the growth of this Cichlid fish (Omoregie and Okapanchi, 1992)

The presents study investigates the effect of *Azadirachta indica* leaf extract on protein content of muscles and gills in semi fingerlings of *Catla catla*.

### **Materials and Methods**

The semi fingerlings of *Catla catla* were procured from Manjrifish farm Hadapsar Pune in living condition weighting 1-1.55 gm. They were acclimatized to the laboratory condition for period of 15 days. Then all semi fingerlings, were divided into 10 batches in and each batch 10 semi fingerlings selected and were used for the present course of investigation. Whole experiment was carried out in Post Graduate Laboratory department of Zoology Poona College.

### **Preparation of Aqueous Neem Leaf Extract**

*Azadirachta indica* leaves were collected from the surrounding area of the Pune City. The leaves were dried and finely chopped, then dissolved in tap water at concentration of 500gm of dried leaves per liter of water, for 24hrs at room temperature (Cruz *et al.*, 2004). The mixture was filtered and the extract (500g/l) was used immediately in the experiment, in different dilutions.

### **Fixation of sub lethal Concentration and Physiological Biological Assays**

Experiments was set to evaluate acute effect of leaf extract on protein. The semi fingerlings, were distributed in four groups and 4 fishes were kept in each group. It comprises two experimental group with control. Each group was placed into 1 lit of water. Experimental group were exposed for 2 days and 7 days to both concentration of leaf extract corresponding to sub lethal

(1/10<sup>th</sup> and 1/2). The control was simultaneously exposed to dechlorinated tap water and the end of 2<sup>nd</sup> and 7<sup>th</sup> day of exposure.

### **Preparation of Tissue for Protein Estimation**

100mg tissue of muscles and gills of Semi fingerling was taken into a Petri dish. 10ml of 10% TCA was added to it. After which the tissue was homogenized thoroughly. The tissue was centrifuged for 15 min at 3000 rpm.

Supernatant was discarded. The residual solution was diluted with 10 ml of 1N NAOH. From this solution 0.1 ml solution was taken in 3 different test tubes, in each test tube 4 ml reagent 'C' was added. After which 0.4 ml of FCR was added. The solution was kept for 30 min at room temperature in dark condition. Optical density was taken at 540 nm. Estimation of protein was done by (Lowry et. al 1951).

### **Results and Discussion**

The finding of the present study clearly showed that the neem extract reduce the protein content of muscles and gills in semi fingerling of *Catla catla*.

In the present study Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.520 and 0.776 – 0.512 for 2 days. When it was expose with concentration 4.25mg / lit with ½ 24hrs Lc50 respectively. Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.636 and 0.776-0.586 for 2 days. When it was expose with concentration 0.85mg / lit with 1/10th 24 hrs Lc50 respectively.

Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.504 and 0.776 – 0.495 for 7 days. When it was expose with

concentration 4.25mg / lit with ½ 24hrs Lc50 respectively. Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.628 and 0.776-0.578 for 7 days. When it was expose with concentration 0.85mg / lit with 1/10th 24 hrs Lc50 respectively. The overall results were represented in table 1.

Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.495 and 0.776 – 0.479 for 2 days. When it was expose with concentration 3.25mg / lit with ½ 96hrs Lc 100 respectively

Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.628 and 0.776-0.603 for 2 days. When it was expose with concentration 0.65mg / lit with 1/10th 96 hrs Lc100 respectively.

Protein (mg/dl) in muscles and gills decreased from 0.793 – 0.471 and 0.776 – 0.462 for 7 days.

When it was expose with concentration 3.25mg / lit with ½ 96hrs Lc 100 respectively. Total protein (mg/dl) in muscles and gills decreased from 0.793 – 0.603 and 0.776-0.570 for 7 days. When it was expose with concentration 0.85mg / lit with 1/10th 96 hrs Lc100 respectively. The overall results were represented in table 2.

The finding of the present study showed that *Azadirachta indica* extract reduce the protein content of muscles and gills in semi fingerling of *Catla catla*.

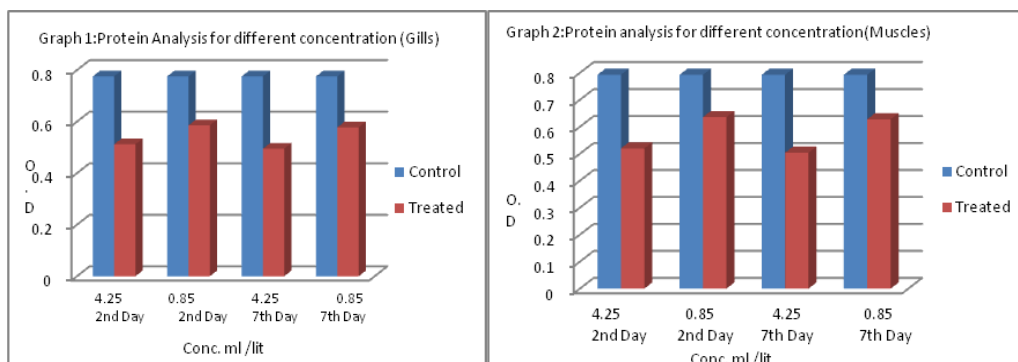
The present finding correlates with report given by (Mamdouh A.A.mousa *et al.*, 2008) showed the effect of *Azadirachta indica* leaf extract on fresh water fishes with respect to significant reduction in total protein content after treated Lc50 of *Azadirachta indica* leaf extract with non – treated one after two and seven days of exposure were recorded.

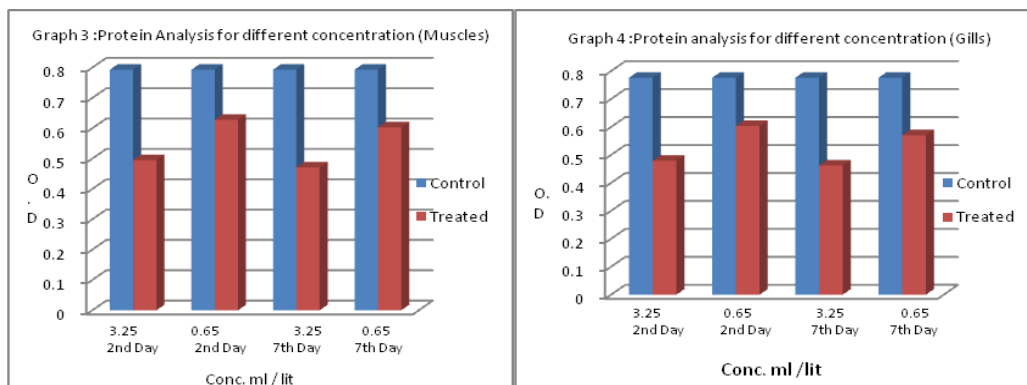
**Table.1** Effect of Sub Lethal Concentration of *Azadirachta indica* Leaf Aqueous on Fingerlings of *Catla catla* for Different Period for Lc50 24 Hrs

Concentration (gm / lit)	Parameter Period (Days)	Total Protein(mg/dl)	
		Muscles	Gills
Control		0.793	0.776
4.25 conc. ½ 24 hrs Lc50	2	0.520	0.512
0.85 conc. 1/10 <sup>th</sup> 24 hrs Lc50		0.636	0.586
4.25 conc. ½ 24 hrs Lc50	7	0.504	0.495
0.85 conc. 1/10 <sup>th</sup> 24 hrs Lc50		0.628	0.578

**Table.2** Effect of Sub Lethal Concentration of *Azadirachta indica* Leaf Aqueous on Semifingerlings of *Catla catla* for Different Period for Lc100 96 hrs

Concentration (gm / lit)	Parameter Period (Days)	Total Protein(mg/dl)	
		Muscles	Gills
Control		0.793	0.776
3.25 conc. ½ 96 hrs Lc100	2	0.495	0.479
0.65 conc. 1/10 <sup>th</sup> 96 hrs Lc100		0.628	0.603
3.25 conc. ½ 96 hrs Lc100	7	0.471	0.462
0.65 conc. 1/10 <sup>th</sup> 96 hrs Lc100		0.603	0.570





The similar finding reported from (Rathod S.H.et.al 2013) as the fishes were exposed to sub lethal conc. of *Azadirachta indica* (14 µl/lit) for different time intervals. The total protein was found to decrease 50.40, 48.18,44.12 and 42.15 in muscles and 31.31, 29.60, 27.21,25.14 in liver tissue at the different exposure period and compare to control.

Similar finding by (Lynch et.al 1969) observed there is significant co relation between RNA and protein deficiency synthesis of a type of RNA should have in reflection in corresponding failure or reduction in protein synthesis. Tivari and Singer, (2006) reported that the decrease protein level in liver and muscles of fish exposed to *Azadirachta indica* extract might have a resulted from high protein hydrolytic activity in both the tissue.

In the present report the decrease of protein level in muscles and gills during sub lethal exposure might have resulted from their degradation and possible utilization from metabolic purpose.

The present study indicates that leaf extract of *Azadirachta indica* has caused significant alternation in protein content of muscles and gills in semi fingerling of *Catla catla* which might be helpful to management and the better usage of aqueous extract of *Azadirachta indica* in aquaculture farms.

## Acknowledgement

The authors would like to acknowledge to Head, Department of Zoology, Poona College for providing necessary facilities during course of work.

## References

- Barton, B.A., G.K.Iwama 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effect of corticosteroid. *Annu.Rev.Fish Dis.*1:3-26
- Biswas, K.I. Chattopadhyay, R.K.Banerjee, and U.Bandyopadhyay.2002. Biological activities and medicinal properties of neem (*Azadirachta indica*) *Curr.Sci.*82:1336-1345
- Cruz, C., J.G. Machado-Neto and M.L. Menezes 2004.Toxicidade aguda do inseticida Paratrimetilico e do biopesticida azadiractina de folhas de neem (*Azadirachta indica*) para alevino e juvenile de pacu (*Piaractus mesopotamicus*) *pesticidas :R Ectotoxicol. e Meio Ambiente* 14:92-102.
- Dunkel,F.V.,D.C.Ricilards.1998. Effect of an azadirachtin formulation on six non target aquatic macro invertebrates, *Environ.Entomol.*27:667-673.
- Harikrishanan, R.,M.N.Rani and C.Balsundram. 2003. Hematological and biochemical parameter in common

- carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *aquaculture* 221:41-50
- ICAR. 1993. World Neem conference souvenir ICAR, Bangalore, India
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). *J. Biol. Chem.*, 193:265
- Martinez, S.O. 2002. NIM-Azadirachta Indica: natureza, usos e propriedades Instituto Agronomico Parana (IAPAR), Londrina, PR.
- Omeregic, E., M.A. Okpanachi. 1992. Growth of *Tilapia zilli* exposed to sublethal concentration of crude extract of *Azadirachta indica*. *Acta hydrobiol.*, 34:281-286
- Senhorini J.A. 1991. Larviculture do pacu *Piaractus mesopotamicus* Holmberg. 1887. (Pisces Characidae) em viveiros com esmorganos fosforados (Folidol 60%) *Boletim Tecnico* CEPTA 4, 11-22.
- Siwicki, A.K., D.P. Anderson and G.L. Rumsey (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Vet. Immunopathol.* 41:125-139.
- Tiwari, S. and Singh, A. 2006. Biochemical stress response in fresh water fish *channa punctatus* induced by aqueous extract of *Euphorbia tirucalli* plant. *Chemosphere*, 64:36-42
- Rathod, S.H. 2013. Effect of *Azadirachta indica* on the total protein of the fresh water cat fish *Heteropneustes fossilis*. *Int. journal of Innovation in Bio-Sciences*. Vol 3(2): 64-67.
- Lynch, M.J., S.S. Raphael, L.D. Mellor, P.D. Spase and M.J.H. In wood. *Medical laboratory Technology and clinical pathology*, 2<sup>nd</sup> Ed. W.B. Saunders Co. Toronto, London.
- Van Der Nat, M.G., KTD. Van Der Sluis & R.P. Labadie. 1991. Ethnopharmacognostical survey of *A. indicajuss* (Maliaceae). *J Ethnopharmacol.*, 35:1-24
- Wan, M.T., R.G. Watts, M.B. Isman and R. Strub. 1996. Evaluation of the acute toxicity to juvenile pacific northeast salmon of azadirachtin, neem extract and neem based product. *Bull. Environ. Contam. Toxicol.*, 56:432-439.

#### How to cite this article:

Rukhsar Shaikh, M.A. Majid and Kalim Shaikh. 2016. Effect of *Azadirachta indica* Leaf extract on protein content in muscles and gills of semi fingerling of *Catla catla*. *Int.J.Curr.Microbiol.App.Sci.* 5(2): 701-706. doi: <http://dx.doi.org/10.20546/ijcmas.2016.502.077>