RESEARCH ARTICLE

Study on the evaluation of the effect of *Azadirachta indica* on the protein expression of fish *Puntius ticto* by SDS-PAGE

Shahid Mir[1*] and Gour Anil J[2]

[1]P. G. Department of Zoology Vidya Bharati Mahavidyalaya, Amravati, Maharashtra, India
[2]Associate professor Bar. RDIK & KD college Badnera, Maharashtra, India
[*]Corresponding author Email: mirshahid1990@gmail.com

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ABSTRACT

Organism and the surroundings in which they live is an ecosystem, whereas the plants and animals living within an ecosystem are interdependent with each other. From the present study, it could be argued that the impact of the *Azadirachta indica* decreases total protein in muscle and liver of the fresh water ticto barb *Puntius ticto*. Proteins are mainly involved in energy and metabolic process due to which it bears upon the physiological and metabolic status of the fish, which means *Azadirachta indica* shows the effect on the survival and growth of the fresh water *Puntius ticto*. The survey also indicates that the application of neem leaf extract can be used to control unwanted organisms in ponds as environment-friendly material instead of deleterious pesticides. Likewise, extensive investigations should be established to provide information for the suitable methods of application in aquatic animal production facilities to be fully explored in future for its safe use in aquaculture.

Keywords: *Azadirachta indica*, Neem, *Puntiusticto,* Deleterious pesticides, Aquaculture

INTRODUCTION

The Ticto barb is a freshwater and briny sub-tropical fish belong to the family Cyprinidae. It is a native of the Bangladesh Salwen, Pakistan, Meklong and upper Charo Phraya basins in the rural areas of Nepal, India, Irrawaddy, Myanmar, Sri Lanka, upper Mekong, Laos, and Thailand. It has frequently been confused with P. Padamya in the aquarium trade, but in that species the male is reddish-orange (lacking in P. ticto). The ticto barb *Puntius ticto* (Hamilton, 1822) is a small, Home-grown fish species of Maharashtra (commonly called Tapri in Marathi) that is widely distributed in the natural waters of Asian countries. *Puntius ticto* is a popular fish species to the consumers of North-India, Eastern India, and North-East India as well. This species supports attractive morphological features skin. For this cause, it looks noticeable. It is natively found in halt, shallow, marginal waters of lakes and rivers, usually with muddy bottoms. It browses shut to the substrate in superficial water. Ticto Barb natively

survives in subtropical locations and prefer water with Ph of 6.0 - 7, a water rigidity of 5 -10 dGH, and a warmth range of 55 - 70 °F (13 - 20 °C). They can exist in equally clean and salty water. Their diet consists of minute crustaceans, insects, and plankton. It reaches to 10 cm (04 inches) in length. (Froese et al. 2013).

Experimental feed-Neem *Leaf Extract (Azadirachta indica):*

Neem; *Azadirachta indica*, is one of the most gifted medicinal plant, having a broad range of biological action, 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, anti-feedant, and sterilant and is thus commercially exploitable (Biswas et *al.*, 2002; Das *et al.*, 2002), and hence, traditionally used to treat large repellent, pesticidal, molluscicidal, ecdysone inhibitor number of diseases (Van der Nat et al., 1991). This eco-friendly native tree of India is perhaps the most researched tree in the world. Water soluble extract of A. indica leaves was found to possess significant hypoglycemic, hypolipidemic, hepatoprotective, anti-fertility and hypotensive actions. In the present study, we determine the toxicity of the aqueous extract of neem leaves on the endurance and health status of *Puntius ticto* and its ecological impacts on the zooplankton community. The chemical elements are found in the leaves of neem as azadirachtin (the insecticide) Nimbin, Nimba-nene, 6-desacetylnim-binene, Nimba N diol, Nim bolide, ascorbic acid, n-hexacosanol and amino acids (Hossain et al, 2013). Neem has been used successfully in aquaculture systems to control fish predators (Dunkel and Ricilards, 1998). Martinez (2002) stated that aqueous extract of neem leaves and other neem-based products have been extensively employed in fish-farms as an alternative for the control of fish parasites and fish fry predators such as dragonfly larvae. Although neem extract is considered of low toxicity towards non-target aquatic life, water extracts of the bark of the neem plant caused respiratory problems in Tilapia Zilli (Omoregie and Okpanachi, 1997), while long exposure to low concentrations of the crude extract of A. indica delayed the maturation of this cichlid fish (Omoregie and Okpanachi, 1992).

MATERIAL AND METHODS

Preparation of aqueous *Neem* leaf extract:

A. indica leaves were obtained from the surrounding area of Vidya Bharati college laboratory, dried and finely chopped, then dissolved in tap water, at a concentration of 500 g of dehydrated leaves per liter of water, for 24 hours at room temperature (as described by Cruz et al., 2004). The mixture was filtered and the extract (500 g/l) was used immediately and directly in the experiments, in different dilutions. So, the aim of the current investigation was to evaluate the effects of leaf extract of neem plant on the survival and health status of *Puntius ticto*. The laboratory determinations of lethal concentrations LC100 and LC50 (Mamdouh et al, 2008) through a static bioassay test. The 24 h LC100 of neem leaf extract was estimated as 25 mg/l, for *Puntius ticto*, at the highest test concentrations, adverse effects were obvious with significant reductions in fish. Some alterations in glucose levels, total plasma protein, albumin, globulin as well as AST and ALT in plasma of treated P. ticto with 1/2 and 1/10 LC50 of neem leaf water extract compared with non-treated one after 2 and 7 days of exposure were recorded and discussed.

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate proteins with relative molecular mass no smaller than 10 KD. SDS-PAGE is widely used to analyze the proteins in complex extracts. The most commonly used methods are derived from the discontinuous SDS-PAGE system. The system actually consists of two gels - a resolving (aka running) gel in which proteins are resolved on the basis of their molecular weights (MWs) and a stacking gel in which proteins are concentrated prior to entering the resolving gel. Differences in the compositions of the stacking gel, resolving gel and electrophoresis buffer produces a system that is capable of finely resolving proteins according to their MWs. (He, 2011; Laemmli 1970).

Chemicals Required:

Pre-stain Protein MW marker, TEMED, Ammonium persulfate, SDS, 30% Acrylamide stock (37.5: 1 acrylamide: bisacrylamide), Bromophenol Blue, Tris Base, Glycine, EDTA, Glycerol Isopropanol, Tris-HCl (pH 6.8), β-mercaptoethanol, 10x running buffer, 2x SDS protein sample buffer

Preparation of Reagents for SDS PAGE

1: Stock solution.

(A) Stock acrylamide solution (monomer solution): (i) Acrylamide = 30% (ii) Bisacrylamide = 0.8 % (iii) 100 ml distilled water. Store the solution in Amber colour bottle at room temperature.

(B) Seperating Gel Buffer (Stock) PH= 8.8

(i) 1.875 M TrisHcl = 27.7 gm (ii) 100 ml distilled water (While preparing dissolve weigh quantity of trisHcl in about 10 to 20 ml then adjust the PH 8.8 and them make up the volume. Store at room temp).

(C) Stocking gel buffer (Stock) PH = 6.8

(i) 0.6 M TrisHcl = 7.26 gm (ii) Distilled water =100 ml.

(D) Electrode buffer (PH = 8.2 TO 8.4) (Stock) (i) 0.05 M TrisHcl = 12 gm(ii) 0.192 M Glysine = 28.8 gm (iii) SDS = 2 gm (iv) Distilled Water = 2 Liters

E) Sample buffer (Stock).

Gel loading buffer

(i) Glycerol = 10 gm (ii) TrisHcl = 10 ml(iii)SDS 20% = 1 gm (iv) Mercaptoethanol = 0.50 ml (v) Bromophenol blue = 2ml (vi) Distilled water = 20 ml

F) SDS Solution 20% (stock)

Dissolve 20 gm of SDS in 100 ml of distilled water and store at room temperature.

G) Protein stain solution (stock)

(i) Acetic acid = 10 ml (ii) Methanol = 40 ml(iii) Commasie brilliant blue = 100 mg(iv) Distilled water = 50 ml

2) Working Solutions

(A) Polymerizing agents (working)

(i) Ammonium persulphate 5%. (0.5 gm of ammonium persulphate is dissolved in 10 ml of distilled water). (ii) TEMED (already available in laboratory).

(B) Destainer solution (working)

(i) Methanol = 40 ml (ii) Acetic acid = 10 ml(iii) Disilled water = 50 ml

(C) Prepration of separating gel (15%)

(i) Stock acrylamide solution = 2.5 ml (ii) TrisHcl (PH = 8.8) = 1.25 ml (iii) Distilled water = 1.2 ml (iv) Ammoniumpersulphate 10% = 70 µl (v) SDS 20% = 100 µl (vi) TEMED = 5 µl.

(Mix carefully and gently, leave to set for 30-60 min)

(D) Prepartion of Stacking Gel (5%)

(i) Stock acrylamide solution = 0.830 ml (ii) TrisHcl (PH = 6.8) = 0.630 ml (iii) Distilled water = 3.45 ml (iv) Ammonium persulphate 10% = 70 µl(v) SDS 20% = 100 µl (vi) TEMED = 5 µl

(E) Preparation of agarose for ceiling of plates (working)

(i) Agarose 1.5% = 450 mg (ii) Distilled water =30 ml. Boil water and then add agarose, immediately ceil the plate

(F) APS7% = 70 mg in 1 ml distilled water

Sample preparation: Sample prep. Prepare the same amount of protein samples according to BCA assay result, see BCA (bicinchoninic acid) protein assay. Add the same volume of 2x protein sample buffer to each protein sample, mix and boil the samples at 95 °C heating block module for 10 min. Spin the samples at the maximal speed for 1 min (samples from some tissue/cell sources may need longer spin) in a tabletop centrifuge and leave the samples at room temperature until you are ready to load onto the gelatin.

RESULTS AND DISCUSSION

In the present study it was observed that control group fishes behaved normally and survived, they were very active, with well coordinate movement and behave as usual manner, but the experimental freshwater fishes *Punitus ticto* kept in *Azadirachta indica* dose treatment for varying exposure period (from 24 hrs to 7 days) the natural activities of fishes get altered such as swimming ability changes, hyperactivity, non-directional movement and opercular movement get increased. Simultaneously it was observed that there was asignificant decrease in the total protein of liver and muscle tissues of tested freshwater fishes *Ponitus ticto* at adifferent exposure period of *Azadirachta indica*. The protein content value was found to be decreased2.033 mg/ 100mg inmuscle and 1.89 mg/100mg in liver tissue respectively compared to control values 3.524 mg/100mg in muscle and 2.63 in liver respectively as given in Table no.1 and Table no. 2 with graphical representation fig 1 & fig 2.

Table 1: Effect of *Azadirachta indica* (Neem) on the total protein observed in muscle of the freshwater fish *P.ticto*.

Concentration of protein in the muscle mg/100mg		
Group	**Control**	**Experimental**
Fish (P.ticto)	**3.524**	**2.033**

Table 2: Effect of *Azadirachta indica* (neem) on the total protein observed in the liver of the fresh water fish *P.ticto*.

Concentration of protein in the Liver mg/100mg		
Group	**Control**	**Experimental**
Fish (P. ticto)	**2.63**	**1.89**

Table 3: Effect of (*Azadirachtaindica*) neem on the total protein observed in liver and muscle of the freshwater fish *P.ticto*

Concentration of protein in *P. ticto* (mg/100mg)		
Sample	**Control**	**Experiment**
Muscle	3.52	2.03
Liver	2.63	

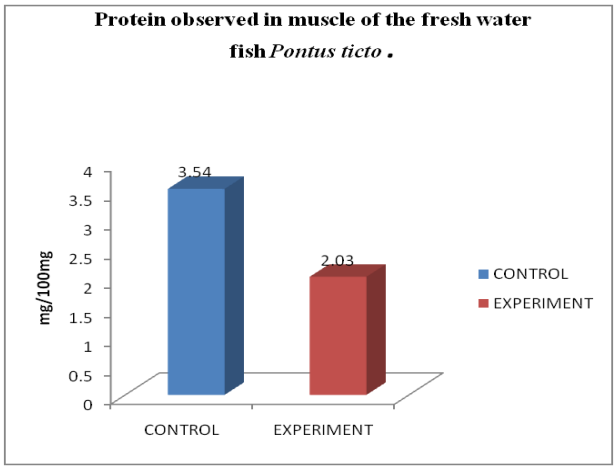

Figure 1:- Effect of *Azadirachta indica* (Neem) on the total protein of the fresh water fish *P. ticto* observed in muscle.

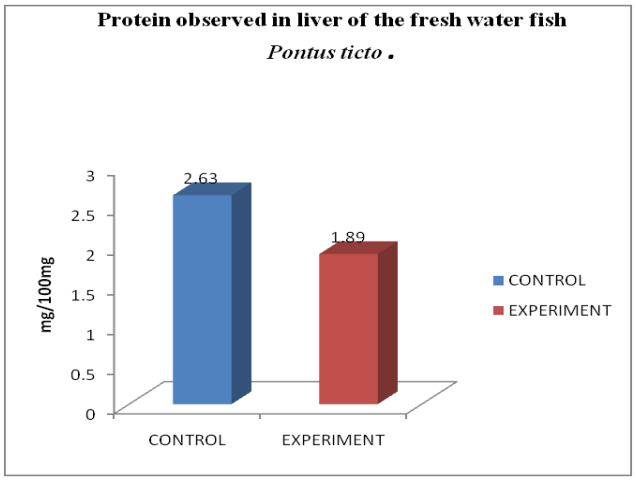

Figure 2: Effect of *Azadirachtaindica*(neem) on the total protein observed in the liver of the freshwater fish *P.ticto*.

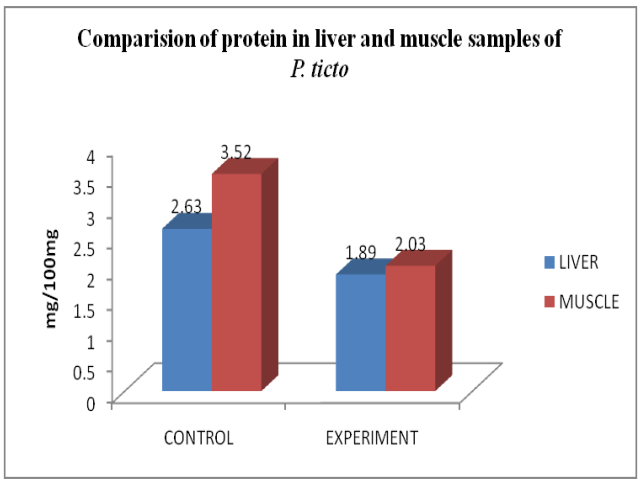

Figure 3: Effect of (*Azadirachtaindica*) neem on the total protein observed in liver and muscle of the fresh water fish *Puntius ticto*

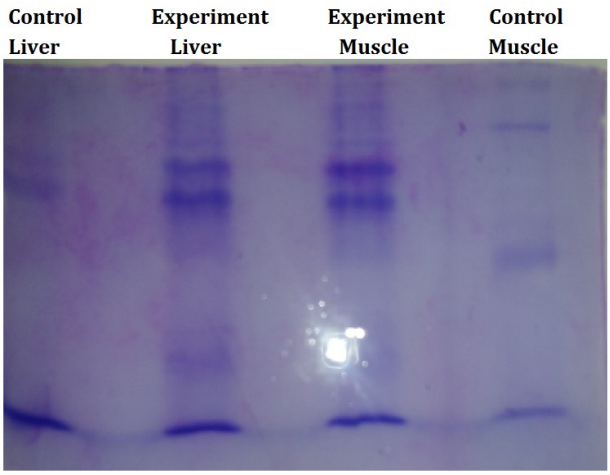

Figure 4: Bands obtained from SDS-PAGE of liver and muscle tissue of freshwater fish *Pontiusticto*.

Conflicts of interest: The authors stated that no conflicts of interest.

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