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Original Article

Neem leaf juice as bio-safe anaesthetic against two live fish, *Anabas testudineus* and *Channa punctata*

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Abstract: Activity of fresh neem leaf juice to immobilize, anaesthetize and fatal effect were studied against two live fish species, *Anabas testudineus* and *Channa punctatus*. The concentrations, 5, 10, 15 and 20% of neem leaf juice affected both the species, but *A. testudineus* was found to be comparatively more susceptible to the treatment than *C. punctatus*. The time required to immobilize were 4 to >6 hours for *A. testudineus* and 7 to 9 hours for *C. punctatus*; to anaesthetize were 6 to >12 hours and 8 to 10.15 hours for *A. testudineus* and *C. punctatus*, respectively; and death occurred 7 to >13 hours in *A. testudineus* and 9 to 10.45 hours in *C. punctatus* after treatment depending on the concentrations. The toxic effects were positively co-related with the concentrations and negatively co-related with the total weight of the fish, though the values of the coefficient of the correlations (r) were low. At the anaesthetic stage, the colour of skin, gills and eye were changed, the fishes showed irritated movement first (*A. testudineus*) and then became sluggish (both species), and instead of the normal fishy smell the fishes gave neem leaf like smell.

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Introduction

Application of plant materials in the management of aquaculture ponds is gaining momentum because they are safe, effective, widely available and comparatively cost-effective (Mousa et al., 2008). In countries like Bangladesh, fish parasites and fish predators, which cause a great economic loss in productivity, are mainly controlled by using toxic chemicals. These chemicals are mostly applied indiscriminately and without training of the fish farmers. As a result, high level of chemical residues has accumulated not only in the fish but also in the pond environment. It is reported that most of these chemicals used in the aquaculture system interfere with the homeostasis of the fish and thus affect their life performances (Barton and Iwama, 1991; Wendelaar Bonga, 1997). To overcome these adverse effects of the traditional chemical treatments extensive research on the alternative materials for fish disease control are ongoing.

Among the plants, Neem tree is a very significant one, because the plant has chemicals which are of medicinal, insecticidal and anti-bacterial values. The major chemical component of the Neem plant (*Azadirachta indica*) is 'azadirachtin', which is one of the most promising natural compound exhibiting antiviral, antibacterial and antifungal properties (Harikrishnan et al., 2003), and has been used successfully in aquaculture system to control fish predators and parasites (Dunkel and Ricilards, 1998; Winkaler et al., 2007) and pathogenic bacteria in culture water (Das et al., 2002). Neem extract has also been found to be potential in controlling bacteria isolated from marine fishes (Dhayanithi et al., 2010). However, Omoregie and Okpanachi (1997) reported that aqueous extract of neem bark caused respiratory problems in *Tilapia zillii*.

Saravanan et al. (2011) reported that as neem

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extracts affect the haematological, ionoregulatory, biochemical and enzymological parameters in fish (*Cirrhinus mrigala*). Therefore, there is a need for establishing safe limit of neem or any other biopesticides against the culture fishes, before introducing these bio-pesticides in culture ponds to eradicate insect pests or pathogens. The present study was designed to determine as a bio-safe anaesthetic, toxic and morphological effects of neem leaf juice against the two live fishes, *Anabas testudineus* and *Channa punctata*.

Materials and Methods

Collection and acclimatization of experimental fish: In the present experiment, two live fish species viz. *A. testudineus* and *C. punctata* were used. Live fishes of both species were collected from the fish landing centre, and carried to the laboratory in plastic containers containing water. In the laboratory, the total length and total weight of each specimen were recorded, and then released them in earthen pots containing pond water. The fishes were acclimatized to the lab condition for three days providing feed, changing the water after 24-hour. The dead fishes were removed immediately.

Preparation of neem leaf juice: Freshly collected neem leaves were thoroughly washed. About 500 g of the leaf was taken with 1 L fresh water in an electrical blender. The juice was then filtered, and the filtrate liquid was considered as the stock solution. Required quantity of the stock solution was mixed with water to prepare 5 L solution, for obtaining the concentrations of the neem leaf juice as 5, 10, 15 and 20%.

Experimentation: Two sets of experiments were done, toxicity test and observation on morphological characters. In each experiment three fishes of each species of similar length and weight, were used separately for each of the concentrations. Each fish was kept in separate plastic container, and the mouth of the container was covered with a piece of mosquito curtain tied with a rubber band to avoid the escaping of the fish. The experiments were carried at room temperature with three replications for each. The fishes under experiments were kept starved for two hours before they were released in the treatment. During the experiment no food was given to the fishes.

Parameters recorded: For toxicity test, the times when the fish became immobilize, anaesthetize and finally died were recorded. To study the effects of the treatment on the morphology of the fish, the skin, gill and eye colours were compared with that of the fishes kept in fresh water only as control. Movement of the treated fish and change in smell were also observed in the treated and control fish. These data were taken when the fishes were anaesthetized due to the treatment.

Statistical analysis: The toxicity data were presented as the average time taken to product the different toxic stages of the fish. Regression equation ($Y=a+bX$) was calculated to find out the correlations between the time required to immobilize and anaesthetize the fishes and the concentrations of the neem leaf juice, and that in between the time required to immobilize and anaesthetize the fishes and the total weight of fish.

Results and Discussion

Toxic effect of neem juice: The concentrations of neem leaf juice used were found to be toxic against both species of the fish. *Channa punctata* showed slightly increased tolerance to the treatment. *Anabas testudineus* were immobilized at 5.17, 6.30, 5.30 and 4.20 hours after treatment with 5, 10, 15 and 20% neem leaf juice, respectively. *Channa punctata* became immobilized at 8.45, 9.05, 8.15 and 7.45 hours after treatment, respectively (Fig. 1).

The fishes were anaesthetized or became senseless at 7.10, 12.10, 6.30 and 6.10 hours (*A. testudineus*), and 10.15, 10.05, 9.25 and 8.28 hours (*C. punctata*) after treatment with the mentioned concentrations, respectively (Fig. 2).

However, the time required for death, from anaesthetic stage was less in *C. punctata* than *A. testudineus*. The time required for death of *A. testudineus* were 9.10, 13.20, 8.00 and 7.30 hours; and that for death of *C. punctata* were 10.45, 10.35,

10.25 and 9.05 hours after treatment with 5, 10, 15 and 20% of neem leaf juice, respectively (Fig. 3).

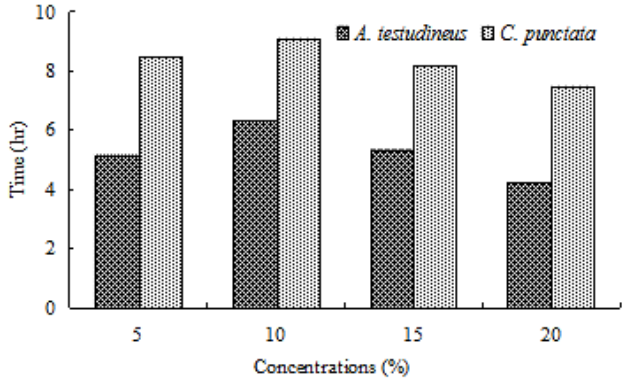

Figure 1. Immobilizing time of *A. testudineus* and *C. punctata* in different concentrations of neem leaf juice.

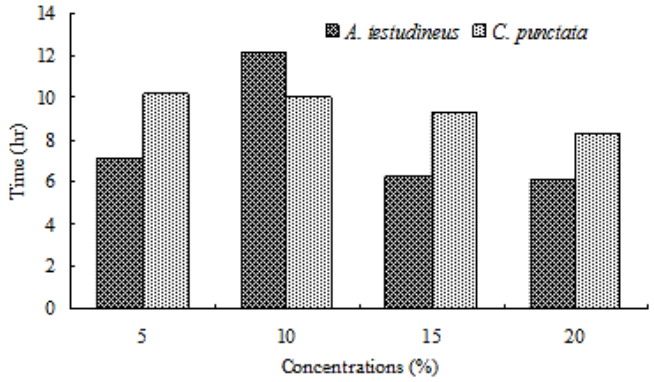

Figure 2. Anaesthetizing time of *A. testudineus* and *C. punctate* in different concentrations of neem leaf juice.

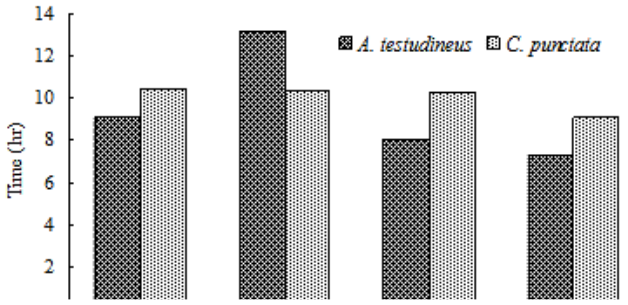

Figure 3. Dying time of *A. testudineus* and *C. punctate* in different concentrations of neem leaf juice.

The time required for immobilize and anaesthetize the fish were found to be negatively correlated with the concentrations of neem leaf juice used, where the coefficient of correlation values were obtained as r=0.59 and r=0.41, respectively (*A. testudineus*), and r=0.76 and r=0.95, respectively (*C. punctata*) (Figs. 4 and 5).

Total weight of the fish was an important factor for the efficacy of the neem leaf juice as anaesthetic. The relations between the total weight and the time

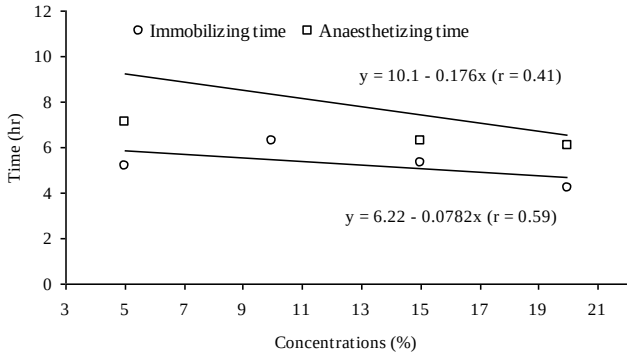

Figure 4. Relationships among concentrations of neem leaf juice with immobilizing and anaesthetizing time of *A. testudineus*.

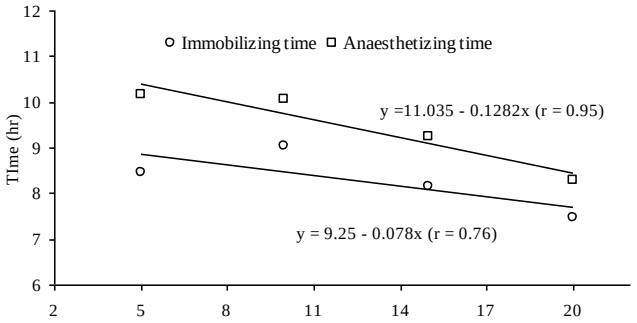

Figure 5. Relationships among concentrations of neem leaf juice with immobilizing and anaesthetizing time of *C. punctata*.

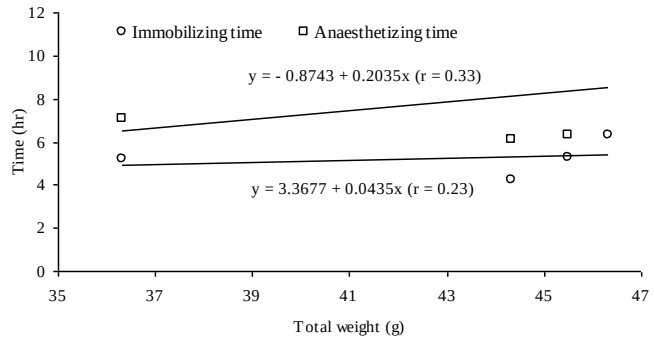

Figure 6. Relationships among total weight and immobilizing and anaesthetizing time of *A. testudineus*.

required to immobilize and anaesthetize the fish were poorly but positively correlated with coefficient of correlation values as r=0.23 and r= 0.33, respectively (*A. testudineus*). Whereas, the correlation were high in case of *C. punctata*, where the r values were obtained as 0.63 and 0.79, respectively (Figs. 6 and 7).

Morphological effects: The change of morphological

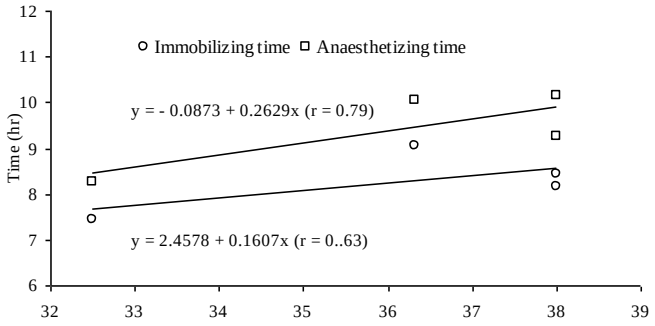

Figure 7. Relationships among total weight and immobilizing and anaesthetizing time of *C. punctata*.

features of the experimental fishes were assessed when they became senseless or anaesthetized due to the treatment with the aforementioned concentrations of neem leaf juice. The normal skin colour of *A. testudineus* was changed to yellowish and pale brown at different concentrations. The skin colour of *C. punctata* was changed to yellowish, but at higher concentrations it became greyish. Eye of the anaesthetized fishes became cloudy, and the normal fishy odour was changed to a smell like neem leaf. Gills of the fresh fishes were deep red in colour, which finally changed either to yellowish or to brownish. Movement of *A. testudineus* was frequent showing some restlessness in the treated water at lower concentration, but the agility was decreased in the higher concentrations. Whereas *C. punctata* showed very slow movement in the treated water at all concentrations. The above mentioned characters of the treated fishes are presented in Table 1.

The present results revealed that the neem leaf juice has anaesthetic effect against both

Table 1. Effect of neem leaf juice on the morphological characters of *A. testudineus* and *C. punctata* with time.

Fish species	Concentration (%)	*Exposure time (hr)	Morphological characters				
			Skin color	Eye	Gill	Movement	Odor
Anabas testudineus	Control (only water)	-	Normal (grayish)	Transparent or clear	Deep red	Normal (frequent)	Normal
	5	4	Yellowish	Transparent	Deep red	Frequent	Smelled like neem leaf
		8	Pale brown	Cloudy	Yellowish	Rapid and restless	Smelled like neem leaf
	10	4	Brownish	Transparent	Deep red	Frequent	Smelled like neem leaf
		8	Yellowish	Transparent	Brownish	Slow	Smelled like neem leaf
		12	Pale brown	Cloudy	Yellowish	Very slow	Smelled like neem leaf
	15	4	Pale brown	Cloudy	Brownish	Very slow	Smelled like neem leaf
	20	4	Yellowish	Cloudy	Yellowish	Immobile	Smelled like neem leaf
Channa punctate	Control (only water)	-	Normal (Blackish)	Transparent or clear	Deep red	Normal (frequent)	Normal
	5	4	Yellowish	Transparent	Pale red	Slow	Smelled like neem leaf
		8	Yellowish	Cloudy	Pale red	Slow	Smelled like neem leaf
	10	4	Yellowish black	Transparent	Pale red	Slow	Smelled like neem leaf
		8	Yellowish	Cloudy	Brownish	Very slow	Smelled like neem leaf
	15	4	Yellowish	Cloudy	Brownish	Slow	Smelled like neem leaf
		8	Grayish	Cloudy	Yellowish	Very slow	Smelled like neem leaf
	20	4	Grayish	Cloudy	Yellowish	Very slow	Smelled like neem leaf

* The exposure time was measured from the time of release up to the time the fish were anaesthetized.

A. testudineus and *C. punctata*, and both these fishes are designated as hardy fish. The time calculated between the anaesthetic stages to death for *A. testudineus* ranged from 1.30 to 2 hours and that for *C. punctata* ranged from 0.35 to around 1 hour at different concentrations of the juice used. However, the time of effectiveness of the neem leaf juice was found to be dependent on the body weight of the fish, i.e., younger fish of both the species will need >5% of the juice for anaesthetization.

Though the fish farmers use neem extract to control fish parasites and fish fry predators, but it has also been reported that longer exposure can be fatal for the fish. Winkaler et al. (2007) reported that LC_{50} of the aqueous extract of neem was estimated as 4.8 gL^{-1} at 24-hour exposure for the juveniles of *Prochilodus lineatus*. The authors also reported that the extract at concentrations of 5 and 7.5 gL^{-1} damaged the gill and kidney tissues, but not influenced the osmoregulatory capacity of the fish. Akinwande et al. (2007) estimated the mortality rate and opercular ventilation under laboratory conditions over 96-hour exposure to aqueous extract of the mesocarp of neem fruit against the hybrid *Heteroclarias* sp. catfish. The LC_{50} value at 96-hour exposure was calculated as 81.28 mgL^{-1}, and the treated fishes exhibited respiratory distress (as they were gasping air), loss of appetite, loss of balance and erratic swimming prior to death. The present results showed that the respiratory distress observed clearly in anaesthetized *A. testudineus*. The species showed somewhat erratic movement at lower concentrations but became lethargic at higher concentrations. *Channa punctata* is normally a sluggish fish, and in treatment its movement became slower.

The LC_{50} values of neem leaf extract were found as 4 and 11 gL^{-1} for the juvenile *Oreochromis niloticus* and *Clarias gariepinus*, respectively, when exposed for 24 hour (Mousa et al., 2008). *Labeo rohita* when exposed to sub-lethal concentrations of neem extract for 25 days, the LC_{50} value was obtained as 1.035 gL^{-1} (Saravanan et al., 2010).

Neem based pesticides like Nimbicidine and Neem Gold were reported to be toxic against the freshwater loach, *Lepidocephalichthys guntea*. The 96-hour LC_{50} values of Nimbicidine, Neem Gold and combination of two were determined as 0.0135, 0.0525 and 0.0396 mgL^{-1}, respectively (Mondal et al., 2007). The authors also reported that the fish under toxicity stress suffered several abnormalities like erratic and rapid movement, imbalance and surface floating, which were correlated with the concentrations of the pesticides used. Kumar et al. (2010) observed severe effects of neem extract on the gills of *Heteropneustes fossilis* exposed to a treatment with 0.2 mgl^{-1} for 28 days. The effects were over secretion of mucous, hyperplasia, fusion of gill lamellae, necrosis of gill epithelial cells, and epithelial uplifting. These changes were time and dose dependent. The result of Mousa et al. (2008) revealed that neem extract is safe to use in fish farm, as it exhibited low toxicity to non-target aquatic life.

The neem extract may have lethal effects but at low concentrations it could be a good anaesthetic if the recovery rate from effect is high and quick. The present results revealed that for handling live fish during vaccination or other purpose, 5-10% neem leaf juice will provide enough time to keep the fishes immobile. Moreover, bathing the fish at these concentrations may provide treatment against the bacterial or fungal diseases, and the fish will not lose its normal morphological characters. Preparation of neem leaf juice is easy, and of low cost. Fish farmers can easily use it for their cultured fish. Further works with neem leaf juice are needed to estimate the recovery rate and recovery time from the anaesthetic state of different species of fish.

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