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EFFECT OF CLOVE OIL AS AN ANESTHETIC ON *LABEO ROHITA* (HAMILTON)

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Abstract: An experiment was conducted in the Wet Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh to study the efficacy of clove oil as an anaesthetic for *Labeo rohita* (Hamilton). Clove oil were used at different doses (0.01, 0.025, 0.05, 0.1, 0.2 and 0.3ml/l) on different size groups (4-6 cm, 9-11 cm, 14-16 cm, 19-21 cm.) of fish. During the experimental period water temperature was 25 to 28°C. The highest 8.35 minutes and lowest 0.48 minutes induction times of clove oil were recorded at the dose of 0.01 ml/l and 0.3 ml/l, respectively for all size classes. Induction times were decreasing with the increasing doses of clove oil. The highest 19.50 minutes and lowest 1.78 minutes recovery times of clove oil were recorded at the dose of 0.3 ml/1 and 0.01 ml/l, respectively for *Labeo rohita* of all size groups.
Key words: Clove oil, *Labeo rohita*, anaesthetic, induction time

Introduction

In Bangladesh, fish anesthetization is a new practice in the field of aquaculture. Anesthetic keeps fish sedate during stripping in artificial spawning, tagging and grading (Anderson *et al.*, 1997) and reduces the physical stress during transportation and handling of fish. Anaesthetic is also used at the time of measuring length and weight of fish (Soto and Burhanuddin ,1995). There are a few anaesthetic agents used for anesthetization of the fish such as 3-aminobenzoic acid ethyl ester methanesulfate (MS-222), benzocaine-hydrochloride, quinaladine, carbondioxide, carbonic acid and clove oil. Among the above anesthetic agents, MS-222 is the most commonly used anesthetic in the fisheries field. Smit *et al.* (1979) investigated the anesthetic potency of MS-222 in *Cyprinus carpio, Sarotherodon mossambicus* and *Salmo gairdneri.* Ferreira *et al.* (1984) induced benzocaine hydrochloride anesthesia in the common carp, C. *carpio;* tilapia; *Oreochromis mossambicus* and rainbow trout, S. *gairdneri.*

Clove oil is a dark brown liquid resulting from the distillation of flowers, flower stalks, and leaves of clove trees *(Eugenia aromatica)* and used throughout the world for applications ranging from food flavouring to local anesthesia in the dentistry profession (Nagababu and Lakshmaiah, 1992). According to Hernani and Tangendjaja (1988), it consists primarily of phenol eugenol (70-90%), eugenol acetate (>17%) and kariofilen 5 (12%). It is considered non-carcinogenic, non-mutagenic and "Generally Recognized as Safe" (GRAS) substance by the FDA (Nagababu and Lakshmaiah, 1992). Clove oil's properties and its status as a GRAS substance make it an ideal candidate as an anesthetic to use in field of fisheries. The present experiment was conducted to assess the effectiveness of clove oil as an anaesthetic using water as solvents on *Labeo rohita* of different size groups.

Materials and Methods

The experiment was conducted for a period of 10 months in 6 Aquaria (33L) which were located in the wet laboratory at the south west corner of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. All the aquaria were rectangular in shape and possessed similar size (45 x 25 x 30 cm). The experimental fishes were collected from the Fish Seed Multiplication Farm, Isharganj, Mymensingh and transported to the wet laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh by poly-ethylene bag with oxygen. The fishes were kept in a number of glass aquaria in the laboratory for a period of seven days at room temperature to acclimatize with the new environment and also with the regular feeding. Total length of the collected fishes were taken and catagorized in to four size groups according to the following manner on the basis of their length (Table 1).

Table 1: Groups of experimental fishes in length (cm).

Group No	Length (cm)
1	4-6
2	9-11
3	14-16
4	19-21

At first any leakage of each aquarium was checked up. The aquaria were cleaned with the help of common salt and were sponged thoroughly and water was scooped out from the aquaria. The aquaria were rewashed and filled almost up to the top level and kept for a day. The water of the aquaria was scooped out in the following day. The aquaria were again washed with the help of common salt to destroy the micro-organisms or other harmful elements. Then the aquaria were sponged and were filled with tap water and scooped out and then dried. Aquaria were placed on the iron made self in a row and filled with fresh water. One aerator (bubble contact aerator) was set up in each of the five aquaria arranged for static system. Prior to weighing fish were caught with a fine mesh scoop net and their group wise length and weight were recorded to the nearest (0.1) centimeter and nearest (0.1) gram respectively. The total length (cm) and weight (g) of the individual fish were carefully recorded. A wooden measuring board was employed for measuring the length. The total body weight of individual fish was determined by a sensitive electric balance and the fishes were kept in a number of glass

aquaria in the laboratory for a period of seven days to acclimatize the fishes with regular feeding.

This experiment was conducted for *Labeo rohita* (Hamilton) of different size groups (4-6 cm, 9-11 cm, 14-16 cm and 19-21 cm) at a concentration of clove oil doses 0.01, 0.025, 0.05, 0.1, 0.2 and 0.3 ml/l of water. The above experimental fish were acclimated to laboratory conditions for 2 hours containing 30 litre water-holding aquaria with deep tube well water under laboratory facilities. Required amount of clove oil was taken to fresh water containing plastic bottle and was shacked vigorously. This solution was transferred to the test tank and mixed thoroughly with water to make 0.01 concentration of clove oil.

Same procedure were maintained for others five doses of clove oil. Then acclimated fish were transferred to the test container by scoop net. Then serially various size group of fishes were tested. The air supply to the anesthetic bath was removed immediately before introduction of a fish so that clear observations could be made on fish behavior during the induction period. The three stages of induction of anesthesia caused by exposure to clove oil under identical experimental conditions are described as follows:

Induction 1: The onset of erratic opercular movement.

Induction 2: Partial loss of equilibrium and erratic swimming.

Induction 3: Total loss of equilibrium.

Induction and recovery times within the different size group were judged visually and measured with a stopwatch to the nearest second. Values for induction and recovery times were expressed as mean time in minutes A minimum of 24 fish was tested for each size group at different concentration.

Statistical data analysis was performed by one way analysis of variance (ANOVA) to test the significance of variation between the treatment means. Standard error of treatment means were calculated from the residual mean squares in the analysis of variance (Zar, 1996).

Results

The results of the present studies for measuring the effects of different doses of clove oil as an anaesthetic agent on various size group of fishes were presented below-

Rohu (*Labeo rohita*)

Induction: Induction time to anaesthesia of rohu was high (8.35±0.03 min.) at lowest dose (0.01 ml/1) of clove oil for the largest (19-21 cm) group of fish. Induction time to anaesthesia of same species was low (0.48±0.00 min.) at highest dose (0.3 ml/l) of clove oil for 4-6 cm size group of fishes. The smaller size group of fishes was more susceptible than the larger size group (Table 2). Induction time was decreased when the dose of clove oil was increased (Figs. 1-4). No fish was died at any dose of anaesthetic agent.

Recovery: Recovery time from anaesthesia of rohu was low (1.775±0.17 min.) at low dose of clove oil (0.01 ml/1) and was high (19.50±4.91 min.) at dose of clove oil (0.3 ml/1) all fishes were recovered at different doses of anaesthesia.

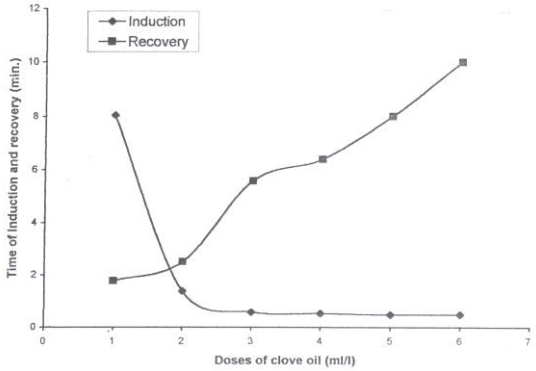

Fig. 1. Induction and recovery time for Rohu of size group-1 (4-6cm) at different doses of clove oil

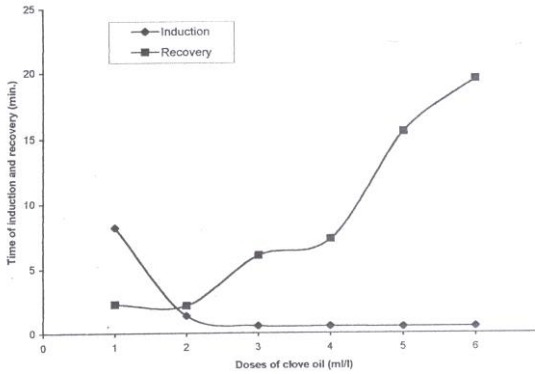

Fig. 2. Induction and recovery time for Rohu of size group-2 (9-11cm) at different doses of clove oil

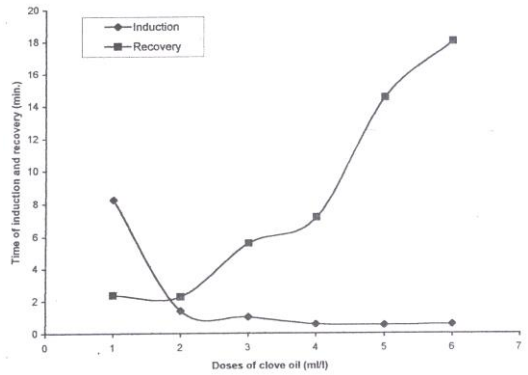

Fig. 3. Induction and recovery time for Rohu of size group-3 (14-16cm) at different doses of clove oil

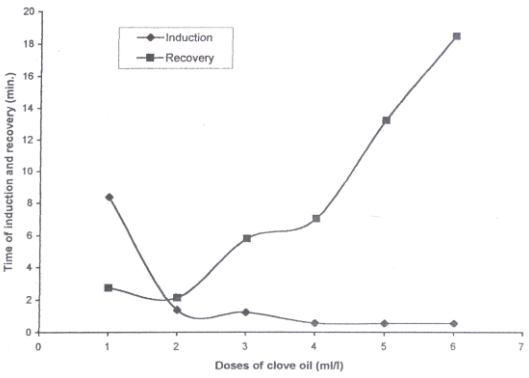

Fig. 4. Induction and recovery time for Rohu of size group-4 (19-21cm) at different doses of clove oil

Table 2: Induction and recovery times of rohu(*Labeo rohita*) at different doses of clove oil with various size groups

Dose (ml/l)	Size group-1 (4-6cm)		Size group-2 (9-11cm)		Size group-3 (14-16cm)		Size group-4 (19-21cm)	
	Induction	Recovery	Induction	Recovery	Induction	Recovery	Induction	Recovery
0.01	**8.05±0.03**^{a}	1.77±0.17 ^{a}	8.15±0.03^{b}	2.30±0.08^{b}	8.25±0.03^{c}	2.35±0.03^{b}	8.35±0.03^{d}	2.75±0.03^{c}
0.025	1.37±0.00^{a}	2.50±0.18 ^{b}	1.38±0.00^{a}	2.20±0.11^{ab}	1.39±0.00^{b}	2.25±0.03^{ab}	1.39±0.03^{b}	2.15±0.09^{a}
0.05	0.56±0.02^{a}	5.57±0.10 ^{a}	4.50±0.01^{a}	6.00±0.03^{b}	1.00±0.00^{b}	5.57±0.08^{a}	1.25±0.03^{c}	5.82±0.09^{ab}
0.100	0.52±0.00 ^{a}	6.40±0.07 ^{a}	0.54±0.00^{b}	7.25±0.19^{b}	0.55±0.00^{b}	7.15±0.23^{b}	0.58±0.00^{c}	7.05±0.23^{b}
0.200	0.47±0.00 ^{a}	8.00±4.62 ^{a}	0.50±0.01^{b}	15.50±0.29^{b}	0.51±.00^{b}	14.50±0.29^{ab}	0.56±0.00^{c}	13.25±0.49^{ab}
0.300	0.48±0.00^{a}	10.00±5.00 ^{a}	0.52±0.00^{b}	19.50±4.91^{a}	0.55±0.00^{b}	18.00±5.20^{a}	0.56±0.00^{d}	18.50±5.48^{a}

* Figures with the same superscript within a column are not significantly different ($p<0.05$)

Discussion

The maximum dose of clove oil was 0.3 ml/l and the minimum dose of the same was 0.01 ml/l. It was found that induction time were low (0.48 minutes) at higher doses 0.3 ml/l of clove oil for *Labeo rohita* and induction time was high (8.35 minutes) at low dose of clove oil 0.01 ml/l. However, the recovery time was low (1.78 minutes) at low doses 0.01 ml/1 of clove oil and recovery time was high (19.50 minutes) at doses 0.3 ml/l for Rohu.

Marking and Meyer (1985) outlined desirable time scale (3 and 5 minutes) for the induction and recovery from anaesthesia for fish respectively. Anderson *et al.* (1997) stated that induction and recovery times for adult rainbow trout following exposure to clove oil at a concentration of 120 ml/1 or 40 ml/l, induction to anaesthesia was rapid (about 1 minutes) but recovery time exceeded that recommended by Marking and Meyer (1985), Soto and Burhunuddin (1995) have been reported for the marine golden spot spine foot anaesthetized in clove oil at 100 ml/l . Furthermore, juveniles rainbow trout exposed to FA-100 also exhibited anaesthasia induction times similar to those in the Anderson *et al*. (1997) study. It was found that the catla were more susceptible to clove oil. Catla is a surface feeder and it lives in well oxygenated part of the water body which might be the causes of more susceptibility. It was also found that the induction times to anaesthesia at different size group of same species at same doses increases with the increasing size group, which might be due to the accumulation rate of clove oil in comparison to their body weight was high incase of small fishes than the large fishes.

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