

EFFECT OF CLOVE OIL AS AN ANAESTHETIC IN FRESH WATER FISH *Labeo rohita***Mayur V. Darbeswar***

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Abstract

Clove oil containing the active ingredient eugenol has been used as an anesthetic for fish. The experiments were conducted to study the efficacy of clove oil as an anesthetic for fresh water fish *Labeo rohita*. *Labeo rohita* were exposed to different concentrations of clove oil. In this study, fish anesthesia phases and its recovery period were studied. The lowest concentration which is suitable for long exposure to anesthesia was studied and finally its oxygen consumption of anesthetic fish (experimental fish) compared with the normal fish (control fish).

Keywords: Anaesthesia, Clove oil, *Labeo Rohita*

Introduction

Anaesthesia plays an important role in aquaculture, fish farming and fishery research, being used to facilitate various procedures such as weighing, marketing, tagging, sorting, handling practices, sampling, length measurement, biopsy, induced breeding, biometric evaluation, application of hormonal preparations, health state checking and veterinary treatment (Smith 1990, Kazun, Siwicki 2001).

In aquaculture operations, anesthetics are very important because they minimize the stress on fishes and reduce physical injury during various handling practices. Anesthesia is widely used in routine aquaculture activities to reduce the incidence of stress by sedating and immobilizing fish before performing any task in aquaculture. Anesthesia abolishes pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness (Summerfelt and Smith, 1990). Anesthetics act at various intensities, driving fish into general anesthesia, resulting in loss of consciousness, inhibition of reflex activity, and reduced skeleton muscle tone (McFarland 1960).

According to many authors, an ideal anesthetic fulfills such requirements as a quick induction of an anesthetic together with its gradual recovery. Anesthetics must not be toxic to fish, must not develop any problems in handlers, must be quickly metabolized and excreted from organisms, must not produce any residues and must be cheap. It is a natural product and is considered neither toxic nor carcinogenic to humans and other animals (Fisher et al., 1990; Zheng et al., 1992; Lee and Shibamoto, 2001).

Material & Method**Clove oil**

Clove oil is a slightly yellowish and or brownish liquid strongly refracting the light; it has a characteristic pleasant smell and burning taste. It is extracted from dry blossom buds of *Jambosa caryophyllus* / *Eugenia caryophallata* tree.

Preparation of 5% Clove oil stock solution:

The 10 ml clove oil is mixed with 50 ml ethanol and then diluted by shaking with 150 ml distilled water. This will create a stock solution. Keep this solution in a dark bottle, so this fresh stock solution was protected from direct sunlight to limit photo and thermal degradation.

Clove oil which is available in the laboratory was used in this experiment. A 5% stock solution of clove oil was used. The experiments were carried out using *Labeo rohita*. Live fishes of medium size with an average weight of 22 to 58 grams and an average total length of 4 cm to 14.5 cm were collected from Aquaculture Bhategaon Reservoir and Seed unit, Bhategaon Tq. Ardhapur Dist. Nanded. Before initiating the experiment, the fishes were acclimated to laboratory conditions. The fishes were kept in a glass aquarium (size 92cm×30cm×39) for 3 days in 70 lit. Aerated fresh water prior to trial at normal room temperature. The glass tank was covered with a plastic roof-like a pad-to avoid fish jumping out of the tank and the recovery tank. The induction of anesthesia and its recovery were studied in 3-liter small glass aquaria at normal room temperature. After the experiment, the fish were transferred to a 40-liter freshwater aerated tank.



Precaution & Ideal Condition for Fish before Inducing Anesthesia

The most common anesthetic technique in fish is adding the anesthetic agent to the water. This is similar to inhalation anesthesia in terrestrial species as the drug is inhaled through the water enter the arterial blood and the remainder of metabolites are excreted via the gills, kidney and skin. This will affect the nervous systems of fishes. Once the fish are placed in the recovery bath, the analgesic effects of the anesthetic drugs wear off. The ideal anesthetic agent should have a rapid induction and recovery time, with the least possible stress. The

anesthetic agent should provide adequate immobilization and analgesia for the duration of the procedure and have a wide safety margin. An anesthetic agent that is easy to administer and water-soluble, harmless to the environment and operator is preferred.

Anesthesia Phases:

The effect of anesthesia was studied at different stages. The fishes (sizes 04 to 14.5 cm) were anesthetized in small batches (2 in number) in small experimental aquaria. The effect was recorded on the basis of the visual movement and breathing of their body. The two individual fish were parallel used when monitoring the course of anesthesia. After stocking the randomly sampled fish from the storage tank, the starting times were recorded. After 10 min. exposure, the fish were transferred into clear aerated water of the same temperature. The concentration of clove oil used for anestahesial experiments ranged from 0.5 ml to 3 ml. The induction time for four stages of anesthetization and recovery were noted and recorded for all experiments.

In anesthetic trials, the onset of individual phases of unconsciousness to recovery rates was studies and evaluation mode as follows.

Sedative Event	Recovery Event
➤ Larger mouth opening	➤ Increased opercular activities
➤ Caudal fin and Pectoral fin movements	➤ Attaining equilibrium
➤ Settling at bottom	➤ Increase in the Pectoral and Caudal fin movements
➤ Loss of equilibrium	➤ Movement of body
➤ Breathing	➤ Swimming
➤ Reduced reflexes to external stimuli	➤ Regain control
➤ Vertical position (mouth was kept upwards)	➤ Response to tactical stimuli
➤ Slowed opercular movement	➤ Response to external stimuli
➤ No reflexes to tactile stimuli	➤ Normal activities, Normal Swimming
➤ Inverted position, Breathing very slowly	

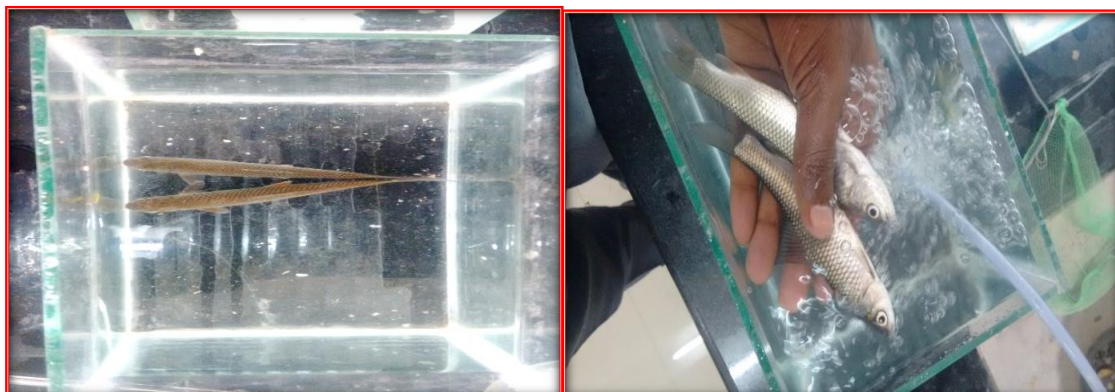


Fig: Showing no reflexes to tactile stimuli

Table: Showing experimental observation of anesthetic dose of clove oil (different concentration)

Clove Oil Dose	Temp	Weight	Time	Anaesthetical Event	Recovery Period
3ml / 3lit.	24 ⁰ C	22.40 g 22.40 g	3.19PM 3.20PM 3.21PM 3.23PM 3.24PM 3.25PM 3.26PM 3.27PM 3.28PM 3.29PM	Clove oil immersion Larger mouth opening Caudal fin & pectoral fin movement increases Caudal fin & pectoral fin movement decreases Loss of equilibrium Vertical Position (Mouth was kept upward) Slow opercular movement Settling at bottom Inverted position No reflexes to tactile stimuli	3.30 PM: The fishes were transferred to fresh aerated recovery tank. It was recovered at 3.36 PM.
2ml / 3lit.	26 ⁰ C	16.41 g 4.43 g	4.29PM 4.31PM 4.33PM 4.34PM 4.36PM 4.37PM 4.38PM 4.41PM 4.42PM	Clove oil immersion Larger mouth opening Caudal fin & pectoral fin movement decrease Loss of equilibrium Mouth was kept upward Slow opercular movement Settling at bottom No reflexes to tactile stimuli Inverted position	4.43PM: The fishes were transferred to fresh aerated recovery tank. It was recovered at 4.48 PM.
1.5ml / 3lit.	25 ⁰ C	19.72 g 16.93 g	4.44PM 4.45PM 4.46PM 4.47PM 4.49PM 4.51PM 4.53PM 4.56PM 4.58PM 4.59PM	Clove oil immersion Caudal fin & pectoral fin movement increases Movement of mouth opening increases Caudal fin & pectoral fin movement decrease Vertical position Loss of equilibrium Settling to bottom Slow opercular movement Inverted position No reflexes to tactile stimuli	5.05PM: The fishes were transferred to recovery tank. It was recovered at 5.05PM.
1ml / 3lit.	27 ⁰ C	20.14 g 25.16 g	11.16PM 11.18PM 11.19PM 11.21PM 11.23PM 11.26PM 11.29PM 11.31PM 11.35PM 11.36PM	Clove oil immersion Caudal fin & pectoral fin movement increases Caudal fin & pectoral fin movement decrease Larger mouth opening Loss of equilibrium Vertical position Slow opercular movement Settling to bottom Inverted position The fish opercular movement slow. It continuous exposure up to 2.00 PM	2.01PM: The fishes were transferred to recovery tank. It was recovered in 2.06PM.
0.75ml / 3lit.	26 ⁰ C	19.28 g 22.20 g	11.45PM 11.52PM 11.60PM 12.08PM 12.19PM 12.23PM 12.41PM 12.56PM 1.03PM	Clove oil immersion Caudal & pectoral fin movement increases Caudal & pectoral fin movement decreases Large mouth opening Vertical position Loss of equilibrium Settling at bottom Slow opercula movement Reduced reflexes to external stimuli The fish still exposure to anaesthesia tank for 2 days.	21 Jan, (2.01PM): The fishes were transferred to fresh water recovery tank. It was recovered at 2.07 (6min.)

0.5ml / 3lit.	26 ⁰ C	20.16 g 15.94 g	7.45AM	Clove oil immersion Caudal fin & pectoral fin movement increases Reduces the movement Slow breathing Reduces reflexes to external stimuli Loss of equilibrium Vertical position The fish was settled down. Mobility very slow.	The fish were transferred to fresh water tank 2.00 PM It was recovered at 2.08 PM
			8.03AM		
			8.16AM		
			8.23AM		
			9.06AM		
			9.47AM		
			11.04AM 12.46AM		

Result & Conclusion

Determining the lowest effective concentration of clove oil

The main aspect of this experiment was to determine the lowest effective concentration of clove oil suitable for induction of general anesthesia. After doing experiments with different concentrations of clove oil, we can conclude that the lowest concentration is 0.5ml and 0.75ml for 3 liters of water. This concentration is suitable for the induction of general anesthesia in *Labeo rohita* fish. To find this concentration of clove oil for induction of anesthesia, we tried different concentrations, such as 3ml, 2ml, 1.5ml, 1ml, 0.75ml and lastly, 0.5ml for 3lit. Both 0.5ml and 0.75ml/3 liter of water are sufficient to fish for the induction of anesthesia for a long duration. It is “generally regarded as safe”. In contrast to some other anesthetics, a withdrawal period for clove oil is considered unnecessary, as it does not pose any environmental hazard. Clove oil (0.5 ml and 0.75 ml/3lit) appeared to be a suitable agent for anesthesia in this species of fish. As we obtained acceptable induction and recovery times and negligible mortality even at the very highest tested concentration (3ml/3lit) in our study, we have also concluded that clove oil can be effectively used as an anesthetic, especially at levels lower than 0.5ml and 0.75 ml/3 liter of water for 04 – 14.5 cm sized fishes.

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