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RESEARCH ARTICLE.....

Effectiveness of clove oil as an Anesthetic on Mozambique tilapia (*Oreochromis mossambicus*)

PRAKASH V. PARMAR, REKHA P. NANJIYANI, NAYAN L. KAMALIYA **AND** HITESH V. PARMAR

ABSTRACT..... Anesthesia in aquaculture is generally used during sampling, weighing, disease diagnosis and transportation. Total 99 fish (mean length 6.55 cm±0.04; mean weight 3.64 g ±0.05) were used for this experiment. This experiment was carried out in triplicate using ten different concentration of clove oil (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/l) along with control (without clove oil). For the experiment, 1 litre tanks were used as induction tank and 10 litre tanks were used as recovery tank. Clove oil is not soluble in water so stock solution was prepared using absolute ethanol in the ratio of 1:10 before experiment and directly added to induction tank and induction time was measured. The fish werethen transferred to recovery tank and three different stages of recovery were measured. Clove oil concentration 10 mg/l did not produce any stage of anesthesia while 20 mg/l produced only two stages of induction and recovery. As the concentration of clove oil increased from 30 mg/l to 100 mg/l, time of induction decreased and time of recovery increased. 100 mg/l was found to be an ideal concentration as it produced induction in less than 3 min. The result of this experiment suggested that clove oil can be used as an effective anesthetic on tilapia which produced minimum stress without mortality.

KEY WORDS..... Aquaculture, Anesthetic, Clove oil, *Oreochromis mossambicus*, Induction time, Recovery time

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Author for Corresponding -

PRAKASH V. PARMAR
Department of Aquaculture,
College of Fisheries Science,
Junagadh Agricultural University,
VERAVAL (GUJARAT) INDIA
Email : pvparmar@gmail.com

See end of the article for
Coopted authors'

INTRODUCTION.....

Anesthesia is a biological state that affectby external agent which responsible for partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non-chemical anesthesia (Summerfelt and Smith, 1990). Anesthesia in aquaculture is generally used during sampling, weighing, disease diagnosis and transportation. To produce minimal stress, chemicals and anesthetics are usually administered to fish prior handling (Ross *et al.*, 1983).

The mozambique tilapia (*Oreochromis mossambicus*) is a tilapiine cichlid fish native to southern Africa. It is a popular fish for aquaculture. Due to human introductions, it is now found in many tropical and subtropical habitats around the globe. There are many anesthetic used in aquaculture like clove oil, sodium

bicarbonate, carbone dioxide gas, metomidate, benzocaine, tricanemethan sulphonate (MS-222), 2-phenoxyethanol and quinaldine (Massee *et al.,* 1995; Bowser, 2001 and Palic *et al.*,2006). Whenever selecting anesthesia there are some important deliberations like efficacy, coast, availability, side effects on fish, human and environment (Marking and Meyer, 1985). An ideal anesthetic have some characteristics like they should be nontoxic, inexpensive and easy to handle and result inrapid induction and calm recovery (Treves-Brown, 2000).

Clove oil is collected from the distillation of the stems, leaves and flowers of *Eugenia aromatica* and *Eugenia caryophylata* trees and its active component is eugenol (Soto and Burhanuddin, 1995). It is low in price, it has less environmental effect, some adverse effect like photo sensitivity for fish and amphibians and it is safe for handling (Cho and Heath, 2000). Clove oil is good economic alternative compare to normally used chemical for fish anesthetic and it also used for several species of fish (Ross and Ross, 2008).

RESEARCH METHODS.....

Fish:

A total of 99 tilapia (*Oreochromis mossambicus*) juvenile fish (mean length 6.55 cm±0.04; mean weight 3.64 g ±0.05) were collected from the Western Indian Fish Hatchery, Bhuj, Gujarat. Fish living condition was maintained in plastic tank with continues aeration at room temperature and fed twice a day with commercial feed.

Anesthetic agent:

Clove oil (Himedia Laboratories Pvt. Ltd. Cat No. GRM 340-100G) was used for this experiment. Since clove oil is not soluble in water, fresh stock solution was prepared using absolute ethanol in the ratio of 1:10 before experiment.

Induction and recovery stages of anesthesia:

Fish were acclimation for two weeks and then transferred to holding tanks with fresh and aerated water. A preliminary study on tilapia (*Oreochromis mossambicus*) with random concentrations of clove oil was carried out. Based on the result, 10 different concentrations of clove oil (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/l) were selected for this experiment. Three individual fish were used for ten different concentration of clove oil anesthetic. This experiment was performed in triplicate along with control (without clove oil) to verify results. Different concentration of clove oil was directly added to induction tank (1 lit.) already containing fish, mixed well and time to reach three different stages of induction was measured using stop watch (Table 1). Then fish were transferred in recovery tank (10 lit.) and time for three different stages of recovery was measured (Fatih and Kaya, 2013). Reaction against strong stimuli was checked by pressing pointed object in tail region. Water quality parameters (pH 7.73±0.019; DO 5.47 mg/l±0.089 and temperature 25.03^{o}C±0.10) were maintained throughout the experiment.

Statistical analysis:

The data obtained were collected and expressed as Mean ± Standard error (SE).

RESEARCH FINDINGS AND ANALYSIS.....

Stages of induction and recovery time of tilapia was measure after exposed to various concentration of clove oil. Different stages of induction and recovery as mentioned in Table 1 and 2. Variable results have been reported after anesthetizing different species to clove oil. Lower concentration of clove oil failed to produce any stage of anesthesia. Anesthetic effect of clove oil initiated from 30mg/l onwards (Table 2). In present study

Table 1: Induction and recovery stages of anesthesia in *Oreochromis mossambicus*

Induction stages	
Stage-I	Loss of balance, partial inhibition of reactions to external stimuli
Stage-I I	Total loss of equilibrium. Fish still react to strong stimuli
Stage-III	Total loss of reflexes and movement, Fish lay on bottom of the tank
Recovery stages	
Stage-I	Start of movements. Fish still lay on the bottom of the tank
Stage-I I	Regular breathing. Reaction to strong stimuli. Irregular balance
Stage-III	Total recovery of equilibrium. Reaction to slight stimuli. Normal swimming

increasing the concentration clove oil result in decresed induction time and increased recovery time. The same results were observed by Hoskonen and Pirhonen (2004); Roubach *et al*. (2005); Ogretmen *et al*. (2014). In seven species of tropical reef teleosts (Cunha and Rosa, 2006), sockeye salmon (Woody *et al*., 2002) and cobia, *Rachycentron canadum* (Gullian and Villanueva, 2009) has been reported that the increase recovery time with increasing concentration level of anesthetic. However, Mylonas *et al*. (2005) reported that in european sea bass (*Dicentrachus labrax)* and gilthead sea bream (*Sparus aurata*), decreased recovery time when concentration of clove oil and 2-phenoxyethanol (PE) increased.

Effective concentration of anesthetic for induction time should be in 3 min and recovery time should be 10 min (Gilderhus 1990 and Weyl *et al.*, 1996). Hence, the present study shows that 100mg/l concentration is ideal concentration for fish anesthetic. Velisek *et al*. (2005) obsereved that 30mg/l clove oil could not produce any effect in rainbow trout, while in our study only 10 mg/l clove oil failed to show effect. While in 20 mg/l showed only two stage of induction and recovery. Waterstrat (1999) also observed that 100mg/l clove oil as safe concentration for fish.

Eugenol, being main active constituents of clove oil is generally recognized safe. It is used as a food additive and an analgesic and disinfectant in dentistry (Schnick *et al*., 1986). So, tilapia treated with clove oil is safe for human consumption.

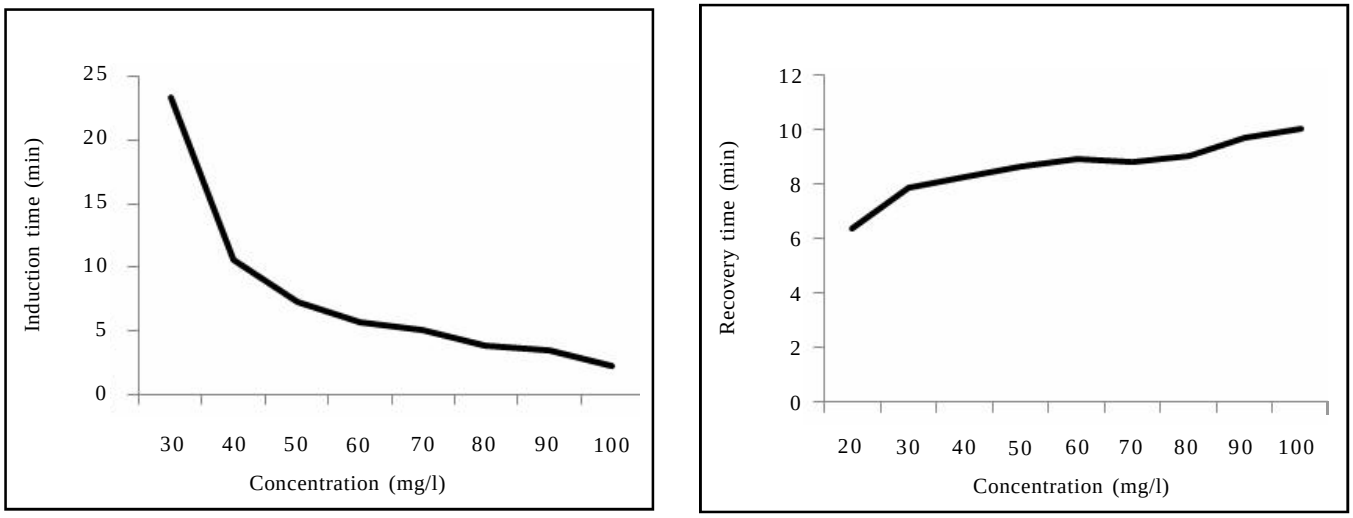

Fig. 1 : Induction and recovery times relation to clove oil concentrations in *Oreochromis mossambicus*

Table 2 : Induction and recovery times of *Oreochromis mossambicus* anaesthetized with ten different concentrations of clove oil as an anesthetic agent **Data are presented as mean±sd**

Concentration (mg/l)	Induction time (min)			Recovery time (min)		
	Stage -I	Stage -II	Stage -III	Stage -I	Stage -II	Stage -III
10	-	-	-	-	-	-
20	5.24±0.15	9.53±0.27	-	-	4.95±0.24	6.33±0.59
30	4.25±0.49	5.85±0.45	23.34±0.65	3.61±0.44	5.26±0.65	7.86±0.75
40	2.21±0.14	3.40±0.45	10.54±0.27	5.22±0.57	5.52±0.61	8.22±0.54
50	1.32±0.16	2.43±0.07	7.24±0.56	6.03±0.49	6.74±0.60	8.60±0.25
60	1.28±0.02	2.06±0.48	5.75±0.72	6.16±0.04	7.22±0.55	8.90±0.66
70	1.22±0.18	1.73±0.26	5.13±0.04	6.31±0.58	7.98±0.49	8.78±0.65
80	1.13±0.05	1.63±0.36	3.86±0.22	6.98±0.34	7.88±0.16	9.01±0.31
90	0.72±0.19	1.39±0.07	3.49±0.85	7.36±2.11	8.47±0.87	9.66±0.34
100	0.66±0.17	1.25±0.10	2.26±0.47	7.57±0.75	9.15±0.29	10.02±0.39

Conclusion:

The findings of this experiment showed that clove oil was effective, producing minimum stress and no mortalities and therefore, can be recommended as an effective anaesthetic for use in aquaculture. Clove oil is readily available and it is inexpensive compare to other anesthetics. Clove oil is very safe and 100 mg/l concentration is sufficient for anesthetize tilapia.

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COOPTED AUTHORS' –

REKHA P. NANJIYANI, NAYAN L. KAMALIYA AND HITESH V. PARMAR, Department of Aquaculture, College of Fisheries Science, Junagadh Agricultural University, VERAVAL (GUJARAT) INDIA

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