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INDUCTION AND RECOVERY FROM ANESTHESIA IN *CYPRINUS CARPIO* (LINNAEUS, 1758) IMMERSED IN DIFFERENT CONCENTRATIONS OF 2-PHENOXYETHANOL (CLOVE OIL)

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ABSTRACT

The sedative effects of eugenol (2-phenoxyethanol) commonly called clove oil were studied in common carp, *Cyprinus carpio*. The effects include percent mortalities, induction and recovery from sedative agent. *C. carpio* weighing 0.66 ± 0.047g (0.54 – 0.97 g) were immersed to 0.1, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.25 mL/L of clove oil. The fish exhibit longer duration of induction and recovered within a minute without any mortality. The induction time proportionally decreased as concentration increased. The recovery time was longer due to heavy mixing of eugenol with water and require more time to flush off from gill lamella and blood. Mortality rate became high with increased concentration probably due to deposition of eugenol into different organs like liver, spleen, brain and kidneys. These findings suggest that eugenol could be an effective anesthesia in *C. carpio* for transportation, tagging, induced breeding and minor surgery. Its advantages include lower cost, lower required dosage, and improved safety for both fish and the aquacultrists.

KEYWORDS: Fish anesthesia, Clove oil, Fish transportation, Sedative agents in aquaculture, Eugenol.

INTRODUCTION

Uses of chemicals in aquaculture are presently under strict control with respect to their residuals as well as efficacy (Tylor and Roberts, 1999). The chemicals which target on animals by sequentially loss of equilibrium, movement, conciousness and respond against external stimuli (Summerfelt and Smith, 1990). These chemicals are categorized as anesthetics or tranquillizers and frequently used on both farmed reared and wild fishes when they are subjected to handle or transport for induce breeding, tagging or any other purpose like to conduct research. A widely accepted fish anesthetic must full fill major considerations such as highly soluble in water, short induction time, non toxic for both fish and human, permit as ad lib intensification with an immediate recovery and zer residues in fish body (Brozova and Svobodova, 1986; Ross and Ross, 1999).A variety of anesthetics have been tried including Carboinic acid (Gelwicks *et al*., 1998), sodium bicarbonate (Peake, 1998), quinaldine, benzocaine, and clove oil, 2 phenoxyethanol (Gilderhus and Marking 1987; Iwama *et al*., 1989).

Clove oil is used as an efficient anesthetic when fish are subjected to handle for breeding, obtaining ova and milt, blood sampling or any other veterinary objectives. Loss of mobility via anesthetics facilitates handling of too big or too agile species (Wagner *et al*., 2002). Clove oil is as extract of lowers, stalks and leaves of clove oil tree *Eugenia aromatica* (Sato and Burhanuddin, 1995). According to Isaacs (1983), Briozza *et al*., (1989) and Keene *et al*., (1998). Clove oil may also distilled from stems, leaves and flowers, buds of *Eugenia caryophyllata,* and its active ingredient is Eugenol (4-allyl-2-methoxypheno;) which comprises of acetate (<17%) and Kariofilen 5(<12%) are also available in clove oil as an additional additives. The rout of entry within the body is mainly through gills (Houston and Woods, 1976; Ferreira *et al*., 1984). Eugenol along with its compounds and metabolites may instantly removed from blood (Fisher *et al*., 1990) and tissues of treated fish (Zheng *et al*., 1992) and traces of these substances in musculature of fish or other animals are not considered toxic or mutagenous (Philips, 1990).

The focus of present study was to find out the level at which tested fishes respond immediately with respect to induction time (INT) and recovery time (RET) followed by mortality rate as well as clove oil exposure to person who engaged in these kinds of anesthetic trials.

MATERIALS AND METHODS

Active eugenol of clove oil introduced in water as by Brousse (1974) caked anesthesia by immersion. Working solution was prepared with extract of engenol aromatic and methanol by volume (10:10mL/L). The working solution then distributed into serial concentration of 0.1, 0.5, 0.7, 1.0, 1.25, 1.5, 1.25, 2.0 and 2.25mL/L of clove oil. Then individuals of *Cyprinus carpio* of 0.66 ± 0.047 g (0.54 – 0.97 g) in weight were immersed into treatment tank (30 × 30 cm glass aquarium). Parallel to this set up, another same sized glass aquarium was placed as recovery tank with air line access. In anesthetic trials, the onsets of individual phases of unconsciousness recovery rates were studies as induction and recovery time. Evaluations were made in four consecutive phases:

1. Rapid opercular movements followed by partial loss of reactivity to external stimuli.
2. Opercular moments very slow, loss of balance in treatment tank but still reactive against strong stimuli.
3. Completely un-reactive, fish are lying at tank bottom with no response to eternal stimuli.
4. Opercular movement completely stop, fish die if transferred in recovering tank for longer duration.

The relationship of body weight and concentration of anesthesia was also recorded. Statistical analysis was carried out by using MINIITAB 11 to find out relationship of clove oil with induction, recovery time and mortality.

RESULTS

Behavior of tested fishes: *Cyprinus carpio* immersed in differential concentrations (0.1-2.25 mL/L) of clove oil exhibited a variety of behavioral responses as follows:

Behavioral response	Clove oil concentration (mL/L)	Remarks
Normal sedation	(0.1-0.5)	Reactive to external stimuli; opercular rate and muscle tone normal
Light sedation	(0.75)	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
Deep sedation	(1-1.75)	Total loss of reactivity to all but strong erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
Total loss of equilibrium	(2-2.25)	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
Loss of reflex reactivity	(>2.25)	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes.
Medullary collapse	(>2.25)	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes

Induction time (INT) of clove oil: The time taken for unconscious is presented in Table 1. When the experimental fishes immersed in low concentration (0.l mL/L) of clove oil, all respond slowly to become anesthetize and time taken for it was 1120 ± 208.0 sec. This was because of the induction of active ingredient of clove oil i.e. 2-phenoxyethanol into blood stream via gills as the mixed water wash the gill filament. There was inverse relationship between INT and concentration of clove oil (Table 2 & Fig. 1).

Table 1. Anesthetic response of *Cyprinus carpio* immersed to different concentrations of clove oil.

Concentration (mL/L)	N	Weight (g)	Induction time (Sec)	Recover time (Sec)	Mortality (%)
0.1	10	0.76 ± 0.32	1120 ± 208.0	22.9 ± 16.91	00
0.5	10	0.97 ± 0.92	42.8 ± 12.72	59.2 ± 17.07	00
0.75	10	0.54 ± 0.25	85.2 ± 47.5	178.8 ± 95.3	10
1.0	10	0.56 ± 0.32	54.10 ± 18.5	124.7 ± 100.9	30
1.25	10	0.58 ± 0.24	16.8 ± 6.11	93.7 ± 59.3	40
1.5	10	0.59 ± 0.22	13.2 ± 2.78	599 ± 508	40
1.75	10	0.66 ± 0.3	9.0 ± 0.667	233.2 ± 202.9	50
2.0	10	0.55 ± 0.41	9.9 ± 1.72	150.0 ± 157.8	60
2.25	10	0.74 ± 0.30	9.50 ± 1.35	58.8 ± 90.2	90

Table 2. Regression analysis (Log_{10}) of different clove oil concentrations (X) with induction time (Y_1), recovery time (Y_2) and % mortality.

S.No.	Regression equation	R-Sq	$R\text{-}Sq_{(adj)}$	F-value	P-value
1	$Log_{10}Y_1 = 1.45-1.56\ (Log_{10}X)$	92.7%	91.6%	8.44	0.000
2	$Log_{10}Y_2 = 20.8+0.628\ (Log_{10}X)$	42.1%	33.8%	5.08	0.059
3*	% Mortality =-13.8+40.0 concentration	92.8%	91.7%	89.85	0.000

*Not transformed into Log_{10} because first two observations were zero

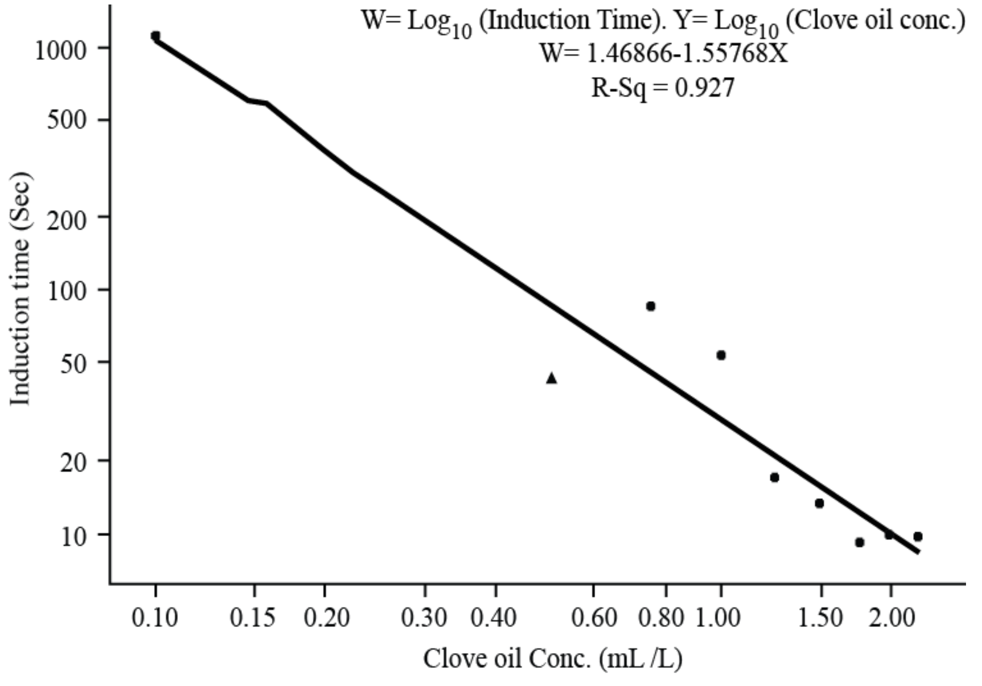

Fig. 1. Fitted line plot between clove oil concentration and induction time.

Recovery time from sedation: When the tested fish placed into fully aerated, de-chlorinated and clove oil free water tank the water start to flush off the 2-phenoxyethanol from the gill filaments. The recover time for lower to higher concentration is found to be directly proportional to Clove oil concentration (Fig. 2). The reason behind this result may be concluded that heavy amount of 2-phenoxyethanol enter into blood stream when the fish immersed to higher concentration and ultimately require more time to recover from sedation. Several factors may affect the recovery time from sedation such as circulatory collapse and nervous breakdown.

Mortality: Mortality had direct relationship with the concentration of clove oil (Fig. 3). During the recovery from an experimental clove oil anesthetic bath, no mortality occurred in 0.1 & 0.5 mL/L of concentration. Therefore, the sedation rate was normal. Mortalities of 10, 30, 40 & 50% occurred when the fishes were immersed in 0.75, 1, 1.25, 1.5 & 1.75 mL/L of concentration respectively. These fishes have undergone in deep sedation. Clove oil of 2 & 2.25 mL/L concentration caused total loss of equilibrium while the mortalities occurred 60 and 90 % respectively. Higher clove oil concentrations affected reflexes and caused medullary collapse. It may be concluded that if 2-phenoxyethanol introduced into blood stream in more than tolerable doses, heavy mortality should occur.

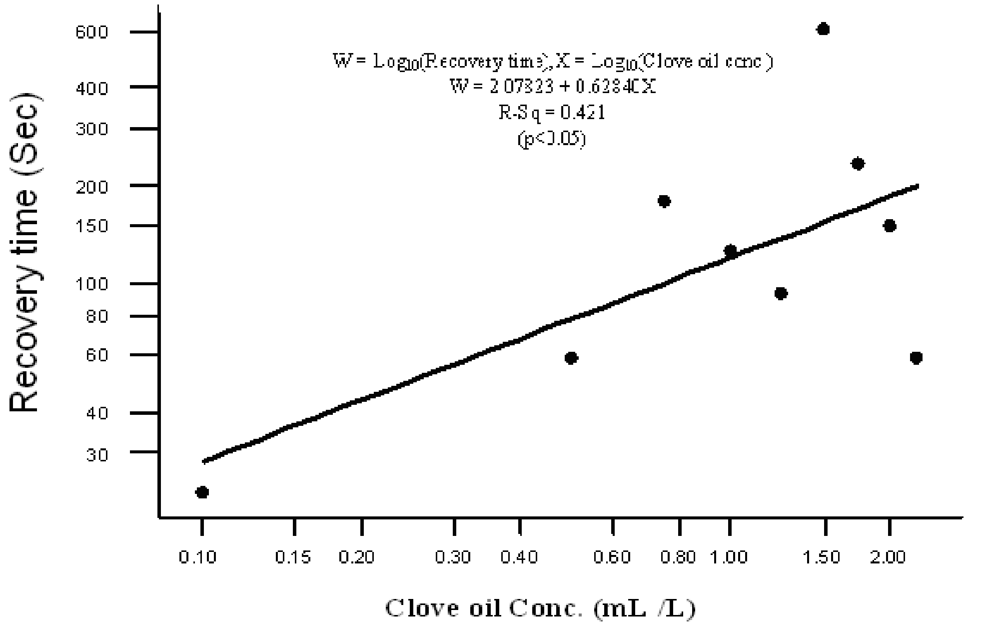

Fig. 2. Fitted line plot between clove oil concentration and recovery time.

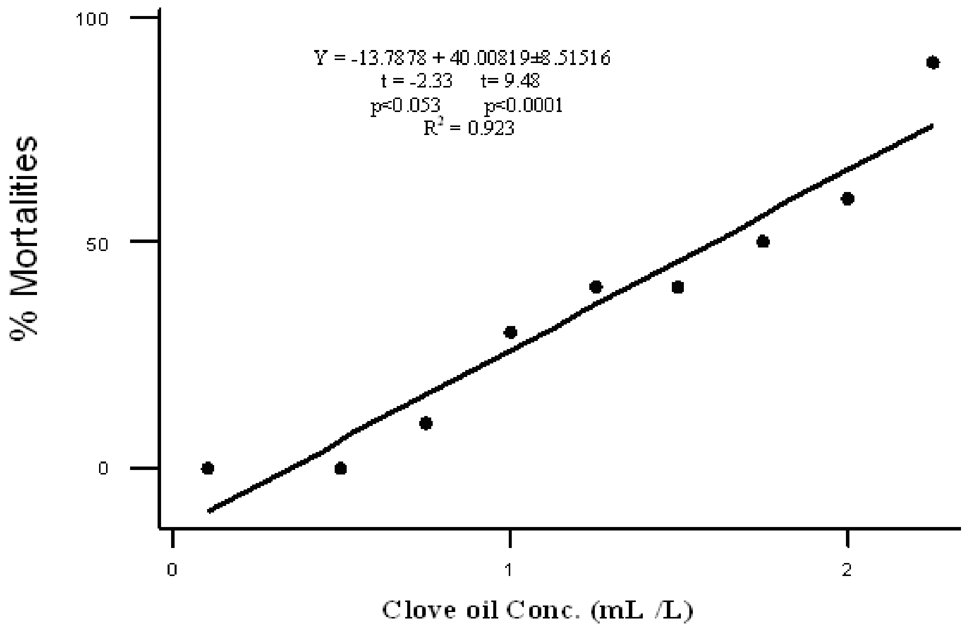

Fig. 3. Fitted line plot between clove oil concentration and % mortalities.

DISCUSSION

A considerably increased studies were carried out since 1990s to expand aquaculture fishery management and pet aquaria industry. Aquaculture is now a sub unit of agriculture and grow substantially to meet the demand of globally increased population (Anon., 2002a, Anon., 2001). Since several years clove oil has been used to anesthetize fish. (Hiksa *et al*., 1986).

Engenol compound ($C_{10}H_{12}O_2$) are active infriedients and derivatives of phenols (Taylor and Roberts, 1999). Other chemicals like quinaldine and ms-222 are frequently focusing to reduce stress and avoid injury during capturing, hand ling, grading and transporting wild and farmed fishes. Naturally fish likes to move feely and may stressed when they restrict to move freely there for the hazard cause health and welfare problems. Physiologically excessive handling is related with secretion of adrenocorticotropic cortisol (Anon, 2002b, Kreiberg, 2000; Wedemeyer *et al*., 1990).

The normally used anesthetics are not only expensive and difficult to procure easily they may also harmful side effects on human particularly in condition where the anesthetic is squister by anglers to capture fishes. (Munday and Wilson, 1997). Therefore use of clove oil as an alterative fish anesthetic is none appropriate and considered as safe. It is inexpensive and non toxic to humans. A lean or stressed fish will less capable to fight against several opportunistic pathogen, water molds bacteria and parasites (Powell 2000). Releasing cortisol under stress conditions also suppress immune system as suggested by Wendelaor-Bonga (1997). In comparison with msl22 clove oil is highly effective even at low concentration (Table 1) of 0.1 and 0.5 mL/L. There was no mortality in 0.1 and 0.5 mL/L concentrations of clove oil. Mortality in our experiment increased in the increasing concentration of clove oil and reached to 90% in 2.25 mL/L concentration of clove oil. Higher concentration of 1.75 and 2.25 mL/L proved toxic and caused 50-90% mortalities. Keene *et al*., (1998) has reported 40% mortality in rainbow trout (*Oncorhynchus mykiss*) in 40 ppm eugenol aqueous solution. Prince and Powell, (2000) also reported clove oil to be well effective in anesthetizing adult rain bow trout.

Clove oil not only meets seven out of eight criteria for an ideal aesthesis (Marking and Meyer, 1985) but it is low priced anesthetics with desirable results. The use of clove oil however demands general principles of safe handling. It was noted that a stay in a poorly ventilated room where clove oil is used cusses headache, nausea and fatigue in vulnerable person. Therefore it is necessary that if trials conducted in airy laboratories in cross ventilated room the clove oil is proven to much effective. The therapeutic index (TI) of Svobodora and Vykusova (1999) was relatively low indicated the advantage of clove oil.

Clove oil meets seven out of eight criteria for an ideal anesthetic (Marking and Meyer, 1985). Its main advantage is its low price. The use of clove oil, however, requires that general principles of safe handling of chemicals be observed. It was observed that a stay in a poorly ventilated room where clove oil is used causes headache, nausea and fatigue in vulnerable persons. The same problems were noticed when the anesthetic trials were carried out. However, if the trials are conducted in airy laboratories or in the place where cross ventilation possible the clove oil is proven to be much effective. The therapeutic index (TI), Svobodva and Vykusova, 1999 was relatively low indicated the advantage of clove oil.

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