The Bangladesh Veterinarian (2009) 26(2) : 68 – 73

Clove oil anaesthesia in singhi (*Heteropneustes fossilis*) and lata (*Channa punctatus*) fish

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Abstract

Three different concentrations of clove oil were applied to two different size groups of singhi (*Heteropneustes fossilis*) and lata (*Channa punctatus*) fish to observe the anaesthetic effect and to find an optimum dose. Two groups each consisting of 10 fish was exposed to 0.01, 0.02 and 0.03% clove oil. All fish of either size anaesthetized with 0.01% clove oil exhibited longer induction and shorter recovery period. Exposure to 0.03% concentration of clove oil produced shorter induction and longer recovery period. The smoothness of induction of anaesthesia and recovery was the best in all groups with 0.02% clove oil. There was no mortality encountered with 0.01% and 0.02% concentration. Exposure to 0.03% of clove oil produced 20% mortality in larger singhi. In smaller and larger lata, the mortality rate was 60 and 50%, respectively. Clove oil (0.02%) appeared to be a suitable agent for anaesthesia in these two species of fish. (*Bangl. vet*. 2009. Vol. 26, No. 2, 68–73)

Introduction

Anaesthesia abolishes pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness (Summerfelt and Smith, 1990). Anaesthetics in fish farms are used to minimize motility during handling and transport. This may reduce susceptibility to pathogens and infection (Woody *et al.,* 2002). Anaesthetics are also used in fish during artificial spawning, weighing, tagging, grading and surgical procedures (Anderson *et al.,* 1997). Anaesthesia in fish may be produced by different agents, mainly tricaine methanesulphonate (MS-222), quinaldine sulphate, benzocaine and phenoxyethanol, which are hazardous, expensive and not very effective (Munday and Wilson 1997; Erdmann 1999). Clove oil is considered to be a potential fish anaesthetic (Woody *et al.,* 2002). This oily substance is distilled from buds, leaves and stems of clove tree (*Eugenia aromatica)*. The main chemical ingredient of clove oil is eugenol (70-98%; Taylor and Roberts, 1999), which is reported to possess high antibacterial and antifungal activity (Karapmar and Aktug, 1987; Briozzo *et al*., 1989). It is non-carcinogenic and non-mutagenic (Nagababu and Lakshmaiah, 1992). Eugenol has been successfully used as an anaesthetic in rabbitfish (Soto and Burhanuddin, 1995); gold fish, crucian carp (Endo *et al*., 1972) and Indian major carps (Farid, 1999).

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The information relating to the use of eugenol in native fish appears scanty. The present research was carried out to study the efficacy of clove oil as an anaesthetic for indigenous singhi and lata fish. The effect of different concentration of clove oil on induction, recovery period and mortality in different size groups is presented.

Materials and Methods

Singhi and lata of different size groups (Table 1) were used from April to June 2006.

Collection and acclimatization of fish

Live fish were collected from the local fish market of Mymensingh district and transported to the laboratory using polyethylene bag with clean water aerated by agitation with fingers during transportation. The fish were kept in plastic buckets in the laboratory and acclimatized in laboratory conditions for a week.

Measurement of experimental fish

Prior to measurement, an individual fish was caught with a fine mesh scoop net and its length recorded in centimetres using a steel measuring scale.

Table 1. Size groups of fish

Species	Size group	Measurement (cm)
Singhi (*Heteropneustes fossilis*)	Smaller	12-16
	Larger	17-21
Lata (*Channa punctatus*)	Smaller	12-15
	Larger	17-20

Water temperature

Water temperature of the plastic containers was recorded daily with a thermometer (YSI, Model 58, USA) and was found within desirable range (29 to 30°C; Boyd, 1979).

Experimental design

The fish were anaesthetised with three concentrations of clove oil (Table 2).

Preparation of stock solution

To prepare anaesthetic stock solution, 10 ml of clove oil (Hilltech Canada Inc. Vankleak Hill. Ontario, Canada) was taken in a volumetric flask and 90 ml of ethyl alcohol was added to prepare a stock solution of 10% clove oil.

To make 0.01%, 0.02% and 0.03% clove oil, 1 ml, 2 ml and 3 ml of stock clove oil solution was made up to one litre of distilled water.

Experimental trial

Experimental trials were carried out in rectangular steel trays of 10 litres capacity containing five litres of tap water. The tray was kept on a table for observation.

Table 2. Experimental design

Size group	Fish species	Clove oil concentration (%)	Observed parameters
Smaller	Singhi (12-16 cm)	0.01 (n=10)	i. Induction period (minutes)
		0.02 (n=10)	ii. Recovery period (minutes)
		0.03 (n=10)	iii. Mortality rate
	Lata (12-15 cm)	0.01 (n=10)	
		0.02 (n=10)	
		0.03 (n=10)	
Larger	Singhi (17-21 cm)	0.01 (n=10)	
		0.02 (n=10)	
		0.03 (n=10)	
	Lata (17-20 cm)	0.01 (n=10)	
		0.02 (n=10)	
		0.03 (n=10)	

Three trays were used. The first contained tap water and was the pre-induction tray, the second contained desired concentration of clove oil and was the induction tray and the third was for recovery and contained only tap water. Normal behaviour of fish was closely observed. When the fish became immobile and in lateral position, they were considered as anaesthetized. Immediately after induction, the fish were transferred to the recovery tray to regain consciousness and normal movement. Ten replications were used for each concentration.

Induction and recovery period

Time taken from putting the fish into the induction tray until it became immobile was considered induction period. The recovery period extended from transferring the fish into the recovery tank until reappearance of mobility.

Statistical analysis of data was performed by paired *t* test and one-way analysis of variance (ANOVA) to test the significance of variation between the treatment means by using SPSS programme. Standard deviation of treatment means were calculated from the residual mean squares in the analysis of variance (Zar, 1984).

Results and Discussion

Induction and recovery period in singhi

Induction and recovery period with different concentrations of clove oil in different sizes of singhi is presented in Table 3. Mean induction period in smaller singhi with 0.01, 0.02, and 0.03% clove oil were 6.5 ± 1.2, 5.2 ± 0.8 and 2.8 ± 0.4

minutes, respectively. The induction periods in larger singhi were 6.8 ± 1.2, 6.1 ± 1.1 and 4.0 ± 0.6 minutes, respectively. These differences between concentrations were significant (P<0.05). Mean recovery periods in smaller singhi with 0.01, 0.02, and 0.03% clove oil were 7.9 ± 2.0, 11.6 ± 1.0 and 15.2 ± 4.8 minutes, respectively. The recovery periods in larger singhi were 7.0 ± 1.1, 10.5 ± 1.3 and 36.1 ± 12.7 minutes, respectively. The differences between concentrations were significant (P<0.05).

Table 3. Effects of different concentrations of clove oil on induction and recovery period (mean ± standard deviation) in singhi

Size of fish	Induction period (min) with different concentration of clove oil			Recovery period (min) with different concentration of clove oil		
	0.01%	0.02%	0.03%	0.01%	0.02%	0.03%
Smaller (n=10) 12-16 cm	6.5 ± 1.2^{a}	5.2 ±0.8^{b}	2.8 ± 0.4^{c}	7.9 ± 2.0^{a}	11.6 ± 1.0^{b}	15.2 ± 4.8^{c}
Larger (n=10) 17-21 cm	6.8 ± 1.1^{a}	6.1 ± 1.1^{b}	4.0± 0.6^{c}	7.0 ±1.1^{a}	10.5 ±1.3^{b}	36.1 ±12.7^{c}

Values in the same row with different superscripts vary significantly

The induction period was shorter at higher concentration (0.03%) and longer at lower concentration (0.01%) of clove oil in smaller and larger size groups of singhi. The induction period with 0.02 and 0.03% concentration of clove oil in smaller singhi was significantly shorter than in the larger size. The recovery period was shorter at lower doses (0.01%) and longer at higher doses (0.03%) of clove oil in smaller and larger groups. The recovery period with 0.03% concentration of clove oil was significantly higher in larger size of fish. This might be due to higher accumulation of clove oil in the body of smaller fish than that of the larger ones. Similar observations have been reported by Farid (1999).

Induction and recovery period in lata

Induction and recovery period with different concentrations of clove oil in different size of lata is presented in Table 4. Mean induction time in smaller lata with 0.01, 0.02, and 0.03% clove oil were 9.4 ± 2.5, 3.2 ± 0.7 and 1.8 ± 0.4 minutes, respectively. The induction times in large lata were 10.7 ± 2.9, 5.8 ± 0.9 and 2.1 ± 0.5 minutes, respectively. The differences between concentrations were significant (P<0.01). Mean recovery period in small lata with 0.01, 0.02, and 0.03% clove oil were 7.1 ± 1.4, 9.3 ± 2.7 and 10.3 ± 2.2 minutes, respectively. The values in larger lata were 6.7 ± 1.7, 8.7 ± 2.9 and 11.3 ± 3.0 minutes, respectively. The differences between concentrations were significant (P<0.05).

The induction period of smaller lata was shorter than that of large fish, but the difference was significant only at 0.02% clove oil concentration. The recovery period of smaller lata was longer than the larger in 0.01 and 0.02% concentration of clove oil.

Table 4. Effects of different concentrations of clove oil on induction and recovery period (mean ± standard deviation) in lata

Size of fish	Induction period (min) with different concentration of clove oil			Recovery period (min) with different concentration of clove oil		
	0.01%	0.02%	0.03%	0.01%	0.02%	0.03%
Smaller (n = 10) 12-15 cm	9.4 ± 2.5[a]	3.2 ± 0.7[b]	1.8 ± 0.4[c]	7.1 ± 1.4[a]	9.3 ± 2.7[b]	10.3 ± 2.2[c]
Larger (n = 10) 17-20 cm	10.7 ± 2.9[a]	5.8 ± 0.9[b]	2.1 ± 0.5[c]	6.7 ± 1.7[b]	8.7 ±2.9[ab]	11.3 ± 3.0[a]

Values in the same row with different superscripts vary significantly

Mortality rate of fish with different concentration of clove oil

There was no mortality with 0.01 and 0.02% concentrations of clove oil. Exposure to 0.03% of clove oil, however, produced 20% mortality in larger singhi, 60% in smaller lata and 50% in larger lata (Table 5). Higher concentration of clove oil has also been reported to produce mortality in salmon (Woody *et al*., 2002).

Table 5. Mortality of fish with different concentration of clove oil

Fish species	Size of fish	Clove oil concentrations and mortality rate (%)		
		0.00%	0.02%	0.03%
Singhi	Smaller (n = 10) 12-16 cm	-	-	-
	Larger (n = 10) 17-21 cm	-	-	20
Lata	Smaller (n = 10) 12-15 cm	-	-	60
	Larger (n = 10) 17-20 cm	-	-	50

- = No mortality

Clove oil enters the gills through water (Brousse, 1974). The active principle eugenol is then carried to the central nervous system through blood circulation (McFarland, 1960).

Behavioural changes during induction and recovery

When the fish were put into induction tray containing clove oil, they became excited and hypermotile followed by bubbling. The gill and fin movement progressively decreased, the fish lost equilibrium and started swimming laterally. Finally the fish became immobile with full loss of equilibrium and consciousness. After transfer to recovery tray reappearance of gill movement was noticed first. This was followed by fin and tail movement. The fish started moving laterally. Gradually full equilibrium was regained and normal behaviour was restored. Similar

behavioural changes during induction and recovery from anaesthesia have been reported elsewhere (McFarland, 1960).

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