ISSN: 2277-5536 (Print); 2277-5641 (Online)

EFFICACY OF CLOVE OIL AS A FISH ANAESTHETIC AGAINST FOUR FRESHWATER HARDY FISHES

M. Manjurul Alam[*], Md. Kamrul Ahsan[**] and Selina Parween[***]

[*]Department of Fisheries, University of Rajshahi, Rajshahi 6205, Bangladesh

[**]Department of Zoology, New Govt. Degree College, Rajshahi 6000, Bangladesh

[***]Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh

[*]E-mail: mamillat@yahoo.com

ABSTRACT

Clove oil at concentrations of 0.10, 0.25, 0.50, 1.00 and 2.00 ml L^{-1}, acted as a potential anaesthetic against four freshwater hardy fishes, *viz.*, *Anabus testudineus*, *Mystus vittatus*, *Channa punctatus* and *Channa orientalis*. Time taken to anaesthetize (induction time) the fishes was 0.55±0.03 to 3.78±0.08 minute (*A. testudineus*), 0.40±0.02 to 3.48±0.18 minute (*M. vittatus*), 0.33±0.02 to 2.86±0.04 minute (*C. punctatus*) and 0.32±0.02 to 3.03±0.06 minute (*C. orientalis*), depending on the concentrations of the oil. Recovery rate of the fishes was ranged from 80-100%. Comparatively longer recovery time was recorded for fishes as 8.80±0.63 to 72.40±0.72, 17.30±0.45 to 84.14±1.82, 16.10±0.35 to 75.88±1.22 and 14.30±0.37 to 71.25±0.82 minute for *A. testudineus*, *M. vittatus*, *C. punctatus* and *C. orientalis* respectively. Clove oil at concentrations from 0.25 to 0.50 ml L^{-1} acted as effective anaesthetic against the studied fishes.

KEY WORDS: *A. testudineus*, Anaesthetics, Clove oil, induction time, recovery time, *M. vittatus*, *Channa.*

INTRODUCTION

Anaesthetization of fish is essential to reduce the stress of handlings (Ross and Ross 2007). Handling stress may affect immune system of the fish and make them vulnerable to disease or often cause death. Anaesthesia is usually require to carry out diagnosis procedures in fish, for injection and topical dressings of the diseased fish, for insertion of tags, taking measurement data, collection of scale samples, etc. (Woody *et al*., 2002; Ross and Ross, 2007). Fish may also be needed to anaesthetize while handled during transportation, counting and stocking in the pond (Gunn, 2000) to minimize mortality rate.

A number of drugs are used as anaesthetics during fish handling and transportation (McFarland, 1960, Munday and Wilson, 1997). The ideal anaesthetic used in the laboratory instigates induction in three minutes or less, and recovery within five minutes, and not toxic to fish or human, and soluble in aqueous solution (Gunn, 2000). Commonly used chemical fish anaesthetics are carbon dioxide gas, quinaldine and 3-aminobenzoic acid ethyl ester methanosulphonate (MS–222), which are comparatively safe for both fish and human (Griffiths 2002, Woody *et al*. 2002). However, there are some drawbacks associated with these chemicals, so search for alternative anaesthetics are needed, which would be effective with safe recovery, and would also be environment friendly. In this context, plant materials such as clove oil is using in the fish laboratories, because it has been used for centuries as topical anaesthetic for humans (Woody *et al.*, 2002). Clove oil, containing the active ingredient eugenol has been reported to be an inexpensive and effective fish anaesthetic.

The objective of the study is to establish the lowest effective concentration of clove oil for the successful anaesthetization and assessing the time taken to recover from the state of anaesthesia of four live fishes *viz*. *Anabas testudineus* (Bloch, 1792), *Mystus vittatus* (Bloch 1794), *Channa punctatus* (Bloch, 1793) and *Channa orientalis* (Hamilton, 1822).

MATERIALS AND METHODS

The method used consisted of introducing the active ingredient of clove oil into the fishs' gills through the water, *i.e.* 'anaesthesia by immersion' (Brousse, 1974). The substance is absorbed through the gills and travels through the bloodstream to the central nervous system. The fish then goes through several anaesthesia stages ranging from balance loss to total motionless and ventilatory arrest (McFarland, 1960).

Clove oil was mixed with tap water at rates of 0.10, 0.25, 0.50, 1.00 and 2.00 ml L^{-1}. The experiments were carried out on wild-caught four species of live fishes *A. testudineus*, *M. vittatus*, *C. punctatus* and *C. orientalis*. In the laboratory, total length and total weight of each individual fish were recorded. The specimens were acclimatized in glass aquaria with aeration for 24 hours prior to the commencement of experiments. The stages of anaesthetization were recorded according to the characteristics given in Table-1.

Table 1. Designated stages of anaesthesia used for clove oil efficacy tests

Stages	Characteristics
1	Opercular movement visibly slows or becomes erratic
2	Sporadic loss of equilibrium, difficulty maintaining position while at rest
3	Complete loss of equilibrium; inability to regain upright position
4	No reaction to handling or a sharp prod in the peduncle
Recovery	Ability to remain upright, normal swimming behaviour

The efficacy criteria of the clove oil were: i) ability to handle fish within three minutes, ii) fish recovery within 10 minutes and iii) survival of a 15 minutes exposure trial. Fifty fishes of each of four species were used separately for testing with each of five concentrations. Fish were placed in the anaesthesia tank and stages of anaesthesia were visually monitored, time was recorded, and classified according to the Table 1. Once a fish had reached a state where it did not react to handling (stage 4), it was removed from the anaesthetic bath. Fully anaesthetized fish were placed in an aerated freshwater recovery tank and monitored until complete recovered.

RESULTS

The range and mean total length and total weight of the fishes used in this experiment are provided in Table 2.

Table 2. Range and mean±SE of total length and weight of the specimens

Species	Doses (ml/l)	Range of Total Length (cm)	Total Length (cm)	Range of Total Weight (g)	Total Weight (g)
A. testudineus	0.10	10.50-13.00	11.80±0.25	21.00-38.00	28.11±2.03
	0.25	10.80-13.60	12.01±0.27	24.00-38.30	30.63±1.80
	0.50	10.90-13.50	12.16±0.26	22.00-38.00	29.85±1.96
	1.00	10.00-12.50	11.77±0.25	24.00-34.00	29.48±1.39
	2.00	10.00-13.00	11.67±0.30	25.00-34.00	29.98±0.95
M. vittatus	0.10	6.50-9.10	7.59±0.26	6.00-10.00	7.77±0.40
	0.25	6.90-9.20	7.71±0.24	6.80-10.00	7.85±0.34
	0.50	7.50-10.00	8.60±0.29	8.00-11.00	8.91±0.31
	1.00	7.00-10.00	8.24±0.26	7.00-9.80	8.47±0.27
	2.00	7.00-10.00	8.32±0.27	8.00-11.50	9.17±0.33
C. punctatus	0.10	15.00-18.90	16.90±0.44	45.20-69.00	55.54±2.52
	0.25	15.50-18.40	16.63±0.27	16.50-64.50	47.77±4.05
	0.50	14.80-19.00	16.61±0.47	40.50-70.50	53.78±3.19
	1.00	14.50-18.60	16.50±0.41	40.50-70.00	52.85±3.10
	2.00	14.50-19.00	17.01±0.42	41.00-71.20	56.78±3.16
C. orientalis	0.10	11.00-15.00	12.94±0.43	22.50-37.00	29.78±1.86
	0.25	10.00-15.50	13.17±0.53	19.00-38.20	30.38±2.22
	0.50	11.00-15.00	12.80±0.40	19.50-42.60	29.82±2.42
	1.00	11.30-14.00	12.78±0.31	21.40-39.60	28.29±2.25
	2.00	12.00-15.70	13.44±0.36	23.80-46.00	32.74±2.37

Results of this experiment showed that all the concentrations of clove oil used were perfect for anaesthesthetizing the experimental fish species except *M. vittatus*. The average time taken to anaesthetized (the induction time) by different concentrations of clove oil were 0.55±0.03 to 3.78±0.08 min in case of *A. testudineus*, 0.40±0.02 to 3.48±0.18 min in case of *M. vittatus*, 0.33±0.02 to 2.86±0.04 min in case of *C. punctatus* and 0.32±0.02 to 3.03±0.06 min in case of *C. orientalis* (Figure-1). The anaesthetizing time required was found to decrease with the increased concentration of the clove oil. However, the recovery time from anaesthesia ranged from 8.80±0.63 to 72.40±0.72 min in *A. tetsudineus*, 17.30±0.45 to 84.14±1.82 min in *M. vittatus*, 16.10±0.35 to 75.88±1.22 min in *C. punctatus*, and 14.30±0.37 to 71.25±0.82 min in *C. orientalis* (Figure-1). Recovery time was found to inversely relate to the concentration used. Hundred percent recoveries of the experimental fishes were achieved in *A. testudineus* at all concentration. The next hardy species were *C. punctatus* and *C. orientalis*. Recovery rate of both species was 100% at all concentrations except 2 ml/l (80%) (Figure-1). Analysis of variance test of the results showed that the induction and recovery times varied significantly with the concentrations of clove oil ($F = 80.938$, $p<0.001$ and $F = 5.233$, $p<0.05$ respectively).

All concentrations of the clove oil were found to be more or less equally effective against the fish species. F-values obtained for induction time was 0.177 and that for recovery time was 0.04 (p = non-significant). The degree of induction time was obtained as shortest in *C. punctatus* and then *C. orientalis*<*M. vittatus*<*A. tetudineus*; and that of

recovery time as *A. tetudineus*< *C. orientalis*< *C. punctatus*< *M. vittatus*. Length and weight of the fishes were probably did not act as limiting factors against the efficacy of the clove oil as fishes having more or less same length and weight were used in the experiment (Table-2).

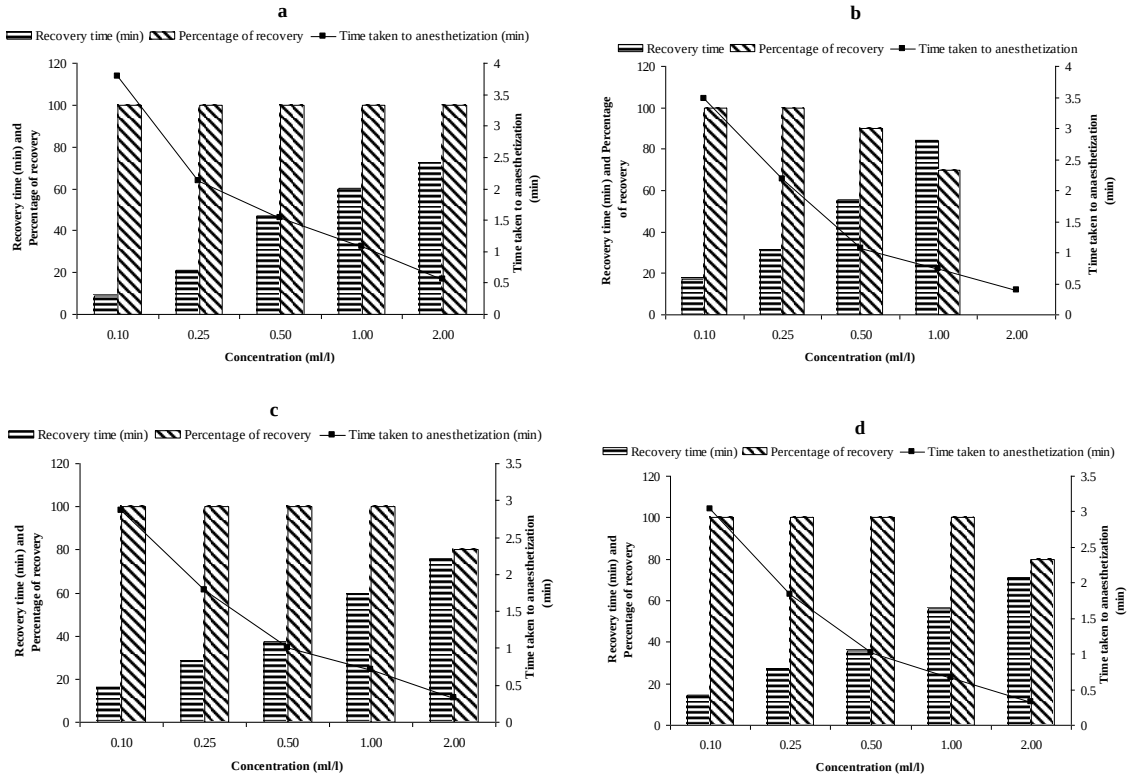

Figure- 1. Potentiality of clove oil in anaesthetizing *A. testudineus* (a), *M. vittatus* (b), *C. punctatus* (c) and *C. orientalis* (d).

DISCUSSION

Effectiveness of clove oil as fish anaesthetic has been reported in a number of literature. Short reduction time and long recovery period with increased concentration, are usually the common pattern for clove oil action (Cunha and Rosa, 2006; Griffiths, 2000; Prince and Powell, 2000; Woody *et al*., 2002). However, either induction time or recovery time is not affected by the variation of size (total length) of the fish (Cunha and Rosa, 2006).

Longer induction and shorter recovery times using low concentrations of clove oil, were recorded in hardy fishes like *Heteropneustes fossilis* and *C. punctatus* (Matin *et al*., 2009) The authors mentioned that a concentration of 0.02% clove oil could be suitable to anaesthetize these two fish species. In the present study, 0.25-0.50 ml L^{-1} concentrations were found to produce effective induction with a satisfactory recovery rate in live fishes including *C. punctatus*. Number of published reports showed that clove oil also acts as a potential anaesthetic against different fish groups. Efficacy of this oil can be obtained at low doses, and Erdmann (1999) claimed that it provides much calmer induction compared to other traditional chemical anaesthetics. Because of low effective doses, clove oil was reported to be less expensive (Keene *et al*., 1998), and for the longer recovery period, it is safe for use in the laboratory research, handling and transportation of fish (Erdmann, 1998).

CONCLUSION

The present results revealed that for handling, transportation and experimental purposes, clove oil is an effective anaesthetic for live fishes. The quick induction, long recovery time and high recovery rate were obtained at concentrations between 0.25 – 0.50 ml L^{-1} of clove oil. This oil is equally effective against the catfish *M. vittatus*. So, clove oil being a user-safe, eco-friendly and alternative to chemical fish anaesthetic, can be use in the aquaculture practice.

ACKNOWLEDGEMENTS
The author wishes to thank the Chairman, Department of Zoology, for providing laboratory facilities.

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