Global Veterinaria 6 (2): 180-184, 2011
ISSN 1992-6197

Size-Relative Effectiveness of Clove Essence as an Anesthetic for Kutum (*Rutilus frisii kutum*)

[1]Amin Farahi, [1]Milad Kasiri, [1]Mohammad Sudagar and [2]Mohsen Soleimani Iraei

[1]Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2]Department of Marine Sciences and Technologies, Hormozgan University, Bandarabbas, Iran

Abstract: The purpose of this work was to investigate the size-relative effectiveness of clove essence as an anesthetic for kutum. In total, 30 kutum (*Rutilus frisii kutum*) (two groups of 20-24 and 30-34 cm mean fork length) were anaesthetized at different clove essence concentrations of 50, 200, 400 ppm for kutum. Results showed that the induction time and recovery time among experimental groups were more size-dependent. The hematological parameters were not significantly influenced by the anesthetic at all concentrations used ($p>0.05$). As a result, clove essence was effective, producing minimum stress and zero mortality and can be recommended as an effective anesthetic.

Key words: Clove essence · Anesthetic · Kutum

INTRODUCTION

Caspian kutum, *Rutilus frisii kutum* Kamenskii 1901, is an endemic fish of Caspian Sea and its populations generally recorded along near the coast, from the Trek River the north to the southern part [1]. It consists more than 70% of fishermen catch in Iran coastal of the Caspian Sea [2].

Rapid expansion of the aquaculture industry that occurred in previous decades prompted scientific debates on the potential suffering of fish being handled during common aquaculture procedures or during slaughtering. Research aimed at lessening the suffering of cultured fish is vital to meet the concern for farmed fish welfare [3]. Handling stress and various manipulations in aquaculture can have a negative impact on fish health and their growth [4]. Anesthetics are therefore applied to reduce these negative effects to the minimum. The dosage required to induce general anesthesia varies according to the anesthetic used and other factors such as water temperature, hardness, salinity and oxygen concentration, length of exposure, body weight, the ratio of gill area/body surface area and the species of fish. In general, small fish are more sensitive to anesthesia than larger fish [5].

An ideal anesthetic for fish should induce anesthesia in less than 3 to 5 min, with total loss of balance and muscle tone, allowing an uneventful and rapid (i.e. less than 10 min) recovery with low tissue residues after recovery, thus being safe to users and consumers. The anesthetic should be inexpensive and easy to use [5 -7]. Some traditionally used anesthetics in aquaculture include tricaine methanesulphonate (MS-222), benzocaine, etomidate, metomidate, 2-phenoxyethanol and quinaldine. In farmed fish, MS-222 appeared to be the most widely used anesthetic. However, recently there has been considerable interest in another fish anesthetic, clove oil extracted from the clove tree, *Eugenia caryophyllus* (syn. *Syzygium aromaticum*) which has traditionally been used as human anesthetic [8]. Chaieb *et al.* [9] reported that the main active ingredients of clove oil are: eugenol (88.58%), eugenyl acetate (5.62%), β-caryophyllene (1.39%), 2-heptanone (0.93%), ethyl hexanoate (0.66%), humulenol (0.27%), á-humulene (0.19%), calacorene (0.11%) and calamenene (0.10%). Clove oil is generally considered a reasonable alternative of fish anesthetic with low cost and of no risk to human health. In fact, clove oil is used for local application to reduce pain and promote healing and exhibits antimicrobial, antioxidant, antifungal and antiviral properties [9]. According to the U.S. FDA, the constituting ingredients of clove oil are considered safe (substance that can be used in food industry), but none of them has been approved for fish anesthesia yet [10].

Corresponding Author: Amin Farahi, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Golestan, Gorgan, Shahid Beheshti Avenue, Postal Code: 49138-15739, Iran.
E-mail: farahi2010@yahoo.com.

Clove essence in Iran is used as an effective agent for anesthetizing fish in aquaculture facilities in order to mitigate the handling stress due to the grading, transporting and artificial spawning [11].

The aim of this study was to observe the response of kutum of different sizes to clove oil used as an anesthetic and to observe its effect on selected hematological and biochemical variables.

MATERIALS AND METHODS

Experimental Animals: The experiment was carried out during April 2010 in kutum propagation farm located in Valiabad River, Tonekabon, Iran. Male kutum (n = 30) with an average fork length 29.06 cm (±2.47, SD) were used for independent experiments. The kutum were maintained in a circular 2 m^3 tank connected to a re-circulating system at mean temperature of 18 ± 0.6°C. The kutum were fed daily with carp pellets at 2% of body weight. Oxygen concentrations were maintained above 7.5 ppm in the tanks. Concentrations of pH, nitrate and ammonia were within the physiological/optimal range for this species (pH: 7.5-7.7; nitrate (<0.01 mgl^{-1}), ammonia (<0.03 mgl^{-1}); photoperiod 12 L: 12 D. All fish were starved for 24 h before the experiment. Before the experiment, the fish were observed daily to evaluate their health condition as indicated by their activity and external appearance.

Prior to anesthesia, the kutum were divided into two size groups (20-24 and 30-34 cm) and maintained in different holding tanks. Five of each size group were exposed to three different concentrations of clove essence (n = 5 fish per size group/dosage tested).

Anesthesia Preparation and Procedures: Three different concentrations of clove essence (50, 200 and 400 ppm) were Chosen according to the pervious scientific papers (add references). For preparation the desired dose of clove essence, a 10% stock solution (1 ml clove essence + 9 ml of maintaining water) was made. To make a 50 ppm solution, 0.5 ml of the stock solution were mixed with one liter of water and this procedure applied for the other two concentrations [12]. The various concentrations were prepared and mixed in 20 l observation tanks filled with water from the respective recirculation systems. Fish were randomly caught by hand-net in the holding tanks and transferred immediately into the anesthetic in the observation tank. The fish were observed for opercular movements, balance and thereafter loss of response to stimulus. The fish were removed when they exhibited loss of equilibrium, no spinal reflexes and imperceptible opercular movements.

Induction and recovery times were obtained using a digital chronometer for fish. Induction of anesthesia was assumed as complete when fish lost equilibrium and reflex reactivity. Once a fish reached anesthesia, it was removed from the experimental tanks, dried and measured. Then the fish was transferred to fresh water in identical tank without rinsing to remove traces of the anesthetic. Recovery was considered complete in all groups when fish were able to regain upright position and swim normally. Recovery and induction times were recorded for each fish. Following recovery, the fish were transferred to maintenance tanks and observed for 48 h for potential mortality. Control groups were fish that remained in the rearing tanks and left undisturbed until they were collected for blood sampling.

Blood Sample Collection: Blood was sampled from kutum with heparinized syringes from the caudal vein. Haematocrit was calculated after centrifugation of microhaematocrit tubes (4,000 g for 5 min, Sigma 1-15 microcentrifuge). For plasma biochemical analysis, blood was collected (0.5-1.5 ml) in Eppendorff vials and centrifuged (9,000 g for 13 min, Sigma 1-15 microcentrifuge). The plasma was collected and stored at -80°C for further analysis. Glucose and calcium plasma concentrations in trout plasma were measured by Olympus 600 Medicon Analyzer, using Hexokinase enzymatic UV and Arsanazo III photometric colour tests, respectively.

Statistical Analysis: Statistical analysis of data was done by One-Way ANOVA with Duncan test at the level of 95% using SPSS 16. Statistically significance was set at the level of $p<0.05$ with ± standard deviation (SD).

RESULTS

All fish used in the present study were healthy as was indicated by their activity and exterior appearance. No mortality was observed during the acclimatization period. Furthermore, no deaths or other adverse effects occurred within 48 h following recovery from anesthesia.

Anesthetic Effect of Clove Oil on Kutum: Figures 1, 2 present the results of induction and recovery of kutum exposed to different doses of the anesthetic and

Table 1: Haematocrit and biochemical variables of kutum anaesthetized with clove essence

Concentration of clove essence (ppm)	Haematocrit (%)	Calcium (mmol/l)	Glucose (mmol/l)
0	44.50±2.12	8.34±1.25	5.05±0.74
50	41/00±1.41	8.45±0.48	5.97±0.29
200	41.50±3.54	9.10±1.45	5.27±0.63
400	43.00±2.83	8.67±0.59	5.43±0.88

Means in the respective column are not significantly different, p>0.05

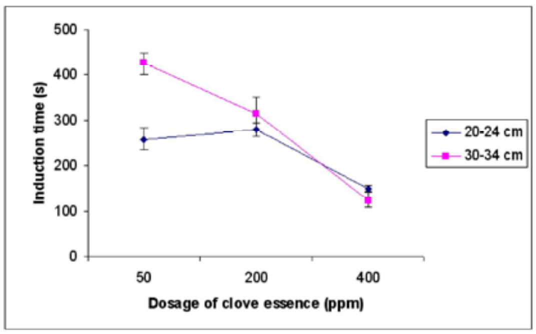

Fig. 1: Induction time (±SD) of kutum anaesthetized with clove essence

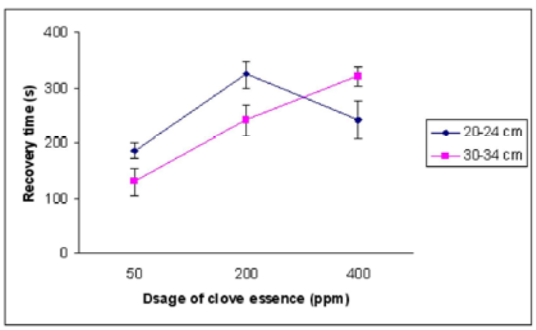

Fig. 2: Recovery time (±SD) of kutum anaesthetized with clove essence

according to their size. Induction time of anesthesia varied with clove concentration, decreasing with the increase of clove concentration (Figure 1). An increase in recovery time with clove concentration was recorded for the larger size class, a decrease for smaller (Figure 2). Induction time was rapid in small-size kutum, irrespective of the clove essence dose applied. Anesthesia was more size-dependent at the lower dose, compared to the higher dose. Smaller fish required less time to lose the upright position than larger ones at the dose 50 and 200 ppm. For the higher dose, induction time was rather uniform in both size groups (p> 0.05).

Recovery time was more size-dependent at the lower dose (50 and 200 ppm), with larger fish requiring less time to recover (Figure 2). However, smaller fish recovered earlier compared to the larger size group at 400 ppm (p<0.05).

Physiological Responses in Kutum: Clove essence did not produce marked changes (p<0.05) in the physiological indicators of experimental kutum compared to control (Table 1). It also didn't cause hyperkalaemia and hyperglycaemia (p>0.05) in the kutum compared to control.

DISCUSSION

In this study, clove essence was found to be effective anesthetic for kutum. The fishes exhibited normal behavior and remained calm during the induction time with no struggling or rapid swimming, which was a positive sign of their welfare. Additionally, no mortalities were observed for a 24 h post-recovery period.

Some species-specific differences were evident in the induction time. This may be the result of a higher uptake of eugenol via the gills [13] in this species. In kutum, induction time was related to the size of fish, with more time needed for larger fish to be anaesthetized. This may be because of higher rate of uptake of the anesthetic through the gills in the smaller fish compared to the larger ones. This is in accordance with the results reported by Woody *et al.* [14] for sockeye salmon (*Oncorhynchus nerka*). The higher dose produced a rapid and uniform response in all size classes, suggesting that a dose of 400 ppm might be well over the effective concentration. Recovery time varied with the concentration of clove oil for various size groups. An increase in recovery time relative to clove concentration was recorded in roach (*Rutilus rutilus*) [15], cobia (*Rachycentron canadum*) [16] and rainbow trout [17] and to 2-phenoxyethanol in rainbow trout [18]. Woody *et al*. [14] and Inoue *et al.* [19] found no direct relationship between clove concentration and recovery times in adult sockeye salmon (*Oncorhynchus nerka)* and matrinxa (*Brycon cephalus*). Results of this study seem to confirm species-specific differences in recovery times of anaesthetized fish.

In kutum, recovery time was slightly longer compared to induction time. This could be explained by the fact that eugenol exerts an inhibitory effect on respiration, reducing the ability of fish to remove the excess of eugenol from the system via the gills [13, 20]. Our results indicate that clove essence concentrations of 50 and 400 ppm induces the minimum induction and recovery times in kutum, respectively; however, the recommended concentrations for kutum may be influenced by environmental factors quite different from those under which this study was conducted.

In some fish species exposed to clove oil, alteration of some blood biochemical and haematocrit values were detected [17, 21, 22]. However, in this work, we didn't observed significant difference among experimental groups. The effects of toxicants on various tissues may vary with dosage, exposure, body size and species. Tort *et al*. [23] reported clove oil altering haematocrit concentrations in rainbow trout. Velisek *et al.* [22] observed the same in rainbow trout and carp, both of them exhibited also significantly increased plasma glucose concentrations. Nevertheless, these findings were observed after longer exposure periods (10 min) compared to 2.08-7.08 min in the present study. These results revealed the significance of exposure time and dosage on some physiological indicators of anaesthetized fish. The short induction time after which blood was collected could explain the lack of biochemical alteration and post-exposure mortality in this study. These results indicate the need to study possible physiological changes occurring in different fish species exposed to different doses of clove essence.

Glucose and calcium plasma concentrations along with cortisol concentrations have been used as stress indicators in fish [24, 25]. In previous studies, clove oil did not induce changes ($p>0.05$) in plasma glucose and calcium concentrations in trout and goldfish used for experiments [21, 26]. These findings agree with our result and mild hyperglycaemia and hypercalcaemia were recorded in treated groups. A rise in glucose concentration is a second order reaction under stress [27] and is mediated by the rise in cortisol concentration induced by stress. Clove was found to block the activity of cortisol, although not completely, in matrinxa (*Brycon cephalus*) [19]. Although the mechanism is not well known, Iverson [28] suggested that it blocks transmission of impulses to the hypothalamus-pituitary interrenal axis (HPI). Cortisol is considered one of the mediators of the increase in plasma glucose under stress [27].

In conclusion, clove essence was found to be safe and can be effectively and easily applied in used dosages to anaesthetize various size groups of kutum with minimal disruption in the physiological indicators studied and with zero mortality. Clove essence was easy to handle, without producing unpleasant or irritating odor. Hence, serious considerations should be given to the use of clove essence as a replacement for synthetic forms of anesthetics.

REFERENCES

1. Shafiei Sabet, S., M.R. Imanpoor, B. Aminian fatideh and S. Gorgin, 2009. Study on sexual maturity and levels of gonad steroid hormones in female kutum, Rutilus frisii kutum Kamenskii, 1901, during spawning season from river Sefid-Rood of the southern Caspian Sea. J. Cell Anim. Biol., 3: 208-215.
2. Sharyati, A., 1993. Fishes of the Caspian Sea region. Iranian fisheries company, Iran, third edition, pp: 77-79 (in Persian).
3. Ashley, P.J., 2007. Fish welfare: Current issues in aquaculture. Appl. Anim. Behav. Sci., 104: 199-235.
4. Hoskonen, P. and J. Pirhonen, 2006. Effects of repeated handling, with or without anaesthesia, on feed intake and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res., 37: 409-415.
5. Ross, L. and B. Ross, 1999. Anaesthetic and sedative techniques for aquatic animals. Blackwell Science, London, pp: 176.
6. Tsantilas, H., A.D. Galatos, F. Athanassopoulou, N.N. Prassinos and K. Kousoulaki, 2006. Efficacy of 2-phenoxyethanol as an anaesthetic for two size classes of white sea bream, Diplodus sargus L. and sharp snout sea bream, *Diplodus puntazzo* C. Aquacul., 253: 64-70.
7. Klimankova, E., K. Riddellova, J. Hajslova, J. Poustka, J. Kolarova and V. Kocourek, 2008. Development of an SPMEGC- MS/MS procedure for the monitoring of 2-phenoxyethanol in anaesthetised fish. Talanta, 75: 1082-1088.
8. Soto, C.G. and S. Burhanuddin, 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). Aquacult., 136: 149-152.
9. Chaieb, K., H. Hajlaoui, T. Zmantar, A.B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A short review. Phytotherapy Res., 21: 501-506.

10. FDA/CVM, 2009. Concerns Related to the use of Clove Oil as an Anesthetic for Fish. FDA Guidance for Industry. No 150. Center for Veterinary Medicine, Food and Drug Administration, USA.
11. Mohamadi, Arani M., 2006. Study on Anestjetiziation of Persian Sturgeon (*Acipencer persicus*) Fingerlings using Clove (*Eugenia caryophyllata*) Oil. Ira. J. Med. Arom. Plants, 22: 188-192.
12. Imanpoor, M.R., T. Bagheri and S.A.A. Hedayati, 2010. The Anesthetic Effects of Clove Essence in Persian Sturgeon, *Acipencer persicus.* World J. Fish and Marine Sci., 2(1): 29-36.
13. Keene, J.L., D. Noakes, R.D. Moccia and C.G. Soto, 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res., 29: 89-101.
14. Woody, C.A., J. Nelson and K. Ramstad, 2002. Clove oil as an anaesthetic for adult sockeye salmon: field trials. J. Fish Biol., 60: 340-347.
15. Sudagar, M., A. Mohammadizarejabada, R. Mazandarania and S. Pooralimotlagha, 2009. The efficacy of clove powder as an anesthetic and its effects on hematological parameters on roach (*Rutilus rutilus*). J. Aquacult. Feed Sci. Nutri., 1: 1-5.
16. Gullian, M. and J. Villanueva, 2009. Efficacy of tricaine methanesulphonate and clove oil as anaesthetics for juvenile cobia *Rachycentron canadum*. Aquac. Res., 40: 852-860.
17. Velisek, J., Z. Svobodova and V. Piackova, 2005a. Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*) Acta Vet. Brno., 74: 139-146.
18. Velíšek, J. and Z. Svobodová, 2004. Anaesthesia of common carp (*Cyprinus carpio* L.) with 2-phenoxyethanol: Acute toxicity and effects on biochemical blood profile. Acta Vet. Brno., 73: 247-252.
19. Inoue, L., L. Afonso, G.K. Iwama and G. Moares, 2005. Effects of clove oil on the stress response of matrinxa (*Brycon cephalus*) subjected to transport. Acta Amaz., 35: 289-295.
20. Guenette, S.A., F.C. Uhland, P. Heulie, F. Beaudry and P. Vachon, 2007. Pharmacokinetics of eugenol in rainbow trout (*Oncorhynchus mykiss*). Aquacult., 266: 262-265.
21. Cho, G.K. and D.D. Heath 2000. Comparison of tricaine methanesulponate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquac. Res., 31: 537-546.
22. Velisek, J., Z. Svobodova, V. Piackova, L. Groch and L. Nepejchalova, 2005b. Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). Vet. Med. Czech, 50: 269-275.
23. Tort, L., M. Puigcerver, S. Crespo and F. Padros, 2002. Cortisol and haematological response in sea bream and trout subjected to the anaesthetics clove oil and 2-phenoxyethanol. Aquac. Res., 33: 907-910.
24. Schreck, C.B., 2000. Accumulation and long term effects of stress in fish. G.P. Moberg and J.A. Mench, (eds.): The biology of animal stress: basic principles and implications for animal welfare, CABI Publishing, Wallingford, pp: 147-158.
25. Sink, T.D., J.S. Richard, G.K. Henry and G.M. Alan, 2006. Linking stress to increased mortality of channel catfish at varying concentrations of Edwardsiella ictaluri. J. Anim Vet. Adv., 5: 208-214.
26. Wagner, G.N., T.D. Singer and R.S. McKinley, 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res., 34: 1139-1146.
27. Barton, B.A. and G.K. Iwama 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish. Dis., 1: 3-26.
28. Iverson, M., B. Finstad, R.S. McKinley and R.A. Eliassen, 2003. The efficacy of metomidate, clove oil, Aqui-S and Benzoak as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts and their potential stress-reducing capacity. Aquacult., 221: 549-566.