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Evaluation of Clove Oil as Anaesthetic Agent in Fresh Water Angelfish, *Pterophyllum scalare*

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Abstract.- Compared to the other anesthetic and sedative agents, clove oil is plant based, does not harm human and animal health, has only a few side effects, is a practical and economic agent. Clove oil is a rather strong and effective sedative and anesthetic agent even though it is used in low doses. In this study, the aim was to determine the most appropriate clove oil dose for a known sensitive aquarium fish. For this purpose; 0.5, 1, 1.5, 2, 2.5 and 3 ml/l doses of clove oil were applied on angelfish which is one of the known stressful species during transportation. Induction and recovery times were measured for each fish separately. Recovery time was longer in the clove oil than the induction time. The shortest induction time was 15±2 s at the dose of 3 ml/1. As for recovery, the shortest time was found to be 343 s (±97,SD) at the dose of 2 ml/1. This dose of shortest induction and recovery times is recommended as the most appropriate amounts for an effective sedative and anesthetic agent in economic terms for aquaculture activities, such as handling, catching with net, transporting to another tank, etc.

Keywords: Ornamental fish, angelfish, *Pterophyllum scalare*, clove oil, eugenol, chemical sedative, anaesthesia.

INTRODUCTION

Many species of ornemental fish are in danger of becoming extinct due to the fact that they are collected from their natural habitats in excessive amounts by inappropriate methods. Thus, a large part, 90 % of the demands of hobbyists in ornamental fish trade is constituted by farm based freshwater fish (Whittington and Chong, 2007; Sanna-Kaisa and Jukka, 2004). Among these species, angelfish (*Pterophyllum scalare*) is a commercially important species that attracts the attention of aquarium sellers with its interesting shape (Rodríguez, 2006; Wikipedia, 2010). Deaths in angelfish observed following the transfer due to stress affect the trade of this fish species negatively (Lim *et al*., 2003). There are many factors causing the fish to get stressed during their hunting, breeding or transfer (Bondad-Reantaso *et al*., 2005; Ross and Ross, 2008; Özmen *et al*., 2007). Stress reduces the body resistance of the fish and this leads to the emergence of various diseases and eventually to death. In aquaculture, sedatives are used in order to lessen stress related negativities. Sedatives and anesthetics decrease the emission of metabolic products impairing the water quality by minimising the vital actions of the fish (Kanyılmaz *et al*., 2007). To this end, such chemicals as Quinaldine Sulphate, Etomidate, 2-phenoxyethanol and natural substances such as clove oil are used in the acuatic medium as sedative and anesthetic.

Clove plant contains 70-90% eugenol (2-methoxy-4-4-(2-propenyl)-phenol). Clove oil is derived from the stem, leaves and buds of the clove tree, *Eugenia caryophyllata* or *Syzygium aromaticum* (*i.e., Eugenia aroaticum*) and it contains the active ingredient eugenol. It is easily dispersible in water at high temperatures by vigorous shaking (Ross and Ross, 2008) and has many advantages as antibacterial, antiviral and analgesic agent (Keene *et al*., 1998; Tort *et al*., 2002). Beside to its these characteristics, it is also preferred since it is cost-effective and does not harm human and animal health (Keene *et al*., 1998). Anesthetic effect of the clove oil on some aquatic organisms was investigated in such cases as its use in the transfer of fish species used in the food sector (Ross and Ross, 2008; Keene *et al*., 1998; Tort *et al*., 2002; Waterstrat, 1999). However, there are not many scientific studies that will determine its use in low doses by maintaning the sedative and anesthetic effect peculiar to the species in freshwater ornamental fish. Therefore, in this study the effect of some doses of the clove oil were observed on an ornamental fish in which is known for its sensitivity

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to transportation.

MATERIALS AND METHODS

Anesthetic agent

In the tests, commercial clove oil was used. Content of the clove oil was analyzed in the laboratory of Pharmacy Faculty of Ege University in Turkey. Its content was reported as 96.1% eugenol (active principle), 2.41% Caryophyllene, 0.29% a-Selinene, 0.27 Isochiapin B.

Maintenance of fish

Angelfish *(Pterophyllum scalare*) (n=105) with mean weight 2.11±0.53g and mean length 4.27±0.34 cm were used. Fish were placed in three glass aquaria each containing 250-liter water (temperature, 24±0.5°C; pH, 7.73 and O_2, 7.88 ppm) for two weeks. Illumination was used in the aquaria for 8 h. Air was delivered to each aquarium through central air engine. Fish were fed with commercial pelleted feed at 2% of body weight once a day. Unused food and metabolic wastes were discharged through siphon every day. Feeding was stopped before the experiment.

pH and temperature were measured through digital pH meter and thermometer. The amount of oxygen in the water was measured via digital oxygen meter.

Experimental procedure

Angelfish were exposed to six doses of clove oil, 0.5, 1, 1.5, 2, 2.5, 3 ml/l. Control group did not receive any clove oil. The concentration of clove oil above 3 ml/l were toxic. Tests were repeated five times for each dose. One fish was used for each dose treatment and one fish was used for control group. Clove oil was added in 1 liter water in the experimental glass pot and mixed for 1 min. This process was repeated for each fish every time. Fish were caught randomly with hand-net and put in the experiment pot. Induction and recovery times were measured via chronometer for each fish. Fish were observed until their opercular did not move (stage 5). After gently drying the fish with a papertowel, the length and weight were rapidly measured and then immediately transferred to the recovery pot and observed until they swam normaly (recovery stage 5). The recovery was measured with the help of a chronometer in terms of second (s). Induction and recovery stages were examined according to Ross and Ross (2008), Yildirim *et al*. (2010) and Ucar and Atamanalp (2010).

After the experiment, the fish were placed in aquaria filled with clean water and proper ventilation until they stabilized and resumed their normal food intakes (Small, 2003). The experimental fish were monitored for 7 days for any abnormal behaviour and or mortality.

Statistical analysis

Length and weight measurements of the fish observed for each dosage were calculated through determinative statistics. When parametric test assumptions were true for each dosage, evaluations were carried out via one-way analysis of variance. In case the assumptions did not come true the statistical evaluations were carried out by non-parametric Kruskal Wallis test and SPSS 11.0 program.

RESULTS

Tables I and II show mean induction and recovery times of the fish for each dose of clove oil. The longest total induction time (250±163 s) was detected in 0.5 ml/l. However, the shortest time of total induction (15±2 s) was observed in 3 ml/1; but 40% fish died at this dose. The fish took longest time (104±100 s) to reach stage 5 at 0.5 ml/l. The shortest time (1±2 s) was taken to reach stage 3 at 1.5 ml/l dose in all stages. The longest time to pass to the induction stage was observed at all stages at clove oil dose of 0.5 ml/1. When all the stages were examined, transitions to the stages 1, 2 and 5 took shortest time at 3 ml/1. Moreover, a difference was found between groups in terms of total induction (p=0.000).

Table II shows recovery time of the fish. The longest time to recovery was observed at 1 and 1.5 ml/1. The shortest time to reach the total recovery stage was detected at the dose of 2 ml/1. When each recovery stage was considered individually, the shortest transitions were recorded at 2.5 ml/1 for stage 4 and stage 5. In total recovery, the longest time 1085 s (±553, SD) was observed at 1.5 ml/1 while the shortest time 343 s (±97, SD) was

Table I.- Effect of different concentrations of clove oil, demonstrated in five different stages on sedation induction time (s) of angelfish.

Clove oil (ml/l)	Stage 1[a] Light sedation (s)	Stage 2[a] Deep sedation (s)	Stage 3[a] Light anaesthesia (s)	Stage 4[a] Deep anaesthesia (s)	Stage 5[a] Surgical anaesthesia (s)	Total induction (s)
0.5	82±46	31±26	24±32	19±33	104±100	250±163
1	18±10	7±2	12±13	17±13	18±15	73±12
1.5	12±6	10±10	1±2	2±8	15±13	42±18
2	7±2	5±3	2±1	2±1	3±3	20±3
2.5	6±1	3±1	3±1	2±1	3±2	18±2
3	4±1	3±1	2±1	2±1	2±1	15±2

[a]Mean± S.E

Stage 1	Light sedation	Slow swimming, decrease in reaction to external stimuli, normal body balance.
Stage 2	Deep sedation	Deep sedation, full reactivity loss except for strong external stimuli, slow down in operculum, partial balance loss in body.
Stage 3	Light anaesthesia	Tilting over one side but still reactive against strong stimuli, continuing opercular movement.
Stage 4	Deep anaesthesia	Non-moving tilting over one side, opercular movement is little or nothing.
Stage 5	Surgical anaesthesia	No reaction even to very strong pushes

Table II.- Effect of different concentrations of clove oil, demonstrated in five different stages on recovery time (s) of angelfish.

Clove oil (ml/l)	Stage 1[a] Light sedation (s)	Stage 2[a] Deep sedation (s)	Stage 3[a] Light anaesthesia (s)	Stage 4[a] Deep anaesthesia (s)	Stage 5[a] Surgical anaesthesia (s)	Total recovery (s)
0.5	125±58	201±146	57±61	39±43	114±118	537±95
1	53±34	319±202	196±132	202±169	295±161	1067±397
1.5	43±34	224±88	190±150	269±169	294±228	1085±553
2	81±58	111±69	60±35	51±28	37±28	343±97
2.5	109±45	146±40	77±38	33±16	34±16	400±80
3	133±33	125±41	82±37	34±17	60±36	435±89

[a]Mean± S.E

Stage 1	Deep anaesthesia	No body movement but opercular movement starts slowly
Stage 2		Regular opercular movement
Stage 3	Deep sedation	Body movements have started.
Stage 4	Light sedation	Appearance in the start of anaesthesia.
Stage 5		Reaching to balance except for very strong stimuli.

observed at 2 ml/1. A difference was found between groups when total recovery was considered (p=0.000). In our study, doses yielding recovery times below 600 s were 0.5, 2, 2.5 and 3 ml/1.

DISCUSSION

Effect of the anesthetic and sedative agents can vary according to weight and length of fish, number of the fish put into the anesthesia bath and water characteristics such as hardness, temperature and salinity (Ross and Ross, 2008). Yıldırım *et al*. (2010) reported that the fish *Cichlasoma nigrofasciatum* weighing 0.54±0.01 g entered in the deep anesthesia stage after 49±1 s at 9.75 ppm quinaldine Sulphate +0.5 ppm diazepam. However, in this study the angelfish fry, weighing 1.02-2.98 g, entered the anesthesia stage in a shorter time. Amend *et al*. (1982) showed that the angelfish (0.3-2.0 g) entered the anesthesia in 78 s at 4.0 mg/l

Etomidae and then recovered in 2400 s.

The ideal concentration must be the lowest dose concentration which enables a transition to general anesthesia in 3 min (180 s) and a full recovery in 10 min (600 s) (Ross and Ross, 2008). In our experiments all doses except for 0.5 ml/1 and 3 ml/l had total induction times below 180 s, hence these dose are recommended for use.

Clove oil shows an immediate effect on the fish even when it is used at low concentrations when compared to such chemicals as MS-222 (Keene *et al*., 1998). However, its recovery time after anesthesia is much longer than the other anesthetics. As the concentration of anesthesia increases, the time of transition to induction stage shortens (Ross and Ross, 2008). Total induction times of the fish vary between 15 and 250 s depending on the dose of clove oil. The longest time were observed in 1 and 1.5 ml/1 in the recovery stage. However, the full recovery took a time from 343 to 1085 s irrespective of the concentration density.

When results of our study are considered, it is clear that the most applicable dose for deep anaesthesia is 2 ml/1 among induction and recovery times.

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REFERENCES

AMEND, D.F., GOVEN, B.A. AND ELLİOT, D.G., 1982. Etomidate: Effective dosages for a new fish anesthetic. *Trans. Am. Fish. Soc*., **111**:337-341

BONDAD-REANTASO, M.G., SUBASINGHE, R. P., ARTHUR, J.R., OGAWA, K., CHINABUT, S., ADLARD, R., TAN, Z. AND SHARIFF, M., 2005. Disease and health management in Asian aquaculture. *Vet. Parasitol*., **132:** 249–272.

KANYILMAZ, M., SEVGILI, H., ERÇEN, Z. AND YILAYAZ, A., 2007. Karanfil Yağının Balık Anesteziği Olarak Kullanımı. *Turk Sucul Yasam Dergisi. Turkish J. aquat. Life*, **5-8:**671-680

KEENE, J.L., NOAKES, D.L.G., MOCCIA, R.D. AND SOTO, C.G., 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, Oncorhynchus mykiss (Walbaum). *Aquacul. Res.,* **29**: 89–101.

LIM, L.C., DHERT, P. AND SORGELOOS, P., 2003. Recent developments and improvements in ornamental fish packaging systems for air transport. *Aquacul. Res*., **34**, 923–935.

OZMEN, I., ATAMANALP, M., BAYIR, A., SIRKECIOĞLU, A.N., CENGIZ, M. AND CENGIZ, M., 2007. The effects of different stressors on antioxidant enzyme activities in the erythrocyte of rainbow trout (*Oncorhynchus mykiss* W., 1792). *Fres. Env. Bull.,* **16**: 922-927.

RODRIGUEZ, R., 2006. Ornamental fish: Economic, ecologic and health implications of the trade. *JAINA Boll. Inf*., **6**:6-27.

ROSS, L. AND ROSS, B., 2008. *Anaesthetic and sedative techniques for aquatic animals*, ISBN-13: 978-1405149389, 240.

SANNA-KAISA, J. AND JUKKA, S., 2004. Sustainable use of ornamental fish populations in Peruvian Amazonia. *Lyonia*, **7**: 53-59.

SMALL, B.C., 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. *Aquaculture,* **218**: 177–185

TORT, L., PUIGCERVER, M., CRESPO, S. AND PADROS, F., 2002. Cortisol and haematological response in sea bream and trout subjected to the anaesthetics clove oil and 2-phenoxyethanol. *Aquacul. Res*., **33**: 907-910.

UCAR, A. AND ATAMANALP, M., 2010. The effects of natural (clove oil) and synthetical (2-phenoxyethanol) anesthesia substances on hematology parameters of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*), *J. Anim. Vet. Adv.*, **9**: 1925-1933

WATERSTRAT, P.R., 1999. Induction and recovery from anesthesia in channel Catfish *Ictalurus punctatus* fingerlings exposed to clove oil. *J. Wld. Aquacul. Soc.*, **30**:250-255

WHITTINGTON, R.J. AND CHONG, R., 2007. Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. *Prev. Vet. Med*., **81**: 92-116

WIKIPEDIA, 2010. Pterophyllum,http://en.wikipedia.org/wiki/Pterophyllum scalare (10.09.2010)

YILDIRIM, Y.B., GENC, E., TURAN, F., CEK, S. AND YANAR, M., 2010. The anaesthetic effects of quinaldine sulphate, muscle relaxant diazepam and their combination on convict cichlid, *Cichlasoma nigrofasciatum* (Günther, 1867) juveniles. *J. Anim. Vet. Adv*., **9**: 547-550

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