JOURNAL OF THE
ARABIAN AQUACULTURE SOCIETY
Vol. 6 No 1 June 2011

Effects of Clove Oil on the Response of Blue Tilapia (*Oreochromis Aureus*) by Transportation Stress

Adel .M .A .Akar
Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt

ABSTRACT

Clove oil has become a popular fish anaesthetic for invasive fisheries research procedures, but few studies have examined the use of low concentrations of clove oil to achieve sedation for aquaculture procedures such as fish handling and transport. The present work evaluated the response of blue tilapia by transportation stress procedures, and using of clove oil as an alternative natural anaesthetic to reduce the stress effects by transportation on blue tilapia(*Oreochromis aureus*). Clove oil solutions were tested at concentrations of 0, 0.2, 1, 2 and 3 mg/L during blue tilapia transportation in plastic bags and sitting in styrfom, supplied with water and oxygen as the usual field procedures in Egypt. Results revealed that clove oil reduced some of the physiological responses such as Cortisol and glucose, which are the most common stress indicators. Also, results showed that clove oil (0.2 mg/l) can mitigate the stress response and high survival rate occurred within group in blue tilapia subjected to transport, wherever high mortality occurred within 24 hrs post transport in 3,0,2 and1 mg/l groups. Preferred to use 0.2 mg/l of clove oil when you move the blue tilapia fish for long distances.

Keywords: Blue tilapia, transport, stress, clove oil.

INTRODUCTION

Modern aquaculture practices frequently expose fish to a variety of acute stressors that have the potential to negatively affect fish performance and survival (Barton, 2000) One method commonly used to minimize or mitigate the effects of stress on fish is the use of anaesthetics (Berka, 1986). Current tilapia fishes packaging systems are characterized by very high fish loading densities and high metabolic wastes in the transport water after shipment. They focus mainly on management of the quality of transport water (Lian *et al*., 2003). Recent studies using the guppy as a model fish showed that post-shipment mortality could be reduced through enhancement off the stress resistance of the fish and hence emphases should also be placed on the preparation of the fish for transport and recovery of the fish after shipment(Lian *et al*., 2003). The mortality in fish has been reported in

many fish farms, and it is usually associated with acute stimulus as handling and transport (Kubitza, 2003). These procedures are necessary, although they elicit many physiological and biochemical responses, collectively known as the stress responses (Iwama *et al*., 1997).

Anesthetics are currently used to minimize the stress associated with aquaculture procedures. The choice of a particular anesthetic depends on the availability, cost and safety to fish and humans(Iwama and Ackerman,1994). Several anesthetics sedate fish facilitating several stressful procedures (Iversen *et al*., 2003; Pirhonen and Schreck, 2003). Clove oil is one of the alternatives. Clove oil is extracted from clove tree, and the main component is eugenol (4-allyl-2-metox-yphenol). In the past, clove oil was used as anti-septic and local analgesic for toothache (Soto and Burhanuddin, 1995). The FDA (US Food and Drugs Administration) considers clove oil as a safe substance (Anderson *et al.,* 1997), and it has been used as a food additive. Clove oil is approved for use in aquaculture facilities in Australia, New Zealand, and other Oceania countries with no withdrawal period for human consumption and release of the fishes in the environment (Kildea *et al.*, 2004). However, in North America the FDA does not approve the use of clove oil for aquaculture purposes (Sladky *et al.,* 2001 and Sinka,. and Nealb,2009). Researchers have investigated the effects of clove oil in fishes (Soto and Burhanuddin, 1995; Keene *et al*., 1998; Cho and Heath, 2000; Sladky *et al*., 2001; Tort *et al*., 2002; Woody *et al*., 2002 and Small, 2004). A study demonstrating the efficacy of clove oil in *O. aureus* broodstock . But, this work only determined the induction and recovery times to anesthesia. The use of a natural anesthetic in blue tilapia transportation would be an alternative to reduce the stress response that is certainly unavoidable, but it claims for new strategies to ameliorate this species management. Therefore, this study aimed to examin the effects of clove oil on the response of blue tilapia by to transportation stress.

MATERIALS AND METHODS

The present study was conducted at Abbassa Fish hatchery, Central Laboratory for Aquaculture Research Abbassa, Abu- Hammad, Sharkia during February 2011. Broodstock *O. aureus* were fed with commercial pellets (30 % protein), twice a day to satiety in earthen ponds . Fish average weight and length were 160.1 ± 17.3 g and 22.1 ± 2.2 cm, respectively . Fish were selected from earthen ponds and after acclimatization for 24 hrs maintained in closed fiberglass tanks with re-circulated water for 48 hrs

before air transport . The tanks were 3-m^3 capacity and the water quality parameters were: temperature 17.7 ± 0.7 ºC, oxygen 5.4 ± 0.1 mg/L and pH 7.0 ± 0.1 . A preliminary study was done to determine whether clove oil would affect cortisol response in *O. aureus* subjected to air transport. Clove oil was obtained from the local market as pharmaceutical grade product, and an alcoholic solution was prepared to evade the clove oil hydrophobic traits. One part of clove oil was diluted in 20 parts of 95 % ethanol alchol. Clove oil density was approximately 1000 mg/mL (National Toxicology Program, 2002), and final clove oil alcoholic solution was 50 mg/mL. Clear plastic bags (50 x 85 cm) were filled with water (10-L), and the respective amount of clove oil (previously diluted in alcohol) was added to obtain the following final concentrations: 0, 0.2, 1, 2 and 3 mg/L. Seventy five broodstock fish divided into five equal groups, whereas fifteen 5 fish / group were netted from the holding tanks and equally distributed to three plastic bags (5 fish / plastic bag) corresponding to approximately 80 g/L, which was used usually as a density to air transport of fish. Pure oxygen was supplied to the bags, which were sealed, placed in a transport truck and driven around for a period of 72 hours . At the end of the transportation, all the fish from each bag were sampled for posterior plasma cortisol and glucose analysis, determinations were done according to Bidinotto et al. (1997). Also, ten fish were sampled from bath of two control groups; one at the beginning of the transportationbefore and other at the end after for the same analysis of plasma cortisol and glucose . Cortisol was measured directly from plasma samples on a microplate reader ([control Y] = 450 nm). Plasma aliquots (100 ml) were disrupted in 1 mL TCA (20 % Tri chlor acetic acid) and centrifuged at 12000 g for 3 min. Liver samples were also collected, and disruption was held in alkaline conditions at 100 ºC. water temperature and dissolved oxygen were measured using YSI model 55 oxygen meter. Also, pH values were measuredby a Oreon model 710A pH meter. Water total ammonia was determined by a colorimetric method according to Gentzkow and Masen, 1942). All data are represented as mean ± SEM. The data were submitted to ANOVA, and the Tukeyes test was used to discern differences among the means ($P<0.05$).

Statistical analysis

Data were statistically analysed according to Bailey (1981). The level of significance between means for the statistical analysis is 0.05.

RESULTS

Plasma cortisol levels were increased significantly (P<0.05) in all groups related with transportation stress (Table 1). The data also showed that fish transported in bags containing clove oil 3 mg/L presented the highest cortisol values among all groups. Fish subjected to transport in bags containing 2 and 1 mg/L clove oil showed significant (p<0.05) decrease of cortisol levels than fish transported in bags with out clove oil addition 0.0 mg/l. However, these cortisol values were increased significantly (p<0.05) than both controls groups (before and after). The results of plasma glucose levels followed the same pattern of cortisol (Table 1).

Plasma glucose levels were significantly (p<0.05) higher in all fish groups related with transportation stress (Table 1). However, plasma glucose levels in fish transported in bags containing (0.2 and1 mg/L) clove oil were significantly lower than fish transported without anesthetic (0 mg/L). Plasma glucose levels in the fish groups transported without clove oil were significantly higher than control groups (before and after) (Table1)

During the transportation total ammonia increased, and pH decreased in all the bags compared with un transported group (before) (Table 2). As previously tested, clove oil addition to the water has no effect on water quality parameters**.**

Results in Table 3 showed that no fish mortality was observed in the 0.2 mg/l in course of transportation after 24 hrs. Highly significant (p<0.05) mortality occurred within 24 hrs post transport in 3,0,2 and1 mg/l group, respectively. Also, results in Table (3) showed that the group 0.2 mg/l were realized higher survival rate significantly than in all groups , even when transportation for 72 hrs.

DESCUSSION

Transport stress has been documented in several fish species. Usually the transport procedures promote changes in the physiological indicators of the stress such as cortisol, glucose, and ions (Wendelaar-Bonga, 1997). As transport is unavoidable in aquaculture, it is important to investigate strategies to minimize the physiological responces associated with this procedure. Results demonstrated that clove oil alleviated most of the measured aspects of the stress response when compared with transported fish with out anaesthetic.

The control group (transport without anaesthetic) showed an increase in plasma cortisol levels when

Table (1) :Effect of clove oil on the plasma cortisol(mg/l) and glucose (mg/l) of the blue tilapia submitted by transportation in plastic bags.

Treatment	Before	0.0	0.2	1.0	2.0	3.0	After
Cortisol	12.1 c ±1.5	26.5 a ±1.3	22.5 b ±2.1	23. b 2±1.8	26. a 1±1.5	31.4 a ±2.3	11.6 c ±1.3
Glucose	94.3 c ±3.2	167.3 a ±3.1	131.1 b ±2.2	134.1 b ±3.2	165.2 a ±3.4	179.2 a ±3.2	94.6 c ±2.6

Values in the same row having the same latter are not significant different (P<0.05).

compared with both unstressed (before) and transported fish in bags containing clove oil solution (0.2 mg/l). Although clove oil attenuated the cortisol response, it did not abolish it completely. In this respect most studies have exposed fish to higher concentrations of clove oil (10-140 mg/L) for shorter periods (1-30 minutes) (Keene *et al*., 1998; Woody *et al*., 2002; Iversen *et al*. 2003 and Small, 2004). Some of these studies have shown that the cortisol response can be prevented when fish are exposed to high doses of clove oil. Atlantic salmon demonstrated that exposure to 10 mg/L of clove oil prevented the cortisol response only during the first 10 min of exposure. After that, plasma cortisol levels significantly increased, but the values were lower (attenuated response) than fish not exposed to clove oil (Iversen *et al*., 2003). Catfish also

Table(2): ***Effect of clove oil on the water quality of the blue tilapia submitted to transportation in plastic bags.***

Treatment	before	0.0	0.2	1.0	2.0	3.0
Ph value	7.1 a ±0.2	6.9 a ± 0.1	6.7 b ± 0.2	6.7 b ± 0.2	6.7 b ± 0.3	6.6 b ±0.3
Temperature C	17.0 a ±0.3	15.0 a ± 0.2	15.2 a ± 0.1	15.0 a ± 0.2	15.1 a ± 0.2	15.1 a ±0.1
Oxygen mg/l	5.0 a ±0.2	3.2 b ± 0.1	3.5 b ± 0.2	3.3 b ± 0.3	3.0 b ± 0.2	3.1 b ± 0.2
Total ammonia mg/l	0.01 c ±0.1	0.04 b ± 0.0	0.03 b ± 0.0	0.04 b ± 0.0	0.08 a ± 0.1	0.05 b ±0.1

Values in the same row having the same latter are not significant different (P<0.05).

Table (3): ***Effect of clove oil on the survival rate(%) of the blue tilapia submitted by different transportation periods in plastic bags***

Treatment	0.0	0.2	1.0	2.0	3.0
24 hrs	66 b ± 0.5	100 a ± 0.0	80 b ± 0.0	72 b ± 0.6	60 c ± 0.0
48 hrs	20 b ± 6.0	52 a ± 0.6	50 a ± 0.6	20 b ± 0.0	20 b ± 0.0
72 hrs	Die	40 a ± 0.0	30 b ± 0.6	Die	Die

Values in the same row having the same latter are not significant different (P<0.05)..

responded in a similar way when exposed to 100 mg of clove oil/L (Small, 2004). The mechanism of clove oil affects the cortisol response is not known, but Iversen *et al*. (2003) speculated that clove oil may block the transmission of sensory information to the hypothalamus, and therefore high concentrations of anesthetics prevent the activation of the hypothalamus-pituitary-interrenal axis more effectively than lower concentrations. So the cortisol response may be prevented (Iversen *et al.*, 2003).

In the present study, using of clove oil prevented the usual increasing in plasma glucose levels associated with fish transportation stress. Similarly, Iversen *et al*. (2003) found that Atlantic salmon did not increase plasma glucose levels during 30 min exposure to different concentrations of clove oil. Cortisol is thought to be one of the mediators of the increase in plasma glucose levels seen in stressful events (Barton *et al.*, 2002). It may be possible that the lack of glucose response in fish exposed to clove oil was due to the attenuation of the cortisol response by clove oil.

It is believed that the use of anesthetics in fish transport may reduce the fish activity and the ammonia excretion through the gills. Consequently, clove oil would provide better water quality for transport, and larger amounts of fish could be transported in the same container (Kubitza, 1998 and Kubitza, 2003). However, the results of the water analysis in this study demonstrated that during transport the water quality deteriorated in all treatments, and the anesthetic addition did not attenuate the water deterioration as expected (Table 2). Water pH decreased after the 72 hrs transport in the plastic bags probably as a result of CO_2 accumulation. The pH influences the toxicity of several substances including total ammonia, which is present in the water as two forms: un-ionized (NH_3 toxic to the fish, it diffuses easily across the gills) and ionized (NH_4). At low pH, un-

ionized ammonia represents a small portion of the total ammonia (Boyd, 1982). Although in the present study total ammonia levels were increased during transportation process (Table 2), but the levels of un-ionized ammonia were decreased due to the reduction in the pH and probably were did not toxic to the fish. Furthermore, water temperature was constant avoiding another variant of ammonia toxicity. Also, results had high dissolved oxygen levels (Table 2), which was due to the fact we saturated the bags with pure oxygen. However, the over oxygen saturation condition in the bags apparently did not have any deleterious effect on *O. aureus* broodstock

In summary, this study suggests that the use of clove oil attenuated the stress response in *O. aureus* Broodsiock during transportation. Overall, from the current results. Further studies are required to understand the physiological responses of broodstock fish exposed to clove oil during other periods of transport and recoveries. Assessments of clove oil for aquaculture purposes have to be encouraged because this natural anesthetic is becoming more evident as a safe and low cost alternative.

REFERENCES

Anderson, W.G.; Mckinley, R.S and Colavecchia, M.(1997): The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Manegement*, 17 (2): 301-307.

Bailey, N. T.(1981) : Statistical Methods in Biology. 2nd ed. (Biological Science Texts).Course on Fresh-Water Fish Diseases and Intoxications. Research Institute of Fish Culture and Hydrobiology, Vodnany, 270 pp.

Barton, B.A.,(2000): Stress In: Stickney, R.R.(Ed.), Encyclopedia of Aquaculture. John wile and Sons, New York,ppp.892-898.

Barton, B.; Morgan, J.D and Vijayan, M.M.(2002): Physiological and condition-related indicators of environmental stress in fish. *In*: Adams S.M. (Ed.). *Biological indicators of aquatic ecosystem stress*. American Fisheries Society, Bethasda. p. 111-148.

Berka, R., 1986. The transport of live fish: a review. FAO Report. EIFAC Technical Paper 48. United Nations,Rome, 52 pp.

Bidinotto, P.M.; Souza, R.H.S and Moraes, G. (1997): Hepatic glycogen in eight tropical freshwater teleost fish: A

procedure for field determinations of microsamples. *Boletim Técnico do Cepta*, 10: 53-60.

Boyd, C.E.(1982): *Water Quality Management for Pond Fish Culture. Developments in Aquaculture and Fisheries Science 9*. Elsevier Science Publishers, Amsterdam. 456 p.

Cho, G.K and Heath, D.D. (2000): Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Research*, 31: 537-546.

Gentzkow, C.J and Masen, J.M. (1942): An accurate method for the determination of blood urea nitrogen by direct nesslerization. *Journal of Biological Chemistry*, 143: 531-544.

Iversen, M.; Finstad, B.; McKinley and Eliassen, R. (2003): The efficacy of metomidate, clove oil, aqui-s and benzoak as anesthetics in atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. *Aquaculture*, 221: 549-566

Iwama, G. and Ackerman, P. (1994): Anaesthetics. In: Hochachka, P.; Mommsen, D. (Eds.). *Analytical techniques in biochemistry and molecular biology of fishes*, 3: 1-15. Elsevier Science, Amsterdan

Iwama, G.; Pickering, A.D.; Sumpter, J.P. and Schreck, C.B. (1997): *Fish stress and health in aquaculture*. Cambridge University Press, 1 ed. Cambridge, 545 p.

Keene, J.L.; Noakes, D.L.G.; Moccia, R.D. and Soto, C.G. (1998): The efficacy of clove oil as an anesthetic for rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, 29 (2): 89-101.

Kildea, M.; Allan, G. and Kearney, R. (2004): Accumulation and clearance of the anesthetics clove oil and Aqui-S^{TM} from the edible tissue of silver perch (*Bidyanus bidyanus*). *Aquaculture*, 232: 265-277.

Kubitza, F. (1998): Técnicas de transporte de peixes vivos. Aqua & Imagem. Campo Grande MS. 44p.

Kubitza, F.(2003): Amenizando as perdas de alevinos após o manejo e transporte. *Panorama da Aqüicultura*, 13: 15-25.

Lian, C . L ; Philippe, D. and Patrick . S.(2003): Recent developments and improvements in ornamental fish packaging systems for air transport. Aquaculture Research, 34, Issue, 11: 923-935.

National Toxicology Program. (2002): Clove oil. Captured on May 23. Online. Available on the Internet,http://ntserver.niehs.nih.gov/

Pirhonen, J. and Schreck, C.B.(2003): Effects of anesthesia with MS 222, clove oil and CO2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). *Aquaculture*, 220 (1-4): 507-514.

Sinka, T. D. and Nealb. W. (2009): Stress response and posttransport survival of hybrid striped bass transported with or without clove Oil. North American Journal of Aquaculture,71:267-275.

Sladky, K.; Swanson, C.; Stoskopf, M.; Loomis, M. and Lewbart, G. (2001): Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetic in red pacu (*Piaractusbrachypomus*). *American Journal of Veterinary Research*, 62(3): 337-342.

Small, B.C. (2004): Effect of isoeugenol sedation on plasma cortisol, glucose and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. *Aquaculture*, 238: 469-481.

Soto, C. and Burhanuddin, S. (1995): Clove oil as a fish anaesthetic for measuring length an weight of rabbitfish (*Siganus lineatus*). *Aquaculture***,** 136: 149-152.

Tort, L.; Puigcerver, M.; Crespo, S. and Padrós, F. (2002): Cortisol and haematological response in sea bream and trout subjected to the anesthetics clove oil and 2-phenoxyethanol. *Aquaculture Research*, 33: 907-910.

Urbinati, E.; Abreu, J.; Camargo, A. and Parra, M. (2004): Loading and transport stress of juvenile matrinxã (*Brycon cephalus*, Characidae) at various densities. *Aquaculture*, 229: 389-400.

Wendelaar-Bonga, S.E. (1997): The stress response in fish. *Physiology Review*, 77: 591-625.

Woody, C.A.; Nelson, J. and Ramstad, K. (2002): Clove oil as an anaesthetic for adult sockeye salmon: field trails. *Journal of Fish Biology*, 60(2): 340-347.

تأثير زيت القرنفل علي استجابة البلطي الآزرق (الآوريا) لاجهاد النقل

عادل محمد عبدالحميد عكر

المعمل المركزي لبحوث الثروه السمكيه بالعباسه شرقيه

أصبح زيت الفرنفل مخدر شعبي واسع المجال للابحاث العمليه في الآسماك لكن الدراسات التي اجريت علي معرفة التركيزات المناسبه لعمليات الاستزراع المختلفه(مسك ومعاملات الاسماك ونقلها) قليله . هذا العمل يقيم مدي استجابة البلطي الآزرق للاجهاد عند اجراء عمليات النقل وأستخدم زيت كمخدر طبيعي القرنفل لتقليل الاستجابه للاجهاد والتركيزات التي استخدمت من زيت القرنفل في هذه التجربه : صفر، ٠,٢ ،١ ، ٢ و٣ ملجم/لتر وذلك اثناء نقل البلطي الآزرق داخل أكياس بلاستيكيه بها ماء واكسيجين وموضوعه في علب فوم ومقفله تماما كما هو متبع في مصر .

النتائج اوضحت ان زيت القرنفل قلل من الاستجابه لبعض العمليات الفسيولوجيه مثل الكورتيزول والجلوكوزحيث انهما مؤشران رئيسيان علي الاجهاد.ايضا ا ظهرت النتائج ان المجموعه ٠,٢ ملجم / لتر كانت الاسماك ذات نشاط وحيويه واضحه مع ارتفاع معنوي في الحيويه و النشاط ، كذلك وجدت معدل نفوق عالي بعد ٢٤ ساعه من النقل في المجموعات ٣، صفر، ٢ ، و١ ملجم/لتر.

لذا ينصح عند نقل اسماك الآوريا لمسافات طويله نستخدم زيت القرنفل كماده طبيعه امنه وبتركيز ٠,٢ ملجم / لتر.