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RESEARCH ARTICLE

IMPACT OF DIFFERENT TYPES OF ANESTHESIA WITH DIFFERENT LEVEL ON SOME CHARACTERISTICS OF COMMON CARP (*CYPRINUSCARPIO* L.)

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ABSTRACT

Handlings and transport of fish are the most stressful procedures in aquaculture. The anesthetic efficacy of two types of natural anesthetics in both forms of powder and oil were evaluated on young common carp (*Cyprinus carpio* L.) with the mean weight of 60±10g for handling and health management experiments. These works were conducted in the laboratory of fish research in the Department of Animal production, Faculty of Agricultural Sciences at University of Sulaimani. The study included three experiments. In the first experiment, safety concentrations of anesthetics type and concentration were assessed by using two types of clove and mustard anesthetics in both forms of powder and oil; each with three replicates for measuring induction times to anesthesia. The induction time of C. carpio which decreased with increasing concentrations of clove powder, the induction time was less than three minutes for a dose of 400mg/L, which was 137.11 sec. was significantly different (P<0.05) from the other dosages (200 and 300 mg/L). As the concentrations of clove powder increased the ventilation rate in stage I, II and III of anesthesia and recovery increased significantly (P<0.05) compared to the control.

INTRODUCTION

Anesthesiais a biological reversible state induced by an external agent, which results in the partial or complete loss of sensation or loss of voluntary neuromotor control, through chemical or non chemical means(Summerfelt and Smith, 1990). Anesthesia is frequently applied in aquaculture being a valuable tool that helps to minimize fish stress and to prevent physical injuries to fish while handling them during routine practices, for example, Anesthesia is required for measuring or weighing fish, sorting and tagging, administrating vaccines, live transport, sampling for blood or gonadal biopsies and collecting of gametes, surgical procedures, to cite some of the main applications (Maricchiolo and Genovese, 2011). When choosing anesthetics, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment and the choice may also depend on the nature of the experiment and species of fish (Mylonas *et al.*, 2005).When the fish is removed from the anesthetic, the recovery should be rapid, the anesthetic should be effective at low doses (Coyle*et al.*, 2004). Dry clove powder (Carnation flowers buds) is a common medicinal plant available in all medicinal plant stores, and is not expensive, thus, it is often used for fish anaesthetizing in research, biometry and injection instead of clove oil, but to date, not enough information exists on its effects on stress response (Hoseini, 2011). Clove oil is derived from the stem, leaves and buds of the *Eugenia caryophyllata* tree (Iversen *et al.*, 2003).

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It has several advantages over other anesthetic agents in fishery research, assessment studies and aquaculture applications. Clove oil is organic, hence no withdrawal period is required for fish intended for human consumption and another advantage of clove oil is that it does not pose a chemical health hazard to the user (Bressler and Ron, 2004). 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). Mustard contains an essential oil (allylisothiocyanate) which, when applied to the outside of the body, increases the circulation and thus helps the elimination of poisons. This makes it of great value in treating a number of complaints, from a simple chill to rheumatism.

Externally, mustard is often applied as a poultice or pack (for example, to ease bronchitis, neuralgia or toothache) but it is also available as an ointment (Minter, 2008). Various genera of this plant, including more than 200 wild and 40 cultivated species are available in the U.S. and Canada. From an economical and practical point of view, three main species are known worldwide, namely yellow mustard, brown mustard and oriental mustard (Milani*et al.,* 2014). Fish are easily stressed by handling and transport and stress can result in immunosuppression, physical injury, or even death. So anesthetics are used during transportation to prevent physical injury and reduce metabolism (DO consumption and excretion). They are also used to immobilize fish so they can be handled more easily during harvesting, sampling and spawning procedures. According to all that the objectives of the project were evaluate the anesthetic effects and safe use of natural agent such as clove and mustard in health treatments research and determine the stress response of common carpto natural anesthesia.

MATERIALS AND METHODS

Common carp fish were chosen to evaluate the efficacy of two anesthetic in the two form (powder and oil) approaches in this study. Common carp were chosen for the study due to their high abundance in Kurdistan, marked tolerance to culture conditions, and the only species that reared in Iraq and Kurdistan. Also have agood survival rates and a highly tolerant for aquarium conditions, making them prime species for the experimental design of this study. All fish used in this study were held in aquarium tanks located at the fish lab in Animal Production Department, Faculty of Agricultural Sciences, University of Sulaimani. Aquaria was equipped with aeration stone consisted of large glass tanks (100cm x 50cm x 60 cm) containingapproximately 150 liters ofwater, Study fish were acclimated for a minimum of two weeks prior to the outset of experiments, ensuring that they had sufficiently recovered from possible capture-related or transport stress. The fish were unfed for 24 hours prior to the experiments (Brown, 2011).

Aquaria water quality parameters were monitored throughout changing of the water biweekly duration of the study. Fish were held for a period of two months following the experiments to assess long-term mortality. In order to reduce observer variability, the same observer monitored progression through the stages of anesthesia and recovery throughout all of the experiments. Each fish in each replicate treated alone. Each anesthetic bath was individually prepared before use (anesthetic and recovery aquaria was 30-L glass tank), water was replaced between each fish treated, and the tank was rinsed before preparation of the next concentration. Each fish was individually monitored from the time of immersion in the anesthetic bath to determine the time required to reach each stage of anesthesia up to stage three, which is characterized by loss of sensation, total loss of swimming motion, and total loss of equilibrium. Induction and recovery times were measured with a video recorder to the nearest second. Following the induction into stage Ш each fish was weighed and transferred to a recovery aquaria that had been filled with aerated freshwater at the same time of preparation the anesthetic baths. In the recovery tub the fish were monitored continuously to determine time to full equilibrium. Recovered fish were promptly returned to their holding system and monitored for survival at 24 and 72 hours post-experiment. Several concentrations of two anesthetic agents in two forms (powder and oil) were tested. Only one anesthetic type was tested at a time.

By using a video recorder, time was recorded for:

- Stage I anesthesia: (partial loss of equilibrium, some body movements and reduced reaction to external stimuli).
- Stage II anesthesia (total loss of equilibrium, no body movements, no reaction to external stimuli).
- Stage III anesthesia as in stage II with decrease in opercula rat.
- Recovery time (Recovery of equilibrium, body movements and response to external stimuli)

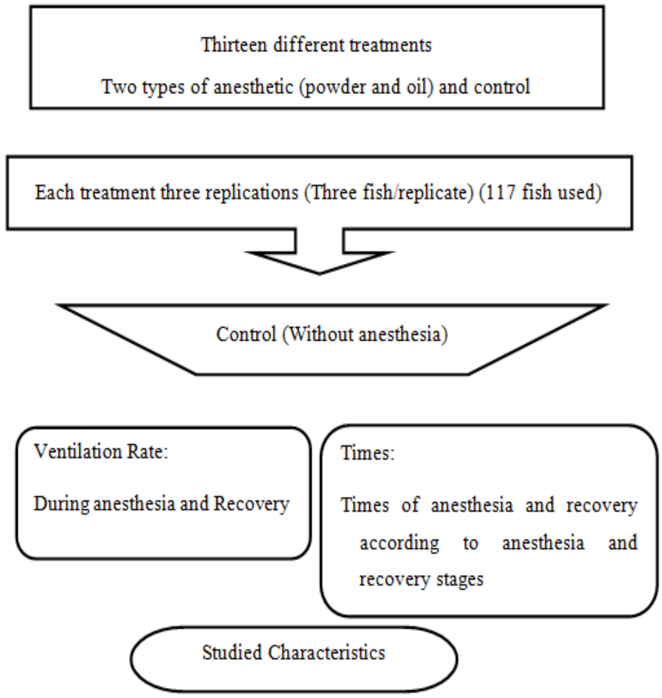


Figure 1. Experimental design of first experiment which includes different types of anesthesia with different concentrations and control

The maximum exposure time to each anesthetic agent was 10 minute. If no anesthetic effect was observed during this 10 min, the concentration of anesthetic was considered insufficient. Individual fish weight (g) were measured in <1 minuet as soon as stage III anesthesia was reached and before fish were moved to the recovery tank (containing clean fresh aerated water). This experiment was representedtwo anesthetic agents with two forms (powder and oil) by three concentrations with 3 replicates. Each aquarium was stocked with three fish. The aquaria (replicates) were randomly allocated to minimize the differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the aquaria.

The clove powder used in the experiment was obtained from local markets in Sulaimani that naturally used for other human purposes. The clove oil that used was EUROSTAR* - Clove oil, clove oil IP 40% base up to 100%, produced in India under technical assistance with Ireland. Mustard powder used was also obtained from local markets in Sulaimani, while the oil obtained from medical plant stores that naturally and locally extracted from the mustard seed. Anesthetic agent's concentrations:The powders concentrations started with 50mg/L and then 100mg/L but during the observation only stage I of anesthesia (sedation) was occurred (for clove) for 24 hours, accordingly the experiment started with 200mg/L. The oils were started with 0.5ml/L and for the same reason that occurred with powders, the oils concentrations for treatments started with 1ml/L.

The following treatments were conducted:

1. Anesthetic: 200, 300 and 400 mg/L of clove powder.
2. Anesthetic: 200, 300 and 400 mg/L of mustard powder.
3. Anesthetic: 1, 1.5 and 2 ml/L of mustard oil.
4. Anesthetic: 1, 1.5 and 2 ml/L of clove oil.

Table 1. Effect of clove powder on anesthesia and recovery stages (Time/sec.) at three stages of anesthesia and recovery of common carp (Mean ± SD)

Clove Powder	Anesthesia stages Time (sec.)			Recovery stages Time (sec.)		
Stages/ Con.	I	II	III	I	II	III
200 mg/L	71.42±0.16^{a}	399.42±0.1^{a}	452.85±0.13^{a}	37.42±0.41^{b}	99±0.43^{a}	199±0.13^{b}
300 mg/L	61.77±0.42^{ab}	129.55±0.55^{b}	186.55±0.32^{b}	57.5±0.38^{ab}	108.37±0.26^{a}	255.37±0.18^{a}
400 mg/L	49.88±0.21^{c}	96.44±0.35^{c}	137.11±0.26^{c}	80±0.38^{a}	111.66±0.23^{a}	290±0.18^{a}

Mean values with different superscripts within a same column are different significantly (*P<0.05*)

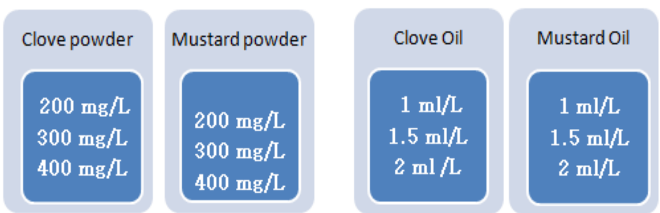
Table 2. Effect of clove powder on ventilation rate of common carp (Ventilation rate/15 sec.) at three stages of anesthesia and recovery (Mean ± SD)

Clove Powder	Anesthesia stages Ventilation rate /15 sec.			Recovery stages Ventilation rate /15sec.		
Stages/ Con.	I	II	III	I	II	III
Control	17.5 ±0.1^{b}	17.5 ±0.1^{b}	17.5 ±0.1^{b}	17.5 ±0.1^{c}	17.5 ±0.1^{c}	17.5 ±0.1^{b}
200 mg/L	28 ±0.06^{a}	20 ±0.09^{b}	19.28±0.05^{b}	22.85 ±0.16^{b}	22.57 ±0.12^{b}	21.57 ±0.1^{a}
300 mg/L	26.66 ±0.1^{a}	23.33 ±0.1^{a}	22.55±0.11^{a}	27.62 ±0.08^{a}	26.62 ±0.08^{a}	23.75 ±0.13^{a}
400 mg/L	28.22 ±0.04^{a}	24.33 ±0.1^{a}	23.33±0.06^{a}	27.88 ±0.05^{a}	27.11 ±0.06^{a}	23 ±0.06^{a}

Mean values with different superscripts within a same column are different significantly (*P<0.05*)

Statistical analysis:First experiment was conducted using thecompletely randomized design CRD, means with significant differences were compared by Duncan's (1955) multiple range tests, according to *P<0.05* significance. Statistical analysis results are shown as mean values in tables. The statistical calculations of the results were completed using XLSTAT. Pro One way (ANOVA). Different letters were given to different treatments. The model of analysis was as follows:

$Y_{ij} = \mu + T_i + E_{ij}$
μ = The overall mean
T_i = The effect of treatment
E_{ij} = The random error



RESULTS AND DISCUSSION

All fish used in the present study were healthy as indicated by their activity and external appearance. No mortality was observed during the acclimatization period. Furthermore, no deaths or other adverse effects were occurred within 48 h following recovery from anesthesia for the short term and long term periods of mortality. Blood sampling was performed while the fish were still deeply anaesthetized. All the fish exposed to different treatments recovered well and returned to normal behavior with response to feeding, surfacing activity, swimming and respond to external stimuli after the anesthetic treatment. Table 1 showed the induction time of C. carpio whichdecreased with increasing concentrations of clove powder. At 400mg/L, the time to reach a complete anesthesia (stage III) (137.11 sec.) was significantly different (P<0.05) from the other dosages (200 and 300 mg/L). There was a clear converse relation of decreasing induction time with increasing concentration of the anesthetic, with the longest induction times for fish in the group exposed to 200mg/ Lof clove powder (452.85 sec.) and the shortest for fish exposed to 400mg/L(137.11 sec.). Also recovery time was negatively correlated to induction time as observed in Table 1., as well. These findings are in agreement with many studies (Gomes *et al*., 2001; Al-Jashami *et al*., 2002; Cunha and Rosa, 2006 and Park *et al.,* 2009) When using anaesthetics, it is expected that there will be a strong negative correlation between the applied concentration and the time required to induce anaesthesia to the desired stage, as observed previously for several fish species (Weber *et al*., 2009; Pawar*et al*., 2011 and Kamble*et al*., 2014).

The recovery time was directly proportional with increasing doses of clove powder. The longest recovery time (290 sec.) was observed at 400mg/L and the shortest time to reach total recovery stage (199 sec.) was detected at 200mg/L as showed in Table 1. Longer recovery time with the increased anesthetic dosage has been reported in (*Hippocampus kuda*Bleeker) (Sajan*et al*., 2012 and Mercy *et al*., 2013). Mylonas *et al.* (2005) pointed out that long exposure to anesthetic led to more anesthetic absorption by fish which, in turn, lengthened the recovery time. The statement is not completely trustworthy otherwise this could be proved with our result becauseif it could be said that longer exposure to low concentration of the anesthetic leads to more anesthetic absorption. It could be said that short exposure to high anesthetic concentration do it as well. On the other hand, Weyl *et al.* (1996) pointed out that compared with anesthesia duration, anesthetic concentration plays more important role on the recovery time. It is believed that the independence of the recovery time from the anesthesia duration, as a resultof that anesthetic, is taken up by the fish through a concentration gradient at the gill interface.Therefore, when equilibrium level established between the gill and anesthetic solution, no further anesthetic will take up by the fish, and during recovery, the anesthetic agent is leaked through such gradient. Therefore, the recovery time is controlled by the anesthetic concentration but not duration of anesthesia (Weyl*et al*., 1996). 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). The effective dosage that induces anesthesia in *C. carpio* is 400mg/L as evidenced by the superior survival at the end of the short term and long term periods of mortality. The results obtained in the present study indicated that clove powder is an effective and safe anesthetic for the handling and transport of *C. carpio* brood stock. The ventilation rate of common carp at the different stages of anesthesia exposed to clove powderwas presented in Table 2, as the concentrations of clove powderincreased the ventilation rate in stage I, stage II and stage III of anesthesia and recovery increased significantly ($P<0.05$) compared to the control (17.5 /15 sec.), and its agree with results of study done by Filiciotto*et al.* (2012). This may be due to the rapid change in the environment of the fish as reported by Al-Obaedy*et al*., (2013). Results obtained in the present study comply with the proposed objectives in which the clove powder was considered as the best effective anesthetic agents for the induction of anesthesia in *C. carpio* with the highest concentration according to their concentration that related to the recovery time, and rapid induction, therefore clove powder might be useful in fish management for short-term work such as handling and sorting rather than longer interferences, such as prolonged transport stress.

REFERENCES

Al- Obaedy, H.J., Al-Biaty, M.N., Daham, H.M., Sabry, S.W. and Al-Jashami, K.J. 2013. Some characteristics of clove (*Eugenia caryophyllata*) used for anaesthesia in some fish carp species. *Al-Nahrain University Journal of Science,* 16(3): 28-32. (In Arabic)

Al-Jashami, K.J., Al-Biaty, M.N., Al-Obaedy, H.J. and Al-Jebury, A.G. 2002. New anesthetic for common carp(*Cyprinus carpio* L.) fish. *Iraqi Journal Agriculture,* 7(1): 111-116. (In Arabic)

Bressler, K. and Ron, B. 2004. Effect of anesthetics on stress and the innate immune system of gilthead seabream (*Sparusaurata*). *Israeli Journal of Aquaculture – Bamidgeh,* 56(1): 5–13.

Brown, L.A. 2011. Anaesthesia for fish.*Vietfish,* 8 (2): 68-70.

Coyle, S.D., Durborow, R.M. and Tidwell, J.H. 2004. Anesthetics in Aquaculture.*Southern Regional Aquaculture Center Publication* No. 3900.

Cunha, F.E.A. and Rosa, I.L. 2006. Anaesthetic effects of clove oil on seven species of tropical reef teleosts.*Journal of Fish Biology,* 69: 1504–1512.

Duncan, D.B. 1955. Multiple Rang and Multiple F-test. *Biometrics*, 11:1-24.

Filiciotto, F., Buscaino, G., Buffa, G., Bellante, A., Maccarrone, V. andMazzola, S. 2012. Anesthetic Qualities of Eugenol and 2-Phenoxyethanol and Their Effect on Same Haematological Parameters in Farmed European Sea Bass (*Dicentrarchuslabrax* L.).*Journal of Animal and Veterinary Advances,* 11 (4): 494-502.

Gomes, L.C., Chippari-Gomes, A.R. and Lopes, N.P. 2001. Efficacy of benzocaine as an anesthetic in juvenile Tambaqui(*Colossomamacropomum*).*Journal World Aquaculture Society,* 32(8): 426–431.

Hoseini, S.M. 2011. Efficacy of clove powder solution on stress mitigation in juvenile common carps, (*Cyprinus carpio* L.).*Comparative Clinical Pathology,* 20(4): 359–362.

Iversen, M., Finstad, B., MacKinley, R.S. and Eliassen, R.A. 2003. The efficacy of metomidate, clove oil, Aqui-S™ and Benzoak® as anaesthetics in Atlantic salmon (*Salmosalar L.*) smolts, and their potential stress-reducing capacity. *Aquaculture,* 221(1-4): 549–566.

Kamble, A.D., Saini, V.P. and Ojha, M.L. 2014. The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity.*International Journal of Fauna and Biological Studies*, 1(6): 01-06

Maricchiolo, G. and Genovese, L. 2011. Some contributions to knowledge of stress response in innovative species with particular focus on the use of the anaesthetics.*The Open Marine Biology Journal,* 5(1): 24–33.

Mercy, T.A., Malika, V. and Sajan, S. 2013. Use of tricainemethanesulfonate (MS-222) to induce anaesthesia in *Puntius denisonii* (Day, 1865) (*Teleostei: Cypriniformes: Cyprinidae*), a threatened barb of the Western Ghats. *India Journal Threatened Taxa*, 5(3): 4414-4419.

Milani, M.A., Mizani, M., Ghavami, M. and Eshratabadi, P. 2014. Comparative analysis of antimicrobial characteristics of mustard paste and powder in mayonnaise.*European Journal of Experimental Biology,* 4(2): 412–418.

Minter, Sue. 2008. Mustard: A Condiment and a Medicine. *North Carolina Testing Program,* 1- 4

Mylonas, C.C., Cardinaletti, G., Igelaki, I. and Polzonetti, M. 2005. Comparative efficacy of clove oil and 2-phenoxyethanol as anaesthetics in the aquaculture of European sea bass (*Dicentrarchuslabrax*) and gilthead sea bream (*Sparusaurata*) at different temperatures. *Aquaculture Research,* 246(4): 467-481.

Park, M.O., Im, S.Y., Seol, D.W. and Park, I.S. (2009).Efficacy and physiological responses of rock bream, (*Oplegnathusfasciatus*) to anesthetization with clove oil.*Aquaculture,* 287: 427–430.

Pawar, H.B., Sanaye, S.V., Sreepada, R.A., Harish, V., Suryavanshi, U., Tanuand Ansari, Z.A. 2011. Comparison of efficacy of four anaesthetic agents in the yellow seahorse (*Hippocampus kuda*Bleeker, 1852).*Aquaculture,* 311(1-4): 155-161

Ross, L.G. and Ross, B. 2008. Anaesthetic and sedative techniques for aquatic animals.3rd Edition.*Blackwell publishing*.

Sajan, S., Mercy, T.V.A.andMalika,V. (2012).Use of an eco-friendly anaesthetic in the handling of *Puntius denisonii*(Day, 1865)-an endemic ornamental barb of the Western Ghats of India.*Indian Journal Fish*, 59(4): 131-135.

Summerfield, R.C. and Smith, L.S.1990. Anesthesia, surgery and related tecniques. In: Schreck CB, Moyle PB, Eds. Methods in Fish Biology. Bethesda. *American Fisheries Society*, pp. 213-272.

Weber, R.A., Peleteriro, J.B., Garcia Martin, I.O. and Aldegonde, M. 2009. The efficacy of 2-phenoxyethenol metomidate, clove oil and MS-222 as anaesthetics agents in the *Senegalese Sule. Aquaculture,* 288(2):147-150.

Weyl, O., Kaiser, H. and Hecht, T. 1996. On the efficacy and mode of action of 2-phenoxyetiianoi as an anaesthetic for goldfish, (*Carassius auratus* L .), at different temperatures and concentrations. *Aquaculture Research,* 27(1): 757–764.

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