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Effect of fish weights and clove powder levels on anesthesia and recovery time in common carp Cyprinus carpio L.

ABSTRACT

This study aimed to demonstrate how different fish weights and clove powder concentration levels together influence anesthesia and recovery time in Cyprinus carpio. Two different body weights classes of common carp (45±5g and 95±5g) were subjected to three concentrations of clove powder (200, 300 and 400mg/L) each replicated four times. At each treatment combination, the anesthetic induction and recovery times were recorded, fish held for a period of one week to two months following the experiments to assess short-term and long-term mortality. Significant differences (P<0.05) appeared in the effect of combination between the different levels of clove powder (mg/L) with the two different weight common carp (C. carpio L.) on anesthesia time, but there was no difference in recovery time between the two size groups. The induction time was less than one minute for doses of 300 and 400 mg/L in lower fish weight 45±5g while in 200mg/L dose with 95±5g of fish weight it needed more than 9 minutes. At all tested concentrations, induction time was significantly weight-dependent (p< 0.05), but the recovery time was not. Significant alterations were recorded in the hematological variables of the fish (RBC, HB, MCH and WBC), no mortality was observed. This study has demonstrated that clove powder can be safely and effectively use in the anesthesia of common carp and anesthesia time affect by doses of clove powder with fish weight.

INTRODUCTION

Fishes face many possible stressors during aquaculture and stocking operations, in the transportation, handling procedures, an extremely crowded and restricted farming environment, possible air exposure, variation in water quality and all causes that can lead organisms to become stressed and have significant effects on fish physiology and survival (Husen and Sharma, 2014). In both capture and culture processes, fish stress and mortality can result in significant resource and productivity losses (Davis, 2010).

Anesthesia is a biological reversible condition caused by an external agent, which results in the partial or total loss of sensation or loss of voluntary neuromata control, through chemical or non-chemical ways (Gholipourkanani et al., 2011). Anesthesia is also used in aquaculture as a valuable method to reduce fish stress and avoid physical injury to fish when handling them during routine practices, such as anesthesia for measuring fish weighing, sorting and marking, vaccine administration, live transport, blood or gonadal biopsy sampling, gametes collection and to cite some of the main applications (Maricchiolo and Genovese, 2011).

Organic farming looks for alternatives to those chemicals that are currently being used in aquaculture, and anesthetics are one such important input, as a result, different chemical anesthetics were studied to compare their effectiveness with natural products (Taylor and Roberts, 1999).

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According to Ross & Ross (2008), anesthesia is acquired by immersing the fish in an anesthetic solution that is drained via the gills and transported to the central nervous system by arterial blood. In general, an optimal anesthetic should cause anesthesia in less than 6 minutes, allow for a rapid recovery in 10 minutes or less, be non-poisonous to fish and humans, and be inexpensive (Mylonas et al., 2005).

Clove powder is produced from the Eugenia aromatic clove tree's dried flowers and flower stalks. Since they are a natural anesthetic (due to the eugenol oil) and a popular medicinal plant that is readily available in all medical plant stores and is inexpensive, their aqueous solution is commonly used for fish anesthesia in the study, handling, and injection instead of clove oil (Hoseini, 2011; Hassan, 2016).

Clove powder (Eugenia caryophyllata) was used by Al-Jashami et al. (2002) as a new anesthetic for common carp C. carpio, different anesthetic concentrations ranged from 120-230 mg/L for fry, fingerling and mature fish. They found that 190 mg/L of clove solution was considered an effective and suitable anesthetic for common carp. This research aims to evaluate and illustrate the relation between various concentrations of clove powder anesthetic with two various sizes of common carp, as well as their impact on some blood parameters.

Material and Methods

This experiment is described by two different weights of Cyprinus carpio (45±5g and 95±5g) with three concentrations of clove powder (200, 300 and 400mg/L) as an anesthetic agent (4 replicates each). All of the fish included in this research were held in the fish lab's aquarium tanks in the department of animal science, college of agricultural engineering sciences, university of Sulaimani/ Iraq. Studied fish were acclimatized for at least 2 weeks before the start of the experiments, ensuring that they recovered adequately from potential capture-related or transport stresses. The fish had not been fed for 24 hours before the experiments.

Following the trials, the fish were held for one to two months to determine short- and long-term morbidity. Each anesthetic bath was prepared separately before use, and water was changed between replication treatments, and the tank was rinsed before preparation of the next treatment. From the moment of immersion in the anesthetic bath, each fish was individually monitored to determine the time needed to achieve stage three of anesthesia, which is characterized by total lack of equilibrium, with slow opercula movement.

The induction and recovery times were recorded to the nearest min using a digital clock. Each fish was quickly moved to a recovery aquarium after induction into the process, which was full of aerated water at the same time as the baths of anesthetics is prepared. The recovered fish were immediately restored to their holding system and were checked for survival at 24 and 72 h post-experiment.

Plan of the Experiment

T1: 200 mg/l of clove powder + 45±5g of common carp
T2: 300 mg/l of clove powder + 45±5g of common carp
T3: 400 mg/l of clove powder + 45±5g of common carp
T4: 200 mg/l of clove powder + 95±5g of common carp
T5: 300 mg/l of clove powder + 95±5g of common carp
T6: 400 mg/l of clove powder + 95±5g of common carp
Totally 96 fish
Each treatment four tanks as replication (4 fish/tank)

Hematological Parameters

The suction of the caudal peduncle was used to collect blood samples from each of the various groups of fish. Whole blood samples were gathered in heparinized vials for the determination of some blood indices. All blood tests were done by using a hematological analyzing device type ACCENT 200 Poland’s origin.

The parameters calculated were as follows: Erythrocyte counts (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hemoglobin (Hb), Leukocyte count (WBC) and % granulocytes, lymphocytes and monocytes

Water Quality

The temperature of the media was measured with a thermometer, the pH of the media was calculated with a Germane pH meter, and the dissolved oxygen was determined with a Dissolved oxygen meter (OAKTON Singapore).

Methods of data analysis

The experiment was conducted using the (Factorial CRD) general linear models (GLM) procedure of XLSTAT. Pro. 7.5 two way (ANOVA). The treated groups' means were compared using Duncan's test. First, the interaction hypothesis must be checked, and only if the interaction result is not significant can the hypothesis of the main effect being tested. Checks for the main effects are pointless if the interaction is significant (Kaps and Lamberson, 2004).

Results and Discussion

In the current study, it was found that clove powder is an effective anesthetic for the common carp. During the induction period, fish behaved normally and remained calm and did not swim quickly, which was a positive sign of their welfare. Also, no mortality during the 24-h post-recovery period was observed.

The physical and chemical properties of the water quality during the experiment were as follows: the water temperature varied from (27-28 °C) and the concentration of dissolved oxygen in the tanks measured ranged from 5-6 mg/l and the pH value of the water ranged from 7 to 8.

Anesthesia and Recovery Time

According to the results observed in table (1), common carp start to stimuli to the anesthesia in the first stage and after that a losing their respond and abnormal swimming and finally in stage 3 total loss of equilibrium without any movement.

Table (1) Behavioral observation of common carp during various stages of anesthesia and recovery

Stages of anesthesia	Observed behaviours
0	Normal
I	Fish lose their equilibrium and swim in an unbalanced way
II	Fish are mildly relaxed on the lateral side, with slow opercula movement
III	Fish are mildly relaxed on the lateral side, with slow opercula movement
Stages of recovery	Observed behaviours
I	There are no body movements, opercula movements begin
II	Starting with normal opercula and body movements
III	The balance of the fish has been restored

The anesthetic effectiveness of clove powder on African catfish fingerlings was investigated by Okey et al. (2018), who found some behaviors in the exposed fish, including opercular motion acceleration, partial loss of reaction to an external stimulus, and loss of equilibrium due to the anesthetic effect of clove powder on the central nervous system.

According to Summerfelt & Smith (1990), Anesthetics focus on the central nervous system of organisms exposed to higher doses, causing relaxing effects, lack of equilibrium, mobility, consciousness, and eventually loss of reflex action. Awareness of the ideal and optimal anesthetic concentration for different fish weights is important since inadequate anesthetic concentrations can lead to adverse effects such as stress (Hoseini and Ghelichpour, 2011), even fish death.

Table (2) indicates significant differences ($P<0.05$) in the effect of combination between the different amounts of clove powder (mg/l) with the two different weight common carp (C. carpio L.) on anesthesia and recovery time. In 200mg/l of clove powder with (95±5g) fish weight, the longest duration of anesthesia (9.44 min.) was shown, while the shortest time (0.47 and 0.56 min.) was observed in 300 and 400mg/l of clove powder with the lowest fish weight. Recovery time was not different significantly ($P<0.05$) between the two size classes in all three concentrations of clove powder.

Table (2) Effects of two different weights common carp with three levels of clove powder (mg/L) on anesthesia and recovery time (mean ± SD).

Groups	Con. W.	Clove powder 200 mg/L	Clove powder 300 mg/L	Clove powder 400 mg/L
Anesthesia Time (min.)	Fish Weight (45±5g)	7.18 ± 0.1[b]	0.47 ± 0.1[c]	0.56 ± 0.1[c]
	Fish Weight (95±5g)	9.44 ± 0.2[a]	1.23 ± 0.1[c]	1.1 ± 0.1[c]
Recovery Time (min.)	Fish Weight (45±5g)	4.55 ± 0.4[a]	6 ± 0.2[a]	4.33 ± 0.1[a]
	Fish Weight (95±5g)	4.1 ± 0.3[a]	6.1 ± 0.1[a]	4 ± 0.2[a]

Mean values with different superscripts within the same groups are different significantly (P<0.05)

As the concentration of clove powder increased, as approved by Farahi et al. (2011) and Hassan (2016), the time for anesthesia decreased. Some individual variations in induction time were obvious. This may be the result of increased clove absorption through the gills. The time of induction was related to the size of the fish, with more time taken to anesthetize larger fish, and this may be related to a higher rate of anesthetic uptake through the gills in smaller fish compared to larger fish, and this is consistent with the findings of the current study.

Jahanbakhshi et al. (2012) studied the efficacy of 2-phenoxyethanol as an anesthetic for two sizes of Persian sturgeon, Acipenser persicus (100 and 400 g). 2-phenoxyethanol failed to induce deep anesthesia in small fish at concentrations 0.1 and 0.3 ml/L, but at a dosage of 1.1 ml/L, all of the fish were anesthetized within 3 minutes of exposure. Larger fish were not anesthetized at low concentrations of 2-PE (0.1 ml/L), but at a dose of 0.9 and 1.1 ml/L, all fish were anesthetized within 3 minutes, this showed that bodyweight of fish has an effect on anesthesia time at a lower concentration of anesthetic agent but the effect of body weight would disappear with increasing concentration and the effects of anesthetic doses would be dominant, the findings of this study agree with that.

The metabolic rate and oxygen intake divided by bodyweight of a fish decrease as the fish's body weight increases, meaning larger fish intake less oxygen compared to their body size than smaller fish (Zahl et al., 2011), this seems to be the case of induction time weight-dependent when clove powder is used as an anesthetic in the current study.

Reports on the value of body size for anesthetic effects vary. Increased efficacy and sensitivity with increasing body size have been observed in white seabream (Diplodus sargus L.) exposed to 2-phenoxyethanol (Tsantilas et al., 2006), Senegalese sole (Solea senegalensis) exposed to 2-phenoxyethanol, metomidate and clove oil (Weber et al., 2009), and clove oil in Turbot (Psetta maxima) in Aydın et al. (2015). In these studies, weight dependence on induction and recovery times was recorded in larger fish needing more dosages or having longer induction and recovery times. However, the reverse has also been found.

Results of the research of Santos et al. (2013) indicate that with increasing water temperature, anesthetic performance increases but is not influenced by body weight for juvenile marbled spine foot. Furthermore, in long-finned eel (Anguilla reinhardtii) exposed to clove oil, no effects of size on induction and recovery time were observed (Walsh and Pease, 2002).

In general, induction time was significantly weight-dependent for all concentrations tested (P< 0.05). However, there was no significant difference in recovery time (P<0.05) between the two size classes in all three concentrations of clove powder as showed in table 2, This result is in line with the findings of other research that have been conducted involving Persian sturgeon and marbled rabbitfish (Jahanbakhshi et al., 2012; Hoseini et al., 2013).

It is well accepted that the recovery time is unrelated to the period of anesthesia since the anesthetic is absorbed by the fish through a dosage gradient at the gill interface. As a result, until an equilibrium stage between the gill and the anesthetic solution is reached, the fish will not absorb any further anesthetic. The anesthetic agent is leaked through such a gradient during recovery; as a result, the recovery period is determined by the anesthetic dosage rather than the period of anesthesia (Hoseini et al., 2013). When anesthetized with clove oil, Woody et al. (2002) discovered that induction time was dependent on the size of migrating sockeye salmon (Oncorhynchus nerka), but not recovery time. Finally, Zahl et al. (2009) discovered that there was no significant relationship between body weight and recovery times in Atlantic cod (Gadus morhua) exposed to benzocaine, MS-222, metomidate, and 2-phenoxyethanol.

Hematology

The effect of various concentrations of clove solution on certain blood parameters in two different fish weights (RBC, Hb, MCH and MCV) are shown in Table 3 in both weights of C. carpio. RBC, Hb and MCH levels were significantly (P<0.05) high in all groups in comparison to the control, while there were no significant differences for MCV level.

Table (3) Effects of two different weights common carp with three levels of clove powder (mg/L) on some hematological parameters (Mean ± SD).

Groups	Con. W.	Control 0 mg/L	Clove powder 200 mg/L	Clove powder 300 mg/L	Clove powder 400 mg/L
RBC (10^{12}cells/L)	Fish Weight (45±5g)	0.93 ± 0.3 ^{c}	1.15 ± 0.04 ^{ab}	1.32 ± 0.11 ^{a}	1 ± 0.1 ^{b}
	Fish Weight (95±5g)	0.91 ± 0.1 ^{c}	1.18 ± 0.08 ^{ab}	1.25 ± 0.09 ^{ab}	0.97 ± 0.05 ^{b}
HB (g/L)	Fish Weight (45±5g)	66.1 ± 0.11 ^{c}	93 ± 0.12 ^{b}	100.77 ± 0.07 ^{a}	104.84 ± 0.01 ^{a}
	Fish Weight (95±5g)	68.7 ± 0.08 ^{c}	95.68 ± 0.11^{b}	98.67 ± 0.08 ^{ab}	100.22 ± 0.06 ^{a}
MCH (pg/cell)	Fish Weight (45±5g)	70.39 ± 0.18 ^{c}	80.95 ± 0.16 ^{b}	80.23 ± 0.12 ^{b}	133.89 ± 0.23 ^{a}
	Fish Weight (95±5g)	74 ± 0.1 ^{c}	88.12 ± 0.06 ^{b}	84.86 ± 0.1 ^{b}	108.2 ± 0.14 ^{a}
MCV (fL)	Fish Weight (45±5g)	237.9 ± 0.08 ^{a}	233.73 ± 0.02 ^{a}	233.87 ± 0.06 ^{a}	225.4 ± 0.11 ^{a}
	Fish Weight (95±5g)	249.3 ± 0.03 ^{a}	233 ± 0.03 ^{a}	234.53 ± 0.04 ^{a}	211.93 ± 0.94 ^{a}

Mean values with different superscripts within the same groups are different significantly (P<0.05)

Hematological parameters are linking to the sensitivity of fish to abnormal environmental conditions and evaluating the health status of fish (Akinrotimi et al., 2010). In general, this study revealed a slight change in the blood parameters reported at higher levels 300-400 mg/L of clove powder in both tested fish weights, this finding was consistent with Farahi et al. (2011) and Imanpoor et al. (2010), both of which recorded a significant rise in PCV, RBC, and Hb when clove essence was exposed to Prussian carp (Acipenser persicus) and kutum (Rutilus frisii kutum) respectively.

Ascertain anesthetics including eugenol, cause hypoxia as a result of the reduced breathing activity, it is important to improve the oxygen-carrying ability of the blood to counteract the effects of eugenol (Hoseini and Ghelichpour, 2011). This state allows the levels of Hct, Hb and RBC to rise. Hematological indices (MCV, MCH and MCHC) could vary following these changes since they are linked to Hct, Hb and RBC levels and it agrees with the results of this study. In great sturgeon (Huso huso) juveniles exposed to higher concentrations of clove solution, Mohammadizarejabad et al. (2009) observed increases in Hct, Hb, and RBC levels but no change in MCV, MCH, and MCHC levels.

Besides these observations, in comparison, Okey (2019) reported a decrease in PCV, RBC and Hb with increasing concentration of clove powder as an anesthetic for Heterobranchus bidorsalis Juveniles. Besides, Sudagara et al. (2009) reported that 7 min exposure to 175, 225, 275 and 350 mg/l clove powder had a significant decrease in the Hct, Hb and RBC after anesthesia on roach (Rutilus rutilus). However, in the exposure of goldfish (Carassius auratus) to clove oil, Abdolazizi et al. (2011) reported no variations in the hematological parameters, this may be related to the introduction of fish to safe doses of clove oil.

A significant increase (P<0.05) was recorded in the values of WBC counts and lymphocytes in each treatment group compared to control. However, in both control and treated groups, monocytes and granulocyte percentages were within the same amount as showed in table 4.

Table (4) Effects of two different weights common carp with three levels of clove powder (mg/L) on some differential leukocyte count (Mean ± SD).

Groups ↓	Con. / W.	Control 0 mg/L	Clove powder 200 mg/L	Clove powder 300 mg/L	Clove powder 400 mg/L
WBC (10^9cells/L)	Fish Weight (45±5g)	129.1 ± 0.25 ^{b}	167.25 ± 0.19 ^{a}	173.86 ± 0.24 ^{a}	175 ± 0.21 ^{a}
	Fish Weight (95±5g)	127.53 ± 0.2 ^{b}	169.58 ± 0.18 ^{a}	161.66 ± 0.25 ^{a}	174.4 ± 0.19 ^{a}
Granulocytes (%)	Fish Weight (45±5g)	64.1 ± 0.11 ^{a}	58.6 ± 0.38 ^{a}	60.2 ± 0.13 ^{a}	64.37 ± 0.09 ^{a}
	Fish Weight (95±5g)	67.9 ± 0.5 ^{a}	62.04 ± 0.06 ^{a}	61.1 ± 0.13 ^{a}	64.04 ± 0.08 ^{a}
Lymphocytes (%)	Fish Weight (45±5g)	1.3 ± 0.5 ^{b}	8.27 ± 1.33 ^{a}	6.5 ± 0.53 ^{a}	4.53 ± 0.47 ^{a}
	Fish Weight (95±5g)	2.01 ± 0.22 ^{b}	5.96 ± 1.38 ^{a}	5.6 ± 0.54 ^{a}	4.62 ± 0.4 ^{a}
Monocytes (%)	Fish Weight (45±5g)	34.6 ± 0.03 ^{a}	33.13 ± 0.07 ^{a}	33.3 ± 0.14 ^{a}	31.1 ± 0.14 ^{a}
	Fish Weight (95±5g)	30.09 ± 0.3 ^{a}	32 ± 0.07 ^{a}	33.3 ± 0.15 ^{a}	31.34 ± 0.12 ^{a}

Mean values with different superscripts within the same groups are different significantly (P<0.05)

The white blood cells in fish are varied in response to stressors. An increased or decreased number of white blood cells is a normal response to exposure to anesthesia (Shahi and Singh, 2011). Increased numbers of leukocytes (WBC) following anesthetization demonstrate unexpected changes in the state of living water or external material damage (Fast et al., 2008).

In this experiment, the rise in WBC level and lymphocytes reported agreed with the research by Okey (2019), who studied the anesthetic effects of clove powder on hematological and biochemical parameters of Heterobranchus bidorsalis Juveniles and Akinrotimi et al. (2015), who worked on the blood of African Catfish (Clarias gariepinus) exposed to clove seed anesthetic. In the present study, a rise in WBC level may have occurred due to the activation of the protection mechanism of the fish to fight the anesthesia effect (Akinrotimi et al., 2015).

Imanpoor et al. (2010), who reported that stress is thought to be responsible for comparatively low leukocyte counts, exposure to clove essence resulted in a decline in WBC in the testing of exposed Persian Sturgeon. This was largely attributed to a rise in the concentration of plasma cortisol, which is a glucocorticoid hormone that can act as an immunosuppressive agent (Fast et al., 2008). Their observations contrast with those of this report.

Lymphocytes are the must-number white blood differential part that acts as a protection against infection in the creation of antibodies and chemical substances (Iheanacho et al., 2017). The increase in lymphocytes noted in this research may be linked to the rise in the creation of antibodies to protect the cells from damage.

On the other hand, the previous work on anesthesia, revealed no significant change in WBC and differential count of leukocytes following exposure to clove solution (Mohammadizarejabad et al., 2009; Gholipourkanani et al., 2011). That may be as a result of a brief period of anesthesia-induced stress (maximum 10 min).

Conclusions

In conclusion, clove powder was found to be healthy and can be effectively and easily applied to anesthetize different size groups of common carp with minimal disturbance of the studied behaviors and with zero mortality in used dosages. This research also showed that the powder could be safely applied at 300 mg/L, which was enough to anesthetize common carp of various sizes.

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تأثير أوزان الأسماك ومستويات مسحوق القرنفل على التخدير ووقت الإستعادة في أسماك الكارب العادي

Cyprinus carpio L

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الخلاصة

هدفت هذه الدراسة إلى توضيح كيف تؤثر أوزان المختلفة من الأسماك ومستويات تركيز مسحوق القرنفل معًا على التخدير ووقت الإستعادة في .Cyprinus carpioتم إخضاع صنفين مختلفين من أوزان الكارب العادي (5 ± 45 غم و 5 ± 95 غم) لثلاث تركيزات من مسحوق القرنفل (200، 300 و 400 ملغم / لتر) تم تكرار كل منها أربع مرات. في كل معاملة، تم تسجيل وقت التخدير والاستعادة، وتم الاحتفاظ بالأسماك لمدة أسبوع إلى شهرين بعد التجارب لتقييم النفوق على المدى القصير والطويل. ظهرت فروق ذات دلالة إحصائية (P <0.05)في تأثير الدمج بين المستويات المختلفة لمسحوق القرنفل (ملغم / لتر) مع نوعين مختلفين من وزن الكارب العادي (C. carpio L) على وقت التخدير ، ولكن لم يكن هناك فرق في وقت الإستعادة بين مجموعتي الحجم. كان وقت التخدير أقل من دقيقة واحدة لجرعات 300 و400 ملغم / لتر في وزن سمكة 5 ± 45غم بينما في جرعة 200 ملغم / لتر مع 5 ± 95 غم من وزن السمكة يحتاج إلى أكثر من 9 دقائق. في جميع التركيزات المختبرة ، كان وقت التخدير يعتمد بشكل كبير على الوزن (p <0.05) لكن وقت الإستعادة لم يكن كذلك. تم تسجيل تغييرات في المتغيرات الدموية للأسماك RBC ، HB ، MCH و WBC ، ولم يلاحظ أي نفوق. أظهرت هذه الدراسة أن مسحوق القرنفل يمكن استخدامه بأمان وفعالية في تخدير الاسماك الكارب العادي ووقت التخدير المتأثر بمستويات مسحوق القرنفل مع وزن السمك.