فعالية زيت القرنفل كمخدر بديل على أسماك الكارب العادي (*Cyprinus carpio*, L.)

نادر اسكندر حموي* نور عدنان علي باشا حمام التاجر*** طوني فرح******

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الملخص:

يعتبر زيت القرنفل مخدراً طبيعياً يمكن أن يساعد في تخفيف الإجهاد أثناء العمليات البيولوجية والعلاجية مثل التكاثر الاصطناعي. لم تسجل أية حالة نفوق بين الأسماك الخاضعة للاختبارات المحددة في هذا البحث سواء في مرحلة التخدير أو الإنعاش، وتم تسجيل ردود أفعال أسماك الكارب العادي لفترة التخدير بأربع مراحل ولفترة الإنعاش بثلاث مراحل، حيث لم يلاحظ أي تأثير للتراكيز (5 و10 و20 ppm) في تخدير الإصبعيات أو الأفراد البالغة، بينما نجحت التراكيز (60 و80 و100 و120 ppm) في تخدير الإصبعيات بالإضافة للتركيز 40 ppm في تخدير الأفراد البالغة. تراوح زمن التخدير للإصبعيات من 1.04 دقيقة عند التركيز 60 ppm إلى 2.28 دقيقة عند التركيز 120 ppm، وتراوح زمن الإنعاش من 4.44 دقيقة عند التركيز 80 ppm إلى 11.45 دقيقة عند التركيز 120 ppm. في حين تراوح تخدير الأفراد البالغة من 1.03 دقيقة عند التركيز 40 ppm إلى 1.45 دقيقة عند التركيز 120 ppm، مع فترات إنعاش تراوحت من 4.21 دقيقة عند التركيز 40 ppm إلى 10.18 دقيقة عند التركيز 120 ppm. أثبت التركيز 80 ppm أنه الأفضل عن بقية التراكيز السابقة (60 و100 و120 ppm) للاستخدام في تخدير كلا الإصبعيات والأفراد البالغة معاً ($P>0.05$).

الكلمات المفتاحية: الكارب العادي، *Cyprinus carpio*، زيت القرنفل، التخدير، الإنعاش.

*أستاذ، مخبر الأسماك، كلية الطب البيطري، جامعة حماه، حماه، سورية.

**طالبة دكتوراه، مخبر علوم البحار، كلية الزراعة، جامعة تشرين، اللاذقية، سورية.

***طالب ماجستير، مخبر الأسماك، كلية الطب البيطري، جامعة حماه، حماه، سورية.

****طالب ماجستير، مخبر الأسماك، كلية الطب البيطري، جامعة حماه، حماه، سورية.

The efficacy of Clove oil as an alternative anaesthetic on common Carp (*Cyprinus carpio*, L.)

Nader Hamwi* Nour Ali-Basha Hamam Al-Tajer*** and Tony Farah****

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Abstract:

Clove oil is a natural anaesthetic that can help to calm stressed fish throughout both biological and therapeutic processes such as artificial spawning. No mortality was recorded among the fish subject to the tests specified in this research whether in the anaesthesia or recovery phase. The reactions of common Carp were recorded for the anaesthesia period with four phases and for the recovery period with three phases where no effects were observed for the concentrations (5, 10 and 20 ppm) in anaesthetizing fingerlings or adult individuals while concentrations (60, 80, 100 and 120 ppm) succeeded in anaesthetizing fingerlings, In addition to a concentration of 40 ppm in anaesthetizing adult individuals. The anaesthesia time for fingerlings ranged from 1.04 minutes at a concentration of 60 ppm for 2.28 minutes at a concentration of 120 ppm. The recovery time ranged from 4.44 minutes at a concentration of 80 ppm for 11.45 minutes at a concentration of 120 ppm, While anaesthesia for adult individuals ranged from 1.03 minutes at a concentration of 40 ppm for 1.45 minutes at a concentration of 120 ppm with recovery periods ranging from 4.21 minutes at a concentration of 40 ppm for 10.18 minutes at a concentration of 120 ppm. The concentration of 80 ppm proved to be better than the rest of the previous concentrations (60, 100 and 120 ppm) for use in anaesthetizing both fingerlings and adult individuals together ($P>0.05$).

Keywords: *Cyprinus carpio*, common Carp, Clove oil, Anaesthesia, Recovery.

*Professor, Laboratory of Ichthyology, Faculty of Veterinary medicine, Hama University, Hama, Syria.

**PhD. Student, Laboratory of Marine Sciences, Faculty of Agriculture, Tishreen University, Lattakia, Syria.

***Master Student, Laboratory of Ichthyology, Faculty of Veterinary medicine, Hama University, Hama, Syria.

****Master Student, Laboratory of Ichthyology, Faculty of Veterinary medicine, Hama University, Hama, Syria.

Introduction:

Inhibiting the nervous system with anaesthesia and thereby losing the sense of movement facilitates many practical measures that used in fisheries and aquaculture (transporting, sorting, tagging) and minimize metabolism (O_2 consumption, excretion), and also prevent physical injuries to fish, as anaesthesia leads to reducing stress and inhibiting fish movement so that it is easy to catch and handle during harvest and laboratory sampling for various biological experiments, and also during artificial spawnings, such as fertilization and sexual product extraction.

A good anaesthetic is defined by its ability to rapidly anaesthetize and dramatically reduce stress or excessive movement activity very significantly, as well as its efficacy at low doses and a wide margin of safety between the toxic dose and the effective dose, and the organism's recovery after the anaesthesia phase must be rapid (Coyle *et al.,* 2004). The majority of anaesthetic agents induce several phases of anaesthesia, as follows: 1. Sedation that is not too powerful. 2. Sedation at a high level. 3. There has been a partial loss of equilibrium. 4. The equilibrium has been completely lost. 5. Reflex reactivity is lost. 6. Asphyxia is a condition in which an organism is unable to breathe. Clove oil has been applied as a mild loci anaesthetic to help alleviate headaches, toothaches, and joint pain, as well as to sedate or euthanize fish, since ancient times (Gary, 2018; Gary *et al.,* 2014), clove oil seems a yellow liquid with a strong fragrance and flavor, made up of a complex mixture of compounds such as Eugenol (85 - 95%), Isoeugenol and Methyleugenol (FDA/CVM, 2007), clove oil is made from the blossoms, stems and leaves of the clove plant *Syzygium aromaticum* (such as *Eugenia aromaticum* or *Eugenia caryophyllata*).

This study aims to discover a safe and less costly natural anesthetic that can be used to anaesthetize fingerlings and adult individuals of common Carp that are locally farmed as an alternative to commonly used and expensive commercial anaesthetic substances, by deciding the best concentrations of clove oil for anaesthetizing common Carp (fingerlings - adults) and times of anaesthesia and recovery of these fish, to relieve fish stress during the biological processes of artificial spawning as well as the various therapeutic and required processes that occur within the research laboratories and economic productive farms spread across the country.

2-Materials and Methods:

One hundred common Carp (*Cyprinus carpio*) with a weight ratio (W = 30 – 90 g) of fingerlings and thirty adults (W = 450 – 580 g) were collected from a private farm and transported to the Ichthyology Laboratory on the faculty of Veterinary medicine in special containers containing water from the same basin with 2/3 oxygen pumped into it and placed in a large acclimatization pool before the start of the experiment.

- Clove oil is sold in small bottles (50 ml capacity) at perfume shops in the local markets.

1. The experiment was conducted during the first week of December 2020, where necessary measurements were recorded to every fish (total length, TL (cm); total weight, TW (g)), as well as Temperature (19 C°), Dissolved oxygen (DO = 8.6 mg/l), pH (7.5) and Total dissolved solid salts (TDS = 581 ppm) of water used in aquaria.

2. The first stage is split into two sections:

a. Fingerlings were anaesthetized in eight aquaria (each with a capacity of 40 litres) with eight fingerlings in each aquarium, with three replications for each treatment of clove oil concentrations (5, 10, 20, 40, 60, 80, 100, 120 ppm).

b. Recovery of fingerlings: For each of the previous concentrations in the first stage (a). eight aquaria were prepared with fresh water (40 litres capacity), good ventilation, and no clove oil .

3. The second stage is split into two sections:

a. Adult anaesthesia: prepare eight aquaria (each with a capacity of 40 litres) and put three fish in each, with three replications for each treatment of clove oil concentrations (5, 10, 20, 40, 60, 80, 100, 120 ppm).

b. Adult Recovery: For each of the previous concentrations in the second stage (a), eight aquaria were prepared with fresh water (40 litres capacity) good ventilation, and no clove oil .

For both the fingerlings in the first stage and the adults in the second stage, the anaesthesia and recovery times were determined, and the data were statistically analyzed using Excel and SPSS programs using the One-Way ANOVA and the Two-Way ANOVA, and the variations between the averages were determined using Scheffé's multiple comparison tests, as well as Pairwise comparison at the Two-Way ANOVA.

Results:

There was no mortality among the fish subjected to the tests specified in this research, whether during the anesthesia or recovery stages. The following stages of the experimented fish's reactions were observed during the period of anaesthesia, according to visual observation and video recording of the experimented fish (both fingerlings and adults):

1. Slowing down the body's movement and swimming.
2. Slowing the movement of the fins while speeding up the movement of the operculum.
3. Loss of balance and swimming to one side of the body with a slight turn of the caudal fin.
4. The body and the caudal fin stop moving entirely, the operculum moves irregularly, the ability to correct the body's posture is lost, and it is easy to understand it without showing any reaction.

The following stages of its reactions were observed during the recovery period:

1 .The operculum moves in a normal pattern again.
2 .The caudal fin and all fins return to normal movement, as well as the body's equilibrium.
3 .Consistent body movement and incremental routine swimming.

The monitoring was carried out for more than 15 minutes without any signal different from the regular movement of the fingerlings, no effect of concentrations (5, 10, 20, and 40 ppm) was recorded in anaesthesia of fingerlings when part (a) of the first stage was applied, While at concentrations of 60, 80, 100, and 120 ppm, it was affected at a time rate varying from 1.04 minutes at 60 ppm to 2.28 minutes at 120 ppm (Table 1) and it was discovered that when part (b) of the first stage was applied, the fingerlings recovered at a pace varying from 4.44 minutes at 80 ppm to 11.45 minutes at 120 ppm (Table 1).

Table 1: Anesthesia and Recovery times of common Carp fingerlings at different concentrations of Clove oil.

Concentration (ppm)	Total Weight, Tw (g)	Total Length, TL (cm)	Standard Length, SL (cm)	Anesthesia Time (min.)	Recovery Time (min.)	No.
5	41 ± 0.52	12.5 ± 0.11	10.3 ± 0.14	0	0	8
10	63 ± 1.30	15.7 ± 0.12	11.5 ± 0.24	0	0	8
20	55 ± 0.52	13 ± 0.29	10.3 ± 0.16	0	0	8
40	71 ± 1.57	15.9 ± 0.21	11.7 ± 0.15	0	0	8
60	80 ± 0.49	16 ± 0.76	11.5 ± 0.16	1:04 ± 0.01	5:41 ± 0.01	8
80	60 ± 0.64	15.5 ± 0.13	11.8 ± 0.11	1:25 ± 0.04	4:44 ± 0.01	8
100	80 ± 0.52	16 ± 0.99	11.5 ± 0.12	1:58 ± 0.02	6:51 ± 0.01	8
120	40 ± 0.74	12.6 ± 0.15	10.4 ± 0.16	2:28 ± 0.02	11:45 ± 0.02	8

The results of the effect of the concentrations (60, 80, 100, 120 ppm) showed that there are noticeable differences between them when anaesthesia and recovery of fingerlings (One-Way

ANOVA; Scheffé's multi comparisons method, $P < 0.05$). A strong relationship ($R^2 = 0.896$) between periods of anaesthesia and recovery in fingerlings (Fig. 1).

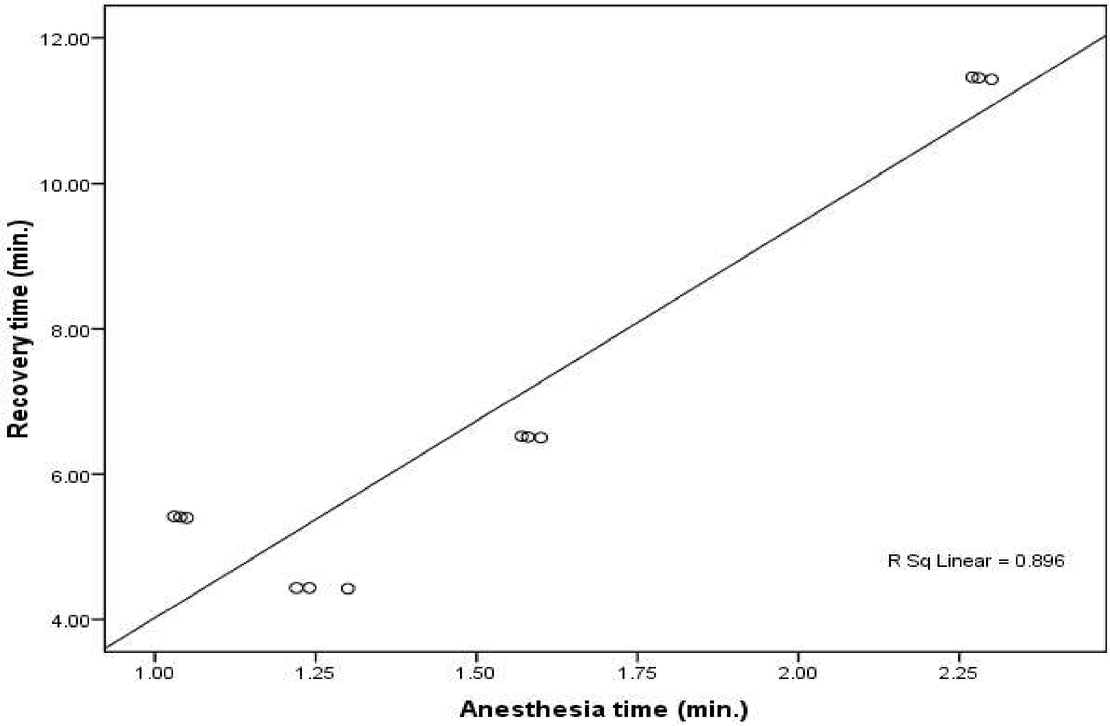


Figure 1: The correlation between Anesthesia and recovery times of common Carp fingerlings.

Furthermore, the adult individuals of the common Carp were exposed to the previous concentrations of clove oil to determine their impact on the anaesthesia process (part (a) of the second stage), and no effect of the concentrations (5, 10, 20 ppm) in causing anaesthesia was observed, as it was monitored for more than 15 minutes without occurring any signal separate from regular movement of adults, while adult individuals were anaesthetized at concentrations (40, 60, 80, 100, 120 ppm) with varying periods ranging from 1.03 min. at 120 ppm to 1.45 min. at 40 ppm (Table 2). The adults recovered from 4.21 minutes at 40 ppm to 10.18 minutes at 120 ppm when part (b) of the second stage was applied (Table 2). Although there were no significant differences between the concentrations (40, 60, 80, 100, 120 ppm) in adult anaesthesia (One-Way ANOVA; Scheffé's multi comparisons method, $P > 0.05$), and no significant difference between the concentrations (40, 60, 80, 100 ppm) in adult recovery (One-Way ANOVA; Scheffé's multi comparisons method, $P > 0.05$), while the 120 ppm concentration showed a significant difference from the other concentrations for adult recovery (One-Way ANOVA; Scheffé's multi comparisons method, $P < 0.05$), indicating a weak relationship ($R^2 = 0.186$) between anaesthesia and recovery periods in adults (Fig. 2).

Table 2: Anesthesia and Recovery times of common Carp adults at different concentrations of Clove oil.

Concentration (ppm)	Total Weight, Tw (g)	Total Length, TL (cm)	Standard Length, SL (cm)	Anesthesia Time (min.)	Recovery Time (min.)	No.
5	475 ± 3.62	28 ± 0.30	22 ± 0.16	0	0	3
10	469 ± 4.40	28.6 ± 0.25	22.8 ± 0.10	0	0	3
20	455 ± 3.78	30.2 ± 1.15	24.6 ± 0.20	0	0	3
40	470 ± 2.38	29.2 ± 0.20	24.5 ± 0.27	1:45 ± 0.03	4:21 ± 0.14	3
60	565 ± 3.93	29.8 ± 1.12	25.7 ± 0.46	1:44 ± 0.05	4:88 ± 1.04	3
80	505 ± 3.09	30 ± 1.20	25 ± 0.06	1:11 ± 0.01	4:30 ± 0.11	3
100	480 ± 4.13	29.5 ± 0.30	24.3 ± 0.22	1:04 ± 0.01	4:41 ± 0.17	3
120	575 ± 3.85	31.5 ± 0.71	26.4 ± 0.16	1:03 ± 0.45	10:18 ± 1.19	3

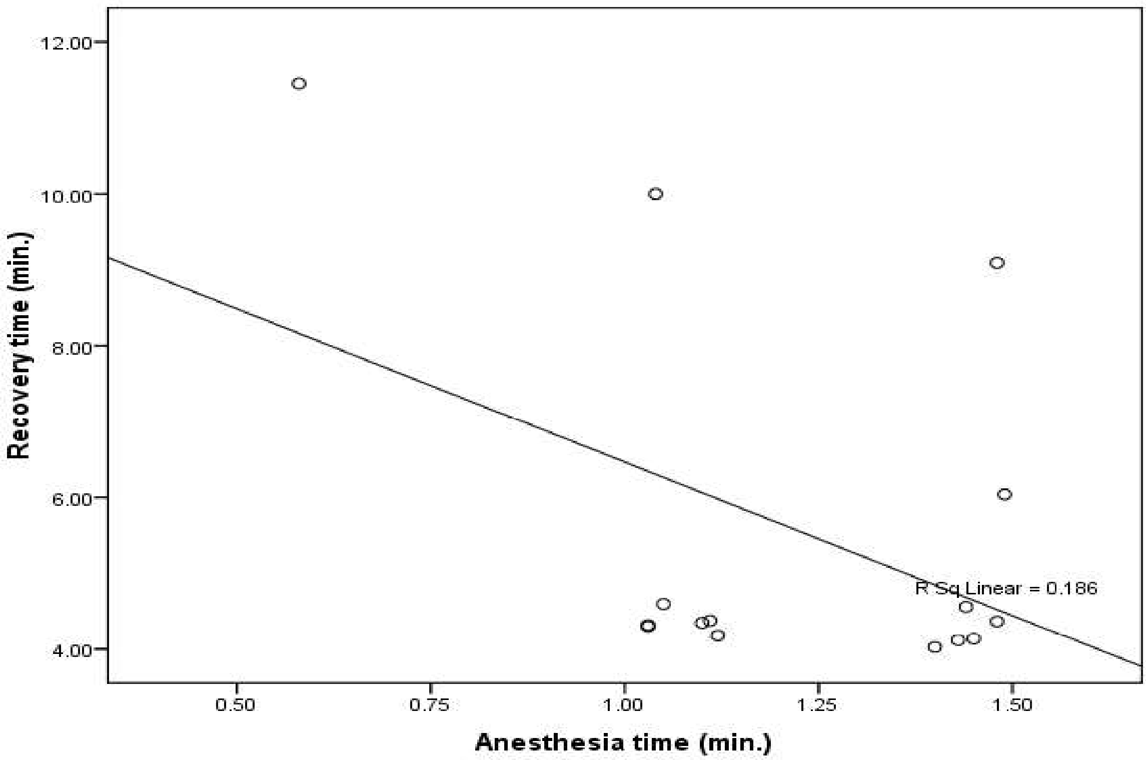
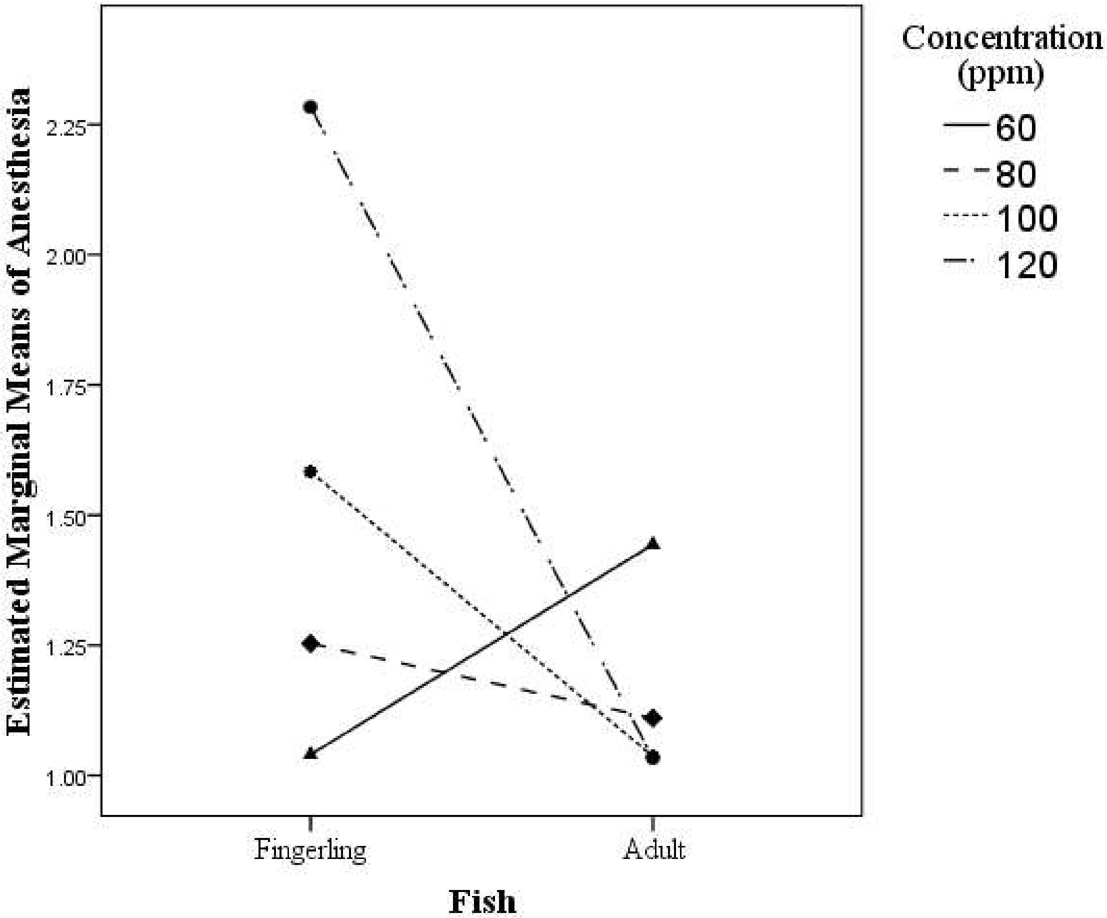
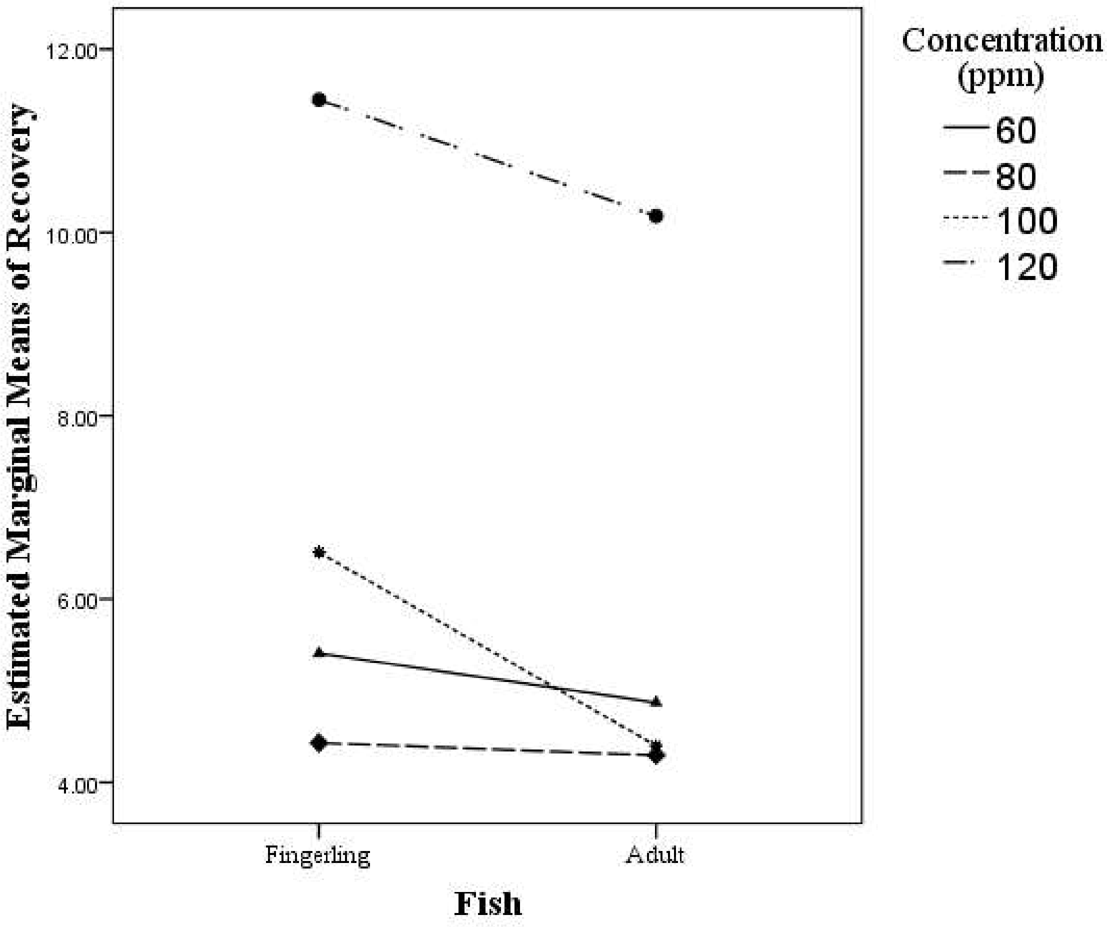


Figure 2: The correlation between Anesthesia and recovery times of common Carp adults.

By trying to understand the impact of concentrations (60, 80, 100, 120 ppm) in anaesthetizing both fingerlings and adults, and then the recovery process, it was found that there is a clear significant difference between the concentration of 120 ppm and previous clove oil concentrations in anaesthesia and recovery of both fingerlings and adults (Two-Way ANOVA; Scheffé's multi comparisons method, $P < 0.05$) (Fig. 3; a, b). Only in the recovery of both fingerlings and adults was there a significant difference was also found between the concentrations 80 ppm and 100 ppm (Two-Way ANOVA; Scheffé's multi comparisons method, $P < 0.05$). And in both fingerlings and adults, a weak relationship ($R^2 = 0.27$) was found between the periods of anaesthesia and recovery.



(a)



(b)

Figure 3: Comparison impact of concentrations (60, 80, 100, 120 ppm) in anaesthetizing (a) and recovering (b) of common Carp (both fingerlings and adults).

A Pairwise comparison method was used in the two-way ANOVA to find out the most appropriate or preferred concentration for use among the previous concentrations of clove oil in terms of anaesthesia and recovery of both fingerlings and adults of common Carp. A significant P> 0.05, the concentration 80 ppm was found to be the most appropriate or preferred over the previous concentrations (60, 100, 120 ppm) for use on common Carp (both fingerlings and adults), while the previous concentrations showed a clear significant difference regarding between them (P <0.05).

Discussion:

In this study, the reactions of common Carp were reported and divided into four stages during the anaesthesia period and three stages during the recovery period based on our findings, while Yousefi *et al.* (2018) and Kamble *et al.* (2014) identified five stages during the anaesthesia period and one stage in the recovery period in their observations on the reactions of common Carp, whereas Akinrotimi *et al.* (2015) determined the reactions of the African Catfish (*Clarias gariepinus*) to five stages for both the anaesthesia and recovery periods.

The increase in the anaesthesia time of the fingerlings with the increase in the concentration of clove oil (60 – 120 ppm) and corresponding directly to their recovery time was due to the small surface of their gills exposed to the absorption of the substance and proportional to the body weight (Coyle *et al.,* 2004), and the state of recovery was consistent with Rotllant *et al.* (2001) and Skjervold *et al.* (1999). Unlike adult individuals that did not show a significant difference in the time affected by anaesthesia when increasing the clove oil concentration (40 – 120 ppm), as well as their recovery time, except at the concentration of 120 ppm, it required a longer time to recovery than the rest of the previous concentrations, due to the large surface of its gills, which is exposed to the anaesthetic effect of clove oil which absorbs more and faster than fingerlings, but adults, especially when exposed to high concentrations of the drug (such as 120 ppm in this case) need a longer recovery time to get rid of the large amount absorbed.

Contrary to what Neiffer and Stamper (2009) found that the appropriate concentration of anaesthesia for common Carp ranges between (25 – 100 mg/l), the concentrations (5 - <40 ppm) used in this study did not affect common Carp (fingerlings and adults). The range of concentrations (40 – 120 ppm) affecting anaesthesia of fish in this study differed significantly from what Hoseini and Nodeh (2013) used in very high concentrations (700 – 5000 ppm) in anaesthesia of common Carp (140 – 160 g), who found that using a higher concentration of clove oil and a shorter time in anaesthesia was more effective than using lower concentrations and a longer effect time, however, despite the positive anaesthesia impact on fish at concentrations (40 – 120 ppm), our finding indicates that the concentration (80 ppm) is the best for use among the previous concentrations of clove oil in terms of anaesthesia and recovery on common Carp (both fingerlings and adults). Although Hajek *et al.* (2006) found that anaesthesia occurs more often at concentrations of (30 – 200 mg/l), that the lowest concentration induces general anaesthesia (40 mg/l), and that the range of concentrations (30 – 50 mg/l) is the safest and most efficient for common Carp.

Conclusions:

1. Four and three stages of common Carp (fingerlings and adults) for anaesthesia and recovery process, respectively were recorded.

2. The anaesthesia time of common Carp fingerlings was less than 2.5 minutes and less than 1.5 minutes for adults.

3. The concentrations (5, 10, 20, 40 ppm) did not affect the anaesthesia of fingerlings.

4. There is no influence of concentrations (5, 10, 20 ppm) on the induction of anaesthesia in adults.

5. For anaesthesia of fingerlings and adults, A variety of concentrations (60, 80, 100, 120 ppm) was found to be appropriate .

6. For use on common Carp (both fingerlings and adults), the concentration (80 ppm) was the most appropriate or preferred over the previous concentrations (60, 100, 120 ppm).

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