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# Uses of Cloves *Eugenia caryophyllus* to Anesthetize Young Silver Carp *Hypophthalmichthys molitrix*

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## ABSTRACT
This study tested the effectiveness of clove extract *Eugenia caryophyllus* as an anaesthetic for silver carp *Hypophthalmichthys molitrix* using five concentrations (80, 90, 100, 120, and 140mg/ L). These concentrations were applied to two groups of fish with different weights. The first group weighed between 5 and 5.5g, and the second group was between 9 and 10.5g. The objective was to evaluate the effect of anesthesia and to follow up the stages of recovery. The results showed that all tested concentrations were effective in inducing anesthesia, as the duration of loss of consciousness for fish weighing 5-5.5g ranged between 12 and 25 minutes, and for fish weighing 9-10.5g, the duration was between 10 and 20 minutes when using a concentration of 80mg/ L. The recovery time ranged between 15 and 19 minutes for the first group and between 12 and 27 minutes for the second group at a concentration of 140mg/ L. The study indicated a direct relationship between increasing the extract's concentration and the anesthesia's effectiveness; the duration of the onset of anesthesia decreased with increasing concentration, while the recovery period increased. It was also shown that the last parts of the body affected by anesthesia are the first to regain movement during recovery. In contrast, concentrations less than 80mg/ L did not show a clear effect in causing anesthesia. This study recommends the possibility of using clove extract as an anesthetic substance for transporting silver carp fish or using it in artificial reproduction operations, provided that the concentration is controlled to ensure safety, without causing harmful effects.

## INTRODUCTION

The use of toxic and anesthetic substances is one of the oldest and most common fishing methods worldwide. Several anesthetics have been developed to anesthetize fish and to reduce stress during live transport **(Al-Shawi, 2000)**. Anesthesia is a state that prevents the perception of pain and other sensations and occurs under the influence of various substances such as analgesia, hypnosis, or relaxation, which prevent unwanted movement or excessive muscle activity, which is required in the field of fish anesthesia **(Sneddon, 2012)**.

Clove (*Eugenia caryophyllus*) is a commonly used substance in human nutrition and medicine, containing 10% volatile oils, most of which is Eugenol, the active ingredient in the anesthetic process **(Pandey *et al.*, 2022)**. Fish are exposed to stress due to multiple factors such as fishing, transportation, overcrowding, restricted environments, and changes in water quality, especially low dissolved oxygen **(Hurst *et al.*, 2007; Zahl *et al.*, 2012)**. These factors significantly affect the physiological condition of fish and their survival rates **(Harmon, 2009)**, increasing the possibility of disease outbreaks in fish farms and leading to significant losses in the production of farmed fish. Narcotics are substances that have a direct effect on the nervous system, causing loss of sensation and movement, which facilitates the handling and transportation of fish. When fish are placed in an anesthetic solution, they absorb it primarily through the gills and partly through the skin, causing general anesthesia **(Neiffer and Stamper, 2009; Mugimba *et al.*, 2021)**.

Various substances have been used in the field of fish anesthesia, such as tricaine methane sulphonate (MS222), propanidid, and sodium bicarbonate. **Al-Hamdani *et al.* (2020)** studied the effectiveness of poppy (*Papaver nudicaule*) extract in anesthetizing common carp (*Cyprinus carpio*), and **Al-Niaeem *et al.* (2017)** studied the effect of nutmeg (*Myristica fragrans)* powder on anesthetizing juvenile common carp, which has shown success in anesthetizing fish, is considered a safe, inexpensive, and effective substance, and meets the general specifications of narcotic substances. The narcotic effect is due to the volatile oil that contains a narcotic substance known as Myristicin, which is the active ingredient. The effect of this compound is similar to the effect of both Amphetamine and Mescaline, which are two powerful stimulants of the central nervous system (**Asghar & De Souza, 1989; Kalam, 2013)**. Researchers have identified some basic specifications that must be available in anesthetics used in fish, such as being water-soluble, inexpensive, safe, and fast-acting, not leaving harmful side effects on fish or humans, and being tolerable by fish **(Plumb *et al.*, 1983)**. **Al-Shawi (2000)** used some chemicals to anesthetize golden carp (*Carassius auratus*) fingerlings, while **Al-Jashmi *et al.* (2002)** resorted to using cloves to anesthetize common carp, as the success of using cloves means benefiting from its specifications as it is a locally available, cheap and safe substance. Cloves can also be used to anesthetize aggressive fish when extracting their eggs during the artificial propagation process, especially grass and silver carp. MS222 was also used in Iraq as a limited anesthetic substance due to its low availability, high price, and lack of information about it among fish farmers.

The current study aimed to identify the appropriate clove concentrations for anesthetizing silver carp using different weight categories and to determine the appropriate time and concentration for these categories.

## MATERIALS AND METHODS

Silver carp *Hypophthalmichthys molitrix* specimens were obtained from the experimental fish farming station of the Marine Science Center. They were divided into two groups according to weight, where the average weight of the first group ranged between (5 - 5.5 ± 2.04) g, while the average weight of the second group ranged between (9 - 10.5 ± 1.02) g. The fish were placed in glass tanks with dimensions of 50 × 30 × 30 cm to adapt them to the experimental conditions.

The required concentrations of clove powder were prepared, which was ground using an electric grinder, where concentrations of (80, 90, 100, 120, and 140mg/ L) were prepared after dissolving them in water at a temperature of 26°C, according to **Okey *et al.* (2018)** upon using (*Eugenia caryophyllata*) powder as anaesthesia on African catfishes *Clarias gariepinus* and *Heterobranchus bidorsalis* fingerlings. Six fish from each group were introduced into each concentration of the prepared solutions, and the fish behaviors, such as swimming pattern, fin movement, and gill cover movement, were monitored from the moment they were exposed to the different solutions.

The time taken for signs of loss of balance to appear, followed by body flipping and complete cessation of movement, was recorded. After this stage, the fish were transferred directly from the solutions containing the anesthetic substance to a clean tank free of the anesthetic substance, where recovery behavior was monitored. The time taken for the fish to regain consciousness and to return to a normal behavioral state was determined.

## RESULTS

Tables (1) and Figs. (1, 2) show the average duration of anesthesia for the two groups of silver carp "*Hypophthalmichthys molitrix*" used in this study in correspondence with different concentrations. The longest duration of anesthesia for fish was 25 minutes for the first group (5-5.5)g at a concentration of 80 mg/L. In comparison, the shortest duration of anesthesia was 10 minutes at a concentration of 140 mg/L. The longest duration of regaining consciousness was 27 and 19 minutes at a concentration of 140 mg/L for the first and second weight groups, respectively, and the shortest duration of recovery was 12 and 15 minutes at a concentration of 80 mg/L for the two weight groups, respectively. Concentrations of 120 and 140 mg/L showed the shortest duration for fish to enter a state of unconsciousness.

**Table 1.** Concentrations of the anesthetic substance (cloves) and the average time of anesthesia and recovery for silver carp fish of different weights

| Concentration mg/L | 5-5.5 g | | 9-10.5g | |
|---|---|---|---|---|
| | Average anesthesia time (min) | Average recovery time (min) | Average anesthesia time (min) | Average recovery time (min) |
| 80 | 25 | 15 | 20 | 12 |
| 90 | 20 | 17 | 15 | 12 |
| 100 | 18 | 17 | 12 | 16 |
| 120 | 15 | 19 | 10 | 20 |
| 140 | 12 | 19 | 10 | 27 |

When fish were exposed to anesthetic concentrations ranging from 80 to 140mg/ L, the average anesthetic time for the 5-5.5 g weight group ranged from 12 to 25 minutes, while the average anesthetic time for the 9-10.5 g weight group was between 10 and 20 minutes. The results of the correlation coefficient showed a strong inverse correlation between the concentration of the anesthetic substance and the duration of anesthesia, as the value of (r) reached -0.9647 for the first group, which weighed between 5 - 5.5 g, and -0.86608 for the second group, which weighed between 9 - 10.5 g, and a strong positive correlation between the concentration and the recovery time, as the value of r reached 0.9181 and 0.983979 for the first and second groups, respectively. Furthermore, the results showed a strong inverse relationship between the recovery time and the duration of anesthesia, as it reached -0.9659 for the first group and -0.7871 for the second group.

The results of the F-test showed no significant differences ($P>0.05$) in the average anesthesia time (min) and average recovery time (min) in all concentration.

## Fish behavior

The behavior of the fish when placed in the anesthetic solution was observed to include a marked calmness and reduced movement, with continued fin movement and an increase in the number of gill openings. The fish also showed signs of confusion and staggering, as they tended to settle in one place and then turn over on their heads, making them easy to catch, while a decrease in respiratory movements was observed. These signs and signals increased with increasing concentration of the anesthetic substance. Upon recovery, the fish begins to move the caudal fin while remaining stable at the bottom.

After a while, the caudal fin begins to move and increase, then the movement of the gill cover increases, and the tail rises while the head remains hanging down. After being transferred to water free of anesthesia, the fish remains slightly swaying and swims normally. The signs of anesthesia and return to consciousness (normal state) appear in reverse.

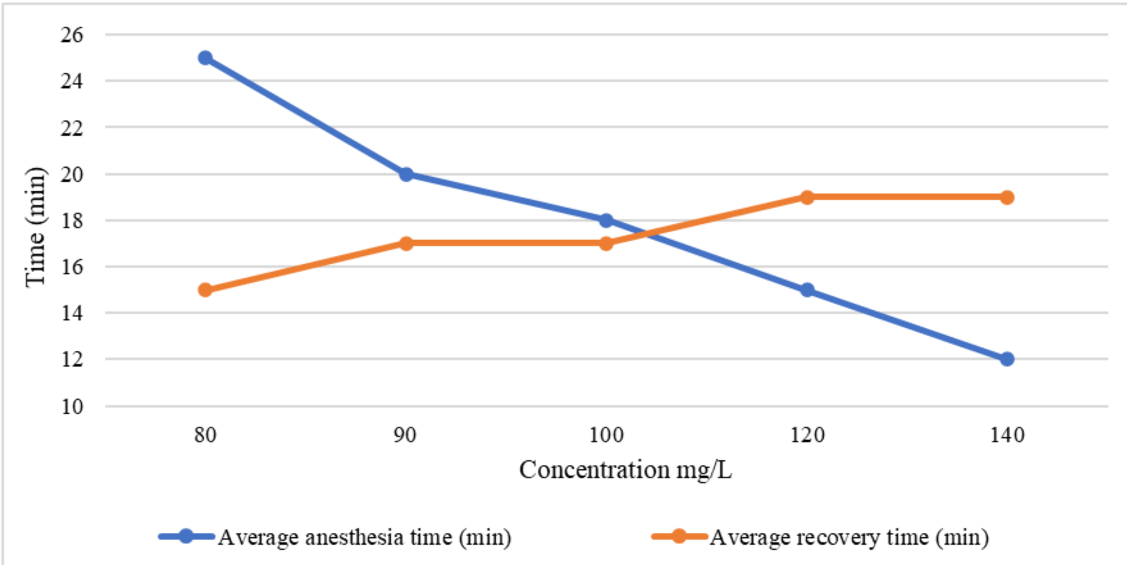


**Fig. 1.** The effect of clove concentrations on the time of anesthesia and recovery of young silver carp weighing (5-5.5g)

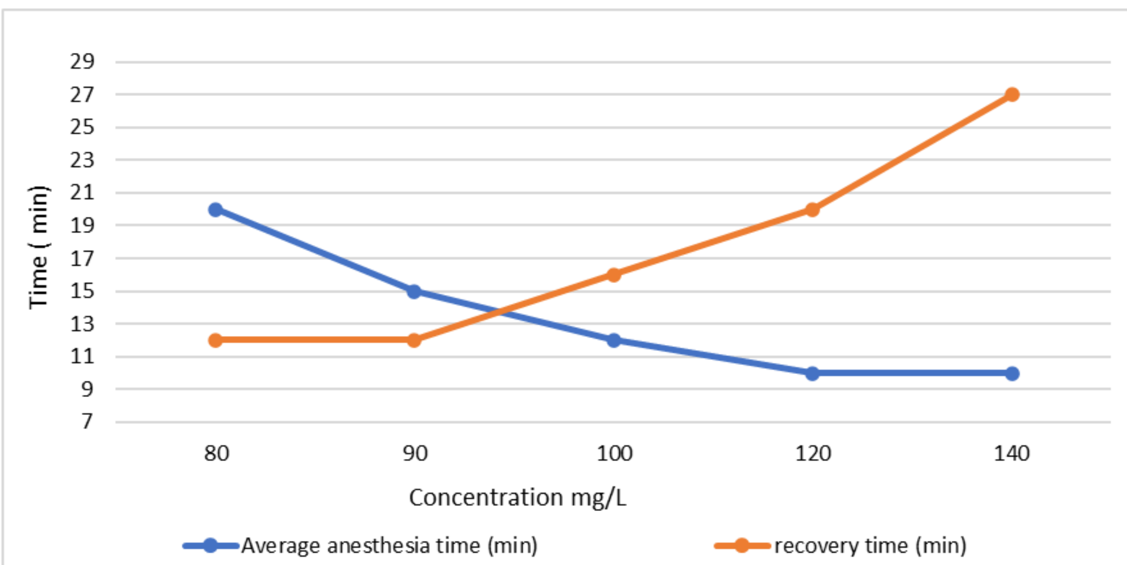


**Fig. 2.** The effect of clove concentrations on the time of anesthesia and recovery of young silver carp weighing (9-10.5g)

## DISCUSSION

Anesthetics have been used to relieve stress in fish in various aquaculture activities **(Neiffer & Stamper, 2009)**, facilitating various routine procedures such as catching, handling, transport, marking, grading, and measurements that often cause harm or physiological stress. To reduce these adverse effects as well as reducing stress levels and preventing mortality, the optimal solution is to use anesthesia during these routine operations in fish farming **(Gholipour *et al.*, 2011)**. The results of the anesthesia experiments showed that using appropriate concentrations of anesthetics in anesthetizing fish is an effective procedure to facilitate transportation and handling operations, which enhances the accuracy and safety of conducting biological studies, such as measuring length, weight, and morphological analyses. Cloves are highly effective as an anesthetic substance for fish, as they are characterized by their safe specifications, high effectiveness, and low cost. Cloves are consistent with the scientific standards required for anesthetics in this context, as indicated by **Fernandes *et al*. (2017)**.

Concentrations between 80 and 140 mg/L induced anesthesia in fish, with varying durations of anesthesia. Previous studies have shown the use of various concentrations and anesthetic agents, for example, **Al-Shawi (2000)** reported that a loss of balance occurred after 60 minutes when using a solution containing one ml/L of tertiary amino alcohol in the golden carp. **Al-Jashmi (2002)** indicated that a concentration of 190 mg/L is suitable for inducing carp anesthesia. In addition, **Sylvester (1975)** showed that a loss of balance occurred at a concentration ranging from 40 to 120 mg/L of the anesthetic MS-222 in the juveniles of *Mugil cephalus*.

These behavioral signs vary in intensity depending on the type of fish and the concentrations and types of anesthetic substances. These observations were also recorded in the study of **Sado (1985)** on three species of tilapia fish. Similar notices were documented in the study of **Booke *et al*. (1978)** on two types of the trout and common carp, where the average recovery period ranged between 15-19 minutes for the weight group 5-5.5 g, while it ranged between 12-27 minutes for the weight group 9-10.5 g. Upon recovery, the fish individual moves the caudal fin while remaining stable at the bottom. After a while, the caudal fin begins to move and increase, the gill cover movement increases, and the tail rises while the head remains hanging down. After being transferred to water free of anesthetic, the fish remains in a slight swaying state and then swims normally. The signs of anesthesia and return to consciousness (normal state) appear in reverse; this is consistent with what is mentioned by **Al-Jashmi (2002)** and **Al-Shawi (2000)**, elucidating that the last part to show signs of anesthesia is the first part to return to movement. Many researchers have observed such results on some fish species **(Post, 1979; Alvarez & Moreno, 1982; Sylvester & Holland, 1982)**. The anesthesia effect is due to the decrease in the concentration of hydrogen ions in the anesthesia solutions **(Sado, 1985)**.

The current study showed a direct relationship between the concentration of the anesthetic substance used and the time of partial and general anesthesia occurrence. In contrast, an inverse relationship was recorded with the time of occurrence of total awakening, which is consistent with what **Sado (1985)** showed in his study on the effect of Quinaldine on some species of tilapia fish, as well as what was indicated by **Hoskonen and Pirhonen (2006)** in their study on the effect of clove oil doses in anesthetizing tilapia fish in the presence of an inverse relationship between the time of total anesthesia and the time of total recovery. In addition, it agrees with what was indicated by **Booke *et al*. (1978)** in the relationship between the concentration used of the anesthetic substance and the time of anesthesia (partial and total) on fish during his study on the effect of MS222 on the trout (*Oncorhynchus mykiss*) and common carp (*C. Carpio*). **Sylvester and Holland (1982)** also obtained a similar result when using the same anesthetic substance (MS222) on the young common carp fish, noting the difference in the concentrations used, and **Fernandesa *et al*. (2017)** in a study on small - sized tropical fishes.

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