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The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity

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Abstract
An experiment was conducted to study the efficacy of clove oil as an anesthetic for common carp. The different doses of clove oil (0.04, 0.05, 0.06, 0.07 & 0.08 ppm) were used in static waters. The highest (15.10 min) and lowest (2.20 min) induction time were noticed at the dose of 0.04 and 0.08 ppm respectively. The induction time significantly ($p<0.05$) decreased with increasing concentration of clove oil. The respective highest (7.10 min) and lowest (0.58 min) recovery times of clove oil were observed at the dose of 0.08 and 0.04 ppm. The metabolic activity (OCR, COR, RQ and AQ) in clove oil anesthetized common carp showed a significant ($p<0.05$) reduction. Still the highest rate of metabolic activity reduction was in higher dose of 0.08 ppm clove oil treated fish than other treatments.

Keywords: Anaesthetic, Aquaculture, Clove oil, Common carp, Metabolism

1. Introduction
In aquaculture operations, anesthetics are very important because they minimize the stress in fishes and reduce physical injury during various handling practices like weighing, length measurement, tagging, sampling *etc* [1]. Anesthetics can help significantly in mitigating physiological stress, reducing metabolic rates, thus reducing oxygen consumption and ammonia and carbon dioxide excretion [2]. The use of anesthetics becomes essential in the transportation medium especially for lowering the metabolic activities [3]. Clove oil is a highly effective fish anesthetic with no side effects. The advantages are that it is locally available and inexpensive [4]. According to Osborn [5] the reduction in consumption of oxygen is caused by an anesthetic that seems to increase the ability of fish to withstand lower concentrations of dissolved oxygen. The gills decrease their functions under anesthesia due to the depression of opercular movements and associated nervous control of breathing [6]. Durve [7] stated that the metabolic rate in anesthetized fish was lowered by nearly half and thereby the weight of fish per unit volume of water could double during transportation.
Anesthetics are widely used in routine aquaculture activities to reduce incidence of stress by sedating and immobilizing fish before performing any task in aquaculture. The desirable attributes of anesthetics used for fin fish include, short induction and recovery time, non-toxic to fish and humans, no lasting physiological effects, rapid clearance from the body, high solubility in fresh and salt water, availability and cost effectiveness [8]. Biologists and aquaculturists alike have been searching for alternative anesthetics that are less toxic, readily available, efficacious and safe for humans.
A wide variety of anesthetic agents have been used in fish. Two of the more commonly used anesthetics today are tricaine methanesulfonate (MS-222) and eugenol. Isoflurane, an anesthetic used in vaporizers for gas anesthesia of mammals and birds, may also be mixed into water for fish anesthesia, although precise dosing and volatilization are problems. Tricaine is the most commonly used fish anesthetic. It comes as a fine white powder which can be weighed for the proper dose, or premixed in a convenient stock solution for addition to the anesthesia tank by volume. Tricaine solutions are acidic and they should be buffered prior to use with sodium bicarbonate (baking soda).An alternative to tricaine is eugenol, the active ingredient of clove oil. Eugenol has been used widely in koi for minor procedures, but has a limited history of use for major surgery.

Clove oil is a dark brown liquid resulting from the distillation of flowers, flower stalks, and leaves of clove trees *(Eugenia aromatica)* and used throughout the world for applications ranging from food flavouring to local anesthesia in the dentistry profession [9]. It consists primarily of phenol eugenol (70-90%), eugenol acetate (>17%) and kariofilen 5 (12%). It is considered noncarcinogenic and non-mutagenic [10]. Clove oil's properties and its status as a GRAS substance make it an ideal candidate as an anesthetic to use in the field of fisheries. In view of this; the present study was conducted to understand the respiratory metabolism [oxygen consumption, carbon dioxide output, ammonia-nitrogen excretion, respiratory quotient (RQ), and ammonia quotient (AQ)] in relation to clove oil application. The main objective of this study was to assess the efficacy of clove oil in aquacultural operations.

2. Material and Methods

2.1. Experimental Fish

Common carp (*Cyprinus carpio communes*) fry with an average weight (0.20±0.01 gm) and average total length (21±0.01 mm) were obtained from Aquaculture Research & Seed Unit, Directorate of Research, MPUAT, and Udaipur. Before initiating final experiments, the fishes were acclimatized to laboratory conditions. For this purpose the fishes were kept in a fiber glass tank (3 x 1 x 0.75 m) filled with freshwater.

2.2. Clove Oil

Laboratory grade clove oil (Hi-MEDIA) was used for present studies. Clove oil is a pale yellow liquid distilled from the leaves, buds and stems of the clove tree (*Eugenia caryophyllus*). Its active ingredients are eugenol [α-methoxy-4-2 (2-propenyl)-Phenol] and isoeugenol (4-propenyl-2-methoxy phenol), which can comprise 90–95% of clove oil by weight. Clove oil concentrations were expressed as ppm and those concentrations were made based on the active ingredient. Fresh stock solutions were prepared prior to each experiment and were protected from a direct sunlight to limit photo and thermal degradation.

2.3. Water Quality Analysis

The selected **w**ater quality parameter such as temperature, pH, dissolved oxygen, carbon dioxide and ammonia were analyzed following standard methods of APHA [11].

2.4. Estimation of clove oil dose for fish Anesthetization

Preliminary experiment was conducted to determine whether clove oil would effective or not as anesthetic for aquacultural operations. Clove oil was obtained from the local market and diluted solution was prepared. One part of clove oil was diluted in 99 part of distilled water. This solution was stored in glass bottles. The range of concentration of clove oil used in the present study was selected after conducting the initial anesthetization tests. The concentration of clove oil used for the initial anesthetization experiment ranged from 0.04 ppm to 0.08 ppm (0.04, 0.05, 0.06, 0.07 & 0.08,). The induction time for four stages (Table 1) of anesthetization and recovery were noted and recorded for all initial anesthetization experiments. The concentration of anesthetic at which the common carp attained sedation in the least induction time along with the highest recovery time and 100% survival was chosen as the maximum level of clove oil for aquacultural operations. After selecting the optimum concentration of clove oil, experiments for assessing their effect on the respiratory metabolism of the common carp were initiated.

Table 1: Different stages of anesthetization in common carp

S. No	Stages	Characteristic Behaviour
1	Stage 1	Sedation ,Onset of erratic opercular movement
2	Stage 2	Partial loss of equilibrium and erratic swimming
3	Stage 3	Total loss of equilibrium
4	Stage 4	Medullary collapse: Respiratory movement or opercular activity cease and fish death
5	Recovery	Ability to remain upright ,regain control of equilibrium and normal swimming behavior

2.5. Estimation of Respiratory Metabolism

The selected anesthetics dose was mixed with water in glass aquaria ($1f^3$) to achieve the desired concentration. Fish collected from the rearing tank were introduced (10 fishes in each aquarium) into the experimental aquaria with a scoop net to avoid stress. The time of induction of anesthesia was noted and when the fish reached the surgical plane (stage IV) of anesthesia, they were then transferred to the recovery tank containing the well aerated water. The rearing tank was covered with a glass lid without any air bubble and sealed with grease to avoid contact with external atmosphere. The opercular movement of the control and treated fish was noted for every one minute with an interval of 15 minutes of visual observation during the entire period of experiment. The respiratory metabolic parameters such as OCR, COR, and AER were estimated within durations of 1 hour and 3 hours. The dissolved oxygen, free a carbon dioxide and ammonia-N level in the experimental tank water was estimated by adopting standard methods described by APHA [11]. The rate of oxygen consumption, carbon dioxide output, and NH_3-N excretion was estimated using the following formula.

$$OCR,/COR,/AER= (I-F)\ V/1000 \times 1000/G \times 60/t$$

Where I = Initial level; F = Final level; V = Volume of water; g = Weight of the fish in grams; t = Experimental duration in minutes

The respiratory quotient (RQ) and ammonia quotient (AQ) were calculated using the following formula:

RQ = volume of CO_2 output/ volume of O_2 consumption

AQ = volume of NH_3 -N excretion/ volume of O_2 consumption

3. Results

3.1. Induction and Recovery Time

Table 2 and Figure 1 show incubation and recovery time of the fish for each dose of clove oil. The largest total induction time (15.10 min) was detected in 0.04 ppm dose of clove oil. However, shortest time of total induction (2.20 min) was observed in 0.08 ppm dose. The shortest (0.30 min) and longest (10.7 min) time to reach light sedation (stage 1) was noticed in dose 0.08 and 0.04 respectively. Similarly, the highest and lowest time taken to reach stage 2 (partial loss of

equilibrium) and stage 3 (total loss of equilibrium) was also recorded in 0.08 ppm and 0.04 ppm dose of clove oil. When all the four stages were examined, transition to stages 1, 2, 3 and 4 took shortest time at highest (0.08) dose. Figure 1 shows recovery time of the fish, the longest time to recovery was noticed at 0.08 ppm dose of clove oil. The shorter recovery time was observed in 0.04 ppm dose. The times of recovery in other doses were 4.59, 3.50 and 1.10 minute in 0.07, 0.06, and 0.05 ppm doses respectively. The recovery time in different doses of clove oil was statistically significant at 5 % level ($P < 0.05$) of probability (Table 2). The survival percentage of experimental fish exposed to different concentrations of clove oil (0.04 to 0.08 pp) is given in Table 2. It is evident from this table that there was no mortality in 0.04, 0.05 & 0.06 ppm clove oil treatment. However 10 and 18 % mortality was noticed in 0.07 and 0.08 ppm dose respectively.

3.2. Effect of Clove Oil on Opercular Activity

In clove oil anesthetization, the opercular beat showed a general decreasing trend from the beginning to the end of the experiment, except in the 0.07 ppm dose. The opercular beat in control was initially highest. Thereafter, the value remained almost constant as compared to clove oil treated fish. The opercular beat in treating fish was lowest 28/min at 1.45 and 2.3 hours (Fig 2). The respective highest (98/min) and lowest (38 min) values in 0.04 ppm dose was recorded at 0.00 and 2.15 hours. In 0.05 ppm dose, the maximum number of opercular beat was (99/min) at 0.00 hours, while lower (30/min) was noticed after 2 hours of anesthetization. The respective lowest (28/min) opercular beat in 0.06 ppm dose was noticed at 2.30 and 0.00 hours (Fig. 2) The statistical analysis reveals that the opercular beats of common carp fry varied significantly ($P < 0.01$) between experimental duration and treatments. In all the doses of clove oil, the opercular beats of experimental fish up to the first 15 minutes were less than that of the control fish , subsequently, the opercular beating showed decreasing trends among the control and all the anesthetized fishes.

3.3. Effect of Clove Oil on Respiratory Metabolism

The effect of different doses of clove oil on oxygen consumption rate (OCR), carbon-dioxide output rate (COR) and Ammonia excretion rate AER are shown in Table 3. The level of OCR in fish anesthetized with clove oil were significantly lower ($P < 0.05$) than control. As such the lowest oxygen consumption rate (12.5±0.15 mg/kg) was at 1 hour in 0.05 ppm dose of clove oil.

The OCR , COR and AER of test fish fry (common carp) in controlled condition showed the highest values during the first and three hours test . Similarly these values were higher during 1 hour and lower during 3 hours (Table 3). The highest COR (106.05 ±0.37 mg/kg) was recorded at three hours in 0.04 ppm doses , while lowest being (46.5±0.15 mg/kg) at 2 hours in 0.08 ppm dose of clove oil . The respective higher values of COR in 0.05 , 0.06 , and 0.07 ppm dose were 104.7±0.015 , 103±0.015 and 102±0.015 mg/kg of 3 hours in all the treatments.

The level of ammonia excretion rate was highest at 1 hour test in all the treatment and control group. This was significantly reduced with increasing test duration. However, the higher reduction rate was noticed in clove oil anesthetized group than control (Table 3). As such the highest (7.6 mg/kg) and lowest (2.58 mg/kg) the level of AER was in 0.04 ppm dose of clove oil. The statistical analysis revealed that all parameters showed significant difference ($P < 0.05$) between the time and treatments.

A comparative view of respiratory and ammonia quotient in relation to time and clove oil level is given in (Fig. 3) it would be seen from this (Fig. 3) that the highest level of respiratory quotient (6.96) was in control at 1 hours test. On comparing values of RQ at 1, 2 & 3 hours test it was noticed that the level of RQ was initially high, which decreased at 2 hr and subsequently increased at 3 hours. The results obtained from ammonia quotient (AQ) in control and clove oil anesthetized group are given in Fig. 4. It is evident from this figure, that the AQ has significant relationship with time and clove oil doses. The levels of AQ were initially high in all the treatment groups and control as compared to 2nd and 3rd hour test. At 1 hour test, the highest (0.64) and lowest (0.36) values of AQ were recorded in control and 0.07 ppm dose of clove oil respectively. More or less similar trends were obtained at 2nd and 3rd hour tests.

4. Discussion

The most commonly used fish anesthetic is tricaine methane sulfonate (MS- 222) [8]. However, this anesthetic is regarded as a carcinogenic and also a 21-day withdrawal period is required if the fish is intended for human consumption. One option to anesthetize fish is clove oil, which is relatively new as a fish anesthetic. Clove oil is readily available and it is inexpensive when compared to MS-222.

The qualities required of an anesthetic agent in sedating fish varies, depending on the nature, mode of application and species of fish. Most importantly, a quick induction and recovery time which allows for maximum manipulations of fish in culture medium is desirable by many aquaculturists. Despite this, anesthetics in aquaculture should be cheap, safe, easy to handle, readily available and accessible to the fish farmers in different parts of the country. In the present study, the maximum dose of clove oil was 0.08 ppm and minimum of the same was 0.04 ppm. It was found that induction time and clove oil had a direct relationship as the induction time significantly ($p<0.05$) reduced with increasing concentration of clove oil (Table 2).On the other hand, the recovery time increased with increasing concentration of clove oil (Fig.1).While working on clove oil and other anesthetics, more or less similar trends were also reported by other scientists [7, 12, 13].

Clove oil has shown to immobilize fish effectively at low dose [1, 14, 15, 16]. Keene *et al.* [14] showed that rainbow trout (weight 20.5 gm) reached loss of equilibrium stage within 1.8 – 0.6 min at concentrations of 20–100 ppm. Taylor and Roberts [17] pointed out that white sturgeon (16 gm in weight) exposed to 25 ppm of clove oil required less than 3 min for the induction time. Juvenile chinook salmon (*O. tshawytscha*) reached anesthesia or loss of reactivity after 2 min at a 20 ppm clove oil [1]. The 25 ppm of clove oil anesthetized juvenile mullet *Valamugil cunnesius* (9 gm in weight) in less than a minute [16]. Nevertheless, Anderson *et al.* [18] found concentrations of 60 and 120 ppm were needed to anesthetize rainbow trout using MS-222, higher concentrations than with clove oil. In this study, rapid induction time (less than 3 min) in which fish lost their equilibrium at low dosage (0.08 ppm) was obtained.

Regarding recovery time, common carp fry exposed to 0.04 – 0.08 ppm of clove oil demonstrated recovery within 0.5 – 7.1 min. It indicates that anesthetization with clove oil required

longer recovery time. Munday and Wilson [19] noted that recovery time after anesthesia with clove oil was two to three times longer than recovery from quinaldine, benzocaine, MS 222 and 2-phenoxyethanol. In addition, the recovery times for rainbow trout exposed to clove oil were six to ten times longer than in those exposed to similar concentrations of MS 222. According to Sladky *et al.* [20], the longer of recovery time is caused by the increased duration of exposure or the physical properties of clove oil. Because clove oil or eugenol is oil, it has the physical properties whereby it coats anatomic structures, which may be particularly important when it persist on gill epithelia. Consequently, there is prolonged exposure to the chemical and the potential for sustained anesthetic effects. In several studies, although recovery times for fish exposed to clove oil were generally longer than fish exposed to other anesthetics such as MS- 222, benzocaine, quinaldine or 2-phenoxyethanol, the times were not excessive or less than 10 min [14, 19, 21, 22, 23]. Therefore, in present study, clove oil, at 0.04, to 0.05 ppm met the criteria from Gilderhus and Meyer [24] in term of recovery time.

The efficacy of an anesthetic is dependent on several factors. Stehly and Gingerich [15] reported that a temperature increase is likely to increase the efficacy in several anesthetics. Size also seems to be positively correlated with anesthetic efficacy. The efficacy normally increases with increasing size [17]. However, Durville and Collet [16] and Walsh and Pease [23] both reported that there was no significant difference in efficacy of clove oil between juvenile and adult fish. The efficacy of clove oil is also influenced by species difference. A study conducted by Stehly and Gingerich [15] revealed that a concentration of 20 ppm clove oil gave an induction time ranging from 5.3 min for channel catfish (*Ictalurus punctatus*) to 1.2 min for bluegill (*Lepomis macrochirus*).

The OCR, COR and RQ of clove oil anesthetized common carp showed a decreasing trend for experimental duration of one hour to two hours (Table 3). The lowest value was observed for 0.08 ppm clove oil treated fish. Similar observation was made by Beacker [24] that OCR and COR was the lowest in clove oil anesthetized Angle fish. The reduction in OCR, COR and RQ values of clove oil anesthetized common carp could be due to the reduced metabolic activity of the fish. Ferrira *et al.* [26] have also reported reduced OCR, COR and RQ in anesthetized *O. mossambicus.* Pandit and Ghosh [27] opined that the anesthetics affected the brain either directly or through the blood which influenced the changes in behavior of fish as well as the metabolic.

In the present study, clove oil anesthetized common carp showed the lower opercular activity (Fig. 2) as compared to control. Pandit and Ghosh [27] stated that the difference in reduction in opercular movement may be due to the short term adaption of fish and this is true in the case of clove oil anesthetized common carp. Graham and Iwama [28] reported that Coho Salmon and Rainbow trout recovered the pre-anesthesia values of ventilation rates within 1-2 hours with ketamine hydrochloride. Forgan and Forster [29] also recorded similar reduction in ventilation frequencies of Blue Code. Iwama [30] observed reduction in opercular activity in anesthetized Coho salmon, *O. kisutch*.

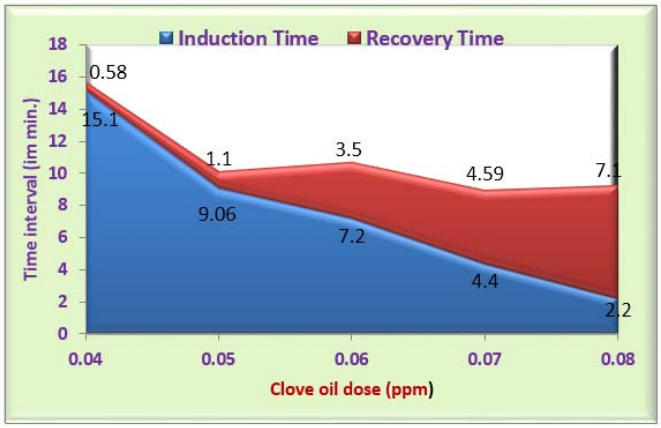

Fig 1: Induction and recovery time of common carp at different doses of clove oil

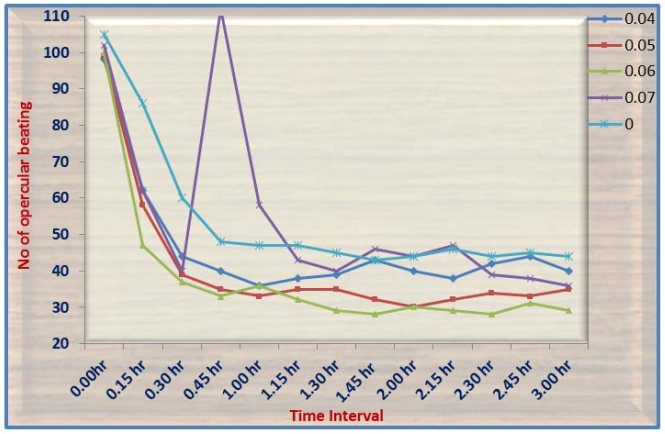

Fig 2: Effect of clove oil on opercular beating of anesthetized and control fish, common carp (fry), *Cyprinus carpio*

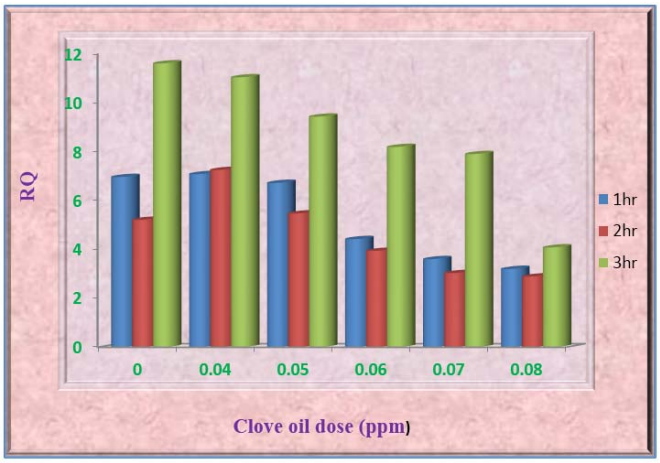

Fig 3: Respiratory quotient values at different time intervals in clove oil treated fishes

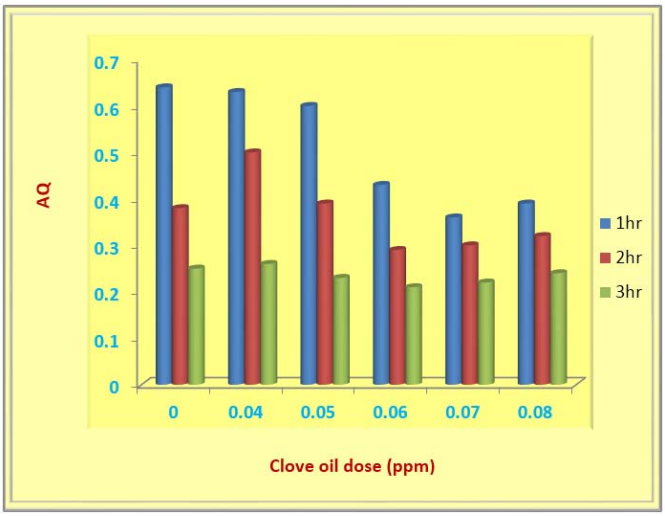

Fig 4: Ammonia quotient values at different time intervals in clove oil treated fishes

Table 2: Induction and recovery time of common carp fry at different doses of clove oil

S. No	Clove oil dose(ppm)	Induction Time (min.)				Recovery Time(min.)*	Fish Survival (%)
		LS	PLE	TLE	MC		
1	0.08	0.30	1.05	2.10	2.20	7.10^{a}	82
2	0.07	1.09	2.11	3.05	4.40	4.59^{b}	90
3	0.06	3.46	4.50	6.50	7.20	3.50^{c}	100
4	0.05	5.10	6.58	8.19	9.06	1.10^{d}	100
5	0.04	10.7	12.38	13.58	15.10	0.58^{e}	100

LS: Light Sedation, PLE: Partial loss of equilibrium, TLE: Total loss of Equilibrium; MC: Medullar collapse
*Values superscript with different letters are significantly ($p<0.05$) different

Table 3: Respiratory metabolism of control and anesthetized common carp *Cyprinus carpio* at different time intervals

Time Interval	O_2 level (mg/l)	O_2 consumption rate (mg/kg/hr)	CO_2 level (mg/l)	CO_2 output rate (mg/kg/hr)	NH_3-N level (mg/l)	NH_3-N Excretion rate (mg/kg/hr)
Control						
1 hour	6.11±0.01	13.5±0.152	1.88±0.015	94.0±0.015	0.173±0.001	8.65±0.015
2 hour	5.91±0.015	11.75±0.015	2.45±0.015	61.25±0.015	0.18± 0.002	4.5±0.152
3 hour	5.626±0.01	11.4±0.152	8.83±0.07	132.45±0.015	0.193± 0.003	3.89±0.015
Clove oil(0.04)						
1 hour	6.14±0.01	12±0.015	1.70±0.015	85.0±0.015	0.152± 0.001	7.6±0.152
2 hour	6.06±0.02	8±0.015	2.30±0.152	58.0±0.015	0.161± 0.002	4.02±0.015
3 hour	5.74±0.01	9.6±0.152	7.07±0.0152	106.05±0.375	0.172± 0.002	2.58±0.015
Clove oil(0.05)						
1 hour	6.11±0.011	12.5±0.152	1.68±0.015	84.0±0.015	0.163± 0.001	7.15±0.015
2 hour	5.96±0.02	7.5±0.152	2.30±0.015	57.5±0.152	0.166± 0.002	4.15±0.015
3 hour	5.62±0.020	8.1±0.0152	6.98±0.161	104.7±0.015	0.177± 0.002	2.65±0.015
Clove oil(0.06)						
1 hour	6.00±0.015	9±0.015	1.68±0.015	74.0±0.015	0.164±0.004	7.02±0.152
2 hour	5.8±0.020	6.5±0.152	2.29±0.015	56.25±0.015	0.172±0.002	4.3±0.152
3 hour	5.55±0.01	7.45±0.015	6.80±0.015	103±0.015	0.182±0.002	2.73±0.015
Clove oil(0.07)						
1 hour	5.92±0.011	9±0.015	1.66±0.015	73.0±0.015	0.167±0.002	7.03±0.015
2 hour	5.82±0.015	4.6±0.015	2.26±0.015	56.5±0.015	0.173±0.007	4.32±0.015
3 hour	5.52±0.015	5.75±0.015	6.80±0.015	102.0±0.015	0.194±0.001	2.91±0.015
Clove oil(0.08)						
1 hour	5.90±0.02	8.15±0.015	1.54±0.015	67.0±0.015	0.181±0.002	7.05±0.015
2 hour	5.80±0.02	4.5±0.152	2.26±0.015	46.5±0.015	0.186±0.001	4.65±0.015
3 hour	5.61±0.02	5.05±0.152	6.20±0.015	93.0±0.015	0.185±0.003	2.77±0.015

5. Conclusion

Based on the result, the appropriate concentration of clove oil for common carp should be 0.06 ppm. This concentration did not because fish mortality, ensured fish stay calm without loss of equilibrium and reduced the metabolic rate causing reduction of oxygen consumption, carbon dioxide and total ammonia in the water. Clove oil does appear to have promise as an effective and safe anesthetic for use on food fishes. However, until further studies are conducted regarding physiological effects, it should be used with caution and at the lowest concentration necessary to induce anesthesia.

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