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Evaluation of Clove (*Eugenia caryophyllata*) and Tobacco (*Nicotiana tabacum*) powders for common carp *Cyprinus carpio* L. anesthesia

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Abstract. *The anesthetic efficacy of two kinds of natural anesthetic Clove (Eugenia caryophyllatata) and Tobacco (Nicotiana tabacum) powders were evaluated on common carp (Cyprinus carpio L.) for handling and health management experiments. The concentrations of anesthetics were assessed by using two types of clove and tobacco anesthetics; each with three replicates for measuring induction times to anesthesia. Concentrations of 100, 200, 250, and 350 mg/l at the ratio 1:1 of the aqueous extract of both Clove (Eugenia caryophyllatata) and Tobacco (Nicotiana tabacum) to evaluate their effect on common carp anesthesia were used. The aqueous extract anesthetized fish partially in the high levels. No significant differences appeared on blood glucose, ALP, ALT and AST among different levels of the anesthesia concentrations used. As the anesthesia concentration increased, the Red blood cell (RBC) count and hemoglobin decreased.*

Keywords: anesthetic effect, aqueous extract, blood parameters, concentration, *Cyprinus carpio* L., *Eugenia caryophyllata*, *Nicotiana tabacum*

Introduction

Common carp (*Cyprinus carpio* L.) is the main aquaculture species in many European, Asian and Latin American countries (Aprodio et al., 2012). Carp contributes more than 70% of the inland aquaculture production in Asia and the world and it is considered as the major provider of fish protein through aquaculture (Acosta and Gupta, 2005). Common carp is extensively cultured all over the world due to its fast growth, omnivorous feeding nature and tolerance to wide water quality and temperature ranges, this fish species can feed in any part of any water system (Jain, 2002).

During aquaculture and stocking activities, fishes are faced with several potential stressors in particular ways such as transportation, capture and handling procedures, a highly crowded and confined farming environment, possible air exposure and variation in water quality are all factors that may increase the stress level of organisms (Zahl et al., 2012) and these have significant effects on the fish physiology and survival (Harmon, 2009). Fish stress and mortality can cause significant losses of resources and productivity in both capture and culture systems (Davis, 2010).

Anesthesia is a biological reversible state induced by an external agent, which results in partial and complete loss of sensation or even loss of voluntary neuromotor control, though chemical or non-chemical (Summerfeld and Smith, 1990). Anesthesia is frequently applied in aquaculture that being a valuable tool that helps to minimize fish stress and prevent physical injuries to fish while handling them during routine practices, for example, it is required for measuring or weighing fish, sorting and tagging, administering vaccines, live transport, sampling for blood or gonadal biopsies and collecting of gametes and finally surgical procedures to cite some of the main applications (Maricchiolo and Genovese, 2011).

Organic farming of agricultural and horticultural crops is being used as a popular venture in the direction of sustained and eco-friendly food production activity. Organic farming looks for alternatives to those chemicals that are currently being used in aquaculture, and the anesthetics are one such important input, as a result, different natural anesthetics were investigated to compare their effectiveness with chemical products (Taylor and Roberts, 1999).

Dry clove powder (Carnation flowers buds): is a common medicinal plant available in all medicinal plant stores, and it 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). The results reported by Hassan (2016) conclude that the 300 mg/l of clove powder was considered as the best effective anesthetic agent for the induction of anesthesia in *C. carpio* with the highest concentration according to their concentration that related to the recovery time, rapid induction, circulating cortisol and glucose, 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.

The lowest cortisol level of clove powder anesthesia was found in the low temperature degree (9±1°C) with 300 mg/l of clove powder. The lowest and the highest glucose levels were found in a low temperature degree with 300 and 400

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mg/l of clove powder. Clove powder of 200 mg/l with normal temperature degree (27±1°C) showed the lowest level of red blood cells (Abdulrahman et al., 2018).

Carp exposed to doses of clove powder showed clear adverse relation between induction time and concentration of the anesthetic (452 s in 200 mg/l and 137 s in 400 mg/l). Recovery time was negatively correlated to induction time and was directly proportional with increasing doses of clove powder (290 s at 400 mg/l and 199 s at 200 mg/l). The ventilation rate increased significantly in all stages of anesthesia and recovery compared to control (17.5/15 s) (Hassan et al., 2016). Results of Al-Niaeem et al. (2017) showed that anaesthesia time decreased with increasing concentrations of nutmeg powder, and there were significant differences ($p<0.05$) in anaesthesia time between the different concentrations.

The use of aqueous and alcoholic extracts of tobacco as an anesthetic for fish handling procedures was investigated using the Nile tilapia, *Oreochromis niloticus*. An effective concentration of the anesthetic was defined as one giving a sure state of anesthesia for 75% of the fish after exposure time of less than 5 min. The effective concentration of the aqueous extract was 4/4.5 g/l. For the alcoholic extract it was 6/7 ml/l. There is a highly significant correlation ($r= -0.98$, $p<0.01$) between concentration and recovery time with the aqueous extract. Recovery times with both preparations were short, 2-5 min with the aqueous extract and an average of 10 min with the alcoholic extract. Anesthesia with the aqueous extract was recommended over the alcoholic extract as it had a lower effective dose and a comparable recovery time (Agokei and Adebisi, 2010).

According to all that, the objectives of the study were to evaluate the anesthetic effects and the safe use of natural agents such as clove and tobacco in health treatments research and determine the stress response of common carp (*C. carpio* L.) to natural anesthesia.

Material and methods

Object of study

For the purpose of the study as natural materials used in fish management and handling, we used clove (*Eugenia caryophyllata*) and tobacco (*Nicotiana tabacum*) powder that is commercially found in markets as anesthesia for common carp (*Cyprinus carpio* L.).

Experimental design

Common carp were obtained from fish farms at the University of Basrah, Basrah province, Iraq, putting the fish in an aquarium (50*30*30 cm) for three days for acclimation at 24ºC temperatures ensuring that they had sufficiently recovered from possible capture-related or transport stress. The experiment was carried out in fish diseases laboratory at the College of Agriculture, University of Basrah. The used concentrations and experimental groups in carp treatment with clove and tobacco were as follows:

- Clove: Control group (T1): without clove; Experimental groups: (T2) - 15 mg/l, (T3) - 20 mg/l, and (T4) - 25 mg/l;
- Tobacco: Control group (T1): without tobacco; Experimental groups: (T2) - 50 mg/l and (T3) - 75 mg/l;
- Clove + Tobacco at ratio 1:1: (100, 200, 250 and 350 mg/l);
- Clove + Tobacco at ratio 1:2: (200, 250, 300 and 350 mg/l).

Induction and recovery times were measured with a video recorder to the second stage. Following the induction into stage III each fish was weighed and transferred to a recovery aquarium that had been filled with aerated freshwater at the same time of preparation of the anesthetic baths. In the recovery tub the fish were monitored continuously to determine time to full equilibrium.

Monitored parameters of the carp

Blood parameters: The blood samples from each fish of the different groups were collected by suction of the caudal peduncle. Whole blood samples were collected for determination of Red blood cell, RBC ($x10^5$/ul), White blood cell, WBC ($x10^3$/dl) and Hemoglobin, Hb (g/dl).

Biochemical parameters: Alanine aminotransferase activity (ALT, IU/L), Aspartate aminotransferase activity (AST, IU/L) and Glucose (mg/dl). All blood tests were done by using a hematological analyzing device type ACCENT 200 Poland's origin. The levels of enzymes were assayed according to the instructions provided with the corresponding enzymatic kits.

Behavior of the fish: A video recorder was used to monitor the behavior of carp.

Statistical analysis

The trial was conducted by one way (ANOVA) with completely randomized design (CRD) and general linear models (GLM) procedure of XLSTAT 2016 Version.02.28451. Duncan's test was used for comparison among treatment means at $p\leq0.05$.

Results and discussion

No significant differences were observed in each of the fish weight and length ($p>0.05$), the glucose ratio was significantly different among treatments in which the third treatment with (20 mg Clove/L) was significantly higher ($p<0.01$) as shown in Table 1.

Table 1. Effect of aqueous solution of Clove powder in common carp blood glucose

Treatments	Fish weight, g	Fish length, cm	Glucose, mg/dl
T1 Control	3.733 ± 0.033^a	8.133 ± 0.017^a	27.333 ± 0.020^c
T2 (15 mg Clove /L)	3.500 ± 0.015^a	6.000 ± 0.018^a	15.300 ± 0.012^d
T3 (20 mg Clove /L)	3.243 ± 0.019^a	6.250 ± 0.014^a	75.000 ± 0.017^a
T4 (25 mg Clove /L)	4.500 ± 0.031^a	7.750 ± 0.019^a	58.000 ± 0.012^b
Significant	No	No	Yes

*Data represent as mean ± SD; Data in the same columns with different superscript are significantly different ($p<0.01$)

The results in Table 2 show that the addition of clove had an effect on the anesthesia time of common carp, as the concentration of clove increased, anesthesia time decreased in either partial or total anesthesia. For the recovery time the T3 with (20 mg Clove/L) was higher significantly than other treatments ($p<0.01$).

Table 2. Effect of aqueous solution of Clove powder in common carp anesthesia and recovery

Treatments	Partial anesthesia, min	Total anesthesia, min	Partial recovery, min	Total recovery, min
T2 (15 mg Clove /L)	15.000± 0.011[a]	35.000± 0.014[a]	3.000± 0.013[c]	8.000± 0.016[b]
T3 (20 mg Clove /L)	7.000± 0.013[b]	10.000± 0.017[b]	15.000± 0.014[a]	18.000± 0.014[a]
T4 (25 mg Clove /L)	5.500± 0.012[c]	10.500± 0.011[b]	5.500± 0.016[b]	9.500± 0.012[b]
Significant	Yes	Yes	Yes	Yes

*Data represent as mean ± SD; Data in the same columns with different superscript are significantly different ($p<0.01$)

No significant differences were observed in the use of tobacco powder as anesthesia in common carp. The weight, length and blood glucose content were as shown in Table 3. Numerically T2 with (50mg Tobacco/L) was higher in blood glucose content compared to the control and T4 (75mg Tobacco/L) treatment, but the differences were not statistically significant ($p>0.05$).

Table 3. Effect of aqueous solution of Tobacco powder in common carp blood glucose

Treatments	Fish weight, g	Fish length, cm	Glucose, mg/dl
T1 Control	3.615± 0.012	7.700± 0.014	27.500± 0.011
T2 (50 mg Tobacco /L)	3.710± 0.015	6.850± 0.013	31.000± 0.014
T3 (75 mg Tobacco /L)	3.950± 0.016	7.775± 0.011	27.500± 0.012
Significant	No	No	No

The results for the effect of the aqueous solution of tobacco powder in common carp anesthesia and recovery are presented in Table 4. The data show that tobacco powder is not suitable to be applied as anesthesia in common carp, and we do not recommended this powder to be used for these purposes.

Using 50 or 75 mg/l of tobacco powder as anesthetic agent for common carp by putting the extraction in the refrigerator for 24 hours and testing their effect on the fish anesthesia showed that the test was without any results, which means that the fish did not respond to those treatments (Table 5).

Test interpretation, H0: The difference between them is equal to 0; Ha: The difference between them is different from 0, as the computed p-value is higher than the significance level alpha= 0.05, one cannot reject the null hypothesis H0. The risk to reject the null hypothesis H0 while it is true is 9.89% for partial anesthesia, 5.90% for totally anesthesia, 59.23% for partial recovery. For total recovery, as the computed p-value is lower than the significance level alpha= 0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis (Ha). The risk to reject the null hypothesis H0 while it is true is lower than 0.03%.

Table 4. Effect of aqueous solution of Tobacco powder in common carp anesthesia and recovery

Anesthesia stage	Difference	Test (Observed value)	Test (Critical value)	DF	p-value (Two-tailed)	alpha
Partial anesthesia, min	4.500	2.141	2.776	4	0.099	0.05
Total anesthesia, min	2.500	0.658	2.776	4	0.546	0.05
Partial recovery, min	-0.503	-0.581	2.776	4	0.592	0.05
Total recovery, min	-3.503	-12.135	2.776	4	0.000	0.05

Table 5. Effect of aqueous solution of Tobacco powder (50 and 75 mg/l) in common carp anesthesia and recovery in hot water

Anesthesia stage	Difference	Test (Observed value)	Test (Critical value)	DF	p-value (Two-tailed)	alpha
Partial anesthesia, min	4.500	2.141	2.776	4	0.099	0.05
Total anesthesia, min	5.500	2.617	2.776	4	0.059	0.05
Partial recovery, min	-0.503	-0.581	2.776	4	0.592	0.05
Total recovery, min	-3.503	-12.135	2.776	4	0.000	0.05

The 100, 200, 250, and 350 mg/l at ratio 1:1 of the aqueous extract of both clove and tobacco were used to determine their effect on common carp anesthesia, the aquarium water temperature was 23ºC, pH 7.6 and the salinity 3.5 ppt. Table 6 showed that the partial anesthetic effect happened in the higher concentrations and failed in total anesthesia.

Table 6. Effect of different levels of aqueous extract of Clove and Tobacco mixture (1:1) in common carp anesthesia

Concentration, mg/l	Fish weight, g	Total recovery	Partial Recovery	Total anesthesia	Partial anesthesia
100	No	23	----	----	----
200	No	21	----	----	----
250	No	24	----	----	----
350	1.15	19	No	----	----

The results of the present study revealed that the mixture of clove and tobacco 1:1 did not anesthetisize fishes in the concentration 100, 200, 250 and 350 mg/l and they recovered rapidly, but it anesthetized common carp at the ratio of 1:2. These results are consistent with the results of Hoskonen and Pirhonen (2006) who reported that clove buds contain eugenols that are regarded as an anesthetic agent.

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 because if it could be said that a long exposure to low concentration of the anesthetic leads to more anesthetic absorption by fish, the results obtained revealed that the short exposure to high anesthetic concentration does it as well. On the other hand, Weyl et al. (1996) pointed out that compared with anesthesia duration, anesthetic concentration plays a more important role on the fish recovery time. It is believed that the independence of the recovery time from the anesthesia duration, as a result of that anesthetic, is taken up by the fish through a concentration gradient at the gill interface. Therefore, when equilibrium level is established between the gill and the anesthetic solution, no further anesthetic will be taken up by the fish, and during recovery, the anesthetic agent is leaked through such a gradient. Therefore, the recovery time is controlled by the anesthetic concentration but not by the duration of anesthesia exposition (Weyl et al., 1996).

The aqueous extract of clove and tobacco mixture were used at concentration of 200, 250, and 300 mg/L at ratio 1:2 to study their effect on anesthesia of common carp reared in water with temperature 25ºC, pH 7.4 and salinity 3.7 ppt, as explained in Table 7, the extracts anesthetized fish partially and totally in high concentrations.

Table 7. Effect of different levels of aqueous extract of Clove and Tobacco mixture (1:2) in common carp anesthesia

Concentration, mg/l	Fish weight, g	Total recovery	Partial Recovery	Total anesthesia	Partial anesthesia	Glucose, mg/dl
200	11	1:14	1:17	1:20	1:23	90
250	20	1:16	1:20	1:24	1:28	85
300	15	9:21	9:25	9:29	9:32	100
350	6	9:21	9:25	9:29	9:32	105

In experiment of Hassan (2016) the concentration of glucose was significantly increased in anesthesia with clove powder compared with that in the control fish. Wagner et al. (2003) found that the increase in the blood glucose concentration demonstrated the response of exposed fish to metabolic stress, rapid increases in plasma glucose are mediated by the release of catecholamines, which increase (presumably in response to the hypoxia caused by cessation of respiration) in the plasma of the anaesthetized fish. The present findings agree with those of Holloway et al. (2004) who also detected an increase in glucose concentration in rainbow trout (*O. mykiss*) following clove oil anesthesia and Ortuno et al. (2002) who reported also an increase of glucose in *Sparus aurata* anaesthetized with 2-phenoxyethanol.

Table 8 shows that the 300 and 400 mg/l affect significantly the Red blood cell (RBC) count and Hemoglobin ($p<0.01$), no significant difference observed in White blood cell (WBC) ($p>0.05$). Hematological parameters provide the required information on the physiological status of fishes; help the aquaculture and research personnel to make proper decisions to increase the survival of fishes (Gholipourkanani and Ahadizadeh, 2013). Sudgaran et al. (2009) observed that the anesthetic effect of clove powder on roach (*Rutilus rutilus*) after 7 min anesthesia, at the concentration of 175, 225, 275 and 350 mg/l immediately and 24h post anesthesia had no effect on hematological parameters, and Gholipourkanani and Ahadizadeh (2013) reported the same results when they used different dosages of Propofol as anesthetic agent for Gold fish (*Carassius auratus*).

Some authors report different from our results change in WBC. Hassan (2016) observed exhibited marked decrease in WBC in anesthetic treated fishes with the concentrations of 200, 300 and 400 mg/l. The same tendency in WBC decrease was reported by Abdolazizi et al. (2011) who studied the effect of two levels of clove oil concentrations (0.75 and 150 mg/l) on goldfish (*C. auratus*). Imanpoor et al. (2010) also found that anesthesia by clove essence declined in WBC in *Acipenser persicus*.

Table 8. Effect of anesthetic types after recovery on some blood indices

Parameter	Red blood cell ($x10^5$/ul)	White blood cell ($x10^3$/dl)	Hemoglobin (g/dl)
Control	54.23 ± 2.25^a	4.20 ± 0.22^a	32.42 ± 2.42^a
200 mg/l	53.04 ± 2.16^a	4.31 ± 0.20^a	38.13 ± 2.44^a
250 mg/l	55.52 ± 2.06^a	4.15 ± 0.35^a	36.31 ± 1.35^a
300 mg/l	63.32 ± 1.31^b	4.03 ± 1.32^a	45.24 ± 2.43^b
350 mg/l	63.45 ± 1.42^b	4.23 ± 1.43^a	47.13 ± 2.52^b

*Clove and tobacco mixture 1:2; Data represent as mean ± SD; Data in the same columns with different superscript are significantly different ($p<0.01$).

No significant differences between all treatments were observed in blood enzymes ALP, ALT and AST as shown in Table 9. The best concentration was 350 mg/l as the total anesthesia occurred after 8 min and total recovery after 6 min. After recovery the RBC and Hb increased, no effects on WBC and the blood enzymes.

Table 9. Effect of aesthetic types after recovery on some blood enzymes

Parameter	ALP	ALT	AST
Control	41.53 ± 3.25	4.10 ± 1.32	34.52 ± 2.52
200 mg/l	45.04 ± 2.26	4.50 ± 1.20	34.04 ± 1.54
250 mg/l	44.32 ± 2.24	5.05 ± 1.25	36.10 ± 1.25
300 mg/l	45.40 ± 2.24	4.53 ± 1.23	35.13 ± 2.12
350 mg/l	47.20 ± 2.24	5.53 ± 0.23	37.03 ± 1.02

*Clove and tobacco mixture 1:2; Data represent as mean ± SD.

The effect of different levels of aqueous extract of clove and tobacco mixture on common carp behavior is described in Table 10. In 200 mg/l fish start to swim abnormally and

go back to normal swimming after 20 min. At 250 mg/l the ventilation rates increased leading to total anesthesia after 17 min, while in 300 mg/l there was slow swimming; rapid ventilation rate after 10 min; partial anesthesia occurred after 9 min as the ventilation rate was slow with slow swimming vertically and near surface; total anesthesia after 15 min with very slow ventilation rate and laying in the bottom, but in 350 mg/l there was slow swimming, rapid ventilation rate; partial anesthesia occurred after 4 min as the ventilation rate was slow and vertical swimming near surface; total anesthesia occurred after 8 min when fish was laying on surface and there very slow ventilation rate.

Table 10. Effect of different levels of aqueous extract of Clove and Tobacco mixture (1:2) in common carp behavior

Concentration, mg/l	Behavioral observations
200	Abnormal swimming; Back swimming; trying to swimming to water surface, going back to normal swimming after 20 min.
250	Slow swimming; increase in ventilation rate after 10 min of anesthesia; the total anesthesia occurred after 11 min as fish's ventilation rate was very slow and they swim vertically towards the surface. Total anesthesia occurred after 17 min the fish's layout at aquarium bottom and very slow ventilation ratio. Partial recovery occurred after 3 min while the total recovery after 6 min. No significant ratio of blood glucose between fish after total recovery and the control.
300	Slow swimming; rapid ventilation rate after 10 min; partial anesthesia occurred after 9 min as the ventilation rate was slow with slow swimming vertically and near surface; total anesthesia after 15 min with very slow ventilation rate and laying in the bottom; partial and total recovery happened after 3 and 6 min, respectively. No significant ratio of Blood glucose between fish after total recovery and the control.
350	Slow swimming, rapid ventilation rate; partial anesthesia occurred after 4 min as the ventilation rate was slow and vertical swimming near surface; total anesthesia occurred after 8 min when fish laying on surface and very slow ventilation rate. Partial and total recovery happened after 3 and 6 min, respectively. No significant ratio of blood glucose between fish after total recovery and the control.

Conclusion

The use of natural materials for anesthesia of common carp (*Cyprinus carpio* L.), such as clove and tobacco powder have effect on fish anesthesia and recovery so they can be used in management and handling of fish. The best result was observed in using the combination between clove and tobacco in 1:1 with 350 mg/l having rapid anesthesia and recovery.

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