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Using clove and mint oils as natural sedatives to increase the transport quality of the Nile tilapia (*Oreochromis niloticus*) broodstock

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ABSTRACT

The present study was conducted to create a new strategy for increasing the transport efficiency of Nile tilapia (*Oreochromis niloticus*) brood-stock using clove and mint oils for 3.5 h. The fish study was exposed to conditions simulating those normally used in transporting. This study was performed in a fish research center of the Arish University. The average initial weight of the brood-stock was 117.07 ± 09.07g (± standard error). The brood-stock was randomly distributed into 10 clear plastic aquaria (30 liters capacity) with a stocking density of one brood-stock/3.75 liters of water. The present study examined two concentrations of clove oil as a deep anesthetic for 5 min and two concentrations of mint oil as a sedative. It included five treatments in duplicate, namely, the control group, G1: clove and mint oil at 100 and 20 µL/L, respectively; G2: 100 and 10 µL/L of clove and mint oil, respectively; G3: 50 and 20 µL/L of clove and mint oil, respectively; and G4: 50 and 10 µL/L of clove and mint oil, respectively. Blood samples were taken from all groups before transporting from a random sample and after transporting. Results confirmed that G1 had the best parameters of water quality and blood indicators with the highest survival rate followed by G2. Moreover, using clove and mint oil improved the transport efficiency and survival rate after 24 h of fish transporting compared to that of the control group.

INTRODUCTION

Aquaculture production in Egypt surpassed 79% of the total fish production. The production of the cultured tilapia represents 70% of the aquaculture production (**GAFRD, 2018**), which reflects the importance of aquaculture in general and the cultured tilapia in particular. Providing fry for farms in the optimum time with suitable amounts and a high survival rate is considered one of the success factors of the cultured tilapia in Egypt. Therefore, foundation hatcheries near rearing ponds encourage providing seeds before starting the production season in high quality to avoid transportation costs. The transportation of the tilapia brood-stock from collection sites to the hatcheries needs some precautions to reduce the associated stress during transportation, such as the shock of the transportation means, the small volume of transport tanks or plastic bags, the high density, the decrease in the level of dissolved oxygen (DO), the increase in the total ammonia and carbon dioxide, and the accumulation of metabolic and organic wastes. Strategies that are employed to reduce stress during fish transport have great economic importance. Exposing the broodstock to transport stress not only increases the mortality rate, but also delays the spawning season (**Delbon & Paiva, 2012; Abdel-Aziz, 2013**). The use of sedatives is one option to help reduce the stressful conditions and metabolism rates during fish transport. Sedatives have been tested as an additive during the shipment of live fish to help alleviate stress (**Cooke *et al*., 2004**). The following are the two types of commercial anesthetics: natural and synthetic anesthetics or sedatives. Synthetic sedatives are banned owing to safety issues and residues; however, natural sedatives are safer and were thereby developed and are expected to occupy a great position in the future. The following are several synthetic agents used to anesthetize the fish: eugenol (**Cupp *et al*., 2012**), etomidate (**Readman, *et al*., 2017**), tricaine methanesulfonate (MS-222), and phenoxyethanol (**Bahrekazemi & Yousefi, 2017)**. Several natural sedatives are used to transport fish, such as the essential oil of *Lippia alba* with catfish (*Rhamdia quelen*) (**Salbego *et al*., 2014**), lavender oil and clove powder with convict cichlid fish (*Cichlasoma nigrofasciata*) (**Raisi *et al*., 2020**). Mint oil is another option which is considered a good anesthetic agent for aquatic animals, such as the fat snook (**Souza *et al*., 2019**). In addition, clove oil is the most common sedative with a good reputation for immobilizing the fish during transport. Clove oil is consisted of beta-caryophyllene (13%) and eugenol, which is an effective component, and represents >80% of clove oil that can be extracted from the flower stems or leaves of cloves (*Eugenia aromaticum* or *Eugenia caryophyllata*) **(Putri *et al*., 2014)**. On the other hand, mint oil is consisted of l-menthol (37.0%), isomenthone (7.48%), limonene (6.75%), menthone (20.25%), menthyl acetate (4.60%), isopulegone (1.81%), pulegone (1.39%), carvone (0.08%), and cineole (0.34%) (**Ostrensky *et al*., 2016**). Determining the optimum used doses of sedative for fish transporting depend on several factors, such as fish type, fish size, transport period, fish density, water quality, and transport timing. Furthermore, sedation overdose may cause negative effects on fish and human health. In this context, clove oil has a relatively low safety margin, and hence, slight differences in dose may kill the fish (**Kamble *et al*., 2014**).

The present study aimed to collectively assess the two essential oils as natural sedatives to reduce the stressful conditions and minimize possible injuries associated with the transport of the Nile tilapia brood-stock. In this study, clove oil was used with a high dose as a pre-deep anesthetic agent, while mint oil was used with a low dose as a sedative.

MATERIALS AND METHODS

This study was performed to evaluate the strategy of using clove oil as a deep anesthetic agent before fish transport and mint oil as a sedative during the fish transport on physiological indicates and survival rate (SR) of the broodstock Nile tilapia (*Oreochromis niloticus*). The broodstock was subjected to conditions simulating those normally used in transporting. This work was accomplished in a fish research center of the Arish University, Egypt.

Anesthetic and sedative preparation

Clove and mint oil were brought from a local market of medicinal and herbal products. The oils were diluted in ethanol alcohol 95% (part oil: 10 parts alcohol) and mixed with the transport water. The final clove and mint oil alcohol solution was 100 µL/mL.

Fish and experimental design

Broodstocks were captured from hatching ponds of the fish research center, with an average weight of 117.07 ± 09.07 g (SE). They were randomly distributed in 10 clear plastic aquaria with water volume of 30 liters/aquaria at a stocking rate of one brood-stock/3.75 liters. The transport duration was 3.5 h. This study included five treatments using two levels of clove oil as a pre-deep anesthetic agent (50 and 100 µL/L, previously diluted with alcohol) for 5 min. Afterwards, the water in all aquaria was exchanged; two different levels of mint oil as a sedative with 10- and 20-µL/L concentrations (previously diluted with alcohol) were added. The followings were the formulated treatments: G1, the control group, fishes in this group were not exposed to any anesthetic or sedative; G2, 100-µL/L clove oil as a deep anesthetic agent for 5 min, then 20-µL/L mint oil; G3: 100-µL/L clove oil with 10-µL/L mint oil; G4: 50-µL/L clove oil with 20-µL/L mint oil; and G5: 50-µL/L clove oil with 20-µL/L mint oil. Each treatment was duplicated.

Water quality parameters

Physiochemical parameters of the transport water were recorded after every hour of transporting duration. Temperature, pH, and DO were measured by a multi-parameter water quality analyzer, and the total ammonia was verified using the color methods.

Biochemical blood parameters

At the beginning and the end of the transporting period, blood samples were randomly collected from the caudal vein of the fish using 3-mL syringes and emptied into two tubes. The 1st tube contained ethylenediaminetetra acetic acid (anticoagulant 10%) to prevent coagulation and estimate the hemoglobin (Hb) content. While, the 2nd tube did not contain ethylenediaminetetra acetic acid to spectrophotometrically measure the serum parameters (plasma cortisol, glucose, GOT, GPT, urea, and creatinine) using commercial kits. Then, the sample tubes were immediately transported to a hematological laboratory.

Statistical analysis

Data were analyzed using one-way analysis of variance at a 95% confidence limit, and SPSS software, version 19. Waller-Duncan test was used to compare means, and F values from the analysis of variance were significant at $P < 0.05$.

RESULTS

Means of water quality indicators are presented in Table (1) indicating significant differences among groups ($P < 0.05$). The control group had the highest temperature and total ammonia (25.20°C and 0.22 mg/L, respectively) as well as the lowest pH and DO (6.03 and 3.40 mg/L, respectively) compared to other groups. No significant difference was noted in water temperature before transporting between the groups treated with clove and mint oils.

Table 1: Averages of physiochemical parameters of treatments before and during the transportation

parameters	before	control	G1	G2	G3	G4	PSE*	f-value
Temperature ºC	25.037^{b}	25.20^{a}	25.030^{b}	25.038^{b}	25.032^{b}	25.03^{b}	0.02	12.89
pH	6.93^{a}	6.03^{c}	6.60^{b}	6.56^{b}	6.50^{b}	6.40^{b}	0.07	4.97
DO, mg/l	6.97^{a}	3.40^{d}	4.63^{b}	4.70^{b}	3.93^{c}	3.86^{c}	0.28	73.41
Total ammonia, mg/l	0.023^{c}	0.22^{a}	0.057^{bc}	0.08^{b}	0.08^{b}	0.09^{b}	0.015	23.50

Means within the same row with different superscript letters are significantly different at $P < 0.05$
*, Pooled standard error

The pH values of G1 (6.60), G2 (6.56), G3 (6.50), and G4 (6.40) did not significantly change; however, they were significantly higher and significantly lower before transporting than that of the control group.

The DO (in mg/L) varied among groups. G1 (4.63) and G2 (4.70) had a higher level of DO than G3 (3.93) and G4 (3.86). The level of these groups was significantly higher than that of the control group and lower than before transporting (6.97 mg/L). In the same manner, the total ammonia value of fish pre-transporting was the lowest (0.023 mg/L), followed by G1 (0.057 mg/L), which was the best group in comparison with those treated with oils. However, the control group recorded the highest total ammonia value (0.22 mg/L).

Hematological parameters

Blood parameters are shown in Table (2). Significant differences ($P < 0.05$) were noted in all parameters among groups, except for GPT. The highest Hb g/dl content was recorded by the pre-transport group (6.55 g/dl), followed by G1 (6.20 g/dl), which had the highest Hb content compared to those of the treated groups with oils, followed by G2 (5.85 g/dl), G3, and G4. Notably,the control group had the lowest Hb content (5.30 g/dl). In addition, the control group had the highest plasma glucose level of 145 mg/d, followed by G4 (128 mg/d), G3 (117.5 mg/d), G2 (86 mg/d), G1 (74 mg/d), and pre-transport groups (54 mg/d). The cortisol values were 17.21, 15.17, 13.74, 9.17, 8.36, and 6.70 mg/l for the control, G4, G3, G2, G1, and pre-transport groups, respectively, whereas cortisol did not significantly differ between G1 and G2, with the same trend of glucose.

Table 2: Mean values of blood parameters of treatments before and during the transportation using clove oil and mint oil as deep anesthetic and sedative

Parameters	before	control	G1	G2	G3	G4	PSE*	F-value
Hb g/dl	6.55^{a}	5.30^{c}	6.20^{ab}	5.85^{bc}	5.45^{c}	5.55^{c}	0.142	7.72
Glucose mg/d	54^{f}	145^{a}	74^{e}	86d	117.5^{c}	128^{b}	9.63	289.90
Cortisol, mg/l	6.70^{c}	17.21^{a}	8.36^{c}	9.17^{c}	13.74^{b}	15.17^{ab}	1.20	21.34
Urea mg/dl	11.95^{c}	15.35^{a}	14.20^{b}	15.15^{ab}	14.95^{ab}	14.85^{ab}	0.36	15.69
Creatinine, mg/dl	0.11^{e}	0.39^{a}	0.16^{d}	0.38^{a}	0.34^{b}	0.29^{c}	0.31	128.48
GOT U/l	24^{b}	68^{a}	30^{b}	25^{b}	35^{b}	28^{b}	4.55	31.43
GPT U/l	6	12.5	7.5	6	6	6.5	0.89	1.89

Means within the same row with different superscript letters are significantly different at P < 0.05
*, Pooled standard error

Insignificant differences were noted among G2, G3, and G4 in terms of the urea value, though they were significantly higher than that of G1 (14.20). The highest urea, mg/dl was recorded in the control group (15.35) and the lowest was obtained in the group before transfer (11.95). The highest creatinine (mg/dl values were 0.39 and 0.38) was noted in the control and G2 treatment groups, respectively, followed by G3 and G2. The lowest values were 0.11 and 0.16, which were recorded in fish before transfer and G1 groups, respectively. Insignificant differences were noted among G1, G2, G3, G4, and pre-transport groups in terms of GOT, U/l; however, those groups had significantly lower GOT levels than those of the control group (68) ($P < 0.05$). GPT, U/l did not significantly differ among groups; however, the control group had the highest value of GPT (12.5).

SR (%)

After directly transporting, the SR of broodstocks significantly varied between the groups treated with clove and mint oils and the control group. However, the control group had a lower SR than that recorded in the other groups.

Table 3: Survival rate after transporting directly and after 24 hours

parameters	control	G1	G2	G3	G4	PSE*	F-value
Survival rate** after transporting directly	75^{b}	100^{a}	100^{a}	100^{a}	100^{a}	3.41	25.00
Survival rate after 24 hr of transporting	50^{c}	100^{a}	100^{a}	87.5^{ab}	77.5^{b}	6.46	13.21

Means within the same row with different superscript letters are significantly different at P < 0.05
*, Pooled standard error
**, (number of fish at the end of experimental period/ number of fish at start)×100

After 24 h of transport, the SR significantly differed among groups. The highest SR was observed with G1 and G2, followed by G3 and G4, whereas the SR of the control group decreased to 50%.

DISCUSSION

It is worthy to mention that, fish suffer from several stressful factors during transport, such as high density, low water volume, reduced DO, the accumulation of suspended solids, and the increase in the total ammonia (**Emmanual *et al*., 2014**). Table (1) shows a decrease in DO and pH level as a result of carbon dioxide accumulation with increasing total ammonia for the transported fish compared to that of the pre-transport group. Similarly, **Aker (2011)** reported that, water quality deteriorated when fish were under transport-induced stress. However, the obtained results of water quality parameters revealed that the treated groups with clove and mint oils had better water quality indicates than the control group during the transportation. This was attributed to clove oil applied as a deep anesthetic for 5 mins and mint oil as a sedative, which resulted in immobilizing the fish and reducing the metabolic activity; therefore, the rate of the total ammonia excretion and oxygen demand decreased. Moreover, it was observed that a similar trend of using sedatives in fish transport reduces the fish activity and excreted ammonia through gills (**Singh *et al*., 2004; Aker, 2011**). However, information regarding the use of natural oil sedatives to attenuate stressful conditions of live fish transport is scarce, although determining the doses of these oils need more than one study and research. Accordingly, Table (1) determines that G1 and G2 had better water quality than G3 and G4, wherein the used doses of clove and mint oil in G1 were more effective than those used in G2, G3, and G4.

For the physiological parameters, there are two types of physiological responses of fish to stressful conditions, including primary and secondary responses, wherein each type is associated with changes in specific hematology indicates (**Barbosa *et al*., 2007; Zahl *et al*., 2009**). Therefore, the cortisol concentration is an important primary response, whereas the glycemic level is affected and hematological alterations are typically secondary responses (**McDonald & Milling, 1997**). Table (2) reveals that the control group recorded the highest level of cortisol compared with the treated groups with clove and mint oils. In addition, the G1 treatment group had a lower level of cortisol, followed by G2, G3, and G4. In the same trend, the results of plasma glucose values were consistent with the results of cortisol concentrations, wherein using the oils of clove and mint led to a decrease in the glucose level of the transported fish compared with those transported without the use of these oils. This finding agrees with that of **Pottinger and Pickering (1997)** suggesting that glucose and cortisol levels are continuously employed as signs of a stressful fish. **Martinez-Porchas *et al*. (2009**) reported that the cortisol concentration increased in the fish exposed to stressful conditions. Furthermore, the previous authors found that glycogenolysis and gluconeogenesis can regulate the plasma glucose level of fish in response to a stressful condition; an increase in the plasma glucose level appears to be an immediate response to the increased metabolic and respiratory rates in muscle cells. Additionally, **Aker (2011)** mentioned that the plasma glucose level of fish decreased in response to the exposure to clove oil; this response is the result of cortisol level alterations.

The treated groups with oils increased the Hb content compared to the control group. G1 recorded a higher Hb level than G2, G3, and G4, respectively. These results are consistent with the observations of **Bolasina (2006)** who noted an increased Hb content and a hematocrit value after anesthesia; the use of benzocaine as an anesthetic agent for fish results in increased erythrocyte and Hb concentrations.

Regarding urea and creatinine, as determinants of kidney function, their concentrations decreased in fish serum treated with oils compared with those in the control group. Moreover, the fish in the G1 treatment group recorded the lowest values of urea and creatinine compared to those in the transported fish groups, thereby clearing the role of clove and mint oil and their used doses. While, increasing these parameters in fish may be indicative of a change in the gill epithelium, because the gills appear to have more responsibility in the excretion of ammonia than the kidneys (**Shih *et al*., 2008**). Moreover, under the stressful condition, the increased ammonia extraction caused osmotic imbalance with ion loss, leading to overload on the gills, thereby increasing the urea concentration. Similarly, **Mazandarani *et al*. (2017)** found that the increased serum glucose, cortisol, and urea concentrations decreased the serum ions of the transported fish. Remarkably,creatinine is the end product of energy used by muscle tissues. Creatinine is formed from creatine, and its blood concentration can be changed at different levels of muscle activity (**Thrall *et al*., 2004)**. Thus, reducing the activity and movement of the fish during transportation by using sedatives resulted in the reduction of serum urea and creatinine.

Concerning liver functions, it was found that the control group recorded higher GOT and GPT activities than the groups treated with oils, whereas the lowest value of GOT was obtained by G1. GOT and GPT activities are considered as determinants of the health of the fish (**Prusty *et al*., 2011**), since injured hepatocytes can induce a release of GOT and GPT into the blood. In the present study, the activities of plasma GOT and GPT increased with increasing stocking density; indicating that the liver was damaged to a certain extent when the fish were reared in the high stocking density (**Wang *et al*., 2019**).

Positive effects of using clove and mint oils directly reflected the SR at the end of the duration transport and after 24 h. The SR was 100% for the group treated with oils, whereas that of the control group was 75% directly after finishing the transport. After 24 h of transport, the SR of G1 and G2 did not decrease, although that of G3 and G4 decreased to 87.5 and 77.5, respectively; the SR of the control group became 50%. Generally, the applied strategy of using clove oil as a deep anesthetic agent for 5 min and mint oil as a sedative is considered an effective tool to reduce the stressful conditions during broodstock transport. This used strategy could reduce general activity stress, oxygen demand, and increase the SR after transport. Several studies agree with the current findings regarding the impact of using clove and mint oils to improve the efficient transport of fish, such as, *Curassiu auratus* (**perdikaris *et al*., 2010**), *Solea senegalensis* (**Weber *et al*., 2011**), *Oreochromis niloticus* (**Aker, 2011; Navarro *et al*, 2016; Rezende *et al*., 2017**), *Amphiprion ocellaris* **(Ostrensky *et al*., 2016)**, and *Cyprinus carpio* (**Saini *et al*., 2018**). The anesthetic mechanism of those oils to prevent physical injury is the reduction in the activation of the hypothalamus-pituitary internal axis associated with the stressful condition that leads to a release of cortisol, and subsequently, the secondary responses of stress, such as the elevation of plasma and lactic (**Hikasa *et al*., 1986**).

The present study showed a new strategy of using different doses of clove and mint oils to mitigate the stressful conditions during live fish transport. The efficiency of sedatives depends on their used dose, whereas overdose causes negative effects. It is noteworthy mentioning that, the toxicity level is associated with the high doses of sedatives resulting in central nervous system depression aligned with respiratory and cardiac failure. Moreover, the low doses do not effectively improve the quality of the transported fish.

CONCLUSION

Using clove oil as a deep anesthetic agent at a concentration of 100 mg/L for 5 min before transport, thereafter changing the transport water and adding the mint oil at 20 mg/L achieved a high quality of the tilapia brood-stock transport, with duration of 3.5 h. This strategy recorded the best water quality, hematological parameters, and highest SR, followed by the transported brood-stock using 100 mg/L of clove oil and 10 mg/L of mint oil. Decreasing the dose of the pre-deep anesthetic agent (clove oil) to 50 mg/L resulted in decreased transport efficiency. The transported brood-stock without the use of sedative oils recorded the worst parameters of water quality, blood indicators, and SR that reached 50% after 24 h of the transport.

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