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Original Article

Assessment of lavender (*Lavandula angustifolia*) essential oil as a natural anesthetic and sodium bicarbonate as a sedative on physiological and histopathological status of *Tilapia zilli* during the transport practices

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ABSTRACT: This study presents a new plan to improve the efficiency of transporting live fish using lavender oil (Lavandula angustifolia) as a deep anesthetic prior to transportation and sodium bicarbonate (baking soda) as a sedative during transporting on physiological indices, histopathological alterations, and survival rate of *Tilapia Zilli* fingerlings. Fish were exposed to conditions simulating those normally used in transporting. This experiment consisted of five treatments using two levels of lavender oil as a pre-deep anesthetic (200 and 400µl/liter, previously) for 5 minutes thereafter water in all aquaria was totally exchanged then adding different two levels of sodium bicarbonate as a sedative with concentrations (2.5 and 5 g/liter) these treatments were compared with the control group and normal fish that were not exposed to stressful conditions in terms of physiological statues, net ion fluxes, and survival rate after directly or 24 hrs transporting. Results affirmed that the control group and treated group with 400 µl/liter of lavender oil as a pre-deep anesthetic with 2.5 or 5 g/liter of bicarbonate of sodium as a sedative led to an increase in ion loss of fish bodies, deterioration in water quality and physiological statues with increasing mortality rate in comparison with treated groups with 200 µl/liter of lavender and 2.5 or 5 g/liter of sodium bicarbonate in special groups that treated group with 200 µl with 5g of lavender oil and bicarbonate sodium respectively.

Key word: Transporting live fish, anesthetic and sedative, sodium bicarbonate, lavender oil, *Tilapia Zilli*, blood parameter, histopathology

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1. INTRODUCTION

Keeping the high survival rate and performance of fish requires minimizing stress during the transport of live fish by minimizing the shock of the transportation means, the volume of transport tanks or plastic bags, the high density, the level of dissolved oxygen (DO), the total ammonia and carbon dioxide, and the buildup of metabolic and organic wastes.

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Improving the efficiency of transporting fish is very important economically (El-Dakar *et al.*, 2021). Fish have been sedated and kept in a stable condition for comfortable handling, catching, transport, measuring, etc. using anesthetics or sedatives (Czerniak *et al.*, 2018). Anesthetics and sedatives are used to restrict metabolic activity, induce changes in blood flow, and promote effective methods of energy production (Nilsson & Renshaw, 2004). As it's known, stress leads to decreased immune system function, resulting in sickness and death (Tacchi *et al.*, 2015). Conversely, anesthesia can reduce general activity, stress or pain, metabolic rate and oxygen demand, and improve ease of handling (Ross *et al.*, 2009). Various natural and synthetic substances are used during the anaesthetic process. The ideal anaesthesia should be inexpensive, easy to use, water-soluble, and leave no trace in fish, people, or the environment (Ross *et al.*, 2009). It should also provide quick anaesthesia and recovery. Different essential oils (Eos), which are thought of as a decent substitute for synthetic anaesthetics, are currently being used more and more frequently, and investigations on the discovery of novel herbal anaesthetics are progressing quickly as a result of some rules pertaining to animal welfare. Although anesthetic effects of Eos originating from plants, such as *Lippia alba (Bushy matgrass or lemon balm), Ocimum gratissimum (alfavaca or tree basil), Aloysia triphylla (Lemon Verbena, Lemon beebrush),* and *Syzygium aromaticum* (clove oil) (da Cunha *et al.*, 2010), (Silva *et al.*, 2012), (T. V. Parodi *et al.*, 2014), (Aydın *et al.*, 2015).

Lavender essential oil (Lavandula angustifolia) stands out among the many different kinds of EOs that can be employed in clinical practise. The *Lamiaceae* family includes the genus Lavandula, which can have more than 100 distinct constituents, including terpenoid and phenolic chemicals. One of the major components of lavender, linalool, is a constituent of several Eos whose depressor activities on the central nervous system are well described in rodents (Dobetsberger & Buchbauer, 2011). In addition to the anesthetic properties for aquatic animals (Mazandarani *et al.*, 2017). It is also acknowledged for having a wide range of pharmacological effects, including those that are therapeutic, sedative, depressive, antiseptic, antifungal, calming, and antiemetic. According to several research, it is regarded as safe (Barbas *et al.*, 2017)

For fish, carbon dioxide gas is a suggested anaesthetic; it is used to sedate them during shipping and enable simple handling (BOWSER, 2001). Its advice depends on the species and the desired end result. Additionally, it is regarded as safe when used with the proper precautions, just like any other anaesthetic. Baking soda is advantageous since it is cheap, easily available, and safe for both humans and fish also it is environmentally friendly (Altun *et al.*, 2009).

With recent awareness of the need for safe aquaculture practices, there is need to use anaesthetics with low environmental and health risks. Due to prohibitive cost and scarcity of conventional anaesthetics (Akinrotimi, 2014), there is need to develop viable alternative anaesthetics. Therefore, the present study was undertaken to create a new strategy for increasing the transport efficiency of brood stock *Tilapia zilli* for 4 hours. This work is the first study in fish transportation use lavender oil with a high dose as pre-deep anesthetics and bicarbonate sodium with a low dose as a sedative to reduce the stressful conditions and minimize possible injuries that associate the transport of *Tilapia zilli* fingerlings**.**

2. MATERIALS AND METHODS

2.1. Preparation of experiment agents

Bicarbonate of soda and lavender (EO) were purchased at a neighbourhood herbal and medical supply store. In order to dissolve lavender oil with transport water, lavender oil

was diluted in ethanol alcohol 95% (part oil: 10 parts alcohol). Final concentration of lavender oil in alcohol solution became 100 µl/ml.

2.2. Animals, experimental design and facilities

Fish were captured from a private farm in Diba Triangle Zone, Damietta, Egypt. With an average weight of 30.97±2.33 and randomly distributed in 10 clear plastic aquaria with a water volume of 40 liter/ aquaria at a stocking rate of one fish/liter. The transport duration was 4 hours. This experiment included five treatments using two levels of lavender oil as a pre-deep anesthetic (200 and 400µl/liter) for five minutes thereafter water in all aquaria was totally exchanged then adding different two levels of bicarbonate sodium as a sedative with concentrations (2.5 and 5 g/liter).

The treatments were formulated as the following: G0, the control group their fish did not expose to anesthetic or sedative; G1, 200 µl/l lavender oil as deep anesthetic for 5 minutes then 2.5 g bicarbonate soda/liter, G2: 400 µl/l and 2.5g/liter; G3: 200 µl/l and 5g/liter; G4: 400 µl/l and 5g/liter of lavender oil and bicarbonate soda respectively. Each treatment was duplicated.

2.3. Water analysis

Parameters of physiochemical of the transport water were recorded before the transporting and after every hour of transporting duration. Temperature, pH, and dissolved oxygen DO were measured by multi-parameter water quality analyzer (MULP-8C) and total ammonia was verified using the chemical methods (APHA, 1995). A 100 sample was taken before the transporting and at the end of trial of each treatment to determined concentration of water sodium and potassium, by using Inductively Coupled Plasma Emission Spectrometer (ICP) (ICAP-6300 Duo) Soil and water analysis laboratory, Fayoum University, Egypt.

2.4. Ions fluxes

Net ion fluxes were determined according to Gonzalez *et al.*, (1998)

$$\textbf{Net} = \frac{V[(ion\ 1)-(ion\ 2)]}{MT}$$

Where [ion1] and [ion2] are the ion concentrations in the water of transport at the start and end of the transport period, respectively, V is the water volume (in L), M is the mass of the fish (in kg) and t is the duration of the transport (in h).

2.5. Blood sampling and assay

At the beginning and the end of transporting period, blood samples were randomly collected from the Caudal blood vessels of fish using a 3-ml syringes and emptied in two tubes one of them contained EDTA (anticoagulant 10% ethylenediaminetetra acetate) to prevent coagulation and estimate the hemoglobin (Hb) and other tube did not contain EDTA to measure the serum parameters (plasma cortisol, glucose, Alanine transaminase (ALT). Aspartate transaminase (AST), and urea were measured spectrophotometric ally using commercial kits. Subsequently, the samples tubes were immediately transported to hematological laboratory.

2.6. Histopathological examination

In total 6 fish were randomly sampled at each treatment for histopathological examination. Samples of gills were collected and fixed in 10% neutral buffered formalin. After 24 h, the tissues were kept at 50% ethyl alcohol solution for 48 h. Then the tissues passed through alcohol series, and they were stored at 65°C temperature in paraffin overnight. The samples were embedded in paraffin for the tissue section process and sectioned at a thickness of 5 µm. In the tissue section process, a deparaffinization process was applied and tissues were exposed to alcohol and xylene series. Tissue sections were stained with hematoxylin and eosin, and fixed with Stellan and histopathologic changes were examined with a light microscope (Kurtoglu *et al.*, 2016).

2.7.Statistical analysis

All data were statistically analyzed using the software SPSS (version 20). The Shapiro-Wilk's and Levene's tests were used to verify variance normality and homogeneity. The differences among the treatments were determined at a significant level (P<0.05) by One-way analysis of variance (ANOVA) and Waller-Duncan's test used to compare means.

3. RESULTS

Water analysis showed significant differences among the groups in pH, DO, and TAN (Total Ammonia Nitrogen) in comparison with the recorded values before the transporting (Table 1). The highest level of TAN was recorded in G4, G3, and control group respectively while the lowest TAN was found with G1 and G2. On the other hand, G4 and G3 had the lowest pH followed by G1, G2 and control. DO did not significantly differ with control, G1, and G2 but these groups were significantly higher than G3 and G4 in DO level. Water temperature and salinity insignificantly varied among all treatment and ranged between (19.45 to 19.70 °C and 15 to 17.2 ppt.) Table 2 presents a comparison between physiological statuses of fish before transporting and after transporting using lavender oil and sodium bicarbonate. Blood indicators showed significant differences with all treatments. G1 and G2 had higher levels in Hb and then control but G3 and G4 had the lowest. Blood glucose did not change among G2, G1, and the sample of pre- transporting but these groups were significantly lower than G4 and G3 in glucose level.

Cortisol concentration was higher in G4 and G3 followed by control, but G2 and G1 had the lowest. G3 and G4 had recorded very low levels in ALT and AST compared to the normal level or the sample of pre- transporting. However, there were no differences between pre-transporting sample and G1, but they recorded higher level than G2. Besides, control had the highest level in AST or ALT. Urea in blood was not significantly differ under the transporting conditions.

Table (3) showed that survival rates (SR) after transporting directly did not significantly vary but G2 and G1 did not record any mortality compared to the other groups. After 24 hours of transporting SR recorded a significant deference among the treatment, wherein G4, G3, and control had higher mortality than G2 and G1 which recorded 100% survival rate.

Table 1. Effect of lavender oil and bicarbonate sodium on averages of physiochemical parameters of water used for transportation of *Tilapia zilli* for 4 hours.

Parameters	Before (Normal)	Control G0	G1	G2	G3	G4	PSE*	*P-value*
Temperature (ºC)	19.55	19.70	19.50	19.45	19.75	19.65	0.71	0.888
pH	7.93^{a}	7.70^{ab}	7.20^{cd}	7.36^{bc}	6.90^{cd}	6.85^{d}	0.12	0.006
Salinity (ppt)	15	15.60	16.1	16.4	16.9	17.2	1.25	0.487
DO (mg/L)	6.8^{a}	4.40^{b}	4.50^{b}	4.70^{b}	3.53^{c}	3.30^{c}	0. 73	0.028
Total ammonia TAN (mg/L)	0.023^{e}	0.15^{abc}	0.077^{cd}	0.080^{bcd}	0.19^{ab}	0.20^{a}	0.07	0.02

Means within the same row with different superscript letters are significantly different at (P <0.05).
***PSE: Pooled standard error**
DO: Dissolved oxygen
TAN: Total ammonia nitrogen

Table 2. Effect of lavender oil and bicarbonate sodium on blood indices of *Tilapia zilli during* transportation for 4 hours.

Parameters	Before (Normal)	Control G0	G1	G2	G3	G4	PSE*	*P-value*
Hb (g/dL)	6.05^{a}	4.87^{c}	5.47^{b}	5.28^{bc}	3.61^{d}	3.45^{d}	0.29	0.00
Glucose (mg/dL)	44.00^{c}	89.01^{b}	45.50^{c}	30.01^{c}	99.01^{ab}	105.50^{a}	9.08	0.02
Cortisol (nmol/L)	68.00^{d}	101.72^{b}	89.50^{bc}	87.50^{bc}	131.02^{a}	137.62^{a}	7.57	0.04
ALT (U/L)	62.50^{b}	73.00^{a}	66.05^{b}	56.04^{c}	17.00^{d}	13.51^{d}	7.16	0.00
AST (U/L)	51.50^{ab}	63.50^{a}	55.00^{ab}	49.00^{b}	15.00^{d}	12.54^{d}	6.11	0.03
Urea (mg/dL)	11.15	17.50	19.30	14.55	15.06	13.02	0.48	0.12

Means identified by different small letters in the rows were significantly different (*P <0.05*).
*** PSE: Pooled standard error**
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase

Table 3. Effect of lavender oil and bicarbonate sodium on Survival rate of *Tilapia zilli during* transportation for 4 hours.

Parameters	Control G0	G1	G2	G3	G4	PSE*	*P-value*
Survival rate after transporting directly	85	100	100	85	85	2.95	0.169
Survival rate after 24 hr of transporting	82^{b}	100^{a}	100^{a}	80^{b}	75^{b}	3.58	0.001

Means identified by different small letters in the rows were significantly different (*P <0.05*).
*** PSE: Pooled standard error**
**** (Number of fish at the end of experimental period/ Number of fish at start) ×100**

The histopathologic observations for both control and the treated groups with representative images of the gill tissues displayed in Fig. (1). Individuals of normal group (Before transporting) did not show any histopathological changes in the gill tissues examined by the light microscope. After applying different treatment, the gills showed pathological alterations that vary from moderate to severe.

Control group G0: gills showed thickening of primary lamellae (*), shorting of secondary lamellae (S) and clubbing at tip of lamellae (arrow). Fish gills in G1 showed some pathological alterations such as mild thinking of primary lamellae with mild leucocytic infiltrations (*), Moderate to severe dilation of

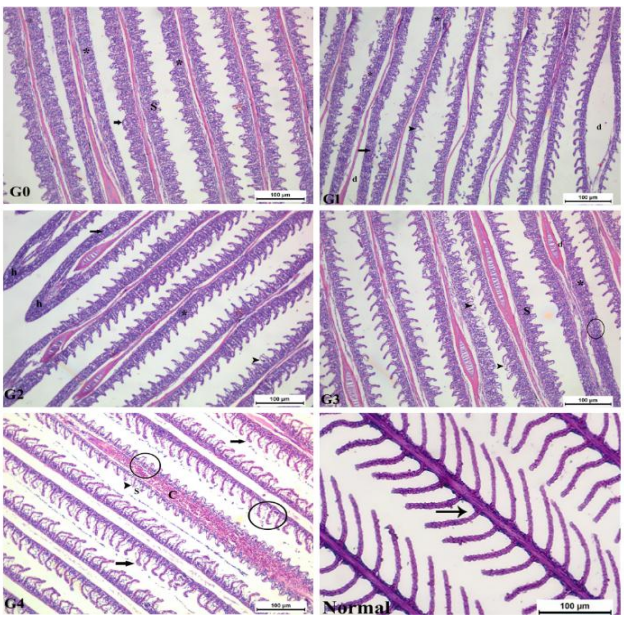

Fig. 1. Histopathological examinations in the gill tissues of *Tilapia zilli* after exposing

lavender oil as a pre-deep anesthetic and bicarbonate sodium as a sedative during 4 hr the transporting period.

Normal: fish were not exposed to transporting conditions; G0, the control group their fish were transported without treated by anesthetic or sedative; G1, 200 µl/l lavender oil as deep anesthetic for 5 minutes then 2.5 g bicarbonate soda/liter, G2: 400 µl/l and 2.5g/liter; G3: 200 µl/l and 5g/liter; G4: 400 µl/l and 5g/liter of lavender oil and bicarbonate soda respectively.

interlamellar space (d). Some of secondary lamellae showed mild epithelial lifting (arrowhead) and lamellar fusion (arrow).

Gills of G2 showed secondary lamellae with moderate epithelial lifting (arrowhead) and fusion of some secondary lamellae (arrow). Tips of lamellae showed hyperplasia and moderate leucocytic infiltrations and erythrocytes (h).

Gills of G3 showed mild to moderate dilation of interlamellar space (d), shortening (S) and degeneration of secondary lamellae (O) with moderate epithelial lifting (arrowhead). The primary lamellae showed thickening with hyperplasia leading to complete lamellar fusions and leukocytic infiltrations (*).

Gills of G4 performed degeneration and necrosis of primary lamellae (O) with severe congestion in interlamellar spaces (C). The secondary lamellae showed atrophy and necrosis (O) and shortening (S) with severe epithelial lifting (arrowhead) with sever clubbing at tips (arrow).

Normal fish: gill tissues did not show any histopathological changes.

4. DISCUSSION

Using plant-based anaesthesia at sedative concentrations is frequently an important tool to reduce sensitivity to visual and mechanical stimuli, swimming activity, and stress in modern aquaculture because they help to reduce stress, which may make fish more susceptible to diseases as a result of resistance during fish handling and transport (Teixeira *et al.*, 2017; de Souza *et al.*, 2019).

In general, EOs in sedative concentrations lower metabolic rates, the intensity of the stress response, and prevent physical harm to fish. EOs supplied to the transport units to enhance water quality, primarily the quantities of dissolved oxygen, while lowering the ammonia nitrogen excretion to the water (Becker *et al.*, 2016; Hohlenwerger *et al.*, 2017).

The physiological effects with EOs were diminished and they protected fish tissues against oxidative damage and mitigated stress responses leading improve or preserve the water quality, mainly the levels of dissolved oxygen, while reducing the ammonia nitrogen excretion to the water (Becker *et al.*, 2017).

One of the most significant essential oils is lavender, which has more than 100 distinct constituents, including terpenoid and phenolic chemicals. The monoterpenoids linalool, linalyl acetate, 1,8-cineole, -ocimene, terpinene-4-ol, and camphor make up the majority of lavender oils (Sharma *et al.*, 2019). The essential oil of *Lavandula angustifolia* has a modest level of camphor and a high concentration of linalool and linalyl acetate. It is one of the best and most sought-after lavender oils in the sectors of aromatherapy and cosmetics. Moreover, Sodium bicarbonate has been shown to be effective as an anaesthetic in common carp (*Cyprinus carpio*) in both cold and warm water conditions (Altun *et al.*, 2009), Rainbow trout (*Oncorhyncus mykiss*) (Keene *et al.*, 1998) and in Nile tilapia (*Oreochromis niloticus*) (Opiyo *et al.*, 2013). Sodium bicarbonate (NaHCO3) instantly dissolves in water and releases carbon dioxide (McFarland & Klontz, 1969). Carbon dioxide gas is a recommended anaesthetic for fishes; it is used to produce sedation during transportation and allow easy handling (BOWSER, 2001).

Our results showed that groups G1 and G2 which were treated with 200 µl/L of lavender oil had the best water quality during the transporting period compared with the control group or G4 and G3. G1 and G2 had the highest DO mg/l and the lowest TAN, mg/l in all the

treated groups with the agent. This may be due to the use of the appropriate dose and combination of deep anesthetic (lavender oil at 200µl/L for 5 min.) and the sedative (2.5 or 5 g/L of sodium bicarbonate). A similar result of using sedatives at the optimum doses in fish transport reduced the fish activity and excreted ammonia through gills (Singh *et al.*, 2004). Furthermore, the addition of this EO to the transport water decreased ammonia excretion in a manner similar of Neiffer & Stamper (2009) found that anesthetics limit the rate at which fish use oxygen because they slow down their metabolic activities. These observations were also supported by the results of a recent study conducted by (Gabriel *et al.*, 2020) and (El-Dakar *et al.*, 2021)

As it is known, essential oils or sodium bicarbonate may block the transmission of sensory information to the hypothalamus, and therefore high concentrations of anesthetics prevent the activation of the hypothalamus pituitary internal axis more effectively than lower concentrations so the cortisol response may be prevented (Iversen & Hydle, 2023). Accordingly determining the effective concentrations,

and the selection of which anesthetic agent to use is also very important. According to (METİN *et al.*, 2018), some anesthetic substances affect fish's immune system, respiratory function, and cardiovascular system.

Negative effects are brought through high concentrations of anaesthetic agents or sedatives, and the toxicity level is linked to large doses of sedatives, leading to central nervous system depression and respiratory and cardiac failure. Additionally, fish may die or take longer to recover when given strong dosages of anesthetic agents or sedatives (Kamble *et al.*, 2014).

From our observations of this trail, fish that were exposed to 400µl/L of lavender oil with 5 g or 2.5 g/ L of sodium bicarbonate had nervous convulsions, accompanied by a state of hyperactivity. So, fishes in G3 and G4 had the lowest pH and DO as a result of increasing CO_2 levels, and they had the highest levels of TAN as a result of increasing the immobilizing the fish and the metabolic activity. According to Altun *et al.* (2009), some potential health risks to fish include hypercapnia (increased systemic CO_2) leading to hyperactivity and then the death. This effect may be doubled when bicarbonate is added with a deep anesthetic in high doses of essential oils. Therefore, physiological indicators of fishes in G3 and G4 were negatively affected by high concentrations of anesthetic agent or sedatives.

Fish in G3 and G4 had the lowest Hb and highest plasma glucose and cortisol in comparison with G1 or G2. Whereas increasing the level of Hb refer to elevating the blood's ability to carry oxygen (Montero *et al.*, 1999). Obviously, levels of blood glucose and cortisol have frequently been employed as indicators of stress in fish (Morgan *et al.*, 1997). In the same context, increasing glucose levels were accompanied by reduction in hematocrit a secondary response of stress (McDonald & Milligan, 1997).

Using the sedative at the optimum doses in fish transport may reduce the fish activity, so increasing plasma glucose levels represents an immediate response to the elevation of metabolic and respiratory rates in muscle cells. Besides, increasing the doses of anesthetics or sedatives indicate physiological responses to augmented energy demand. Ribeiro *et al.*, (2019) cleared that exposure of Nile tilapia to high concentrations of benzocaine led to an increase in plasma glucose.

Actually, cortisol is a major corticosteroid that regulates intermediate metabolite ion levels, osmoregulation, and immunological function has been shown to cause swelling of erythrocytes (Sharp *et al.*, 2004). In comparison to fish treated with sedatives of essential oils at the recommended concentrations, plasma cortisol levels were higher in fish treated without sedatives, according to studies by (T. V. Parodi *et al.*, 2016 and El-Dakar *et al.*, 2021). In the same trend, Akar (2011) found

that control group showed an increase in plasma cortisol levels when compared with both unstressed and transported fish blue tilapia (Oreochromis aureus) in bags containing clove oil. Mazandarani *et al.* (2015) the non-anesthetized female fish of Persian sturgeon (*Acipenser persicus*) had significantly higher cortisol levels than those anesthetized with clove oil.

Changes in the activity of the enzymes that signal liver health in the blood plasma might be a good predictor of stress since the liver is a prominent marker for the endocrine activity that supports fish deal with problems physiologically. Significant changes in AST and ALT indicate tissue damage which may be due to the stress of anaesthetics.

From the aforementioned data, the control group had the highest ALT and AST followed by G1 and G2. It has been suggested that stress induces elevation of the transamination pathway and is likely to have been a factor in the increased transamination activities with control group. But the severe decrease in the level of ALT and AST with G3 and G4 may be due to the used doses of anesthesia or sedative, the high doses of these agents led to damage to the liver and lost its ability to secrete enzymes. According to R. R. Teixeira *et al.*, (2017), high concentrations of anesthesia agents may be uneconomical and may cause undesirable adverse effects. Velíšek *et al.*, (2005) revealed that 2-phenoxyethanol and clove oil significantly reduced AST activity in rainbow trout (*Oncorhynchus mykiss)*. However, Congleton (2006) observed an increase in AST and ALT activities in rainbow trout (*Oncorhynchus* mykiss) treated with MS-222. Akinrotimi, (2014) reported a similar trend in African catfish (*Clarias gariepinus*) brood fish exposed to anaesthetic metomidate.

Definitely, under stressful conditions, 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. Stressful procedures, such as transport, interfere in fish ion regulation because of increased blood gill flow and paracellular permeability (Ashley, 2007). The addition of anesthetics and sedatives in the transport water has reduced the net ion loss in *R. quelen (Pale catfish 0r South American catfish)* (Becker *et al.*, 2016); Parodi *et al.*, 2014) and this response is followed by an increase in the plasma ion levels (Zeppenfeld *et al.*, 2014). Therefore, the high blood ion levels found in the present study could also be related to lower net ion loss. Our results affirmed that G1 and G2 had the lowest ion loss (Fig 2& 3) in comparison with G3, G4, and control. Fish of these groups were less stressed, as indicated by the lower plasma cortisol levels, and thus they probably had a lower catecholamine release, resulting in lower gill blood flow. The main reason to the observed Na^+ and K^+ efflux in transported fish is an increase in gill permeability, which can cause a temporary hydromineral imbalance in the fish (Barton *et al.*, 1998).

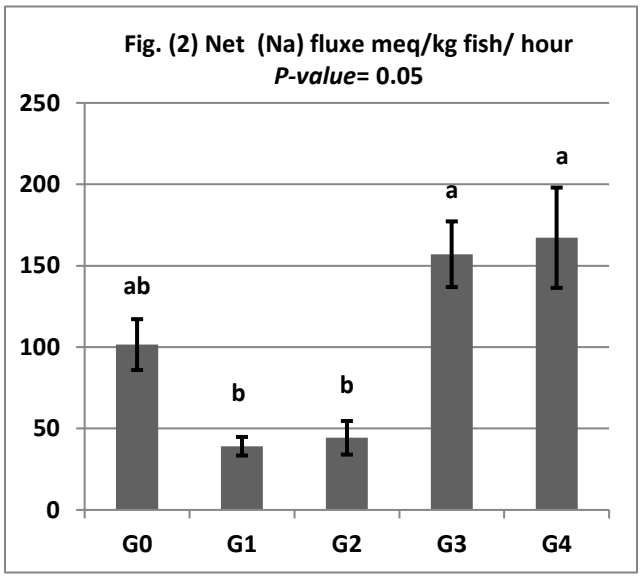

Fig.2 Effect of lavender oil and bicarbonate sodium on net ion (Na) fluxes of the water used for of *Tilapia zilli* 4 hours. Values are means ± SE. Different letters indicate significant differences between treatments for the same ion (*P<0.05*).

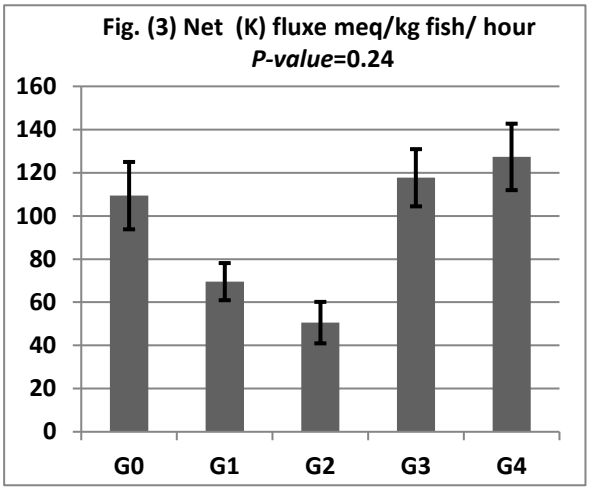

Fig.3 Effect of lavender oil and bicarbonate sodium on net ion (K) fluxes of the water used for of *Tilapia zilli* 4 hours. Values are means ± SE. Different letters indicate significant differences between treatments for the same ion (*P<0.05*).

Histological studies are an important criterion in determining the effects of toxic substances. Histological study showed that negative effect on gills tissue of control and these effects were higher with fish of G3 and G4. Changes in fish gill tissue due to exposure to anesthetic agents were determined by visually comparing experimental groups gill tissue with control group gill tissue. Ak *et al.*, (2022) showed that increased concentration of chamomile oil in rainbow trout caused histological deterioration in gill tissue. Ogueji *et al.*, (2019) explained that ivermectin caused damage to *C. gariepinus* gill epithelium due to physiological stress. The gill damage prompted a reduction in blood values due to hemodilution caused by osmoregulation impairment.

Likewise, in this study, vacuolization, necrosis and epithelial lifting which was detected intensively in gill epithelium can damage osmoregulation mechanism in histopathological examinations of anesthesia groups. As a result of the deterioration in epithelial tissues, a decrease in Hb values occurred. Nitrogenous compounds (e.g., ammonia and nitrite) can damage or alter gill morphology and these alterations can have several impacts in that they may affect the uptake and clearance of inhalant anaesthetics (Neiffer & Stamper, 2009).

CONCLUSION

Not only exposure of fish to anesthesia overdose or prolonged might be cause a stress, but also, use of high or inappropriate doses has the opposite effect and increases the severity of transport stress. Perhaps its effect exceeds the effect of stress on untreated fish (control group). So, it is important to determine the effective anesthetic concentration in terms of fish health, environmental effects and cost. Commonly, using lavender oil as a pre-deep anesthetic at rate of 200 µl/liter for five minutes afterwards water in all aquaria was totally exchanged then adding different bicarbonate sodium as a sedative with concentrations (2.5 or 5 g/liter) during the transporting *Tilapia zilli* reduced stressful conditions associated with transportation and maintained water quality, physiological status and survival rate.

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