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## Evaluation of lemon balm (*Melissa officinalis*) leaf powder as a natural anaesthetic during transportation of red belly tilapia (*Coptodon zillii*)

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### Abstract

Transportation of fish is a crucial process in aquaculture but often induces stress that affects physiological stability and survival of fish. This study evaluated the effectiveness of lemon balm (*Melissa officinalis*) leaf powder as a natural anaesthetic during transportation of red belly tilapia (*Coptodon zillii*). Lemon balm leaves were collected, dried and ground into powder. Ninety tilapias with an average weight of 23.1 g were randomly distributed into three treatments: T1 (control, without anaesthetic), T2 (5 drops palm oil/7.5 L water), and T3 (10 g/7.5 L *Melissa officinalis* powder) respectively. Ten fish were stocked in each of nine plastic containers (10 L capacity). Thereafter, the fish were transported over a distance of approximately 210 km (Minna-Suleja route, to and fro). Mild induction time and faster recovery time were observed in treatment T3. No significant difference ( $p>0.05$ ) was recorded in water temperature across the treatments. However, significant differences ( $p<0.05$ ) were recorded in dissolved oxygen, pH, ammonia, and phosphorus levels. The control group had the highest mortality, while T3 showed the highest survival rate. These findings suggest that *Melissa officinalis* leaf powder could serve as a promising natural anaesthetic for tilapia transportation in aquaculture.

**Keywords:** Natural anesthetic, Lemon balm (*Melissa officinalis*), palm oil, fish transportation, induction and recovery

### 1. Introduction

Aquaculture is an important sector in the global food industry, providing a significant portion of the world's fish supply. Tilapia, in particular is a widely cultured fish species due to its adaptability, fast growth, and high market demand. However, transportation of live tilapia from hatchery to grow out farm or farms to markets or processing facilities can be a stressful experience for the fish, leading to negative impacts on their health and overall quality.

The transportation of tilapia is an essential aspect of aquaculture, as it involves moving fish from one location to another. However, the transportation process can subject the fish to various stressors, including handling, crowding, and environmental changes, which can adversely affect their health and overall quality (Arechavala *et al.*, 2022) [2]. To alleviate these stressors and ensure the well-being of tilapia during transportation, the use of anesthetics has been widely explored in aquaculture practices. Anesthetics help in reducing stress, minimizing injuries, and preventing mortalities during transportation by inducing a state of temporary unconsciousness or sedation in fish. One promising natural anesthetic that has gained attention is lemon balm (*Melissa officinalis*).

In recent years, there has been growing interest in exploring natural alternatives as anesthetics in aquaculture practices. Lemon balm (*Melissa officinalis*), an herb from the *Lamiaceae* family, has shown a potential as natural anesthetic due to its soothing and calming properties (2019) [9, 11]. Lemon balm (*Melissa officinalis*) is an herb known for its medicinal properties and calming effects. It contains various bioactive compounds, including polyphenols, terpenes, and calming Flavonoids, which contribute to its

Pharmacological activities. It has also been studied for various applications, including its antimicrobial and antioxidant properties, its potential as an anesthetic in tilapia transportation remains relatively unexplored. Conventional chemical anesthetics used during transportation may have drawbacks, including potential negative effects on fish health and the environment (Li *et al.*, 2022) [5]

Therefore, there is a need to explore alternative anesthetic of natural origin that are effective, safe, ecofriendly, and sustainable, aiming to minimize stress, mortality and enhance the welfare of tilapia during transportation.

## 2.0 Materials and Methods

### 2.1 Experimental Site

The experiment was conducted in two phases (within and outside the University). The first phase was the transportation of the induced tilapia from Minna to Suleja. The second phase (post transportation phase) was on analyses of physical and chemical parameters of water

### 2.2 Experimental Plant

Lemon balm (*Melissa officinalis*) leaves were collected from a farmland and conveyed to the wet and dry laboratory. Upon arrival, they were washed thoroughly with water to remove dirt, insect and other contaminants. The leaves were subsequently sundried and ground into fine powder (<250 $\mu$ m). A sensitive weighing balance (Model 20001) was used to measure 10 g *Melissa officinalis* powder and tea spoon was used for the palm oil measurement.

### 2.3 Experimental Fish

Ninety Tilapia (*Coptodon zilli* sp) (of a high valued aquaculture species comprising both female and male with mean weight of 23.13 $\pm$ 2.43g and mean total length of 12.51 $\pm$ 0.39 cm was purchased and acclimatized for 24 hours. Prior to the transportation, the fish were starved for 12 hours to reduce metabolic waste during transport. A complete randomized design comprising of three treatments and three replicates were used for the experiment and designated as; 10 g *Melissa officinalis*, and five drops of palm oil respectively. 10 Tilapia (*Coptodon zilli* sp) were stocked in each plastic gallons container. The fish were transported to and fro Minna and Suleija covering a distance of (204 km). The induction and recovery time were carefully noted.

### 2.4 Water Quality Parameter

Dissolve oxygen, pH, temperature, phosphate, and ammonia were analyzed before, during, and after transportation using a

thermometer, dissolved oxygen meter, pH meter, ammonia concentration was determined using the nitrogen distillation while phosphate concentration was analyzed using the volumetric method.

## 2.5 Statistical Analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS version 23. DUNCAN multiple range test was used to separate the means at a significance level of ( $p < 0.05$ )

## 3.0 Results

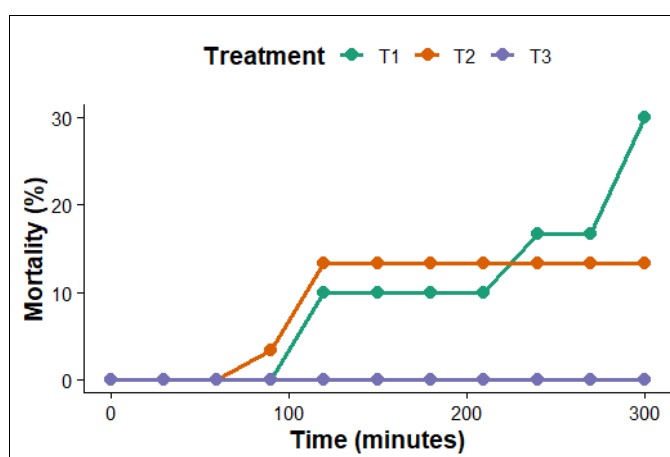
### Table 1: Induction and Recovery Time in *Coptodon zilli* Fingerlings Treated with *Melissa officinalis* and Palm oil as Anaesthetics

Table 1 depict the induction and recovery time of *Coptodon zilli* fingerlings exposed to concentration of *Melissa officinalis* powder (lemon balm) and Palm oil during transportation. Treatment 1 and 2 exhibited normal behavioral activities including body balance, opercular movement, normal movement of tail and fins and swimming. No observable induction and recovery response was recorded in the aforementioned treatment (1 and 2) respectively, while mild induction time was recorded at 16 minutes after administration of the *Melissa officinalis* treatment which lasted for 53 minutes before the full recovery of the fish.

**Table 1:** Induction and Recovery Time in *Coptodon zilli* Fingerlings Treated with palm oil and *Melissa officinalis* Powder

Concentration (g/7.5L)	Overall introduction time (min)	Overall Induction time (min)	Overall Recovery time (min)
0 (control)	10:43	00:00	00:00
1ml Palm oil	10:44	00:00	00:00
10 g of <i>M. Officinalis</i>	10:44	11:05	11:58

Figure 1 shows the mortality rate of fingerlings treated with inclusion of Palm oil and *Melissa officinalis* Powder as anaesthetic. There was mortality recorded in 0 g/7.5l and palm oil inclusion of 1 ml/7.5l, no mortality was recorded in treatment 3 (*Melissa officinalis*) Powder throughout the period of the transportation. The trends of the mortality in treatment 1 increase with exposure and transportation time of the fish.



**Fig 1:** Mortality Rate of *Coptodon zilli* Fingerlings Exposed to Palm oil and 10 g inclusion level of *Melissa officinalis* Powder (0 g, 1 ml palm oil and 10 g *Melissa officinalis* powder)

Table 2 shows the survival rate of fingerlings treated with palm oil and *Melissa officinalis*. The trends of the survival depict higher rate of survival at the inception of the transportation and decrease in trts 1 and 2 with exposure to increase in transportation time.

**Table 2:** Survival Rate of *Tilapia zilli* Fingerlings Exposed to Palm Oil and *Melissa officinalis* Powder (0g and 10g)

Time (min)	T1(0g)	T2 (Palm oil)	T3 ( <i>M. o</i> )
0	100	100	100
30	100	100	100
60	100	100	100
90	100	96.66	100
120	90	96.66	100
150	90	96.66	100
180	90	96.66	100
210	90	96.66	100
240	83.33	96.66	100
270	83.33	96.66	100
300	70	83.33	100

Table 3 presents the mean values of physicochemical parameters of water at different inclusion level of *Melissa officinalis* Powder (0g, 10g,) and Palm oil. At the temperature level, there was significant difference during the transportation ( $p < 0.05$ ). However, the highest mean value was recorded in time variation before transportation ( $30.00 \pm 0.00$ ) while the lowest was recorded after the transportation ( $28.66 \pm 0.57$ ). At pH level, there was also a significant difference ( $p < 0.05$ ) among the time variation during transportation. The highest mean value was recorded before the transportation ( $6.93 \pm 0.10$ ) while the lowest was recorded after transportation ( $6.70 \pm 0.29$ ). In the case of dissolved oxygen (mg/l) level, there was significant difference ( $p < 0.05$ ) among the time variation during transportation. However, the highest mean was recorded during transportation ( $6.22 \pm 0.97$ ) while the lowest was recorded before transportation ( $4.00 \pm 0.00$ ). No significant difference ( $p > 0.05$ ) among the variation in ammonia concentration. The highest was recorded after transportation ( $0.70 \pm 3.05$ ) while the lowest was recorded before transportation ( $0.00 \pm 0.00$ ). However, the variation differs significantly. For phosphate, there was significant difference during transportation ( $p < 0.05$ ). However, the highest value was recorded after transportation ( $0.16 \pm 0.28$ ) while the lowest was recorded before transportation ( $0.00 \pm 0.00$ ).

**Table 3:** Variation in Physical and chemical parameters of Water in treatment 1, 2 and 3

Parameters	Control	Palm Oil	<i>Melissa officinalis</i>
Temp (°C)	$29.25 \pm 0.87^a$	$29.32 \pm 0.66^a$	$29.68 \pm 0.57^a$
pH	$6.90 \pm 0.13^a$	$6.98 \pm 0.38^a$	$6.84 \pm 0.16^b$
DO (mg/L)	$6.61 \pm 1.12^a$	$3.14 \pm 1.47^b$	$2.81 \pm 1.58^b$
Ammonia (mg/L)	$0.03 \pm 0.01^b$	$0.03 \pm 0.02^b$	$0.77 \pm 0.02^a$
PO <sub>4</sub> (mg/L)	$0.02 \pm 1.84^b$	$0.06 \pm 0.25^b$	$0.17 \pm 0.25^a$

#### 4. Discussion

The results of this study revealed that *Melissa officinalis* influenced the induction and recovery responses of *Coptodon zillii*, in addition to affecting the survival rate, and physicochemical parameters of water during transportation. Similar findings were reported by Ja'afar *et al.* (2025) [4] who documented the effectiveness of *Ocimum gratissimum* as an anesthetic during the transportation of *Clarias gariepinus* fingerlings. The use of anesthetics in live fish transportation is

widely recognized as an effective strategy to minimize stress by reducing the activity of fish, lowering oxygen consumption, and minimizing physical injuries associated with handling and crowding. The effects consequently contribute to higher survival rates and improved water quality during transport. The higher survival rate recorded in the present study agrees with the findings of Yang *et al.* (2021) [13] who reported variations in the efficacy of different anesthetic treatments across fish species during transportation. These variations may be attributed to differences in environmental conditions during transport and species-specific physiological responses to anesthetics as well. The efficacy of *Melissa officinalis* as an anesthetic in this study may have been influenced by several factors, including concentration used, the size and species of the fish, and the duration of exposure. The result showed that mortality rate and the survival rate were directly related to the exposure time. No mortality was recorded across all treatments from 0 to 60 minutes of exposure, resulting in a 100% survival rate during this period. However, between 90 and 240 minutes, mortality increased gradually, with the highest mortality recorded at 300 minutes in treatment 2, while treatment 3 depicts comparatively lower mortality values. The increase in mortality observed over time may be associated with prolonged exposure resulting to metabolic stress and possible accumulation of metabolic wastes in the transport medium. These findings contrast with those of Baba *et al.* (2016) [3] and Ngugi *et al.* (2017) [6] who reported a sharp decline in the survival rates of experimental fish at higher concentrations of anesthetics.

Furthermore, the used of *Melissa officinalis* in this study appear not to exert adverse effects on the physiological condition and survival of the fish, as the pH of the water remained within the acceptable range even after transportation. To minimize stress and ensure fish welfare maintaining stable water quality parameters is essential during live fish transport. Similar observations were reported by Wang *et al.* (2021; 2022) [12, 11], who found that exposure of sea bass (*Lateolabrax maculatus*) to different concentrations of aqueous and alcoholic extracts of lemon balm did not compromise fish survival or disrupt water quality parameters. During transportation, the temperature of the water reduced following the application of *Melissa officinalis*. This was expected, as fluctuations in temperature commonly occur during live fish transportation due to the duration of transport and environmental conditions. Nilsson *et al.* (2010) [7], reported the ideal temperature range for fish transportation between 15 °C and 20 °C, which helps reduce physiological stress and maintain fish survival.

There was an observed variation in dissolved oxygen (DO) levels before and after transportation, with lower DO concentrations recorded during transportation and higher levels observed prior to transportation. This shows that DO decrease following the addition of *Melissa officinalis*. This is consistent with the observations of Ja'afar *et al.* (2025) [4], who reported a decline in dissolved oxygen when *Ocimum gratissimum* was used as an anesthetic during the transportation of *Clarias gariepinus* fingerlings. Similarly, the decrease in DO at the concentration of 10 g/7.5 L used in this study corroborates the report of Tadese *et al.* (2022) [10], who noted that dissolved oxygen levels decrease following the introduction of plant extracts into water. This decline may be link with the decomposition of plant-derived compounds and metabolic activities of fish in the transport medium.

The highest pH value was recorded prior to transportation, while the lowest value occurred after the introduction of *Melissa officinalis*, indicating a reduction in pH following the inclusion of the plant extract. Additionally, ammonia levels indicate significant differences before and after the addition of palm oil and *Melissa officinalis* to the water. Phosphate concentration was lowest before transportation but increased after transportation, suggesting that the inclusion of *Melissa officinalis* contributed to elevated phosphate levels in the water. These findings are in consonant with the finding reported by (Wang *et al.*, 2021) <sup>[12]</sup>.

Overall, alternate anaesthetics for use in fish transportation such as *Melissa officinalis* show potential level of effectiveness which is in line with the report of (Okey and Igiri 2021) <sup>[8]</sup>.

## 5. Conclusions

Based on the findings of this study, *Melissa officinalis* demonstrated more effectiveness, indicating better safety and efficacy compared to control and palm oil. The results shows that *Melissa officinalis* has good potential as an alternative anesthetic supporting fish survival during transportation. However, further studies across different species are necessary to evaluate its safety and effectiveness during transportation conditions, and varying concentrations to establish optimal application protocols.

**Data availability** all datasets generated and analyzed are included in this manuscript

**Declarations of Conflicts of interest** the authors declare no competing interests

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