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Organic Dietary Extracted Oils Supplementation: A Convenient Approach to Improve Welfare in Nile Tilapia (*Oreochromis Niloticus*)

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Abstract | The dietary supplementation of extracted oils from lemon (*Citrus limon*) (LEO) and/or sweet orange (*Citrus sinensis*) (OEO) peels in fish culture is initiated to be a potential factor for achieving welfare in Nile tilapia and improving growth performance. The results revealed that the extracted oils treated groups showed a marked increase in the following behavioural patterns such as feeding behaviour and swimming behaviour compared with chemically synthesized zinc oxide nanoparticles (ZnONPs) and control groups. While surfacing, body care, resting and aggressive behaviours with all their patterns were the lowest in the extracted oils treated groups than the other groups. Crossing test showed that fish raised at the extracted oils treated groups were more active and could had the highest values of growth rate than those raised at ZnONPs groups. It was concluded that the addition of extracted oils from lemon and/or sweet orange peels on basal diet enhanced the optimal Nile tilapia fingerlings' behaviour and improved their growth rate. Thus, the usage of extracted oils as a feed additive is very essential to increase growth performance and achieve welfare of Nile tilapia fingerlings.

Keywords | Extracted Oils, Behavioural Patterns, Crossing Test, Growth Rate, Welfare

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INTRODUCTION

Objective of this study was to investigate the effects of extracted oils from lemon (Citrus limon) (LEO) and/or sweet orange (Citrus sinensis) (OEO) peels on Nile tilapia behaviour and performance.To meet their nutritional needs, humans rely on fish culture as a vital source of highly nutritious, easily digested animal protein. Because of its resilience to environmental changes, such as high nitrite and ammonia levels, low dissolved oxygen levels, and large pH swings, tilapia is considered the most suitable species (Ahmed, 2020). Tilapia, the star of fish culture and dubbed as "aquatic chicken," is now consumed more often throughout the world (Youssef *et al*., 2024). Over 65% of all aquaculture productivity is made up of the most common species, tilapia (Dickson *et al*., 2016). The Nile tilapia, which is highly disease-resistant, has a low feed conversion ratio, excellent adaptability to poor water quality, and rapid growth performance, making it one of the most popular freshwater fish in Egypt and the rest of the world (El-Sayed, 2019). Essential oils (EOs) are a blend of organic compounds that are extracted from plants and can be used as food preservatives (Zeppenfeld *et al*., 2016).

They are naturally occurring antioxidants and antimicrobials that can enhance fish growth, behaviour, and immune system function while also lowering production costs. (Souza *et al*., 2015; Mohamed *et al*., 2021).

The majority of essential oils have potent antibacterial and functional qualities. Therefore, adding substances based on essential oils to the food chain may extend the shelf life of food products and improve overall health. (Kumar *et al*., 2020).

Many studies have demonstrated the potential benefits of using extracted oils (Eos), which are taken from specific fruits or plants, as nutritional additions. These benefits include the control of illnesses, enhanced growth performance, improved nutrient absorption, and immunological response in Nile tilapia (Toutou *et al*., 2018; Vicente *et al*., 2019). Citrus essential oil in Nile tilapia diets could increase growth performance and enhancement for innate immune response, so it is considered as a potential substitute to antibiotics for controlling disease in Nile tilapia culture **(**Acar *et al*., 2015**)**. Although the important role of essential oils in fish growth and development is well known, the information on synergistic effects of essential oils extract from bitter lemon (*Citrus limon*) and sweet orange (*Citrus sinensis*) peels on Nile tilapia under aquaculture conditions is very rare (Mohamed *et al*., 2021).

Nanotechnology provides core applications for water treatment to provide a safe habitat for fish breeding. The zinc oxide nanoparticles (CH-ZnONPs) are the most commonly utilized nanomaterials (NMs). Globally, CH-ZnONPs with low concentration have gained special attention due to their environmentally friendly characters with various excellent applications such as anticancer, antimicrobial, and photo catalysis, etc. (Jiang *et al*., 2018).

At lower quantities, zinc (Zn) is necessary for fish growth and development, whereas higher Zn levels are toxic (Murugan *et al*., 2008). One of the biggest threats to the growth of Nile tilapia is environmental pollution, such as the addition of high doses of chemical zinc oxide nanoparticles (ZnONPs). This can negatively impact the production process and stress of Nile tilapia, interfering with their normal physiological and behavioural conditions (Elsabagh *et al*., 2018; Lopes *et al*., 2019).

As nutritional supplements in Nile tilapia culture, therapeutic plants or their by-products (extracted oils) are used because they increase immunity, disease resistance, growth performance, and lowering heavy metal toxicity (Abdel Rahman *et al*., 2020). Dietary supplementation of orange extracted oil (OEO) and/or lemon extracted oil (LEO) decreased tissue damage by any environmental pollution, enhanced resistance to an oxidative stress and accordingly improved the feeding behaviour and the health status of cultured Nile tilapia. (Mohamed *et al*., 2021).

MATERIALS AND METHODS

The current study was conducted at the Zagazig University Faculty of Veterinary Medicine, Fish Behaviour and Management Research Unit, which is part of the department of Behaviour and Management of Animal, Poultry, and Aquatic. It lasted from the beginning of October to the end of November (2022).

Fish Handling and Water Hygiene

Fish handling: A total of 120 Nile Tilapia fingerlings were purchased from a fish farm in Ismailia governorate provided, weighing an average of 25.5±1 g at the beginning of the study. Before the study started, fingerlings were housed for a 14-day acclimatization period (Youssef *et al*., 2024) in 12 aquaria with a capacity of 90 L at the Fish Behaviour and Management Research Unit of the department of Behaviour and Management of Animal, Poultry, and Aquatic (10 fish in each sub group as replicate). The Nile Tilapia fingerlings were divided into six groups: the first group (G1) received a basal diet (Table 1), the second group (G2) received a basal diet containing lemon extracted oil (LEO) at a concentration of 1‰ (‰ = part per thousand); the third group (G3) received a basal diet containing sweet orange extracted oil (*Citrus sinensis*) (OEO) at a concentration of 3‰; the fourth group (G4) received a combination of (LEO 1‰) and (OEO 3‰); the fifth group (G5) received a basal di*et al*ong with chemically synthesized zinc oxide nanoparticles (ZnONPs) in aquarium water at a concentration of 1 mg/L as a high dose; and the sixth group (G6) received a basal diet plus chemically synthesized zinc oxide nanoparticles (ZnONPs) at a concentration of 1 mg/L for three weeks before being treated with feeding basal diet containing a mixture of (LEO 1‰) and (OEO 3‰) till the end of experiment,. Mortality and morbidity data were recorded daily.

Aquaria and aquarium water hygiene: Twelve completely set-up glass aquaria (30 x 40 x 100 cm, two aquaria each group) were utilized. In order to ensure that every aquarium had consistent aeration and that all organic waste materials were removed, electrical aerators and filters were used. A thermostat (a controlled heater) was also used to control the temperature of the water and maintain the ideal temperature for the fingerlings. To facilitate the transportation and handling of fish and stop the transmission of infection, a separate nylon hand net was used for each aquarium, along with a water thermometer to measure the water's temperature every day. In order to maintain fish at the following aquarium water parameters, as Gregory and Grandin (2013), approximately 25% of the water was changed daily and the entire water was changed once per week using dechlorinated water from a water storage tank. Water quality parameters were measured on a daily bases during the experimental period and were kept around the normal average of Nile tilapia, these parameters include: water temperature (26 ± 2°C), pH (8 ± 0.5), dissolved oxygen (DO) (8–10 mg/L), CO_2 (2 mg/L) were measured using multiparameters probe metre (HI9829-03042-HANNA®instruments, www. hannainst.com). Total ammonia (NH_3) (0.02–0.5 mg/L), nitrite (NO_2) (0.01-0.05 mg/L) were estimated using a portable colorimeter (Martini MI 405) and salinity (10–15 ppt).

Medication: Using potassium permanganate (2 mg/L) and oxytetracycline (50 mg/kg b.wt.) to treat fish for columnaris disease, which affects many freshwater fish in stressful situations, as well as NaCl (1 g/L) as a protection against any fish disease twice/week after altering the water of all aquaria (Abdel-Fattah *et al*., 2020).

Feeding Program

Preparation of basal diet: The first step in the feeding program is to prepare the base diet. The Cairo Poultry Processing Company (CPC) produced the basal diet. It was created as dry floated pellets to satisfy the fingerling Nile Tilapia's nutritional needs. Table 1, lists the components and approximate makeup of the baseline diet in accordance with the National Research Council (NRC, 1993).

Extracted oils: The oils extracted from fresh citrus peels were Citrus limon (LEO) and Citrus sinensis (OEO). These were obtained using hydro-distillation with a Clevenger system, following the steps described by Öntaş et al. (2016) and Acar *et al.* (2015), respectively. Scientists from the National Research Center, the Central Laboratories Network, and Egypt used an Agilent Technologies GC-MS-MS system to analyze the oils that had been extracted. This system has a mass spectrometer detector and a gas chromatograph. By comparing the spectrum fragmentation pattern with those found in Wiley and NIST Mass Spectral Library data, many constituents might be identified. Before being used, the orange-extracted oil (Citrus sinensis) and lemon-extracted oil (Citrus limon) were kept separate in dark glass bottles at 4 °C. Table 2, displays the active ingredient components of the LEO and OEO analysis results.

Chemically synthesized Zinc oxide nanoparticles (CH-ZnONPs): ZnONPs were made chemically and the amounts of ZnONPs used in this study were chosen based on previous research (Hao and Chen, 2012).

Table 1: Components of basal diet applied in the study.

Composition	
Fish meal, 66%	20
Soybean meal, 44%	20
DDGS, 28%	10
Yellow corn	15
Corn gluten, 62%	4.55
Rice bran	26.45
Vegetable oil	3.50
L-Lysin HCL 98%	-
D L- Methionine	-
Calcium carbonate	-
Vitamin. mineral premix*	0.50
Total %	100
Calculated composition	
DM, %	86.47
CP, %	32.01
EE, %	11.47
CF, %	4.27
Ash, %	7.60
NFE, %	34.06
Ca, %	0.89
P, %	1.19
Lysin, %	1.85
Methonine, %	0.71
DE, Kcal/ kg**	3007.46
Total %	100

Vitamin and mineral mixture (kg/ diet) (Vit .A 6000 I.U, D3 2.000 I.U, E 500mg, k3 12.0 mg, C. 1.000mg. B1 10mg, B2 15.0mg, B6 7.5mg, B12 0.1mg, Biotin 0.2mg, Folic acid 0.4mg, cholin Hcl 1.0g inosit. 3000.0mg,pantothemic acid 50.0mg, Nicotinic acid 100mg, P-Aminobenzonic acid 50 mg, iron 80mg, copper 5g, zinc 40g, Sodium selenite 100 mg and, potassium iodide 300mg, and cobalt sulphate 100 mg), Digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm according to **(**Essam *et al*., 2020**)**. The different concentration of LEO or/and OEO was dissolved in 100 ml of absolute ethanol and then sprayed evenly on 1 kg of feed to achieve the above concentrations in the experimental diets according to **(**Mohamed *et al*., 2021**)**. To guarantee equal conditions with diets supplemented with LEO or/and OEO, the same amount of ethanol has been added to the control diet. The diets were dried for 1 day in the room temperature before being used to feed the fish.

Table 2: Active components in the *Citrus limon* and *Citrus sinensis* peels

	Lemon (*Citrus limon*) peels			Sweet orange (*Citrus sinensis*) peels		
	Active substance	Relative ratio (%)	Retention time (min)	Active substance	Relative ratio (%)	Retention time (min)
1-	D-Limonene	91.4	10.6	D-Limonene	93.8	10.7
2-	Z-citral	0. 5	17.9	Nonadecane	1.2	36.7
3-	E-citral	0.4	18.8	β-pinene	1.6	11.3
4-	Sabinene	1.5	8.5	γ-terpinene	0.9	33.5
5-	β-pinene	5.1	8.3	Geranyl acetate	0.3	34.1
6-	γ-terpinene	0.3	30.1	α-pinene	1.6	17.2
7-	α-pinene	0.1	19.2	p-cymene	0.4	10.4

Feeding schedule: Fingerlings were hand-fed six days a week at three different times a day: 8:00 AM, 12:30 PM, and 4:00 PM. Each daily supply is divided into three portions at the three times specified earlier. Throughout the study period, the daily food intake was maintained at 3% of the fish's total weight by feeding them exactly as much as they could finish in five minutes. According to Ahmed (2020), every fish in every aquarium was weighed.

The Performance of Live Fish

fish performance was observed during the study, as reported by Said *et al*., (2020) as follows:

1. Average daily gain (ADG):
ADG= (W2-W1)/T, where T is the study's duration in days. While W1, W2 = weight of individual fish at two successive weeks.

2. Specific Growth Rate (SGR):
SGR = [log (W2) - log (W1))/T]. X 100

3. Body weight gain (WG)/g: The following method was used to estimate the body weight gain independently over the course of two weeks.
Weight gain/g, or BWG= W2-W1.
Each fish's weights at two different weeks are W1 and W2.

4. Feed Conversion Ratio (FCR):
FCR = [feed intake (g) / Weight gain (g)] X 100

5. Survival rate (SR): To calculate the survival rate (SR), divide the number of fish that survived by the total number of fish and multiply by 100%.
SR = [Number of survived fish / total number of fish] x 100

6. Mortality rate= (total number of dead fish / study length).

7. Morbidity rate = (total number of ill fish / study length).

Observation and Data Collection

The behavioural observations were recorded as follows: Each group was observed twice a day for ten minutes each, four days a week, at a regularly scheduled time (5 minutes for each aquarium; 43 seconds for each fish identified). Intervals of eight hours per week for each group for the course of the study's eight weeks, according to Abdel-Fattah *et al*. (2020).

In order to facilitate easy observation of seven fingerlings during the study period, short plastic bands of different colors were affixed to the dorsal fins of seven fingerlings using thin wire, designating the Nile Tilapia fingerlings (Ahmed, 2020).

Behavioural observations were conducted twice a day, from 8:00 a.m. to 4:00 p.m., using focal sample technique, a multipurpose counter, a notepad to record behavioural patterns, a video camera, and a stopwatch (Said *et al*., 2020).

All presented behaviours were observed according to (El-Saadony *et al*., 2021):

Ingestive Behaviour

1) (a) Feeding behaviour is the act of consuming food during the feeding time. It encompasses a variety of eating-related behavioural responses, such as feeding patterns, frequency, feeding styles, and food preferences.The feeding behaviour's mean frequency was noted every eight hours. The eating behaviour's mean time (sec.) was noted at 8 hours.

(b) Foraging behaviour is the process by which a fish actively seeks out and utilizes available food sources.The foraging behaviour's mean frequency was noted every eight hours.The foraging behaviour's mean time (in seconds) was recorded at 8 hours.

2) The regularity with which fish periodically come to the surface of the water to breathe is known as "surfacing behaviour."The mean frequency of surfacing behaviour was observed at 8 hours. At 8 hours, the mean time (in seconds) of the surfacing behaviour was noted.

3) Swimming behaviour refers to a fish's movement through the water at the top, middle, and bottom of an aquarium, regardless of speed or direction, and without any kind of behavioural activity.The average number of swimming sessions was noted, lasting eight hours.The swimming behaviour's mean time (in seconds) was noted at 8 hours.

4) Body care behaviour: (a) Body shaking: This refers to the fish rapidly shaking its entire body two or three times in a row.For eight hours, the mean frequency of fish shook their body was recorded.The average duration (in seconds) of the body shaking behaviour was recorded, divided by eight hours.(b) Scratching behaviour, also known as chafing is the action of rubbing a fish's body against something, according to Neto and Giaquinto (2020). For eight hours, the mean frequency of scratching behaviour was observed. Over an eight-hour period, the mean scratching behaviour was measured in seconds.

5) Resting behaviour was assessed when they were stationary and still on the bottom, their eyes open due to the absence of eyelids (Sekiguchi and Kohshima, 2003). Every eight hours, the mean duration (in seconds) and mean frequency of resting have been noted.

6) Aggressive behaviour: Attacking someone or engaging in combat is referred to as aggressive behaviour. According to the definition and observations provided by Brando *et al*. (2018) and Barreto *et al*. (2011), the following aggressive patterns were identified:The average frequency of aggressive behaviour per eight hours was noted.The aggressive behaviour was measured as mean time (sec.) / 8 hours.
• Approach: denotes that two fish are swimming precisely in the same direction.
• Fin spreading: This is the action of a fish spreading all of its fins at once.
• Biting: With its pointed mouth, one fish nips at a bodily portion of another fish.
• Chasing: This refers to fish that swim aggressively after another fish.
• Fleeing: Fish swim away from their enemies.
• Fin tagging is the act of a fish biting another fish's fins with its enticing mouth.
• Butting: a fish smacks another fish's genital papilla with its snout.
• Mouth pushing: This is a behaviour in which two fish regularly stand face-to-face and press their mouths against each other.

7) Number of midline crossings: this is determined by counting the number of times fish cross the midline within each tank over a five-minute period, with the entire aquarium's length divided by an external midline, based on the figures and guidelines provided by Scott *et al*. (2003).

Data Handling Statistical Analysis

The SPSS version 21 Statistical Analysis System package (SPSS Windows, 2012) was utilized for the collection, organization, summarization, and analysis of all study data. The study employed a one-way analysis of variance (ANOVA) test to investigate behavioural factors and differences in fish body weight between groups. Following notable outcomes, Tukey's honesty-significant test was employed. Using a mixed-model ANOVA test, crossing tests were performed for several groups over the course of the subsequent study weeks. Eight weeks into the study, an interaction plot was used to compare means between groups. P values less than (0.05) were deemed significant in statistics. Every outcome was shown as mean ±SE.

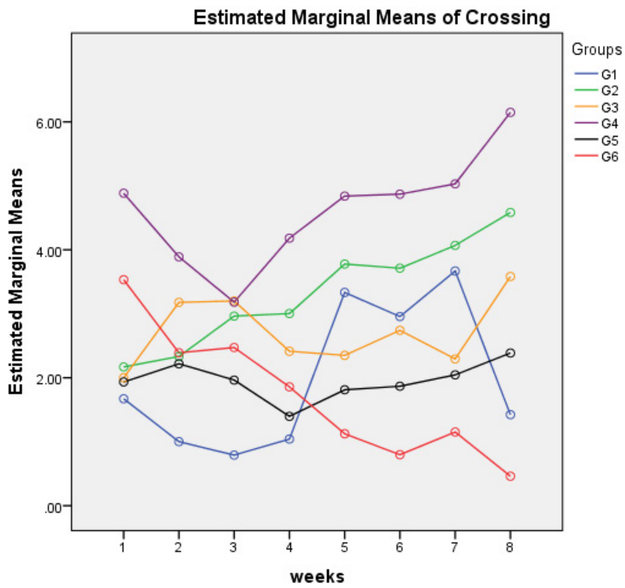

Figure 1: The impact of extracted oils on the crossing test of 6 groups throughout 8 weeks of the study.
G1: Control group; **G2:** Lemon extracted oil group (LEO 1‰); **G3:** Orange extracted oil group (OEO 3‰); **G4:** Mixed extracted oils group (LEO 1‰ & OEO 3‰); **G5:** Zinc oxide nanoparticles (ZnONPs) group); **G6:** Zinc oxide nanoparticles (ZnONPs) group with the mixture of both; oils (LEO 1‰ & OEO 3‰).

RESULTS AND DISCUSSION

Extracted oils (EOs) are a blend of organic compounds that are extracted from plants and can be used as food preservatives. Moreover, EOs are naturally occurring antioxidants and antimicrobials that can enhance fish welfare, immune system function, and growth performance—all of which can lower production costs (Zeppenfeld et al., 2016). As shown in Table 3 and Figure 2, adding extracted oils (LEO 1‰ & OEO 3‰) to the diet had a statistically significant effect (P<0.05) on the feeding and foraging behaviour of the groups that were given the oils over the course of the 8-week study compared to the G1 (control group).

Table 3: The impact of extracted oils on the ingestive behaviour, surfacing behaviour and swimming behaviour of 6 groups throughout eight weeks of study.

	G1	G2	G3	G4	G5	G6	P-value	CI (95%) (effect size)
Feeding Frequency	28.57 ± 2.92 ^{c}	65.43 ± 3.27 ^{b}	60.57 ± 9.28 ^{b}	117.29 ± 6.66 ^{a}	15.57 ± 1.17 ^{c}	34.71 ± 1.89 ^{c}	<0.001	(0.87)
Feeding Time	103.86 ± 8.04 ^{c}	175.71 ± 13.67 ^{b}	199.86 ± 28.11 ^{b}	388 ± 21.88 ^{a}	35.29 ± 2.01 ^{d}	82.14 ± 5.59 ^{cd}	<0.001	(0.89)
Foraging Frequency	50.71 ± 8.58 ^{b}	91.57 ± 11.28 ^{a}	53.86 ± 9.11 ^{b}	88.14 ± 2.32 ^{a}	16.29 ± 1.04 ^{c}	35.57 ± 3.79 ^{bc}	<0.001	(0.63)
Foraging Time	425.86 ± 70.58 ^{a}	689.14 ± 48.76 ^{a}	523.14 ± 160.24 ^{a}	686 ± 55.73 ^{a}	44.29 ± 3.84 ^{b}	106.71 ± 4.57 ^{b}	<0.001	(0.64)
Surfacing Frequency	15.86 ± 1.39 ^{b}	13.71 ± 2.91 ^{b}	15.71 ± 4.02 ^{b}	11.71 ± 2.16 ^{b}	97.86 ± 2.23 ^{a}	94.57 ± 8.14 ^{a}	<0.001	(0.93)
Surfacing Time	171.57 ± 15.16 ^{c}	211.86 ± 55.7 ^{bc}	409.29 ± 122.5 ^{b}	121 ± 24 ^{c}	907.29 ± 24.6 ^{a}	811.29 ± 24.84 ^{a}	<0.001	(0.82)
Surface Swimming Frequency	44.71 ± 4.64 ^{b}	38.71 ± 5.36 ^{b}	52.57 ± 7.6 ^{b}	45.29 ± 2.36 ^{b}	77.57 ± 3.62 ^{a}	85.43 ± 4.95 ^{a}	<0.001	(0.67)
Surface Swimming Time	392.71 ± 36.75 ^{d}	440.14 ± 54.35 ^{bc}	529.43 ± 89.57 ^{ab}	282.86 ±15.67 ^{d}	605.14 ± 34.3 ^{ab}	640 ± 20.79 ^{a}	<0.001	(0.52)
Middle Swimming Frequency	87.71 ± 3.49 ^{b}	96.14 ± 5.96 ^{b}	87.43 ± 6.55 ^{b}	126.57 ± 3.82 ^{a}	30.71 ± 2.91 ^{d}	54.86 ± 2.37 ^{c}	<0.001	(0.88)
Middle Swimming Time	760.14 ± 48.94 ^{b}	1128.7 ± 76.78 ^{a}	899.43 ± 94.29 ^{b}	1150.57 ±38.4 ^{a}	177.86 ± 23.34 ^{c}	349 ± 18.94 ^{c}	<0.001	(0.87)
Bottom Swimming Frequency	79.71 ± 4.34 ^{ab}	96 ± 3.86 ^{a}	74.43 ± 8.89 ^{b}	89.43 ± 5.28 ^{ab}	28 ± 3.15 ^{d}	48.29 ± 3.1 ^{c}	<0.001	(0.78)
Bottom Swimming Time	705.86 ± 33.31 ^{a}	749.57 ± 30.22 ^{a}	631.86 ± 68.32 ^{a}	597.14 ± 49.54 ^{a}	173.86 ± 18.95 ^{c}	332.57 ±14.62 ^{b}	<0.001	(0.82)

When comparing means ^{abc} in the same row with different superscripts, a highly significant difference was seen (P<0.05). While means in the exact same row with the exact same superscripts did not change significantly (P>0.05).The values expressed as Mean ±SE.

The effects were seen in both duration (sec) and frequency (bouts) of feeding and foraging. G4 (mixed LEO 1‰ & OEO 3‰) exhibited the highest and optimal feeding behaviour (time and frequency) at (388 ± 21.88 sec) (117.29 ± 6.66 bout), while G2 (LEO 1‰) demonstrated the highest values of foraging behaviour (time and frequency) at (689.14 ± 48.76 sec) (91.57 ± 11.28 bout). On the other hand, the lowest feeding and foraging behaviour time and frequency were recorded in fish raised at chemically synthesized ZnONPs groups especially G5 (35.29 ± 2.01 sec), (15.57 ± 1.17 bout), (44.29 ± 3.84 sec), (16.29 ± 1.04 bout) respectively, while G6 (ZnONPs group treated with LEO 1‰ & OEO 3‰) showed an increase in both behaviours after adding the mixture of (LEO 1‰) and (OEO 3‰) gradually. The improved behaviour may be due to the impact of extracted oils on digestive processes by increasing feed utilization, improving nutrient digestibility and absorption as well as diet palatability. fish raised in ZnONP groups, especially G5 (35.29 ± 2.01 sec), (15.57 ± 1.17 bout), (44.29 ± 3.84 sec), and (16.29 ± 1.04 bout), may have better behaviour because the oils they are fed affect their digestive systems in ways that make food more digestible and tasty.

These results seem to be similar to those of Mohamed *et al.* (2021), who found that fish fed the diet supplemented with extracted oils showed the highest and best values of feeding behaviour among all treatments, while aquatic creatures fed the control diet showed the lowest values of feeding behaviour. They also agreed with what El-Saadony *et al.* (2021) found: feeding and foraging behaviour (time and frequency) were greatly reduced when a high chemical zinc oxide nanoparticles (ZnONPs) treatment level was used (P < 0.05). The results also corroborated Kasumyan's (2001) theory that chemical metals, such as zinc oxide nanoparticles (ZnONPs), in the aquatic environment can inhibit feeding motivation and cause disruptions to Nile Tilapia's appropriate feeding, food search, and foraging behaviours. These results also correlated with those of Baba *et al.* (2016), who discovered that supplementing *citrus limon* peel-derived oil might improve the eating and foraging habits of cultured Nile tilapia. The management program's resemblance may explain this agreement. These findings may attribute to the bad effect of (CH-ZnONPs) on the growth hormone as reported by (JÖnsson and BjÖrnsson ,2002) while dietary supplementation of

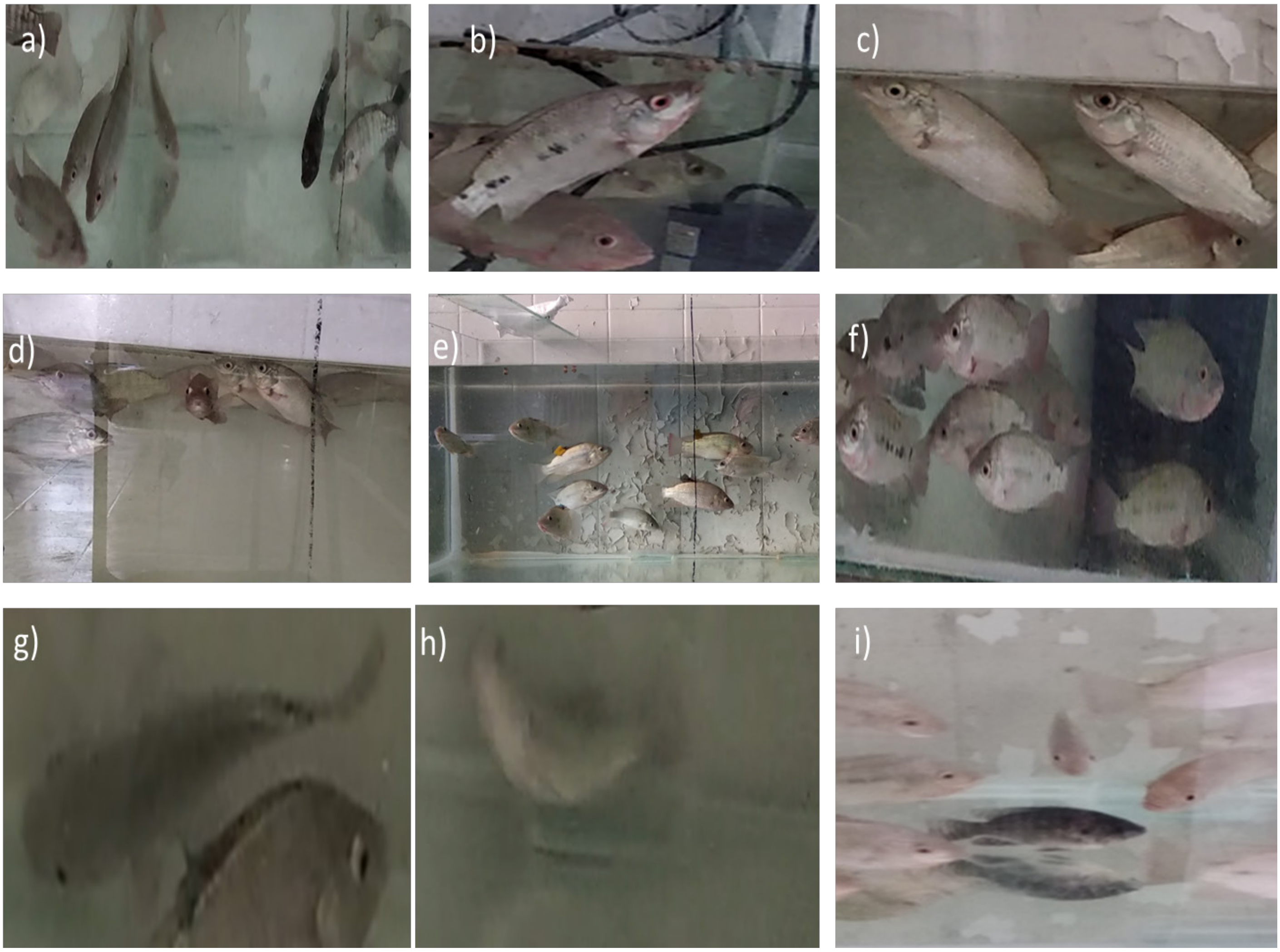

Figure 2: The impact of extracted oils on Nile tilapia fingerlings behaviour during the experiment.
Foraging behaviour, **b)** feeding behaviour, **c)** surfacing behaviour, **d)** Surface swimming behaviour, **e)** middle swimming behaviour, **f)** bottom swimming behaviour, **g)** body shaking behaviour, **h)** scratching behaviour, **i)** Resting behaviour.

both lemon and orange extracted oils may enhance it. The information provided in Table (3) and Figure (2) further demonstrated that the dietary supplementation of extracted oils had a significant impact on the time and frequency of surfacing behaviour ($P<0.05$). G4 exhibited the lowest values of surfacing time and frequency (121 ± 24 sec and 11.71 ± 2.16 bout), respectively. G6's surfacing behaviour decreased after adding the mixture of LEO 1‰ and OEO 3‰. On the other hand, G5 (ZnONPs treated group), had the longest surfacing times (907.29 ± 24.6 sec) and the most frequent bouts (97.86 ± 2.23 bouts). These findings were in line with those of Noga (1996), who noted that fish that have low dissolved oxygen levels in their tanks come to the top more frequently. As a result, fish behaviour in aquariums can be used as a reliable indicator of the water's dissolved oxygen concentration. According to DeMicco *et al.* (2010), any change to the water's parameters, like adding toxic heavy metals like chemical zinc oxide nanoparticles (ZnONPs), could cause hypoxia because of the buildup of zinc in the fish's body and gills. These results also are similar to the data obtained by Mohamed *et al.* (2020) who noted that fish exposed to any toxic agent expressed behavioural abnormalities such as in ability to hardly obtain a sufficient amount of oxygen; this was manifested by enhanced breathing rate along with more frequent visits to surface (increasing surfacing behaviour). These results also coincided with García-Trejo *et al.* (2016) who found that under hypoxic conditions Nile tilapia becomes near to the water surface gasping air for acquiring the oxygen. These results back this up. This could potentially negatively affect the maintenance behaviour of Nile tilapia, leading to an increase in their tendency to surface more frequently. The parallelism in the experiment designs could be the reason for this agreement. The findings in Table 3 and Figure 2 showed that dietary supplementation with extracted oils ($P<0.05$) significantly affects the duration and frequency of swimming behaviours (surface, middle, and bottom). The extracted oil-treated groups, especially G4, had the longest and most frequent middle swimming times (1150.57 ± 38.42 seconds) and bouts (126.57 ± 3.82), compared to the control group G1

Table 4: The impact of extracted oils on body care behaviour, aggressive behaviour and resting behaviour of 6 groups throughout eight weeks of study.

	G1	G2	G3	G4	G5	G6	P-value	CI (95%) (effect size)
Shaking Frequency	6.14 ± 1.44 ^{b}	4.43 ± 1.13 ^{b}	11.43 ± 1.34 ^{ab}	7.43 ± 0.72 ^{ab}	11.57 ± 2.12 ^{ab}	15.71 ± 4.07 ^{a}	0.01	(0.35)
Shaking Time	16.14 ± 5.47 ^{ab}	9 ± 2.35 ^{b}	31.71 ± 5.04 ^{ab}	17.57 ± 1.57 ^{ab}	24.14 ± 5.28 ^{ab}	35.29 ± 9.75 ^{a}	0.02	(0.31)
Scratching Frequency	19.57 ± 2 ^{a}	7.14 ± 1.53 ^{c}	6.57 ± 1.23 ^{c}	9.29 ± 1.58 ^{bc}	8.29 ± 1.25 ^{bc}	14.14 ± 1.91 ^{ab}	<0.001	(0.57)
Scratching Time	52.71 ± 4.6 ^{a}	16.71 ± 3.3 ^{c}	20.29 ± 4.64 ^{bc}	24.14 ± 4.41 ^{bc}	20.29 ± 3.85 ^{bc}	34.57 ± 3.94 ^{bc}	<0.001	(0.59)
Resting Frequency	15.43 ± 2.6 ^{c}	10.14 ± 1.92 ^{c}	7.71 ± 2.51 ^{c}	6.14 ± 0.77 ^{c}	206.14 ± 9.43 ^{a}	135.86 ± 3.55 ^{b}	<0.001	(0.98)
Resting Time	198.43 ± 27.35 ^{c}	66.29 ± 11.58 ^{d}	52.14 ± 14.15 ^{d}	20.86 ± 3.18 ^{d}	1297.71 ± 34.71 ^{a}	861.71 ± 30.78 ^{b}	<0.001	(0.99)
Aggressive Frequency	92.43 ± 6.49 ^{a}	24.71 ± 5.82 ^{c}	61.57 ± 4.67 ^{b}	21 ± 2.73 ^{c}	21.86 ± 3.96 ^{c}	25.86 ± 2.66 ^{c}	<0.001	(0.85)
Aggressive Time	193.71 ± 12.92 ^{a}	50.57 ± 20.42 ^{c}	128.14 ± 13.92 ^{b}	39.86 ± 5.47 ^{c}	42.14 ± 6.87 ^{c}	54.71 ± 5.86 ^{c}	<0.001	(0.78)

When comparing means ^{abc} in the same row with different superscripts, a highly significant difference was seen (P<0.05). While means in the exact same row with the exact same superscripts did not change significantly (P>0.05).The values expressed as Mean ±SE.

and the ZnONPs groups, while the ZnONPs groups had the shortest middle swimming duration (177.86 ± 23.34 sec) and frequency (30.71 ± 2.91 bouts). Meanwhile, G6 showed a gradual increase in middle swimming behaviour time and frequency (349 ± 18.94 sec) and (54.86 ± 2.37 bouts), respectively. The results obtained appear to be similar to those of Martins *et al.* (2012), who showed how any change of water quality could alter swimming behaviour. For example, hypoxia (low dissolved oxygen level), as observed in ZnONP groups, may result in decreased swimming activity and speed. These findings are similar to those found by Ellgaard *et al.* (1995), who found that goldfish exposed to toxic heavy metal concentrations below the lethal threshold, like nickel, slowed down their swimming and became less active compared to the control group. These results also correlated to El-Hawarry *et al.* (2018) who concluded that dietary supplementation of oregano-extracted oil to Nile tilapia fingerlings improved their middle and bottom swimming behaviour. When we looked at the Nile tilapia's body care habits, Table 4 and Figure 2 showed that the ZnONPs groups, especially G6, shook their bodies more often and for longer periods of time (35.29 ± 9.75 seconds and 15.71 ± 4.07 bouts, respectively) than the groups that were given extracted oils. The duration and frequency of scratching behaviour were greater in G1 (the control group) at (52.71 ± 4.6 seconds and 19.57 ± 2 bouts, respectively). These findings were corroborated by Noga (1996), who discovered that one of the clinical signs of fish water poisoning is rubbing a fish's body against the tank's sides and bottom. Thus, an increase in fish body rubbing and shaking is a sign of poor management and hygiene. These results backed up what Mohamed *et al.* (2020) who found that giving Nile Tilapia extra geranium-extracted oil (GEO) while they were exposed to toxins in water decreased behavioural changes (excessive scratching and body shaking) in the co-exposed group more than expected and improved their health. The data in Table 4 and Figure 2 showed that the ZnONPs groups, especially G5, had the longest periods of resting behaviour (1297.71 ± 34.71 sec) and the most bouts (206.14 ± 9.43). G6 showed a decrease in resting behaviour time and frequency (861.71 ± 30.78 sec) and (135.86 ± 3.55 bout) after adding the mixture of LEO 3‰ and OEO 3‰ gradually. These findings were in agreement with those of El-Saadony *et al.* (2021), who showed that a high chemical ZnONPs treatment level ($P < 0.05$) significantly enhanced resting behaviour (time and frequency). These results disagreed with those recorded by El-Hawarry *et al.* (2018) who found that dietary supplementation of oregano-extracted oil to Nile tilapia fingerlings caused an obvious increase in resting behaviour. This disagreement may be attribute to the difference in the types of used extracted oils. Table 4 and Figure 3 depict all aggressiveness patterns. The extracted oil-treated groups, particularly G4, showed the lowest values of aggressive behaviour time and frequency (39.86 ± 5.47 sec) and (21 ± 2.73 bout), respectively, compared to the other groups. A study by El-Hawarry *et al.* (2018) also came to the same conclusion. They discovered that oregano-extracted oil generally reduced anxiety and aggressive behaviours (biting, chasing, and mouth pushing) in fish groups that

Table 5: The impact of extracted oils on growth rate, mortality, morbidity and survival rate of Nile tilapia.

	G1	G2	G3	G4	G5	G6	P-value	CI (95%) (effect size)
IBW(g)	25.56±1.46	25.49±1.41	25.53±1.42	25.5±1.51	25.76±1.22	25.54±1.5	1	(0.002)
FBW(g)	39.21±1.47 ^{ab}	41.7±1.55 ^{a}	42.43±1.68 ^{a}	45.73±2 ^{a}	30.78±0.54 ^{c}	34.55±0.93 ^{bc}	<0.001	(0.85)
WG	13.64±0.73 ^{b}	16.21±0.36 ^{ab}	16.9±0.51 ^{ab}	20.23±0.65 ^{a}	5.02±1.5 ^{c}	9.01±1.63 ^{c}	<0.001	(0.93)
ADG	0.24±0.01 ^{b}	0.29±0.01 ^{ab}	0.3±0.01 ^{ab}	0.36±0.01 ^{a}	0.16±0.03 ^{c}	0.163±0.03 ^{c}	<0.001	(0.88)
SGR	0.33±0.03 ^{ab}	0.38±0.02 ^{a}	0.4±0.02 ^{a}	0.45±0.01 ^{a}	0.14±0.04 ^{c}	0.24±0.05 ^{bc}	<0.001	(0.85)
FCR	14.87±0.92	12.96±0.39	12.4±0.32	10.65±0.13	22.86±4.53	22.06±4.25	0.021	(0.63)
Mortality	0%	0 %	0 %	0 %	20%	10%		
Morbidity	0%	0 %	0 %	0 %	20%	10%		
Survival rate (SR) (%)	100%	100%	100%	100%	80%	90%		

IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, ADG: Average daily Gain, SGR: Specific growth rate, FCR: Feed conversion ratio.

Highly significant difference was shown by means ^{abc} in the same row with various superscripts (P<0.05). While Means in the same row with the same superscripts showed no significant difference (P>0.05).

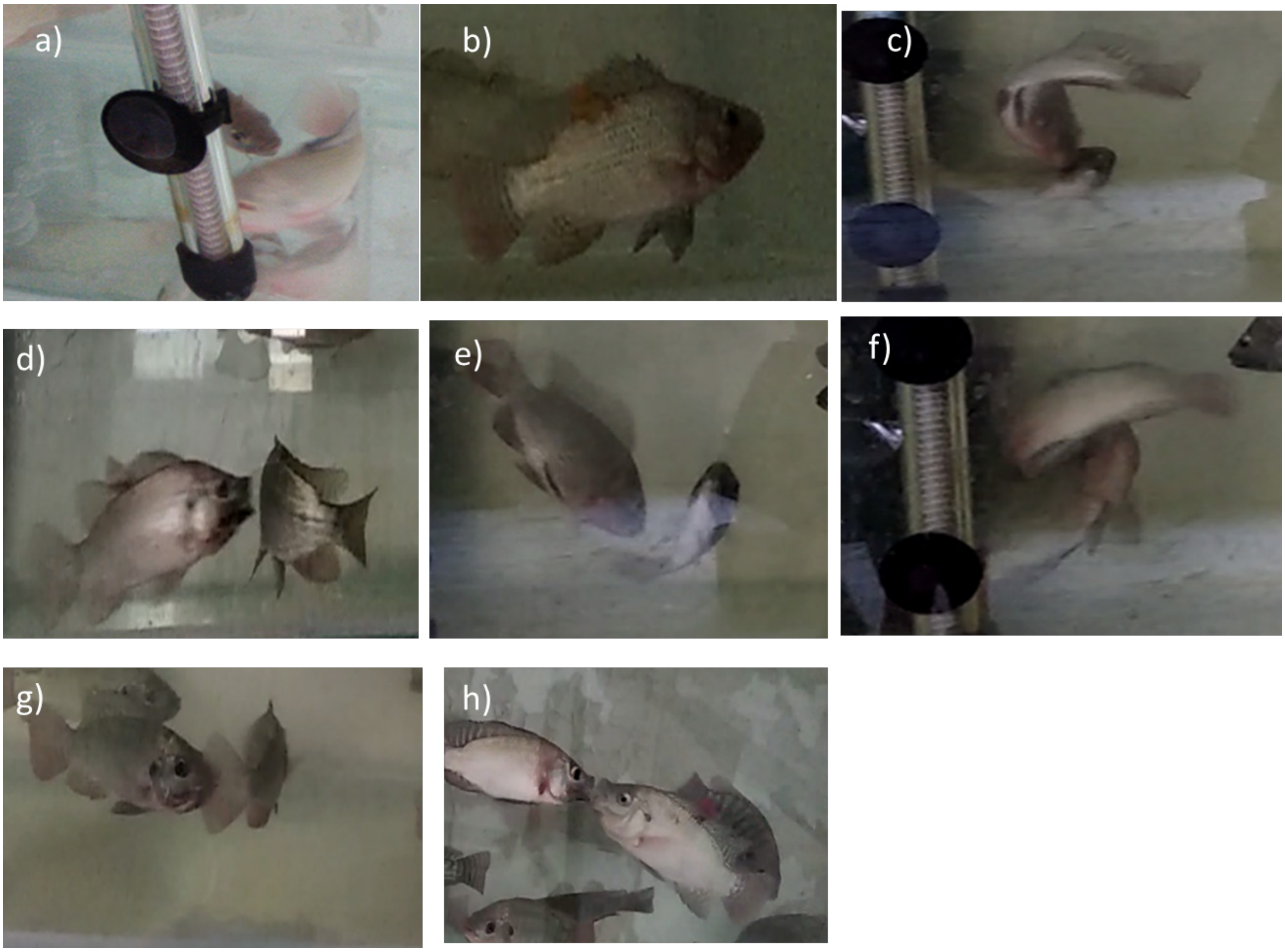

Figure 3: The impact of extracted oils on Nile tilapia fingerlings' aggressive behavioural patterns during the experiment. Approach behaviour, **b)** spreading of fins behaviour, **c)** biting behaviour, **d)** chasing behaviour, **e)** fleeing behaviour, **f)** butting behaviour, **g)** fin tugging behaviour, **h)** mouth pushing behaviour.

were not significantly different. These results were in conflict with those of El-Saadony *et al.* (2021), who noted that when 0.5 mg/L of CH-ZnONPs was administered as opposed to BIO-ZnONPs, aggression increased. The discrepancy could be attributed to varying dosage levels of CH-ZnONPs. Table 5 displays the study's results, which indicate that various extracted oils were added to the diet during the study weeks. Body weight (FBW) was the lowest in the ZnONPs groups as G5 had the lowest FBW at (30.78±0.54g) and the highest in G4 (45.73±2g). However, G6's FBW went up after a mixture of (LEO 1‰ and (OEO 3‰) was added gradually. Adding a mixture of extracted oils made the weight gain (WG), average daily gain (ADG), specific growth rate (SGR), and feed conversion ratio (FCR) in G4 much better than in other groups (20.23±0.65g), (0.36±0.01g), (0.45±0.01), and (10.65±0.13), respectively. These results were in line with studies by Seden *et al*. (2009) and Acar *et al*. (2019) that showed supplementing fish diets with extracted oils could improve the growth performance of a variety of fish species, including Nile tilapia fingerlings. These findings also corroborate the findings of Sutili *et al*. (2016), who reported that the addition of various extracted oils to fish diets enhanced their eating behaviour, thereby improving their growth performance. It was also similar to what Salem and Abdel-Ghany (2018) who found that fish that were fed diets that included different amounts of extracted oils from sweet orange and/or lemon peels grew faster and gained more weight than fish that were fed a control diet. The fish also had a lower feed conversion ratio. They also observed that fish fed mixed extracted oils (OEO and LEO) exhibited a proper growth performance in a dose-dependent manner. The improvement in the growth performance may be attributed to the increased feeding behaviour and appetite caused by extracted oils supplementation. Because of all these factors, nutritional supplements containing mixed extracted oils (LEO 1‰ and OEO 3‰) are preferable for enhancing the welfare of Nile tilapia. The data presented in Table 5 also showed that the different extracted oil dietary supplements had a significant impact on the rates of mortality, morbidity, and survival rate during the study weeks. Specifically, the groups treated with extracted oils did not exhibit any mortality or morbidity, while the ZnONPs groups demonstrated mortality (20%) and morbidity (20%), similar to G5 and G6 mortality (10%) and morbidity (10%). These results agree with those of DeMicco et al. (2010), who said that stress could be seen as a chemical agent, like high levels of zinc oxide nanoparticles (ZnONPs), which could cause reactions that may result in low survival rate and death of aquatic organisms. These results backed up what Baba *et al*. (2016) who found that adding oil from citrus limon peel might improve general immune system functions and lower the death rate of Nile tilapia. This finding fits with what Ngugi *et al*. (2017) who found that feeding fish mixtures with up to 5% essential oil extract from citrus limon fruit peels greatly reduced death and illness and increased survival rates by improving the immune systems of young fish compared to groups that were not treated. The resemblance of the type of used extracted oils may explain this agreement. The results presented in Figure 1 showed that the extracted oils groups, specifically G4, had higher frequencies of midline crossing test that measured the fish activity (6.15 ± 0.34 bouts) than the groups that were given ZnONPs. The link between swimming behaviour and the crossing test could be the reason of these findings. These results resemble those of Mohamed *et al*. (2020), who reported that the co-exposed group's behaviour and activity were enhanced more than anything else when they were given dietary supplements including geranium-extracted oil (GEO) than they were exposed to toxic agent in water. These findings also coincided with those of Kasumyan (2001) who claimed that chemical metals in the aquatic environment, such as chemical zinc oxide nanoparticles (ZnONPs), might suppress fish motivation and activity. This agreement may be due to using the same chemical agent in aquaria's water. Therefore, food supplementation with extracted oils (LEO 1‰ & OEO 3‰) could improve Nile Tilapia's wellbeing.

CONCLUSIONS AND RECOMMENDATIONS

Among the practical applications of the study is that the incorporating natural ingredients such as OEO and/or LEO extracted oils into aqua feed formulations in ponds used to raise Nile tilapia could lead to an increase in growth rate, feed efficiency, and overall fish health. As a result, this study confirms the value of extracted oils as feed additives to improve fish behaviour and growth, which will ultimately result in the welfare of Nile tilapia and a rise in profit and economic return. As a result, extracted oils are recommended as a crucial component in aquaculture feed industries for optimal Nile tilapia's growth performance and welfare, along with full fish harvest in ponds. However, the long term use of such additives should be carefully monitored for any potential adverse effects as this point is among the limitations of the current investigation. The use of more broad concentrations and studying the effects of other environmental factors in the aquaria are among the future perspectives for the study.

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NOVELTY STATEMENT

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AUTHOR'S CONTRIBUTION

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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

Abdel Rahman AN, Mohamed AAR, Mohammed HH, Elseddawy NM, Salem GA, El-Ghareeb WR (2020). The ameliorative role of geranium (Pelargonium graveolens) extracted oil against hepato-renal toxicity, immunosuppression, and oxidative stress of profenofos in common carp, *Cyprinus carpio* (L.). Aquaculture, 517: 734777. https://doi.org/10.1016/j.aquaculture.2019.734777

Abdel-Fattah FA, Ahmed FA, Saleem ASY, Mohammed HH, Youssef MY, Said EN (2020). Effect of the different stocking density on behaviour, performance and welfare of the Nile tilapia (Oreochromis niloticus). Egypt. J. Aquatic Biol. Fisher., 24: 539-560. https://doi.org/10.21608/ejabf.2020.109369

Abdel-Latif HM, Abdel-Tawwab M, Khafaga AF, Dawood MA (2020). Dietary oregano extractedoil improved anti-oxidative status, immune-related genes, and resistance of common carp (*Cyprinus carpio L.*) to Aeromonas hydrophila infection. Fish Shellfish Immunol., 104: 1–7. https://doi.org/10.1016/j.fsi.2020.05.056

Acar Ü, Kesbiç OS, İnanan BE, Yılmaz S (2019). Effects of dietary Bergamot (*Citrus bergamia*) peel oil on growth, haematology and immune response of European sea bass (*Dicentrarchus labrax*) juveniles. Aquacul. Res., 50: 3305–3312. https://doi.org/10.1111/are.14288

Acar Ü, Kesbiç OS, Yılmaz S, Gültepe N, Türker A (2015). Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against *Streptococcus iniae*. Aquaculture, 437: 282–286. https://doi.org/10.1016/j.aquaculture.2014.12.015

Ahmed FA (2020). Behaviour and performance of tilapia nilotica in relation to management. Master Veterinary Science thesis, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Ardjosoediro I, Ramnarine IW (2002). The influence of turbidity on growth, feed conversion and survivorship of the Jamaica red tilapia strain. Aquaculture, 212, 159–165. https://doi.org/10.1016/S0044-8486(01)00881-X

Baba E, Acar Ü, Öntaş C, Kesbiç OS, Yılmaz S (2016). Evaluation of Citrus limon peels essential oil on growth performance, immune response of Mozambique tilapia, Oreochromis mossambicus challenged with Edwardsiella tarda. Aquaculture, 465: 13–18. https://doi.org/10.1016/j.aquaculture.2016.08.023

Barreto RE, Carvalho GGA, Volpato GL (2011). The aggressive behaviour of Nile tilapia introduced into novel environments with variation in enrichment. Zoology, 114: 53-57. https://doi.org/10.1016/j.zool.2010.09.001

Brandão ML, Colognesi G, Bolognesi MC, Costa-Ferreira RS, Carvalho TB, Gonçalves-de-Freitas E (2018). Water temperature affects aggressive interactions in a Neotropical cichlid fish. Neotropical Ichthyol., 16: 170081. https://doi.org/10.1590/1982-0224-20170081

García-Trejo J.F, Peña-Herrejon, GA, Soto-Zarazúa, GM, Mercado-Luna A, Alatorre-Jácome O Rico-García, E (2016). Effect of stocking density on growth performance and oxygen consumption of Nile tilapia (Oreochromis niloticus) under greenhouse conditions. Latin Am. J. Aqua. Res., 44: 177-1831 https://doi.org/10.3856/vol44-issue1-fulltext-20

Demers NE, Bayne CJ (1997). The immediate effect of stress on hormones and plasma lysozyme in rainbow trout. Dev. Comp. Immunol., 21: 363–373. https://doi.org/10.1016/S0145-305X(97)00009-8

DeMicco A, Cooper KR, Richardson JR, White L (2010). Developmental neurotoxicity of pyrethroid insecticides in zebra fish embryos. Toxicol. Sci., 113: 177–186. https://doi.org/10.1093/toxsci/kfp258

Dickson M, Nasr-Allah A, Kenawy D, Kruijssen F (2016). Increasing fish farm profitability through aquaculture best management practice training in Egypt. Aquaculture, 465: 172–178. https://doi.org/10.1016/j.aquaculture.2016.09.015

El-Hawarry WN, Mohamed RA, Ibrahim SA (2018). Collaborating effects of rearing density and oregano oil supplementation on growth, behavioural and stress response of Nile tilapia (Oreochromis niloticus). Egypt. J. Aquatic Res., 44: 173-178. https://doi.org/10.1016/j.ejar.2018.06.008

Ellgaard E, Ashley S, Langford A (1995). Kinetic analysis of the swimming behaviour of the goldfish, Carassius auratus, exposed to nickel: hypoactivity induced by sublethal concentrations. Bull. Environ. Contam. Toxicol., 55: 929–936. https://doi.org/10.1007/BF00209475

El-Saadony MT, Alkhatib FM, Alzahrani SO, Shafi ME, Abdel-Hamid SE, Taha TF, Ahmed NH (2021). Impact of mycogenic zinc nanoparticles on performance, behaviour, immune response, and microbial load in Oreochromis niloticus. Saudi J. Biol. Sci., 28: 4592-4604. https://doi.org/10.1016/j.sjbs.2021.04.066

Elsabagh, M., Mohamed, R., Moustafa, E.M., Hamza, A., Farrag, F., Decamp, O., Dawood, M.A., Eltholth, M. (2018). Assessing the im- pact of Bacillus strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, Oreochromis Niloticus. Aquacult. Nutr., 24(6): 1613–1622. https://doi.org/10.1111/anu.12797

El-Sayed AM (2019). Tilapia culture. Academic Press.

Essam AM, Badawi ME, Yasser HM, Abdelhakeem IE, Ahmed NF (2020). Effect of Chlorella vulgaris enriched diet on growth performance, hematoimmunological responses, antioxidant and transcriptomics profile disorders caused by deltamethrin toxicity in Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol., 102: 422-429. https://doi.org/10.1016/j.fsi.2020.04.061

Feldman BF, Zink JG, Jain NC (2000). Schalm's Veterinary Hematology, 5th ed. Lippincott Williams and Wilkins, New York.

Fitzsimmons K (2005). Tilapia culture. In American Fisheries Society Symposium, 46: 563-590.

Gregory N, Grandin T (2013). Animal welfare and meat production. Wallingford, Oxfordshire, UK Cambridge, MA: CABI.

Hao L, Chen L (2012). Oxidative stress responses in different organs of carp (Cyprinus carpio) with exposure to ZnO nanoparticles. Ecotoxicol. Environ. Saf., 80: 103-10. https://doi.org/10.1016/j.ecoenv.2012.02.017

Jiang, J, Pi, J, Cai, J (2018). The advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg. Chem.

Appl. 3: 1–18. https://doi.org/10.1155/2018/1062562
JÖnsson E, BjÖrnsson, BT (2002): Physiological functions of growth hormone in fish with special reference to its influence on behaviour. Fish Sci., 68(sup1):742-8. https://doi.org/10.2331/fishsci.68.sup1_742
Kasumyan A (2001). Effects of chemical pollutants on foraging behaviour and sensitivity of fish to food stimuli. J. Ichthyol., 41: 76–87.
Kumar, A., Singh, P., Gupta, V., Prakash, B. (2020). Application of nano- technology to boost the functional and preservative properties of es- sential oils. In Functional and Preservative Properties of Phytochemicals (pp. 241–267). Academic Press. https://doi.org/10.1016/B978-0-12-818593-3.00008-7
Lopes JM, de Freitas Souza C, Saccol EMH, Pavanato MA, Antoniazzi A, Rovani MT, Heinzmann BM, Baldisserotto B (2019). Citrus x aurantium essential oil as feed additive improved growth performance, survival, metabolic, and oxidative parameters of silver catfish (*Rhamdia quelen*). Aquacul. Nutr., 25: 310–318. https://doi.org/10.1111/anu.12854
Martins CIM, Galhardo L, Nobl C, Damsgård B, Spedicato MT, Zupa W, Kristiansen T (2012). Behavioural indicators of welfare in farmed fish. Fish Physiol. Biochem., 38: 17–41. https://doi.org/10.1007/s10695-011-9518-8
Mohamed AAR, Rahman ANA, Mohammed HH, Ebraheim LL, Abo-ElMaaty AM, Ali SA, Elhady WM (2020). Neurobehavioural, apoptotic, and DNA damaging effects of sub-chronic profenofos exposure on the brain tissue of *Cyprinus carpio* L.: Antagonistic role of Geranium essential oil. Aquatic Toxicol., 224: 105493. https://doi.org/10.1016/j.aquatox.2020.105493
Mohamed RA, Yousef YM, El-Tras WF, Khalafallaa MM (2021). Dietary extracted oil extract from sweet orange (Citrus sinensis) and bitter lemon (Citrus limon) peels improved Nile tilapia performance and health status. Aquaculture Res., 52: 1463-1479. https://doi.org/10.1111/are.15000
Mohammady EY, Soaudy MR, Abdel-Rahman A, Abdel-Tawwab M, Hassaan MS (2021). Comparative effects of dietary zinc forms on performance, immunity, and oxidative stress-related gene expression in Nile tilapia, *Oreochromis niloticus*. Aquaculture, 532: 736006. https://doi.org/10.1016/j.aquaculture.2020.736006
Murugan SS, Karuppasamy R, Poongodi K, Puvaneswari S (2008). Bioaccumulation pattern of zinc in freshwater fish Channa punctatus (Bloch.) after chronic exposure. Turkish J. Fish Aquat. Sci. 8: 55–59.
Neto JF, Giaquinto PC (2020). Environmental enrichment techniques and tryptophan supplementation used to improve the quality of life and animal welfare of Nile tilapia. Aquaculture Rep., 17: 100354. https://doi.org/10.1016/j.aqrep.2020.100354
Ngugi CC, Oyoo-Okoth E, Muchiri M (2017). Effects of dietary levels of extracted oil (EO) extract from bitter lemon (*Citrus limon*) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in Juvenile *Labeo victorianus* fingerlings challenged with *Aeromonas hydrophila*. Aquaculture Res., 48: 2253–2265. https://doi.org/10.1111/are.13062
Noga EJ (1996). Fish diseases diagnosis and treatment. First edition Mosby electronic publishing.
NRC (National Research Council) (1993). Nutrient requirements of fish. National Academy Press, Washington, DC, 112PP.
Öntaş C, Baba E, Kaplaner E, Küçükaydin S, Öztürk M, Ercan MD (2016). Antibacterial activity of citrus limon peel extracted oil and Argania spinosa oil against fish pathogenic bacteria. Kafkas University Veterinary Faculty Dergisi, 22: 741-749.
Said EN, Ahmed FAA, Saleem ASY, Mohammed HH, Youssef MY, Abdel-Fattah FA (2020). Behavioural response, welfare, and performance of Nile tilapia (Oreochromis niloticus) under different water temperatures. Inter. J. Fish. Aquatic Stud., 8: 1-11.
Salem M, Abdel-Ghany H (2018). Effects of dietary orange peel on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture Stud., 18: 127–134.
Scott GR, Sloman KA, Rouleau C, Wood CM (2003). Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (Oncorhynchus mykiss). J. Stud. Biol., 206: 1779-1790. https://doi.org/10.1242/jeb.00353
Seden MEA, Abbass FE, Ahmed MH (2009). Effect of Origanum vulgare as a feed additive on growth performance, feed utilization and whole body composition of Nile tilapia, Oreochromis niloticus. Fingerlings challenged with pathogenic Aeromonas hydrophila. J. Agric. Sci. Mansoura Univ., 34: 1683–1695. https://doi.org/10.21608/jappmu.2009.116675
Sekiguchi Y, Kohshima S (2003). Resting behaviours of captive bottlenose dolphins (Tursiops truncatus). Physiol. Behav. 79, 643–653. https://doi.org/10.1016/S0031-9384(03)00119-7
Souza, CF., Salbego, J., Gressler, LT., Golombieski, J I., Ferst, JG.,Cunha, M A., … Baldisserotto, B. (2015). Silver catfish submitted to a stressful condition: Effect of dietary addition of the essential oil of *Lippia alba* (Mill.) N. E. Brown on metabolism, osmoregulation and endocrinology. Neotropical Ichthyology, 13, 707–714. https://doi.org/10.1590/1982-0224-20140153
SPSS version 21 (2012). IBM Corp. IBM SPSS Statistics for Windows, Armonk, and NY.
Sutili FJ, Velasquez A, Pinheiro CG, Heinzmann BM, Gatlin DM, Baldisserotto B (2016). Evaluation of Ocimum americanum extractedoil as an additive in red drum (Sciaenops ocellatus) diets. Fish Shellfish Immunol., 56: 155–161. https://doi.org/10.1016/j.fsi.2016.07.008
Toutou MA, Soliman AA, Elokaby M, Ahmed AR (2018). Growth performance and biochemical blood parameters of Nile tilapia, *Oreochromis niloticus*, and thinlip mullet, *Liza ramada*, fed a diet supplemented with lemon (*Citrus aurantifolia*) peel in a polyculture system. Egyptian J. Aquatic Biol. Fish., 22(3): 183– 192. https://doi.org/10.21608/ejabf.2018.23920
Vicente IST, Fleuri LF, Carvalho PLP, Guimarães MG, Naliato RF, Müller HDC, Sartori MMP, Pezzato LE, Barros MM (2019). Orange peel fragment improves antioxidant capacity and haematological profile of Nile tilapia subjected to heat/dissolved oxygen-induced stress. Aquacult. Res., 50: 80–92. https://doi.org/10.1111/are.13870
Youssef MY, Saleem ASY, Ahmed FA, Said EN, Abdel-Hamid SE, Gharib HS (2024). The impact of dietary probiotic supplementation on welfare and growth performance of Nile tilapia (Oreochromis niloticus). Open Vet. J., 14(1): 360. https://doi.org/10.5455/OVJ.2024.v14.i1.33
Zeppenfeld CC, Hernández DR, Heinzmann BM, Cunha MA, Schmidt D, Baldisserotto B (2016). Extracted oil of Aloysia triphylla as feed additive promotes growth of silver catfish (*Rhamdiaquelen*). Aquaculture Nutr., 22: 933–940. https://doi.org/10.1111/anu.12311