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Evaluation of Essential Oil of Fennel and Garlic Separately or Combined with *Bacillus licheniformis* on the Growth, Feeding Behaviour, Hemato-biochemical Indices of *Oreochromis niloticus* (L.) Fry

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Abstract

A total of six treatments, i.e., negative control group (D1), *B. licheniformis* 7×10^7 CFU g^{-1} diet (D2); 1ml essential fennel oil (EFO) kg^{-1} diet (D3); 1 ml essential garlic oil (EGO) kg^{-1} diet (D4); *B. licheniformis* 7×10^7 CFU g^{-1} +1 ml EFO kg^{-1} diet (D5) and *B. licheniformis* 7×10^7 CFU g^{-1} +1 ml EGO kg^{-1} diet (D6) were added to the diets of Nile tilapia, *Oreochromis niloticus* to investigate the effects of the respective treatments on the growth, feeding behaviour, hematological and biochemical indices. Fish (1.88 ± 0.12 g) were distributed at a rate of 20 fish per 100-L aquarium and three aquaria have been assigned for each treatment. At the end of the experiment (84-day), results indicated that the highest survival, weight gain and specific growth rate were recorded by fish fed D5 and D6 being statistically different (P<0.05) from other treatment groups. Whereas, the best feed conversion ratio and protein efficiency ratio were observed in D3, D4, D5 and D6 compared with other treatment groups. Fish feed D6 were higher in mouth wrestling and chasing behavior. Fish fed D5 and D6 significantly higher (P<0.05) hematocrit and hemoglobin values also, was effectively enhanced aspartate aminotransferase, alanine aminotransferase, total protein and globulin in comparison to the other treatments. No significant differences were found in the chemical composition of whole body of fish fed different tested diets.

Keywords: Essential oil; Fennel; Garlic; Growth; Feeding behaviour; Hemato-biochemical; Tilapia

Introduction

Improving the bio-economic efficiency of aquaculture is dependent on advances in biology, nutrition and environmental management of the production cycle. Antimicrobials and other veterinary drugs are administered regularly as additives in fish food, are used as therapeutics or growth promoters [1]. Nevertheless, the use of veterinary drugs is becoming more restricted since they present numerous side-effects for the environment and health safety [2]. Use of antibacterial agents in aquaculture should be avoided as it leads to proliferation of resistant and creates a negative image for aquaculture industry. Use of essential oil extracted from herbs medicine, could be a valuable alternative instead of antibiotics because there are fewer chances for the development of resistant strains [3].

There are many aromatic plants such as oregano, rosemary, sage, peppermint, thymus, fennel and garlic in worldwide, especially in the Mediterranean area [4]. The extracted essential oil from aromatic plants are usually used for antioxidant [5], digestive stimulant [6].

Fennel, *Foeniculum vulgare* is a biennial medicinal plant belonging to the family Apiaceae (Umbelliferae). Essential oils of fennel have hepatoprotective effects [7], as well as anti-inflammatory, and antioxidant activities [8]. Many phytochemical studies have been conducted to study the chemical composition of the essential oil of fennel from different origins and have shown that the major components are phenylpropanoid derivatives and monoterpenoids [9].

Allium is the largest and most important genus of the Alliaceae family and comprises 450 species, which were widely distributed in the northern hemisphere. Among them, garlic *(Allium sativum* L.), is a well-known species being used in traditional medicine and food in many countries [10]. Garlic and its constituents have antimicrobial activity. It was showed that the antibacterial activity of garlic resulted from thio sulfinates, especially allicin, which is responsible for the antimicrobial activity, as well as its flavor and aroma [11]. Five types of garlic preparations are currently available on the market: garlic essential oils, garlic oil macerate, garlic powder, aged garlic extract and fresh garlic [12]. Among them, the antimicrobial activity of garlic oil is 200 times greater than garlic powder and 900 times the strength of fresh garlic [12].

Previous studies revealed that herbs medicine and essential extracted oil from herbs can be successfully used in fish nutrition such as Carum carvi seeds [13], *Foeniculum vulgare* seeds [14], Cuminum cyminum powder [15], ground garlic, *Allium sativum* [16,17], oil of oregano, *Origanum heracleoticum* [18,19], oil of thyme and fennel [20]. Furthermore, some aromatic plants and their extracts have been reported to stimulate the growth of certain bacteria where used a prebiotic, but these studies are scarce [21]. As aforementioned, many authors have attempted to examine various essential oil components from herbal plants, but information regarding fry culture, especially the effects of essential oil of fennel and garlic supplements and their combined with probiotics on fish performance and hemato-biochemical blood parameters, is little. Therefore, the present study was designed to investigate the effect of fennel oil, garlic oil and *Bacillus licheniformis* alone and combined as dietary feed additives on growth

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performance, feed utilization, hematological and biochemical blood parameters of Nile tilapia, *O. niloticus* fry.

Materials and Methods

Fish, facility and feeding trial

The feeding trial was performed at an indoor installation of the Aquaculture Laboratory, faculty of agriculture Benha University. Nile tilapia, *O. niloticus* fries were obtained from fish hatchery of central laboratory for aquaculture research, Abbassa, Abou-Hammad Sharkia, Egypt. Fry were acclimated for two weeks to the experimental conditions in a fiberglass tanks. During this period fish fed daily on the commercial diet to be adapted to pelleted feeds. At the beginning of the experiment, 18 (100-L) glass aquaria were stocked with 20 fish with an initial average weight (1.88 ± 0.12 g). Each aquarium was supplied with compressed air via air-stones from air pumps. Overhead fluorescent illuminating was set at 14:10 (light:dark). All fish were fed, initially, at a rate of 6% of total body weight daily for the first 6-week and then decreased to 4% of total body weight daily for the remaining 12-week [22]. The fish were fed four times a day at 10:00, 12:00, 14:00, and 16:00, 6 days a week, for 84 days. All aquaria were siphoned twice a day to remove faecal materials then replaced by aerated fresh water. Total fish weight in each aquarium was determined every 2 weeks to check their growth and to adjust the feeding rate. Feeding was stopped 24 h before sampling. Water samples for chemical analysis were monitored weekly during the experimental period. Dissolved oxygen and temperature were measured on site with an YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, Co. USA). pH was measured using a pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). During the period of the feeding trial, the water-quality parameters were averaged (± SD): Water temperature was 26.25 ± 0.9°C: dissolved oxygen, 5.7 ± 0.5 mg L^{-1}: pH 8.33 ± 0.4 and total ammonia, 0.07 ± 0.01 mg L^{-1}. All tested water quality criteria were suitable and within the acceptable limits for rearing the Nile tilapia *O. niloticus* [23].

Extraction of essential oils and analysis

Fresh fennel seeds were dried at 35°C and crushed to fine powders using a grinder. Essential oil was obtained by hydrodistillation of the powdered dry seeds using a Clevenger-type apparatus. The essential fennel oil (EFO) was separated, dried over anhydrous sodium sulfate. However, extraction of the essential garlic oils (EGO) from fresh cloves of garlic was performed with a hydrodistiller after mashed. The hydrodistillation lasted 3 hours. Essential oil with an unpleasant strong odor was obtained and dried by adding anhydrous sodium sulfate, filter sterilized through a 0.22-mmfilter. Essential fennel and garlic oil were stored in a dark glass bottle at 4°C until use. The analysis of EFO and EGO were analyzed using gas chromatography-mass spectrometry (GC-MS) to identify their chemical constituents according to Aazza and Douiri et al. [24,25], respectively.

Preparation inoculum of (*B. licheniformis*)

Strain of *Bacillus licheniformis* used was obtained from Microbiological Resources Center (MIRCEN), faculty of Agriculture, Ain Shams University, Egypt. Inoclum was prepared by adding 5 g lyophilized cells of *B. licheniformis* to 100 ml of specific media which containing 5 g L^{-1} peptone, 3 g L^{-1} beef extract at pH 7.0 then was incubation at 37°C for 24 hours. After this period, cells were centrifugation for 15 min (2000 g) then washed with phosphate buffered saline (PBS) with pH 7.3. The washed cells was added dropwise into the ingredients of diet before to pellet [26] until to reach the level 7×10^7 (CFU g^{-1}) of 7×10^7 (CFU g^{-1}) of *B. licheniformis*. The control diet received the same volume PBS to maintain an equal volume of PBS in all experimental diets.

Experimental diets

The basal practical diet was formulated to contain approximately (35% crude protein and 19.96MJ Kg^{-1} gross energy) (Table 1). The basal practical diet was divided into six parts. The first part prepared without feed additives as control diet (D1). Other five parts prepared to contain *B. licheniformis* at level 7×10^7 colony forming unit (CFU g^{-1} diet) diet

Ingredients	g
Fish meal	280
Soybean meal	310
Yellow corn	240
Wheat bran	90
Soybean oil	40
Vitamins and Minerals[a]	35
Stay-C 35[b]	5
Proximate analysis	
Dry matter	952.30
Crude protein	351.40
Crude lipid	81.10
Ash	84.50
Total carbohydrate[b]	483.00
Gross energy (MJ/ kg)[c]	19.96

[a]Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).

[b]L-Ascorbic acid monophosphate (35%).

[c]Total carbohydrate = 100 − (Crude protein + Crude lipid + Ash).

[d]Gross energy calculated using gross calorific values of 0.2363, 0.3952 and 0.1715 MJ/g for protein, lipid and carbohydrate, respectively according to (Brett, 1973).

Table 1: Composition and proximate analysis of basal diet (g/ kg diet).

(D2); 1ml EFO kg^{-1} diet (D3); 1ml EGO kg^{-1} diet (D4); 1ml EFO + *B. licheniformis* 7×10^7 CFU g^{-1} diet (D5); 1ml EGO + *B. licheniformis* 7×10^7 CFU g^{-1} diet (D6). Essential fennel and garlic oil were added separately to soybean oil. All dry ingredients were thoroughly mixed with soybean oil, and vitamins and minerals mixture using a homogenous mixture grinder, and then passing the mixed feed through a laboratory pellet mill (0.5-mm die) in National Institute of Oceanography and Fisheries, Cairo Governorate, Egypt (CMP California Pellet Mill, San Francisco, CA, USA).

Feeding behaviour

The observation of fish behaviour was conducted for 6 days from June 20th until July 21st to study the effects of experimental diets on fish behavior according to Hussein et al. [27] to study the effects of experimental diets on fish behaviour. The types of fish behavior included were mouth wrestling and chasing. Fish were fed manually their daily amount of the experimental diets four times a day at 10:00, 12:00, 14:00 and 16:00. Ten minutes after the third meal (14:00) all aquarium were observed individually and each aquarium was given 3 min to quantify the behavioural interactions (i.e., as mouth wrestling and chasing separately) between fish.

Sample collection and analysis

At the end of the trial a random sample of five individual fish were sampled from each aquarium, then oven-dried 105°C for 24 h, ground, and stored at −20°C for subsequent analysis. Proximate analysis was conducted on diets and fish samples. Dry matter, crude lipids, crude protein and ash content were determined by the standard [28]. Dry matter was determined after drying the samples in an oven (105°C) for 3 h. Crude protein was determined by micro-Kjeldhal method, N × 6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) [28] (method number 984.13), crude lipid by soxhlet extraction with diethyl ether (40-60°C) [28] (method number 920.39) and ash by incineration at 550°C for 12 h [28] (method number 942.05). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid and ash then subtracting this sum from 100.

At the end of the feeding trial all fish were counted and weighed to calculate final body weight (FBW), weight gain (WG), specific growth rate (SGR%), feed conversion ratio (FCR) and protein efficiency ratio (PER) using the following equations:

WG (g/ fish) = FBW - IBW; SGR% = [ln FBW - ln IBW]/t×100, where IBW is initial body weight (g); ln = natural logarithmic; t = time in days. FCR = FI /WG, where FI is feed intake (g); PER=WG/protein intake (g).

Blood samples, hematological and biochemical blood parameters

At the end of experimental period, fish were fasted for 24 h prior to blood sampling. Fish were anaesthetized by tricaine methanesulfonate (MS222) at 250 mg L^{-1} in water. Blood was drawn near caudal peduncle from five fish from each treatment by a sterile syringe and transferred into a heparinized tube for hematological study. Blood was drawn from another five fish from each treatment kept outside without anticoagulant at room temperature (26°C) for few minutes then centrifuged at 5,000 g for 15 min for separation of serum (collected serum was stored at -20°C) for biochemical study. Hematocrit (Hct) was determined as described by Reitman and Frankel [29]. Hb was determined by the hemoglobin kits which is a standardized procedure of the cyanomet hemoglobin method. RBCs were counted under the light microscope using a Neubauer haemocytometer after blood dilution with phosphate-buffered saline (pH 7.2). Serum AST and ALT were determined colorimetrically using spectrophotometer using specific kits according to Reitman and Frankel [29]. Total serum protein and albumin and globulin were determined according to Doumas et al. [30].

Statistical analysis

Data were statistically analyzed by ANOVA using SAS ANOVA procedure (Statistical Analysis System 2004). The data were submitted to one ways classification variance analysis. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed [31], at ($P < 0.05$) level. All percentage data were arc-sin transformed prior to analysis [32], however data are presented untransformed to facilitate comparisons.

Results

Essential oil composition

The chromatographic analysis of hydro distilled oil revealed the presence of fifteen major compounds in EFO, representing 87.24% of the total fennel oil. The compounds present in the fennel oil at concentrations higher than 2% are: estragole (47.94); limonene (20.64); fenchone (7.22); trans-Anethole (4.9) α-Pinene (3.61) and eucalyptol (2.93). The chromatographic analysis of hydro distilled oil represented thirteen different compounds in EGO, representing 83.69% of the total garlic oil. The compounds present in the garlic oil at concentrations higher than 2% are: diallyl trisulfide (33.33%); diallyl disulfide (20.61%); methyl allyl trisulfide (14.11%); propenyl dithiopropanoate (10.21%); dimethyl trisulfide (3.20%) and diallyl tetrasulfide (2.23%). Diallyl tetrasulfide, which is generated from allicin is the main sulfurous constitute.

Growth and feed utilization

Survival at the end of the experiment was high (about 95%) and the effect of dietary treatments was significant (Figure 1). No significant differences in survival of fish ($P>0.05$) were observed among fish groups fed diet (D2, D3 and D4). The survival of fish fed diet D6 (EGO + *B. licheniformis*) was significantly higher that of other treatments. The differences in body weight were first observed after the 4 weeks of the feeding and increased as the feeding period continued (Figure 2).

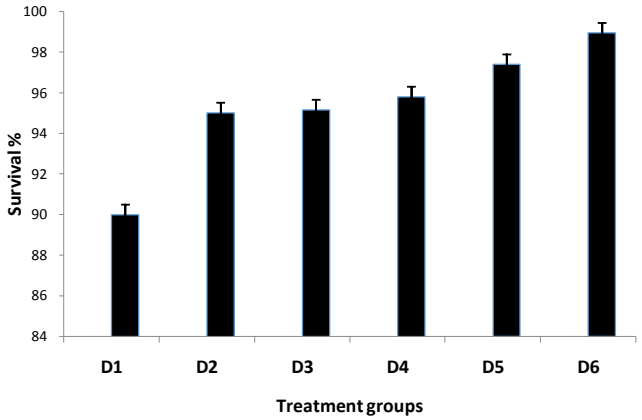

Figure 1: Survival of Nile tilapia fry fed diets containing *B. licheniformis*, fennel oil, garlic oiland their combinedfor 84 days. [D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO +*B. licheniformis*); D6= (EGO + *B. licheniformis*)].

Moreover, a significant reduction in the final body weight of fish control was observed compared with fish fed other diets. Results of growth and feed utilization are presented in (Table 2) and indicated that the highest weight gain (WG) and specific growth rate (SGR) were recorded by fish fed D5 (EFO + *B. licheniformis*) and D6 (EGO + *B. licheniformis*). Furthermore, no significant differences were found in WG and SGR among D2 (*B. licheniformis*), D3 (EFO) and D4 (EGO). The highest FI was observed in fish fed D4 (EGO), D5 (EFO + *B. licheniformis*) and D6 (EGO + *B. licheniformis*). While, the best FCR and PER were observed in D3 (EFO), D4 (EGO), D5 (EFO + *B. licheniformis*) and D6 (EGO + *B. licheniformis*) in comparison to the other treatments groups.

Feeding behavior

The behavioural observation were recorded at week 7 (Figure 3) and the results showed significant differences among all treatments in terms of mouth wrestling and chasing behaviour. It showed that mouth wrestling and chasing behaviour were higher in fish feed D6 (EGO + *B. licheniformis*) resulting in more aggressive for pellets feed than all treatments diets. The opposite trend were observed in D1 (control diet).

Hematological analysis and biochemical blood

The changes in hematological parameters of Nile tilapia fed treated diets are presented in Tables 3 and 4. The highest hematocrit (Hct), hemoglobin (Hb) and red blood cells (RBCs) values were recorded by fish of D5 (EFO + *B. licheniformis*) and D6 (EGO + *B. licheniformis*). No significant differences were found in Hct, Hb and RBCs among D1 (control diet), D2 (*B. licheniformis*), D3 (EFO) and D4 (EGO) treatments groups. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly lower ($P<0.05$) in fish D5 (EFO + *B. licheniformis*) and D6 (EGO + *B. licheniformis*) in comparison to the other treatments, but, no significant differences were found in ALT and AST among D1 (control diet), D2 (*B. licheniformis*), D3 (fennel oil) and D4 (EGO) treatments groups. The results showed the serum total protein and serum globulin was significantly higher ($P<0.05$) in fish fed D5 (EFO + *B. licheniformis*) and D6 (EGO + *B. licheniformis*). There was no significant ($P>0.05$) effect of different treated diets on serum albumin.

Chemical composition

Proximate analysis of whole body of *O. niloticus* fed the experimental diets was presented in Table 5. The chemical composition of whole body appeared that no significant differences were found in dry matter and ash content of fish fed control and treated diets.

Discussion

The present study indicated that the highest growth and feed utilization were obtained at 1ml of EFO and EGO combined with *B. licheniformis* 7×10^7 (CFU g^{-1}). This study of the application of EFO and EGO in diet showed the first report about the growth indices of Nile tilapia. Whereas, fennel seed as a powder was used as the growth

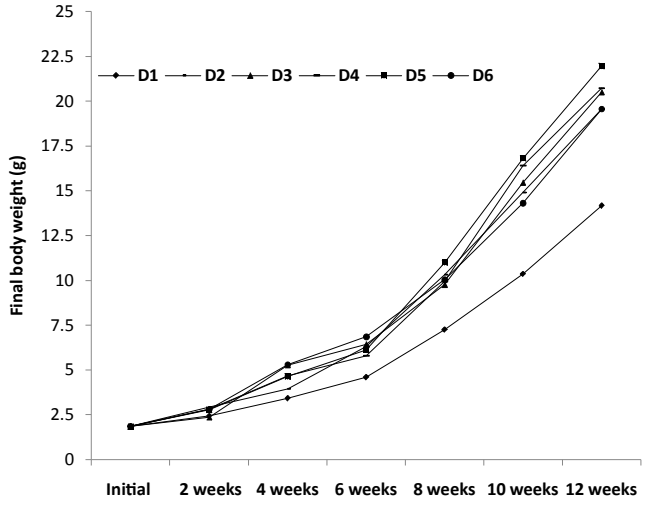

Figure 2: Final body weight of Nile tilapia fry fed diets containing *B. licheniformis*, fennel oil, garlic oiland their combinedfor 84 days. [D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO +*B. licheniformis*); D6= (EGO + *B. licheniformis*)].

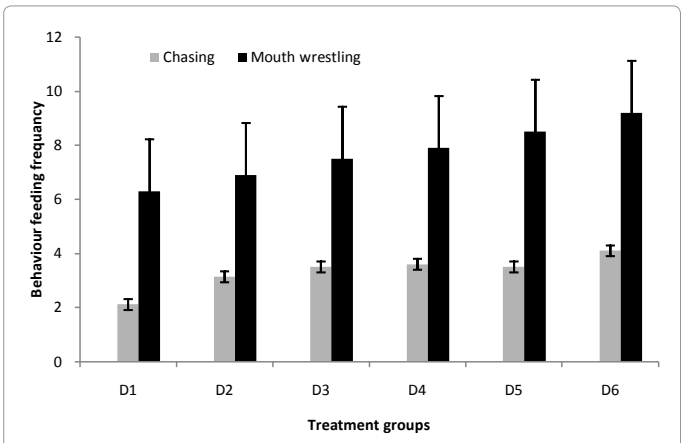

Figure 3: Behaviour feeding Final body weight of Nile tilapia fry fed diets containing *B. licheniformis*,fennel oil, garlic oiland their combinedfor 84 days. [D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO +*B. licheniformis*); D6= (EGO + *B. licheniformis*)].

Parameters	Experimental diets						P value
	D1	D2	D3	D4	D5	D6	
Initial body weight (g)	1.78 ± 0.12	1.90 ± 0.16	1.90 ± 0.11	1.92 ± 0.09	1.93 ± 0.13	1.82 ± 0.14	0.827
Weight gain (g)	12.37 ± 1.55^{c}	17.66 ± 1.23^{b}	18.86 ± 1.43^{b}	18.80 ± 1.52^{b}	20.02±1.47^{a}	19.58 ± 1.61^{a}	0.010
Specific growth rate	2.34 ± 0.04^{c}	2.59 ± 0.10^{b}	2.63 ± 0.03^{b}	2.64 ± 0.43^{b}	2.70 ± 0.05^{a}	2.70 ± 0.062^{a}	0.012
Feed intake (g feed/ fish)	20.69 ± 1.21^{c}	24.70 ± 1.25^{b}	24.64 ± 1.11^{b}	25.48 ± 1.40^{a}	25.95 ± 1.26^{a}	25.09 ± 1.32^{a}	0.001
Feed conversion ratio	1.67 ± 0.66^{a}	1.42 ± 0.68^{b}	1.32 ± 0.14^{c}	1.36 ± 0.14^{c}	1.30 ± 0.58^{c}	1.28 ± 0.66^{c}	0.001
Protein efficiency ratio	2.08 ± 0.11^{c}	2.38 ± 0.18^{b}	2.53 ± 0.14^{a}	2.47 ± 0.17^{a}	2.57 ± 0.13^{a}	2.60 ± 0.21^{a}	0.012
Results are presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($p < 0.05$). D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO + *B. licheniformis*); D6= (EGO + *B. licheniformis*).							

Table 2: Fish performance and feed utilization of Nile tilapia fry fed diets containing *B. licheniformis,* essential oil of fennel and garlic and their combined for 84 days.

Parameters	Experimental diets						P value
	D1	D2	D3	D4	D5	D6	
Hematocrit (%)	17.90 ± 0.52^{b}	17.45 ± 0.63^{b}	17.50 ± 0.75^{b}	17.05 ± 0.28^{b}	21.68 ± 0.85^{a}	23.00 ± 0.74^{a}	0.012
Hemoglobin (g/ dl)	10.80 ± 0.24^{b}	10.90 ± 0.23^{b}	10.25 ± 0.41^{b}	11.00 ± .035^{b}	12.05 ± 0.35^{a}	13.80 ± 0.25^{a}	0.041
Red blood cells (×10^{6} µL^{-1})	1.93 ± 0.02^{b}	1.91 ± 0.01^{b}	2.12 ± 0.05^{b}	2.12 ± 0.04^{b}	2.82 ± 0.02^{a}	2.78 ± 0.06^{a}	0.001
Results are presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($p < 0.05$). D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO + *B. licheniformis*); D6= (EGO + *B. licheniformis*).							

Table 3: Hematological indices of Nile tilapia fry fed diets containing *B. licheniformis,* essential oil of fennel and garlic and their combined for 84 days.

Parameters	Experimental diets						P value
	D1	D2	D3	D4	D5	D6	
Aspertataminotransferase (ul^{-1})	11.80 ± 0.47^{a}	11.90 ± 0.65^{a}	10.95 ± 0.68^{a}	11.20 ± 0.23^{a}	9.15 ± 0.39b	9.40 ± 0.62^{b}	0.014
Alanine aminotransferase (ul^{-1})	10.35 ± 0.36^{a}	10.05 ± 0.45^{a}	9.75 ± 0.13^{a}	9.90 ± 0.75^{a}	8.55 ± 0.63^{b}	8.25 ± 0.58^{b}	0.001
Total protein (g dL^{-1})	2.70 ± 0.12^{c}	2.90 ± 0.14^{c}	3.00 ± 0.16^{b}	3.10 ± 0.15^{b}	3.69 ± 0.11^{a}	3.67 ± 0.15^{a}	0.042
Albumin (g dL^{-1})	0.95 ± 0.02	0.95 ± 0.01	0.99 ± 0.01	1.00 ± 0.04	1.05 ± 0.01	1.01 ± 0.2	0.562
Globulin (g dL^{-1})	1.75 ± 0.13^{c}	1.95 ± 0.17^{b}	2.01 ± 0.12^{b}	2.10 ± 0.13^{b}	2.64 ± 0.11^{a}	2.66 ± 0.12^{a}	0.013
Results are presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($p < 0.05$). D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO + *B. licheniformis*); D6= (EGO + *B. licheniformis*).							

Table 4: Biochemical blood indices of Nile tilapia fry fed diets containing *B. licheniformis,* essential oil of fennel and garlic and their combined for 84 days.

Parameters	Experimental diets						P value
	D1	D2	D3	D4	D5	D6	
Dry matter	275.30 ± 1.23	277.20 ± 1.45	274.50 ± 2.32	275.01 ± 1.54	275.00 ± 1.25	278.52 ± 1.58	0.620
Crude protein	149.20 ± 1.23	152.13 ± 1.51	151.20 ± 1.45	152.35 ± 1.55	153.89 ± 1.63	153.69 ± 1.26	0.452
Crude lipid	64.23 ± 1.10	62.21 ± 0.98	61.13 ± 1.24	61.16 ± 1.44	60.73 ± 1.28	61.12 ± 1.35	0.521
Ash	33.12 ± 1.01	33.99 ± 1.20	34.13 ± 1.21	33.12 ± 1.00	32.95 ± 0.99	32.80 ± 1.01	0.232
Results are presented as means ± SD of triplicate observations. Means in the same row with different superscripts were significantly different ($p < 0.05$). D1= Control; D2 = B. *licheniformis*; D3 = Essential fennel oil (EFO); D4 = Essential garlic oil (EGO); D5 = (EFO + *B. licheniformis*); D6= (EGO + *B. licheniformis*).							

Table 5: Chemical composition of Nile tilapia fry fed diets containing *B. licheniformis,* essential oil of fennel garlic and their combined for 84 days (g/kg wet basis).

promoter for Nile tilapia (*O. niloticus*) and the results are similar with the present study [14,33]. Besides, most of the authors have studied the effect of garlic as a powder form on growth and nutrient utilization [17,26,34,35]. The growth rates for the previous studies were significantly higher in fish fed diets supplemented with garlic than control groups. Garlic contains allicin, which promotes biogenic performance due to its positive effect on the intestinal flora, thereby improving digestion and enhancing nutrients utilization which influences the growth of fish [36]. Steam distillation of mashed garlic produces garlic oil containing methyl and allyl-sulphides of allicin and having the practical advantage of being more stable than allicin itself [37]. Due to the fact that the extracted essential oil from herbal plants has much higher contents of active substance than dried herbal plants resulting in the essential oil is usually used in low percentages in the diet compared to dried herbal plants.

Little information has been reported the effect of application of different essential oil in fish growth. These studies indicated that diets supplemented with essential oil of weet orange peel *(Citrus sinensis)* and *Aloysia triphylla* ([19,38] with different dose could enhance weight gain and specific growth rate. Supplementation of the essential oil of fennel or garlic in diet may improve digestibility, availability of nutrients and lead to a higher protein synthesis, which in turn could explain the better growth in this study. Meanwhile, stimulation of growth by *B. licheniformis* in the present study may attribute to improve digestibility by enhancing the synthesis of vitamins, enzymatic activity [39]. These results are in agreement with *Bacillus subtilis* [40]; carp [41], Tilapia *O. nilotica*.

On the other hand, the increase in growth for the combined application of EFO and/or EGO with *B. licheniformis* may be due to the EFO and EGO promoted the growth of exogenous probionts bacteria *B. licheniformis* in gastrointestinal of fish more than other endogenous populations bacteria, which improved the growth performance of Nile tilapia. The control of the microflora in intestinal fish by addition of oregano essential oil (*Origanum heracleoticum* L) can positively effect on growth performance [18]. The main way of the essential oil work seems to be through the regulation of intestinal microflora [42]. From aforementioned, the essential oil of fennel and garlic are good potential alternatives to the growth promoters.

The feeding behaviour of fish is complex [43], however, the acceptance or rejection of diet is physiologically dependent on inputs from chemoreception [44]. Fish have several chemosensory systems including gustation (taste), olfaction (smell), chemical sensory and chemoreceptor cells. The role of herbal plants for feeding behaviour represented in the flavor, thereby influences of eating patterns, the secretion of digestive fluids and total feed intake [45]. It seems that fish fed EGO combined with *B. licheniformis* could be stimulated the gustatory system is the most important in acquisition and ingestion of food. Allicin has an intense garlic flavor with a strong effect on olfaction in the most aquatic animals such as *Pelodiscus sinensis*, *Ctenopharyngodon idellus, Cyprinus carpio*, *Carassius auratus*, and *Oreochromis niloticus* [45]. Harada [46] indicated that garlic had a strong food calling effecting on loach (*Oriental weatherfish*) and Japanese amberjack, *Seriola quinqueradiata* Temminck et Schlegel.

Hematological variables are good predictors for explaining the health status of fish [47]. The highest Hct, Hb and RBCs count were obtained at D5 (EFO + *B. licheniformis*) and D6 (EGO + *Bacillus licheniformis*). Erythrocytes content in fish blood gives a guide to the health status of fish and can be helpful to determine any abnormalities

arising from the use of immunostimulants. Accordingly, the elevate number of RBCs multiplies the concentration of hemoglobin ultimately resulting in a high capacity for oxygen carrying which improved the health of fish and consequently enhancing growth [48]. Furthermore, Fazllolahzadeh et al. [37] assumed that the increase of blood indices such as, Hct, Hb, and RBCs may be attributable to a defense reaction against garlic which occurs by stimulation of erythropoiesis. The number of RBCs was significantly ($P<0.05$) higher in Asian sea bass, *Lates calcarifer* [49], *Labeo rohita* [50] and Nile tilapia, *O. niloticus* fed diet supplemented with garlic as a powder form. The present study is inconsistent with Soltan and El-Laithy [51]. They reported that fennel seeds and garlic powder did not effect on Hct and Hb values for Nile tilapia, *O. niloticus*. Yılmaz and Ergün [52] reported that garlic oil did not produce any undesired effects on hematological characteristics for Sea Bass *Dicentrarchus labrax*. With regard to the different essential oil addition Acar et al. [19] showed that *O. mossambicus* fed a basal diet containing 1% thyme, rosemary or fenugreek for 45-day increased RBCs count and Hct value.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver enzymes and they have the function of transferring the amino group from alpha-amino acids to alpha-keto acids. A large amount of ALT and AST is released in the blood mostly during liver cell damage [53]. Thus, detection of the serum level of ALT and AST allows monitoring liver cell damage. The diets containing (EFO + *B. licheniformis*) and (EGO + *B. licheniformis*) could decrease the activity of ALT and AST compared to other treatment groups. Similar results were obtained by El-Dakar [33] who showed a significant lower ($P<0.05$) in levels of ALT and AST in Nile tilapia, *O. niloticus* fed fennel seed meal supplemented with diet. Also, Soltan and El-Laithy [51] found that ALT and AST levels were significantly decreased when Nile tilapia fed diets supplemented with 1% garlic powder or 1% fennel seed with compared to other diets. Application of garlic *(Allium sativum)* may cause stabilization cell membrane and protect the liver against deleterious agents and free radical-mediated toxic. This is reflected in the reduction of liver enzymes [37]. Also, Metwally [54] indicated that garlic-addition helps the liver to maintain its normal function by accelerating the generative capacity of its cells. The different active components found in essential oil of fennel and garlic may play an important role in enhancing the activity of liver enzymes but the exact mechanism for this need more advanced study. Globulin fractions are certainly important for maintaining a healthy immune system and immune function in the blood [55]. The increase in total protein and globulin contents is thought to reflect strong innate immunity [17]. In this study, diets supplemented with (EFO + *B. licheniformis*) and (EGO + *B. licheniformis*) showed a significant elevate in total protein and globulin in Nile tilapia. It can be inferred that the EFO and EGO which combined with *B. licheniformis* may enhance the non-specific immune response and more tolerant of stressful conditions of Nile tilapia. These results are similar with [20] who reported that the highest content of total protein was determined in fish fed diet supplemented with mixed oil of (thyme and fennel) with level 100 ml kg^{-1} diet, but serum albumin was not significantly different from the control group. The addition of garlic products, fresh oil and powdered forms increased the total protein in Nile tilapia *O. niloticus*, even at low level 0.25% of garlic oil [54]. Also, Shalaby et al. [16] reported that the highest total protein was determined in Nile tilapia *O. niloticus* when garlic powder was supplemented to the diet at 10 g kg^{-1} diet. Serum total protein and albumin contents decreased in sea bass exposed to garlic oil (0.005 ml L^{-1} water) via bath immersion for 96 h, but serum globulin was decreased at (0.02 ml of garlic oil L^{-1}) [52].

The present study indicated that no significant differences in chemical composition of body of fish fed different treated diets. In this regard, Abd El Hakim [14] found no significant differences in the chemical body composition of Nile tilapia fed diet supplemented with different level of fennel seed. Contrarily, Maniat et al. [56] showed that garlic supplemented in diets induce higher protein and lower fat content in body of benni fish *(Mesopotamichthys sharpeyi)*. Ahmad and Abdel-Tawwab [13] reported that no significant differences ($P>0.05$) in moisture and crude protein in Nile tilapia, *Oreochromis niloticus* body meanwhile total lipids increased by increasing level of caraway seed (belonging to family Apiaceae as fennel). Shalaby et al. [16] reported the highest crude protein and the lowest crude lipid content were obtained by Nile tilapia fed diet supplemented with 30 g kg^{-1} garlic. Furthermore, Talpur and Ikhwanuddin [49] showed that sea bass fed diet supplemented with garlic was decreased in lipid content in body. Also, Metwally [54] showed that crude protein and ash was increased significantly in *O. niloticus* while crude lipid decreased significantly with diets containing different sources of *Allium sativum*.

In conclusion, addition of essential oil of fennel combined with *B. licheniformis* and/or essential oil of garlic combined with *B. licheniformis* resulting in increased growth performance and enhancement of hematological and biochemical blood parameters in Nile tilapia *O. niloticus*.

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