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Antibacterial Activity of Essential Oils and their Effects on Nile Tilapia Fingerlings Performance

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This study investigated the antibacterial activity of five essential oils *in-vitro*. Also, the strongest antibacterial oil (thyme oil) was tested on performance of Nile tilapia (*Oreochromis niloticus*) fingerlings fish. Two experiments were carried out, in the first one antimicrobial activity of essential oils (EOs) of 5 medicinal plants, ginger, black cumin, thyme, clove and watercress were evaluated (*in-vitro*). The screening of antibacterial activity was against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes, Lactococcus lactis* and *Bacillus cereus*. In the second experiment (*in-vivo*) 120 fish were divided in 4 groups. There were 3 replicate glass aquaria (per group) of 10 fish per aquarium. The 1st group fed basal diet (control) and the other groups (2-4) were fed basal diet supplemented with 0.1, 0.25 and 0.5% thyme oil. First experiment showed that thyme oil had the strongest antibacterial activity against all tested bacteria except *Staph. aureus* and *E. coli*. The more sensitive bacteria to thyme oil was *Pseud. aeruginosa* while. *E. coli* was least susceptible to it. Second experiment, showed that thyme oil significantly increase growth rate, feed intake and feed utilization of fish. The best level was 0.25%. Total protein and globulin concentrations in plasma were significantly increased by 0.1 and 0.25% thyme oil. The best economical efficiency was obtained by 0.25% thyme oil.

Key words: Nile tilapia fish, thyme, antibacterial activity

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INTRODUCTION

Improving growth performance and feed efficiency by synthetic and natural feed additives have been widely used (Collington *et al*., 1990; Lee *et al*., 2001). The residue of antibiotics increase bacterial resistance and has bad effect on animal and human health. Therefore, recent researches tended to use alternative natural product like Essential Oil (EO). Therefore, demand for green aquaculture or organic aquaculture has recently been increasing. Natural products like plant extracts contain secondary metabolites. Generally, they can be structured into 3 groups: saponins, tannins and Essential Oils (EO) (Calsamiglia *et al*., 2007). It have been investigated for their therapeutic and prophylactic effects of several fish diseases, inhibited the *Lactococcus garviea* and *Aeromona hydrophila*, fish pathogenic bacteria (Rattanachaikusopon and Phumkhachorn, 2009; Seden *et al*., 2009).

Thymol oil derived from thyme (*Thymus vulgaris*), has demonstrated biological properties such as antimicrobial, antioxidant and antiseptic activities (Lee and Ahn, 1998). It has high activity on inhibition of respiratory tract pathogenic bacteria (Inouye *et al*., 2001). Also, diet supplemented with *Origanum vulgare* (kind of thyme) improve performance of Nile tilapia fingerlings (Seden *et al*., 2009). These effects may change according to the differences in thyme species, cultivation, origin, vegetative stage and growing season of the plants (Milos *et al*., 2000; Martinez *et al*., 2006). The EO recognized as safe admitted by the Food and Drug Administration (FDA, 2004).

However, there are limited studies conducted to investigate the effect of EO on performance of fish. The present study was designed to evaluate the antimicrobial activity of ginger, black cumin, thyme, clove and watercress EO on some pathogenic bacteria (*in-vitro*). From *in vitro* results we choose the best oil (thyme oil) to study its effect on performance of Nile tilapia (*Oreochromis niloticus*) fingerlings.

MATERIALS AND METHODS

The experimental work was carried out in the Aquaculture Research Lab., Abbassa, Abo-Hamad; Department of Animal Production, Faculty of Agriculture and Department of Botany, Faculty of Science, Zagazig Univ., Egypt.

In vitro study

Essential oils: Essential oils of 5 herbs, ginger (*Zingiber officinale*), black cumin (*Nigella sativa*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*) and watercress (*Eruca sativa*) were purchased from El-Hawag Factory, Badr City, Egypt.

Bacterial species: Six bacterial species belonging to Gram-negative and Gram-positive were tested (Table 1). *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* were obtained from Faculty of Medicine, Zagazig University, while *Listeria monocytogenes, Lactococcus lactis* and *Bacillus cereus* were obtained from Faculty of Science, Zagazig University.

Screening of antibacterial activity: Screening of antibacterial activity was performed by standard disc diffusion method (Saeed *et al*., 2007). Fifty sterilized discs of filter paper (6 mm diameter) were soaked in 1 mL of oil, separately for 2 min and then used for screening. The potency of each disc was 10 μL (each 50 discs of filter paper absorbed 0.5 mL). Nutrient agar was used as base medium and Nutrient broth was used for the preparation of inoculums. A sterile cotton swab was dipped into the bacterial test suspension to inoculate entire surface of a nutrient agar plate. Discs of oil were placed on the surface of inoculated plates with the help of sterile forceps. The inoculated plates were incubated at 37°C for 24 h. After incubation inhibition zone diameters of 4 discs for each oil were measured to the nearest millimeter (mm).

Fish study

Experimental design: A total number of 120 Nile tilapia fish (average body weight 13 g) were used in

Table 1: Antibacterial activities of essential oils *(in-vitro)*

	Mean zone of inhibition (mm)±SE				
Tested bacteria	Ginger	Black cumin	Thyme	Clove	Watercress
Gram-negative:					
E. coli	8.0±0.1^{a}	0.0±0.0^{d}	5.7±0.25^{b}	2.0±0.1^{c}	5.3±0.2^{b}
Pseudomonas aeruginosa	6.7±0.15^{c}	0.0±0.0^{e}	13.0±0.5^{a}	2.0±0.15^{d}	10.3±0.2^{b}
Gram-positive:					
Bacillus cereus	6.0±0.25^{b}	5.0±0.1^{c}	7.5±0.15^{a}	5.6±0.3^{bc}	0.0±0.0^{d}
Staphylococcus aureus	0.0±0.00^{b}	0.0±0.0^{b}	0.0±0.0^{b}	4.5±0.15^{a}	0.0±0.0^{b}
Listeria monocytogenes	0.0±0.00^{c}	0.0±0.0^{c}	9.5±0.50^{a}	6.0±0.30^{b}	6.0±0.2^{b}
Lactococcus lactis	5.5±0.25^{d}	7.3±0.15^{b}	11.3±0.1^{a}	6.3±0.20^{c}	0.0±0.0^{e}

$^{a, b, c, d}$Means in the same row bearing different letters differ significantly ($p<0.05$)

Table 2: Chemical composition (%) of the commercial basal diet

	Proximate analysis						
Items	DM	OM	CP	CF	EE	NFE	Ash
As fed	93	75.33	28.64	5.95	4.77	35.97	17.67
On dry matter basis	100	81.00	30.80	6.40	5.13	38.67	19.00

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen free extract

4 experimental groups. There were 3 replicate glass aquaria (per group) of 10 Nile tilapia fish (*Oreochromis niloticus*) per aquarium. The 1st group fed basal diet (control) and the other groups (2-4) were fed basal diet supplemented with 0.1, 0.25 and 0.5% thyme oil, respectively.

Preparation of fish diets: The thyme oil was added to a ground commercials diet which was pelleted again. Commercial diet composed of fish meal, soybean meal meat meal, yellow corn, bone meal and a mixture of vitamins and minerals. The chemical composition of diet (Table 2) was adopted according to AOAC (1984).

Aquarium design and fish rearing: The dimensions of aquarium were 150×150×50 cm, these aquaria were supplied with dechlorinated tap water up to 80% of its highest and continuous aeration was adapted by using an air pump and air stones. Fish wastes were filtered by siphon method each day and the rearing water was completely changed every 3 days. Mean water temperature was 27±2°C. The fish were fed 2 times a day (09:00 and 16:00 h) at a rate of 4% of the total body weight (at two equal meals). The fish were weighted monthly and the feed quantities were readjusted according to the change in live body weight. The period of experiment was 3 months.

Blood samples: At the end of the experiment, blood samples were taken from the caudal vein of 12 fish for each treatment (4 fish/replicate). Blood plasma was separated and stored at -20°C to analysis. Plasma total protein, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed by using commercial kits from Diamond Diagnostics Company, Egypt.

Statistical analysis: Data of the experiment were statistically analyzed using the General Linear Model Program of SAS (1996). Significant differences between treatment means were tested by Duncan"s Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

***In-vitro* study:** Essential oils used in this study exhibited antibacterial activity against some tested bacteria with different degree of inhibition (Table 1). Based on the diameter of inhibition zone, thyme oil had the strongest ($p<0.05$) antibacterial activity against all tested bacteria except *Staphylococcus aeures and E. coli*. These results are similar to those obtained by Viuda-Martos *et al*. (2011) who reported that thyme oil was better than lavender, fennel, parsley and black oil in inhibition of *Listeria innocua*, *Serratia marcescens* and *Pseudomonas fluorescens*. Also, Seden *et al*. (2009) reported that *Origanum vulgare* (kind of thyme) in diet of Nile tilapia fingerlings inhibited fish pathogenic bacteria (*Aeromona hydrophila*). The more sensitive bacteria to thyme oil was *Pseudomonas aeruginosa* while *E. coli* was least susceptible to it. These results agree with those obtained by Inouye *et al*. (2001) who reported that *E. coli* was least susceptible to 14 essential oils. Also, Saeed and Tariq (2008) reported that *E. coli* was the least sensitive to clove oil. Generally, The antimicrobial activity of essential oils against bacteria varies depending on the source of an essential oil and strain of bacteria. The composition of EO can vary among different parts of the same plant (Dorman and Deans, 2000). Also, Martinez *et al*. (2006) observed that the concentration of active components (carvacrol, thymol, p-cymene and Y-terpinene) in thyme EO varied widely depending on the species of the thyme plant. The inhibitory effect of thyme EO against bacteria are due to interacting with bacterial cell membrane (Dorman and Deans, 2000).

Fish study

1- Body weight gain, feed intake and feed utilization: The chemical composition (%) of the commercial basal diet was recorded in Table 2 according to AOAC (1984). All levels of thyme oil (0.1, 0.25 and 0.5%) significantly ($p<0.05$) increase live body weight, body weight gain, specific and relative growth rate (Table 3), feed intake and feed utilization (Table 4) of Nile tilapia fingerlings in all experimental period. However, using 0.25% thyme oil was the best level. These results agree with the findings of Seden *et al*. (2009) who reported that 1% *Origanum vulgare* herb significantly improved growth performance, feed intake, feed conversion, protein and energy utilization of Nile tilapia fingerlings. Also, agree with Al-Kassie (2009) who reported that 200 mg thyme oil kg^{-1} diet significantly improved broiler performance. On the other hand Jang *et al*. (2007) reported that growth

Table 3: Effect of thyme oil on growth performance of Nile tilapia fingerlings

Items	Thyme oil (%) 0.0	0.1	0.25	0.5
Weight (g):				
Initial weight*	13.00±0.10	13.00±0.13	13.10±0.10	13.00±0.12
Weight at 1 month	19.47±0.15^{c}	19.73±0.06^{b}	20.12±0.11^{a}	19.73±0.08^{b}
Weight at 2 months	27.62±0.20^{d}	29.04±0.09^{b}	29.99±0.16^{a}	28.66±0.11^{c}
Weight at 3 months	40.12±0.28^{c}	43.74±0.28^{b}	45.66±0.42^{a}	43.49±0.29^{b}
Body weight gain (g):				
1st month (g)	6.47±0.15^{c}	6.73±0.09^{b}	7.12±0.12^{a}	6.73±0.08^{b}
2nd month (g)	8.15±0.05^{d}	9.31±0.04^{b}	9.87±0.05^{a}	8.93±0.03^{c}
3rd month (3)	12.50±0.10^{c}	14.70±0.36^{b}	15.67±0.31^{a}	14.83±0.21^{b}
Total period (3 months)	27.12±0.28^{c}	30.74±0.28^{b}	32.66±0.42^{a}	30.49±0.29^{b}
Daily weight gain	0.30±0.00^{c}	0.34±0.00^{b}	0.36±0.01^{a}	0.34±0.00^{b}
Specific growth rate (SGR) (%)	1.26±0.01^{c}	1.35±0.01^{b}	1.40±0.01^{a}	1.34±0.01^{b}
Relative growth rate (RGR) (%)	208.62±2.14^{c}	236.46±2.16^{b}	251.23±3.22^{a}	234.54±2.25^{b}

$^{a, b, c}$Means in the same row bearing different letters differ significantly ($p<0.05$)

Table 4: Effect of thyme oil on feed intake and feed efficiency of Nile tilapia fingerlings

Items	Thyme oil (%) 0.0	0.1	0.25	0.5
Monthly feed intake (g):				
1st month	15.60±0.00	15.60±0.0	15.60±0.0	15.60±0.00
2nd month	23.36±0.10^{c}	23.68±0.04^{b}	24.14±0.08^{a}	23.67±0.05^{b}
3rd month	33.14±0.14^{d}	34.84±0.06^{b}	35.99±0.11^{a}	34.39±0.08^{c}
Total period	72.10±0.25^{c}	74.12±0.10^{b}	75.73±0.18^{a}	73.66±0.13^{b}
Feed efficiency (feed/gain):				
1st month	2.41±0.03^{a}	2.32±0.02^{b}	2.20±0.02^{c}	2.32±0.02^{b}
2nd month	2.87±0.01^{a}	2.54±0.01^{c}	2.45±0.00^{d}	2.65±0.00^{b}
3rd month	2.65±0.01^{a}	2.37±0.04^{b}	2.30±0.02^{b}	2.32±0.02^{b}
Total period	2.66±0.01^{a}	2.41±0.02^{b}	2.32±0.01^{c}	2.42±0.01^{b}

$^{a, b, c}$Means in the same row bearing different letters differ significantly ($p<0.05$)

Table 5: Effect of thyme oil on plasma constituent and survival rate of Nile tilapia fingerlings

Items	Thyme oil (%) 0.0	0.1	0.25	0.5
Plasma concentration				
Total protein (g dL^{-1})	4.30±0.03^{b}	4.79±0.23^{a}	4.69±0.11^{ab}	4.55±0.03^{ab}
Albumin (g dL^{-1})	3.00±0.12	3.17±0.19	3.06±0.12	3.10±0.09
Globulin (g dL^{-1})	1.30±0.03^{b}	1.62±0.03^{ab}	1.63±0.28^{a}	1.45±0.06^{b}
AST (UL^{-1})	31.00±1.44^{b}	28.00±0.23^{b}	29.00±1.36^{b}	35.00±0.87^{a}
ALT (UL^{-1})	20.00±0.17^{b}	22.20±0.29^{b}	20.50±0.64^{b}	22.00±0.64^{a}
Survival rate (%)	93.33±5.77	96.67±5.77	93.33±0.58	90.00±10

$^{a, b, c}$Means in the same row bearing different letters differ significantly ($p<0.05$), AST: Aspartate amino transferase, ALT: Alanine amino transferase enzymes

performance, feed intake and feed efficiency were similar among birds fed basal diet and the diet supplemented with 25 or 50 mg commercial blend of EO/kg diet. These differences between researches may be due to the kind, composition of EO which affected by cultivation, origin, vegetative stage and growing season of the plants (Milos *et al*., 2000), nutritional status of animals, infection, diet composition and environment (Giannenas *et al*., 2003). The beneficial effect of EO on fingerlings body weight gain and feed efficiency may be due to: firstly, its antimicrobial and antioxidant effect (Milos *et al*., 2000). Thyme oil improve intestinal microflora (decrease growth of pathogenic bacteria *Aeromonas hydrophila* (Seden *et al*., 2009) and increase growth of beneficial bacteria (*Lactobacilli*) (Jang *et al*., 2007). The pathogenic bacteria increase rate of passage and thickness of intestinal mucosa which reduce nutrient digestibility and absorption (Hu *et al*., 2002; Xia *et al*., 2005). Secondly, enhancing digestibility and absorption of nutrients. EO increase the digestive enzyme activities of the pancreas (trypsin and α-amylase) and intestine (maltase, alkaline phosphatase and leucine aminopeptidase) (Lee *et al*., 2003; Jang *et al*., 2007). Also, EO increase the intestinal microvilli enzymes such as disaccharide, alkaline phosphatase and leucine aminopeptidase. These enzymes are important constituents of the microvillus membrane in the intestinal absorptive cells, where they associated with the degradation and absorption of nutrients from the gut (Ferraris *et al*., 1992). Thirdly, improve immunity and body health including positive effects on cardiovascular diseases, some tumors, inflammatory processes and in general diseases in which the uncontrolled proliferation of free radical is very damaging (Trouillas *et al*., 2003).

Blood parameters: Some fish plasma constituents are shown in Table 5. Total protein and globulin concentrations were significantly increased ($p<0.05$) by 0.1 and 0.25% thyme oil, respectively. These results agree with the findings of Al-Kassie (2009) who reported that broiler diet supplemented with 100 and 200 ppm thyme oil significantly increased plasma total protein. The activity of aspartate amino transferase (AST) and alanine amino transferase (ALT) enzymes significantly ($p<0.05$) increased by addition of 0.5% thyme oil. These results indicate that 0.1 and 0.25% thyme oil improve liver and kidney functions and body health.

Survival rate: The survival rate (Table 5) did not significantly affected by all levels of thyme oil.

Table 6: Effect of thyme oil on economical efficiency

	Thyme oil (%)			
Items	0.0	0.1	0.25	0.5
Total gain (g)	27.12	30.74	32.66	30.49
Total feed intake (g)	72.10	74.12	75.73	73.67
Feed cost (piasters*)	18.03	19.77	22.09	24.56
Gain price (piasters)**	54.24	61.48	65.32	60.98
Profit***	36.21	41.71	43.23	36.42
Relative profit (%)****	100	115.19	119.39	100.58

*Total feed intake x price, The price of 1 kg control, 0.1% thyme oil, 0.5% thyme oil, 1% thyme oil were 250, 266.67, 291.68, 333.35 piaster's respectively (price 2010), One mL thyme oil was 16.67 piaster's, respectively, **Total gain×20 (one kg 2000 piasters), ***Gain price-feed cost, ****Relative profit for treatment/net revenue of control×100

Economical efficiency: As shown in Table 6, the economical efficiency results indicated that the best level was 0.25% thyme oil (119.39%). Generally, the relative profit of control, 0.1, 0.25 and 0.5% thyme oil were 100, 115.19, 119.39 and 100.58%, respectively.

CONCLUSION

This study has shown that all tested essential oils had antibacterial effects on Gram positive and Gram negative bacteria. The most potent oils was thyme oil. Addition of thyme oil specially 0.25% to Nile tilapia fingerlings fish diet improve body weight gain, feed efficiency, blood parameters and economical efficiency. Therefore, thyme oil can be used as an alternative to antibiotics, cheap and safe feed additive.

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