EFFECT OF MYRRAH EXTRACT ON GROWTH PERFORMANCE AND ANTIBCTERIAL INFECTION OF *OREOCHROMIS NILOTICUS (*L.*)*

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ABSTRACT

The main objective of this study was showing the effect of myrrah extract at different levels as growth promoters on growth performance, feed utilization, whole-body composition and *Aeromonas hydrophila*; challenge for all-male Nile tilapia, *Oreochromis nilocticus* fingerlings. Four experimental diets were formulated to contain 0.0 (control) and 0.5, 1, and 1.5% for myrrah. All diets are isonitrogenous (30% crude protein) and isocaloric (4.4 kcal/gm GE). The study was conducted in triplicate treatments. Each aquarium was stocked with 15 fingerlings of an average weight 12±2 gm. Experimental diets were provided at a rate of 3 % of live body weight of total biomass, twice/day, 6 days a week, for 90 days. Diet containing 1% Myrrah resulted significantly ($p < 0.05$) in improvement in growth performance and feed utilization as reflected in gain %, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER), Apparent Protein Utilization (APU) and Energy Utilization (EU). The survival rate was enhanced due to the inclusion of myrrah in fish diets. With regard to body composition, there was no significant difference in dry matter, protein, lipids, and ash ($p > 0.05$) contents which could be related to the levels of mirra meal in the experimental diets. Blood plasma profile showed an improvement in Hemoglobin (Hb), Red Blood Cells (RBCs), hematocrit (PCV), total protein, albumin and globulin while showed a decrease in creatinine, urea, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and glucose in all

treatment groups. Myrrah were found to have antibacterial activity antagonized to *A. hydrophila* in *O. niloticus*.

Introduction

Feed additives are important to improving feed utilization and animal performance, herbs and spices have been added to different types of food to impart flavor as well as to improve storage stability. Many herbs and spices have been shown to impart antioxidant effects in food; the active principles are phenolic **(Shahidi et al., 1992; Scwarz et al., 2001 and Tanabe et al., 2002)**.

A wide variety of phenolic substances derived from herbs and spices possess potent anti-inflammatory, anti-mutagenic, anti-carcinogenic and anti-tumour activities, which contribute to their chemopreventive potential **(Surh, 2002)**.

(Abdel Malak *et al.*, (1995) and Ibrahim *et al.*,(1998) showed successful use of spices and natural herbs in fish nutrition including marjoram, basil, licorice roots, black seeds, peppermint, fenugreek seeds and caraway seeds, and also **El-Saidy (1999)** reported that the dried onion meal can be used In diet in Nile tilapia but in our study, we showed that myrrah extracts are used as diets.

Oleo-gum resins such as myrrah and frankincense are some of the economically and culturally valuable products obtained from trees and shrubs of the genera *Boswellia* and *Commiphora*, respectively. They are important natural plant products used in several industries that include pharmacology, food, flavour, liqueur and beverage, cosmetics, perfumery and others. Moreover, myrrh have several local applications in medicinal, hygienic, and insecticide areas that could be developed through research. They are widely used in traditional medicines of several countries for treatments of a wide variety of ailments from embalming to cancer, leprosy, bronchitis, diarrhea, dysentery, typhoid, mouth ulcers, inflammatory complaints, viral hepatitis, female disorders, infections/wounds, coughs, tumour, and others. Although Ethiopia is one of the few countries that are endowed with large frankincense and myrrh resources (**Abdel Azeem 2006**).

Marjoram plants *Origanum marjorana H.* Or *Marjorana hortensis L.* Or *Origanum vulgare* are considered as an important medicinal

crop in Egypt with high production and great applications. Marjoram has great economic importance which is not only related to their use as a spice. The major essential oil constituents of oregano, which are carvacol, thymol, γ-terpinene and p-cymene, range between 80.2 and 98.4% of total essential oils. The essential oils derived from oregano is known to possess antimicrobial **Adam et al., 1998**, insecticidal **.Botsoglou et al., 2002 and Papageorgrou et al., 2003)** activities, mainly because of its high content of phenolic acids and flavonoids, which is useful in health supplements and food preservation **(Vagi et al., 2005)**. Traditionally, the plant has been used as a folk remedy against asthma, indigestion, headache and rheumatism **(Jun et al., 2001)**.

The main objective of this study was to study the effect of different levels of myrrah supplemented to the diets as natural growth promoter on growth performance, feed utilization, body composition, immunological blood parameters, entropathogenic *A. hydrophila* challenge of all male Nile tilapia *O. niloticus L.* fingerlings.

Materials and Methods

The present study was carried out at the Lab of Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, Egypt, during the year 2013 in order to determine the responses of tilapia fingerlings to Myrrah as extract feeding additives.

Extraction of Myrrah

Myrrah was purchased from the local market; Myrrh leaves were washed thoroughly in running tap water to remove sand and debris. Here after, they were dried by spreading under the sun for 3 days and finally in a hot air oven at 60°C for 8 hours. The dried leaves were crushed to powder in a mortar and pestle and subjected to Soxhlet extraction with 70% ethanol as extracting solvent. The solvent was exhausted from the extract with the help of a rotary evaporator. The extract was stored in a refrigerator until required for use.

Fish and culture technique:

All-male Nile tilapia fingerlings L (monosex) ranged from 10 to 12 gm kept under the same environmental conditions and placed in a fiberglass aquaria for 2 weeks for acclimation period to the laboratory

condition. Acclimated fish were distributed randomly at a rate of 15 fish / 100 L aquaria. Each aquarium was supplied with compressed air via air-stones from air pumps. Settled fish wastes with one half of aquariums water were siphoned daily and water volume was replaced by aerated tap water from the storage tank.

Feed preparation :

Diets were formulated isonitrogenous (30% crude protein and 7% lipid). Dietary formulation and proximate analysis of the experimental diets are shown in table (1). All dry ingredients including myrrah were mixed homogeneously and 100 ml of water was added per kg diet and the mixture were blended using kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through laboratory pellet machine with a (1 mm) diameter matrix. The pellets were dried in a drying oven model (fisher oven 13-261-28A) for 24 hours on 65°C and stored in plastic bags which were kept in a refrigerator at -20°C) during the experimental period to avoid rancidity. The diets were prepared palletized. Experimental diets were formulated to meet the nutritional requirement of fish **(NRC, 1994)**. Diet was given to fish at a rate of 3% of live body weight twice daily at 9:00 and 13:00 hours Each diet was fed to triplicate aquaria of Nile tilapia for a period of 12 weeks. Fish in each aquarium were weighed biweekly and the amounts of given feed were readjusted according to increase in body weight. The dead fish was daily recorded and removed. At the end of feeding trial, each fish weight and length were measured. At the end of the experiments, fish were collected, counted, group weighed per treatments.

Feeding system:

Fish were fed the experimental diets at a rate of 3% of their biomass, twice daily at 9:00 and 13:00 hours. Fish in each aquarium were sampled biweekly and feed amounts were adjusted biweekly interval on the basis of the new fish biomass. Dead fish were daily recorded and removed. The feeding period in experiments lasted 12 weeks.

Table (1): Ingredients and chemical analysis of the experimental diets (on dry matter basis), containing different levels of Myrrh extract.

	Control	**At Myrrh extract % in the diets**		
	0%	**0.5%**	**1%**	**1.5%**
Ingredients				
Fish meal	9.1	9.1	9.1	9.1
Soybean meal	45.5	45.5	45.5	45.5
Ground corn	15.31	15.31	15.31	15.31
Wheat bran	19.21	19.21	19.21	19.21
Starch	4	3.5	3	2.5
Myrrh	***0***	***0.5***	***1***	***1.5***
Cod fish oil	2.23	2.23	2.23	2.23
Corn oil	1.65	1.65	1.65	1.65
Vitamins premix	1	1	1	1
Minerals premix	2	2	2	2
Total	100	100	100	100
Chemical analysis %				
Dry matter	91.68	91.46	91.35	91.69
Crude protein	30.11	30.26	30.38	30.41
Crude fat	7.11	7.22	7.39	7.48
Ash	8.13	8.33	8.17	8.06
Fiber	5.45	5.32	5.55	5.32
NFE	49.2	48.87	48.51	48.73
GE (Kcal/100 gm)	4390.3	4395.7	4403.8	4423
P/E ratio	68.58	68.84	68.99	68.75

1- Vitamin premix (per kg of premix): Thiamine, 2.5 gm; riboflavin, 2.5 gm; pyridoxine, 2 gm; inositol, 100 gm; biotin, 0.3 gm; pantothenic acid, 100 gm; folic acid, 0.75 gm; para-aminobenzoic acid, 2.5 gm; choline, 200 gm; nicotinic acid, 10 gm; cyanocobalamine, 0.005 gm; a-tocopherol acetate, 20.1 gm; menadione, 2 gm; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU **(Jauncey and Rose, 1982)**.

2- Mineral premix (gm/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl, 50; NaCl, 60; $FeC_6H_5O_7.3H_2O$, 25; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03 **(Jauncey and Rose, 1982)**.

3- Nitrogen-free extract (calculated by difference) = 100 – (Protein + Lipid + Ash + Fiber).

4- Gross Energy (GE) was calculated from **NRC (1993)** as 5.65, 9.45, and 4.1 kcal/gm for protein, lipid, and carbohydrates, respectively.

Chemical analysis of fish:

At the beginning of the experiment, fifteen fish were for the body composition analyses. At the end of experimental, five fish from each pond were taken and exposed to the chemical analysis of the whole fish body. The test diets and fish samples were analyzed with 3-replicate according to the standard methods of **AOAC (1990)**.

Analysis of the physico-chemical parameters of water:

Water samples were collected biweekly from each aquarium. Water temperature and dissolved oxygen were measured on site with a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Spring, Ohio, USA). While the pH degree was measured using a pH-meter (Digital Mini-pH meter, model 55, Fisher Scientific, USA). Unionized ammonia was measured using dREL/2 HACH kits (HACH Co., Loveland, Colorado, USA) **(Boyd, 1990)**.

Physiological measurement:

Preparation of blood samples:

Fish were starved for 24 hours immediately prior to sampling. Fish were anaesthetized with buffered MS_{222} (50 mg/L) and blood was collected with a hypodermic syringe from the caudal vein. The blood collection lasted less than 3 minutes in order to avoid cortisol rise induced by the manipulation during sampling. The extracted blood was divided in two sets of pen-doorf tubes. One set contained heparin, used as anticoagulant for hematology (hemoglobin, hematocrit and red blood cell counting). The second set, without anticoagulant, was left to clot at 4°C and centrifuged at 5000 rpm for 5 minutes at room temperature. The collected serum was stored at -20°C for further assays. After decapitation of fish samples of liver and muscle were taken and frozen for further biochemical analysis.

Challenge test:

After 90 days of feeding with different levels of myrrah,the fish of each group were divided into two subgroups; the first subgroup was challenged I/P with pathogenic *A. hydrophila* (0.3 ml of 5 x 10^5 CFU), *A. hydrophila* was obtained from Fish Disease Department, Central Labortory for Aquaculture Research. The second subgroup was injected I/P with 0.3 ml of saline solution as a control. Both

subgroups kept under observation for 10 days to record the daily mortality rate as described by **Schäperclacus et al., (1992)**.

All the experimental infected fish were daily noted for any abnormal clinical signs and mortalities. The dead and clinically diseased fish were subjected to bacterial re-isolation.

Efficiency of Myrrah extract for treatment of infected *O. niloticus* with *A. hydrophilia:*

Myrrah extract was chosen according to the result of in vitro sensitivity test. Eighty *O. niloticus* were allotted into four equal groups. the first group was injected I/P with 0.2 ml of *A. hydrophilia*, the second group was injected I/P with 0.5 ml of Myrrah extract, the third group was injected I/P with 0.2 ml of *A. hydrophilia* and kept for 18 hours and treated by Myrrah extract once intraperitoneal at a dose of 10 mg/kg body weight **(Mahady 2005)**. The fourth group was injected I/P with 0.2 ml of saline as control. All groups were observed for 21 day post-infection.

Statistical analysis:

The obtained data statistically analyses were done using SPSS program version 10 (SPSS, Richmond, USA) as described by Dytham **(1999)**.

Results and Discussion

Effect of myrrah extract on physico-chemical water quality parameters:

Analysis of water quality parameters revealed that water temperature ranged between 26 and 28°C, dissolved oxygen concentrations ranged between 5.8 to 6.7 mg/L, pH 7.4-8.1, and unionized ammonia concentration range was 0.06-0.13 mg/L. The chemical water analysis showed no apparent fluctuation during the experimental period; however, water quality was found to be within the acceptable range for tilapia growth this result agree with **(boyd, 1990)**

Effect of Myrrah extract on growth performance and survival rate of fish:

Data in table (2) show that growth performance of Nile tilapia. The highest final body weight, weight gain and weight gain% (34.12, 21.83 and 188.1 gm/fish respectively) was obtained with diet of 1% Myrrah, as compared to the control diet (30.6, 18.33 and 138.79 gm/fish respectively). Also, Specific Growth Rates (SGR) increased significantly when supplemented diet with different levels of Myrrah extract. The highest values were obtained with 1% Myrrah extract (1.57%/day), while the lowest one was obtained with the control diet (1.24%/day). Also, no significant differences in fish survival rate among different treatments ($p > 0.05$), and its range was 93.3-100%.

The obtained results agree with Shalaby *et al.*, (2003) who mentioned Nile tilapia in aquaria for 70 days and fed on 36% crude protein diets supplemented with licorice roots at rate of 0, 1, 2, 3 and 4% from diet, they showed that roots had positive effect on growth performance specially with 3% level, but in our study, when put Nile tilapia in aquaria for 90 days and fed with 30% crude protein diets supplemented with Artimizia at rate of 0.5, 1 and 1.5%, we showed that both extracts have positive effect on growth performance specially with 0.5% of both extracts. **El-Saidy and Gaber (1997)** reported that dried garlic meal can be used in the diets of Nile tilapia fingerlings at a level of 4% for increasing growth in fish flesh and whole fish. But, in our study, when we added different extracts of Artimizia, we showed no significant difference in fish survival rate among different treatments. **Sakar (2003)** reported that higher significant differences were found in final weight, gain, gain %, and SGR of fish fed different liens of dried Marjoram leaves and dried Basil leaves than control group, but in our study, we showed that the SGR and weight gain and gain % and final body weight are increased than in control group.

Effect of Myrrah extract on feed efficiency and protein utilization:

Table (3) shows feed intake, feed conversion ratio, protein efficiency ratio, protein utilization and energy utilization. There is a significant difference ($p < 0.05$) in Feed Intake (FI) between diets containing different levels of Myrrah extract and control diet.

Table (2): Growth performance (mean ± SE) of Nile tilapia (*O. niloticus*) fed diets containing different levels of Myrrah extract after 90 days.

Items	Control 0%	Myrrah % in the diets		
		0.5%	1%	1.5%
Initial weight (gm/fish)	12.27 ± 0.46	12.33 ± 0.23	12.29 ± 0.32	12.33 ± 0.27
Final weight (gm/fish)	30.6 ^{c}± 0.6	32.14 ^{ab} ± 0.49	34.12 ^{a} ± 0.69	33.5 ^{b} ± 0.71
Weight gain (gm/fish)	18.33 ^{c} ± 0.52	20.81 $^{a\ b}$± 0.97	21.83 ^{a}± 0.33	20.17 ^{b} ± 0.68
Weight gain %	138.79c ± 7.03	159.77 ^{b} ± 8.89	188.1 ^{a} ± 11.3	156.59 ^{b} ± 9.59
SGR (% day)	1.24 ^{c} ± 0.05	1.36 ^{b} ± 0.03	1.57 ^{a}± 0.03	1.32 ^{ab} ± 0.05
Survival rate (%)	93.3 ± 3.8	95.6 ± 2.22	100 ± 0	95.6 ±2.22

Mean the same letter in the same row is not significant different at $p \leq 0.05$.

Table (3): Feed intake, Feed Conversion Ration (FCR), Protein Efficiency Ratio (FER), Apparent Protein Utilization (APU) and Energy Utilization (EU) of Nile tilapia (*Oreochromis niloticus)* fed diets containing different levels of Myrrah extract.

Items	Control 0%	Myrrah extract % in the diets		
		0.5%	1%	1.5%
Feed intake (gm/fish)	34.1 ^{c} ± 0.34	35.8 ± 0.35^{b}	37.8 ± 0.67^{a}	35.66 ± 0.5^{ab}
FCR	2.02 ^{a} ± 0.05	1.79 ^{ab} ± 0.07	1.63 ^{b} ± 0.08	1.85 ± 0.11^{ab}
PER	1.76 ^{b} ± 0.05	1.7 ^{bc}± 0.07	2.1 ^{a} ± 0.09	1.8 ± 0.06 ^{ab}
APU %	27.37 ^{c} ± 0.28	31.63 ^{b} ± 0.35	34.65 ^{a} ± 0.65	31.69 ±0.44^{b}
EU %	16.53 ^{c} ± 0.17	19.21 ^{b} ± 0.19	21.86 ^{a} ± 0.39	19.44 ^{b} ± 0.26

Mean the same letter in the same row is not significantly different at $p < 0.05$.

Nile tilapia fingerlings fed diets contained 1% Myrrah extract exhibited the highest FI (37.8 gm/fish), while the lowest FI was observed for control group (34.1 gm/fish). The best Feed Conversion Ratio (FCR) was observed with 1% Myrrah extract (1.63), while fish fed the control diet showed worse FCR (2.02). PER, APU and Eu increased significantly ($p > 0.05$) with supplemented diets with different levels of Myrrah extract in Nile tilapia diets. The highest values of PER, APU and EU (2.1, 34.65 and 21.86 respectively) were obtained with diet containing 1% Myrrah extract as compared to the control diet (1.76, 27.37 and 16.53 respectively).

Sakar (2003) reported that higher significant differences were found in final weight, gain, gain %, and SGR of fish fed different levels of dried leaves of Marjoram and dried Basil than control group, **Also El-Dakar et al. (2004)** studied the effect of dried Marjoram leaves (0, 0.5, 1 and 2%) on Nile tilapia average 13 gm/fish for initial weight, the best of growth performance was obtained at the 2% level. **Abdel Wahab et al., (2007)** reported that Nile tilapia fed diet containing 0.5% cinnamon showed better growth performance in general compared to other diets (1%). **Abdel Zaher et al. (2009)** found that feed intake, FER, PER, APU and EU increased significantly, while decreased significantly in diet containing 1%, while FCR increased significantly for control diet. But, these results agreed with our result.

Effect of myrrah extract supplementation with body composition:

Data in table (4) show that the whole body composition of Nile tilapia fingerlings at the end of the experiment. No significant difference ($p > 0.05$) was observed in whole body composition (moisture, protein, lipids, and ash contents) for Nile tilapia fed diets containing different levels of myrrah extract. Moisture content has no significant difference due to supplementation diets with myrrah extract and its range was 74.06-74.64%. Protein content, fat content and ash content range were 60-60.71%, 19.25-19.77% and 19.17-19.81% respectively.

Proximate chemical composition of fish at start of this study was 75.22 ± 0.3% moisture; 60.57 ± 0.29% protein; 19.53 ± 0.31% lipid and 19.18 ± 0.52% rash.

Table (4): Effect of Myrrah extract supplementation with body composition and proximate chemical analysis on dry matter basis (mean ± SE) of Nile tilapia ***Oreochromis niloticus***, fed diets containing different levels of Myrrah extract.

Items	Control 0%	Myrrah extract % in the diets		
		0.5%	1%	1.5%
Moisture %	74.64 ±0.78	74.49 ± 0.55	74.27 ± 0.2	74.06 ± 0.31
Crude protein %	60 ± 0.06	60.45 ± 0.3	60.57 ± 0.29	60.7 ± 0.3
Ether extract %	19.72 ± 0.22	19.77 ± 0.3	19.53 ± 0.31	19.25 ± 0.51
Ash %	19.81 ±0.31	19.31 ± 0.63	19.18 ± 0.52	19.17 ±0.09

Table (5): Hematological parameters [Hemoglobin (Hb) (gm/dl), erythrocyte count (RBCs x 10^6/cmm) and haematocrit PCV % (RBCs x 10^6/cmm) and hematocrit of fingerlings Nile tilapia (*O. niloticus*)] fed diets containing different levels of Myrrah.

Items	Control 0%	Myrrah % in the diet		
		0.5%	1%	1.5%
Hb	$4.3^{d} \pm 0.1$	$5^{c} \pm 0.2$	$4.7^{b} \pm 0.2$	$5.1^{a} \pm 0.1$
RBCs	$1.4^{d} \pm 0.02$	$1.5^{c} \pm 0.04$	$1.7^{b} \pm 0.04$	$1.9^{a} \pm 0.04$
PCV	$14^{d} \pm 0.4$	$15.1^{c} \pm 0.5$	$16.8^{b} \pm 0.6$	$18.2^{a} \pm 0.6$

The means with the same letters are not significant in the same column ($p > 0.05$).

Table (6): Biochemical parameters, blood urea (mg/dl), serum creatinine (mg/dl) of fingerlings Nile tilapia (*O. niloticus*) fed diets containing different levels of Myrrah.

Items	Control 0%	Myrrah levels % in the diet		
		0.5%	1%	1.5%
Urea (mg/dl)	17.2 ± 0.1^{a}	15.9 ± 0.1^{b}	14.7 ± 0.1^{c}	13.8 ± 0.1^{d}
Creatinine (mg/dl)	0.5 ± 0.02^{a}	0.4 ± 0.02^{b}	0.4 ± 0.01^{c}	0.4 ± 0.01^{d}
Glucose (mg/dl)	75 ± 2.5^{a}	74.4 ± 3.6^{b}	62.4 ± 3.2^{c}	60.1 ± 2.4^{d}

The means with the same letters are not significant in the same row ($p > 0.05$).

Table (7): Mortality rate (%) of fingerlings Nile tilapia *O. niloticus* fed diets containing different levels of Myrrah for 90 days and challenged by *A. hydrophila* for 10 days.

Items	Control 0%	Myrrah levels % in the diet		
		0.5%	1%	1.5%
Number of injected fish	10	10	10	10
Bacteria dose (5 x 10 CFU)	0.2 ml	0.2 ml	0.2 ml	0.2
Injection route	I/P	I/P	I/P	I/P
Mortality rate (%) after 10 days of injection	100	15	0	0

The same letter in the same row is not significantly different at $p < 0.05$.

Hematological indices of the growing fish:

The results of hematological indicated that Hemoglobin (Hb), erythrocyte count (RBCs) and haematocrit (PCV) in the blood of tilapia (*Oreochromis nilocticus*) increase with increasing Myrrah levels. It is noticed that fish-fed diets contining 1.0 % Myrrah exhibited higher values of RBCs (1.9 UL), Hb (5.1 gm/L and PCV (18.2%) than the control diets (1.4, 4.3 and 14, respectively).

Serum biochemical parameters:

1. Total protein, albumin, and globulin values:

Total protein, albumin, and globulin values increased significantly ($p \leq 0.05$) with Myrrah extract supplementation and the highest values were obtrained at 1% of the diet. The lowest value was obtained at the control diet. Myrrah extract supplementation insignificantly affected the Albumin/Globulin (A/G) ratio .

2. GOT and GPT values:

GOT and GPT values decreased with the increased supplementation by Myrrah extract. The highest values were obtained at the control diet

Determination of urea, creatinine and glucose in fish:

The obtained results showed that urea and creatinine significantly ($p < 0.05$) decreased with increasing levels of Myrrah. There are decreases in glucose fed diet 1.5% (60.1) and 1% (62.4) respectively than in control diet (75).

Hematological studies agreement with **El-Mallah (2003)** who showed that blood plasma parameters showed an improvement in total protein, albumin and globulin and decrease in liver enzymes in addition of some medicinal plants all treatment group. **Also Ali et al. (2005)** indicated that total plasma protein and haemoglobin concentrations were significantly higher ($p < 0.05$) in the blood of lambs fed treated rations (G2 and G3) than those fed control ration. Furthermore, albumin, globulin, and urea concentrations were slightly increased. Meanwhile, the enzyme activities of GOT and GPT were slightly decreased by increasing the chamomile level in treated rations. Also **Abdel Azeem (2006)** showed that, plasma total protein,

albumin, and globulin were increased, while GOT and GPT decreased for groups fed with frankincense) compared to those fed the control diet.

Challenge test:

The results showed that the mortality percent was higher in control group (89%) than all groups of Myrrah. The mortality percent in groups fed diet containing different levels of myrrah was 15, 0 and 0 for 0.5, 1 and 1.5% respectively. So, the Myrrah had antibacterial activity antagonized by *Aeromonas hydrophila* in fresh water. Also, the use of Myrrah, the dose (1%) was enough.

Ahmed et al., (2009) indicated that fish was challenged against *A. hydrophilia* for 10 days and the highest fish mortality was recorded in the first day and decrease by time up to 4^{th} day for each treatment of different levels of cinnamon (***Cinnamomum zeylanicum, NEES)*** except the group fed 0.4 organism that had no fish mortality. No mortality was observed after the 4^{th} day at all treatments. The highest overall mortality was observed at control 80%, also **Sahu et al., (2006)** studied dietary dosage of garlic on the immune response and disease resistance against infection by *A. hydrophilia and some medical plants incorporated into the diet and every 20 day different biochemical and hematological parameters were evaluated, and after 60-day fish were challenged by A. hydrophilia* and mortality was recorded up to day 10 postchallenge. Survival decrease in control group 57% up to day 10 after infection and increase in treatment group and finally indicated that medical plant stimulate the immunity and make *L-rohita* more resistant to infection by *A. hydrophilia.*

Identification of active compound on Myrrah :-

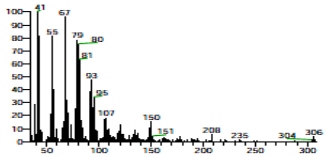
The yellow coloured oil extract obtained from myrrah analyzed separately by gas-liquid chromatography and identified by comparing their retention times with peak area.

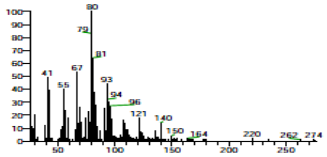
Antibacterial spectrum of the separated principles:

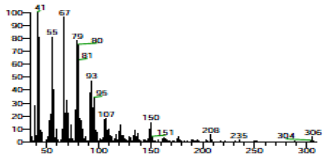
As regard the antibacterial spectrum of the antibacterial agent separated from myrrah plants, the results obtained showed that the Myrra have antibacterial agent .

RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
17.03	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	6.75	103938 8832.26	C20H34O2	306
17.03	3,6-Octadecadienoic acid, methyl ester (CAS)	6.75	103938 8832.26	C19H34O2	294
17.03	8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (CAS)	6.75	103938 8832.26	C20H34O2	306
17.03	R-Limonene	6.75	103938 8832.26	C10H16O3	184
17.03	(-)-Caryophyllene oxide	6.75	103938 8832.26	C15H24O	220

Hit Spectrum

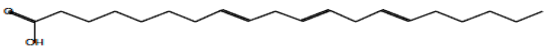




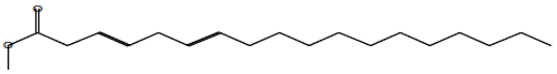


Compound Structure

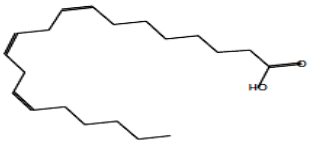
8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
Formula C20H34O2, MW 306, CAS# 1783-84-2, Entry# 1072
cis-8,11,14-Eicosatrienoic Acid



3,6-Octadecadienoic acid, methyl ester (CAS)
Formula C19H34O2, MW 294, CAS# 57156-92-0, Entry# 361875
METHYL 3,6-OCTADECADIENOATE

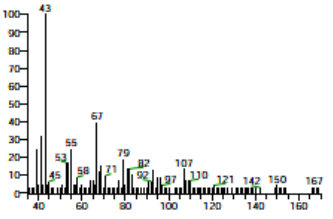


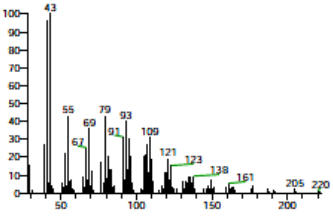
8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (CAS)
Formula C20H34O2, MW 306, CAS# 1783-84-2, Entry# 388157
CIS,CIS,CIS-8,11,14-EICOSATRIENOIC ACID



Hit Spectrum

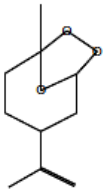
SI 755, RSI 772, mainlib, Entry# 7918, CAS# NA, R-Limonene



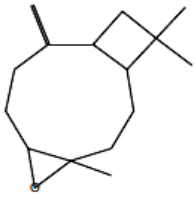


Compound Structure

R-Limonene
Formula C10H16O3, MW 184, CAS# NA, Entry# 7918



(-)-Caryophyllene oxide
Formula C15H24O, MW 220, CAS# 1139-30-6, Entry# 188879
(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,,12-trimethyl-9-methylene-, [1R-(1R*,4R*,6R*,10S*)]- (CAS)



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التاثيرات المناعيه و العلاجية ومعدلات النمو لمستخلص نبات المره فى السيطرة على الامراض البكتيريه فى اسماك المياه العذبه

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الملخص العربى

أجريت هذه الدراسة فى المعمل الرطب بقسم صحة الاسماك ورعايتها بالمعمل المركزى لبحوث الثروة السمكية بالعباسة – أبو حماد – شرقية – مصر وذلك لدراسة تأثير مستويات مختلفة من مستخلص نبات المره المضاف إلى العلائق كدافع نمو طبيعيى على اداء النمو والاستفادة من الغذاء ومكونات الجسم وعينات الدم والمقاومة لبكتريا الايرموناس هيدروفيلا . وهو من النباتات الطبية المعروفة بتأثيرها الطبى على الإنسان واستخدامه كإضافات أعلاف وبعد أقلمة الأسماك لمدة أسبوعين تم توزيعها فى ثمان معاملات وكل معاملة مكونة من ثلاث مكررات وكل مكرر به ١٥ سمكة تم تكوين ثمان علائق تطبيقية متماثلة فى محتواها من البروتين والطاقة وتم تغذية الأسماك بنسبة ٣% من وزنها – مرتين يومياً – لمدة ٦ أيام فى الأسبوع لمدة ٩٠ يوم وتم قياس جودة المياه خلال فترة التجربة . وكمية التغذية اليومية تبعاً للوزن الجديد للأسماك التى يتم ضبطه كل ١٤ يوم وكانت النتائج كالآتى : كانت هناك زيادة معنوية فى كل من متوسط وزن الجسم ومعدل النمو النوعى للأسماك التى تم تغذيتها على مستخلص نبات المرة بنسبة ١% بالمقارنة بمجموعة الكنترول كما وجدت فروق معنوية فى معدل الإعاشة بين المعاملات المختلفة والكنترول .حيث ازداد معدل الاستفادة من العليقة والبروتين وزيادة قيمة البروتين والطاقة بزيادة مستخلص نبات المرة وخاصة بنسبة ١% كما أظهرت هذه العليقة تحسناً معنوياً فى معامل التحويل الغذائى عن باقى المعاملات الأخرى وخاصة عليقة المقارنة. أوضحت النتائج عدم وجود فروق معنوية فى تركيب الجسم بإضافة مسحوق المره والتى أدت التغذية على العلائق المحتوية على مستخلص المرة إلى زيادة تركيز كل من الهيموجلوبين وكرات الدم الحمراء والهيماتوكريت والبروتين الكلى وانخفاض كل من الكرياتينين واليوريا والدهون وإنزيمات الكبد مع الملاحظة بأنه لا يوجد فروق فردية معنوية فى محتوى الجلوكوز فى بلازما الدم . بعد حقن المعاملات ببكتريا الايروموناس هيدروفيلا فى التجويف البطنى للأسماك أوضحت النتائج أن نسبة النفوق تقل بزيادة مستخلص نبات المرة .