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USE OF THYME AND THYMOL AS IMMUNE-MODULATOR FOR *OREOCHROMIS NILOTICUS* CHALLENGED WITH *STREPT.* Sp

By

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ABSTRACT

The present study investigated the effects of thyme and thymol as phytoadditives on hematological and non-specific immune serum biochemical parameters in *Oreochromis niloticus*. In this trial, ninety (90) *O. niloticus* with an average body weight of (50 ± 5) were used. Fish were divided into three groups (30 fish/each group) in triplicate and fed for 8 weeks with (group 1) fed basal diet as the control group, while (group 2) and (3) were fed basal diet supplemented with thyme at 10g/kg diet and thymol at 3g/kg body weight, respectively After 8 weeks of feeding groups (2) and (3) showed particular differences in the immunological and hematological parameters as compared to control group, a significant increase of phagocytic and lysozyme activity were seen in the treated groups. Also, the serum total protein and globulin levels were significantly higher after experimental infection in comparison to the control. The results of the present study indicated that dietary supplementation of 3 g/kg of thymol and thyme in 10g/kg in commercial diets could improve some non-specific immunity and biochemical parameter in *O. niloticus* and increase survival rate against experimental challenge with *Strept* .Sp.

Keywords:

Fish pathogens, herbs (thyme and thymol), *O.niloticus*

INTRODUCTION

Aquaculture industry has shown a rapid growth in recent years. The main objectives of fish industry are both increasing growth rates of fish and improving their health. Antibiotics have long been used for promoting growth and struggling with pathogenic organisms. However, the use of antibiotics in fish culture aquaculture have been restricted or even prohibited in

many countries **(Citarasu, 2010)**. That's why; researchers have been looking for alternatives to antibiotics and the other synthetic chemicals. Herbs or their essential oils can be used for enhancing fish resistance against pathogen bacteria with its antimicrobial and antioxidant features. The features of herbals can be related to terpenoids and phenolic compounds **(Conner, 1993).** It was seen that herbs can promote growth in fish, develop fish health and raise the resistance to illness when added to fish-feed **(Chakraborty, and Hancz, 2011) and (Yilmaz *et al*., 2014)**. The therapeutic potential of thyme rests on contents of flavonoids, thymol,carvacrol, eugenol, aliphatic phenols as well as luteolin, saponins, and tetramethoxylated flavones. **(Javed *et al*., 2013)**. In some studies, a dietary administration of thyme in level of 10 g/kg provided the best survival, growth performance, and feed utilization in tilapia fish **(Ahmad, and Abdel-Tawwab, 2011)**. Some studies have reported that oral administration of combination of thymol and Carvacrol in *Ictalurus punctatus* **(Zheng *et al*., 2009)** and *O. mykiss* **(Ahmadifar *et al* .,2011)** improved growth performance, disease resistance and/or immunity .Diet supplemented with *Thymus vulgaris* (thyme) at 10 g/kg acts as a growth promoter and antimicrobial agent to enhance disease resistance, Also, it can be used as an alternative to antibiotics in controlling streptococcal disease in tilapia culture **(Sevdan *et al*., 2014)**. Therefore, the aim of the present work was to study the influence of phytoadditives thyme and thymol in fish diet on some hematological and the non-specific immune serum biochemical parameters in *Oreochromis niloticus*.

MATERIAL AND METHODS

Fish:

Ninety apparently healthy *Oreochromis niloticus* of an average body weight (50±5 gm) were obtained from the Ismailia fish hatchery, Egypt. Fish were distributed into three group (30 fish/group) stocked in 9 glass aquaria each aquarium was stocked with 10 fish and acclimatized for the experimental conditions for 15 days prior to the start. During that period fish were adapted on feeding of control diet(without any additives) towice daily at 3% of their body weight.Water was changed every week to maintain good water quality. Water temperature and pH were adjusted at 20-25c and 7.4 respectively during the experimental period.

Preparation of fish diet:

1-Thymol powder (W224502, Sigma-Aldrich, and Munich, Germany).

2-*Thymus Vulgaris* from farms of (marsa matrouh "wadi habs"- Elshekh Zoied).

The collected fresh thyme leaves are washed after that, the leaves are Shadow dried at 27-37c and the leaves are made to in powder by using mixture. According to **Sumayaa, and Buvaneswari, (2014)**. The standard commercial pelletized fish food was mixed with different feed additives using a gelatin-2%concentrationasbindingmaterial and appetizer according to **Alina *et al*., (2012)** .study design is : Group (1) was fed basal diet as the control group ,while group (2 and 3) were fed basal diet supplemented with thyme at 10g/kg diet according to **Sevdan *et al*., (2014)** and thymol at 3g/kg body weight according to **(Ahmedifar *et al*.,2011).**

Blood Collection:

Blood samples of six fish/groups were collected randomly from the caudal blood vessels using a vacationer fitted 5 mL. At the last day of 8 weeks. EDTA was used as an anticoagulant for determination of phagocytic index and HB, RBCS, WBCS, PCV, differential leukocytic count. Other blood samples were collected in plain centrifuge tubes and centrifuged at 3000 r.p.m for 15 min for serum separation for determination of liver and kidney function tests, as well as Total proteins and lysozyme. Second set of blood and serum samples were collected again after the experimental challenge.

Determination of Hematological parameter:

Hemoglobin concentration was determined with Drabkin solution according to **(Stoskopf, 1993)**. Natt-Herrick solution was used for red and white cell count according to **(Feldman *et al*., 2000),** Differential leukocytic count. The stained blood film was prepared. The relative and absolute count was estimated according to **(Thrall, 2004).**

Determination of Immunological Analysis:

The phagocytic activity was measured using heat-inactivated *Candida albicans* according to **(Kumari *and Sahoo*, 2006)**, Lysozyme activity was measured using *Micrococcus lysodeikticus* lyophilized Gram positive bacteria.(Sigma®) **(Esteban *et al*., 2001)**,total proteins was carried by a test kit according to biuret method **(Weichsel Baum**, **1946).**

Determination of liver and kidney function:

Estimation of Aspartate transaminases (AST) and Alanine Aminotransferase (ALT) was estimated according to **(Reitchman and Frankel.1957)**, and determination of serum urea and creatinine levels **(Henry *et al*., 1974).**

Challenge tests:

Comparative I.Pchallenges of at the end of experimental period.Ten fish from each group. Injectedwith100 µl Strept.Sp(1x10^8C.F.U/ml) was performed accordingto**(Yılmaz*etal*. 2012**)

identified strain from fish disease department,Vet.Med, [Cairo University.The experimentally infected fish were inspected for seven days' post challenge. The clinical signs were recorded and blood collection for biochemical, immunological parameter and mortalities were monitored for 10 days' post challenge.

Statistical analysis:

The data were analyzed by one-way analysis of variance (ANOVA) to determine the significant variations among the various parameters in the experimental groups. All of analysis was performed using SPSS. **Dytham. (1999).**

RESULTS

Hematological parameter finding:

Hematogram:

The results of hematogram revealed a significant increase in RBCs count, HB. Value, PCV (%)anddifferential leukocytic count in the two groups treated with photobiotic in comparison with control group and the results are represented as means of six samples (Table 1).

Table (1): Hematogram of *Oreochromis niloticus* fed with thyme and thymol before and after challenge with *Strept.* Sp.

Parameters	Before challenge			After challenge		
	Control Group	Thyme Group	Thymol Group	Control Group	Thyme Group	Thymol Group
HB (g/dl)	10.77 ± 0.275 a	13.18 ± 0.206 b	13.40 ± 0.213 b	9.90 ± 0.086 a	12.63 ± 0.230 b	12.35 ± 0.217 b
RBCs x 106	3.22 ± 0.132 a	3.71 ± 0.106 b	3.88 ± 0.159 b	2.51 ± 0.197 a	3.63 ± 0.097 b	3.64 ± 0.119 b
WBCs x 103	84.48 ± 0.735 a	87.52 ± 0.386 b	88.59 ± 0.838 b	89.03 ± 0.424 a	92.05 ± 0.368 b	92.95 ± 0.315 b
PCV %	30.83 ± 0.184 a	33.27 ± 0.323 b	33.27 ± 0.323 b	28.93 ± 0.180 a	32.53 ± 0.228 b	32.53 ± 0.228 b
Lymphocytes x 10^3	27.97 ± 0.182 a	37.10 ± 0.315 b	37.63 ± 0.214 b	29.32 ± 0.194 a	38.08 ± 0.227 b	38.33 ± 0.295 b
Monocytes x 103	1.78 ± 0.048 a	3.17 ± 0.138 b	3.28 ± 0.233 b	1.83 ± 0.033 a	3.32 ± 0.093 b	3.77 ± 0.080 c
Heterophils x 103	9.47 ± 0.115 a	10.44 ± 0.160 b	10.37 ± 0.173 b	9.78 ± 0.039 a	10.63 ± 0.120 b	10.77 ± 0.033 b

± SE.SE= standard error of mean. Row with different letters for each group in the same group are significantly different at P ≤ 0.05.

Biochemical parameters:

Protein profile and liver enzymes.

The results of protein profile and liver enzymes showed a significant increase in total protein and globulin .and significant decrease in albumin, A/G ratio and liver enzymes (ALT and AST) in the two groups treated with phytobiotics in comparison with the control group. These results are illustrated in (Table 2).

Table (2): Serum protein profile, kidney function (urea and creatinine) and activities of liver enzymatic function (ALT and AST) of *Oreochromis niloticus* fed with thyme and thymol before and after challenge with *Strept.* Sp.

Parameters	Before challenge			After challenge		
	Control Group	Thyme Group	Thymol Group	Control Group	Thyme Group	Thymol Group
Total protein (g/dl)	2.62 ± 0.199 a	2.91 ± 0.121 a	2.70 ± 0.233 a	2.09 ± 0.089 a	3.93 ± 0.271 b	3.39 ± 0.220 b
Albumin (g/dl)	1.20 ± 0.073 a	1.06 ± 0.017 a	1.11 ± 0.029 a	0.78 ± 0.047 a	1.03 ± 0.015 b	1.09 ± 0.147 b
Globulin (g/dl)	1.42 ± 0.177 a	1.85 ± 0.129 a	1.50 ± 0.224 a	1.31 ± 0.101 a	2.90 ± 0.266 b	2.30 ± 0.258 b
A/G ratio	0.90 ± 0.104 a	0.59 ± 0.052 a	0.83 ± 0.119 a	0.61 ± 0.068 a	0.37 ± 0.031 a	0.53 ± 0.126 a
Urea (mg/dl)	2.88 ± 0.048 a	2.70 ± 0.156 a	2.78 ± 0.124 a	6.22 ± 0.247 a	2.88 ± 0.048 b	2.96 ± 0.067 b
Creatinine (mg/dl)	0.48 ± 0.018 a	0.47 ± 0.015 a	0.47 ± 0.011 a	1.20 ± 0.037 a	0.52 ± 0.016 a	0.52 ± 0.023 a
ALT (U/L)	18.08 ± 0.598 a	14.61 ± 0.686 b	13.89 ± 0.816 b	28.54 ± 0.203 a	16.52 ± 0.649 b	16.60 ± 0.720 b
AST (U/L)	18.42 ± 0.393 a	16.13 ± 0.794 b	16.35 ± 0.929 b	29.11 ± 0.248 a	18.45 ± 0.983 b	18.03 ± 0.911 b

Data are represented as means of six samples ± SE.SE= standard error of mean. Row with different letters for each parameter in the same group is significantly different at P ≤ 0.05.

RESULTS OF IMMUNOLOGICALANALYSIS

A) Cellular non-specific immune responses (Phagocytic assay):

The phagocytosis percent and phagocytic index were significantly increased in the fish groups (2) and (3) fed on diet supplemented with phytobiotics in comparison with the control group. These values are represented as means of six samples in (Tables 3, 4) and the phagocytic activity of blood monocytes to *Strept.*sp were showed in Fig. (1). (Tables 3, 4).

Table (3): Phagocytic activity% and Phagocytic index in *Oreochromis niloticus* groups before challenge.

	Control group Mean ± SE	Thyme group Mean ± SE	Thymol group Mean ± SE
Phagocytic activity %	**32.87 ± 0.379 [a]**	**40.46 ± 0.619 [b]**	**39.72 ± 0.492 [b]**
Phagocytic index	**1.43 ± 0.040 [a]**	**1.84 ± 0.038 [b]**	**1.76 ± 0.059 [b]**

Data are represented as means of six samples ± SE.

SE= standard error of mean. Row with different letters for each parameter is significantly different at P ≤0.05.

Table (4): Phagocytic activity % and Phagocytic index in *Oreochromis niloticus* groups after challenge with *Strept* .Sp.

	Control group Mean ± SE	Thyme group Mean ± SE	Thymol group Mean ± SE
Phagocytic activity %	**33.31 ± 0.479 [a]**	**49.86 ± 0.466 [b]**	**49.79 ± 0.617 [b]**
Phagocytic index	**1.48 ± 0.027 [a]**	**2.16 ± 0.136 [b]**	**2.05 ± 0.195 [b]**

Data are represented as means of six samples ± SE.

SE= standard error of mean. Row with different letters for each parameter is significantly different at P ≤ 0.05

Serum lysozyme activity:

Lysozyme activity of the treated groups was significantly increased. Before and after experimental infection the fish groups treated with (thyme and thymol) revealed significant increase in lysozyme activity when compared with the control groups. The fish groups treated with thyme and thymol didn't show significant differences.

Table (5): Lysozyme activity in *Oreochromis niloticus* groups before and after challenge.

Parameters	Before challenge			After challenge		
	Control Group	Thyme Group	Thymol Group	Control Group	Thyme Group	Thymol Group
Lysozyme	8.70 ± 0.747 a	15.05± 1.167 b	13.97 ± 1.206 b	15.62 ± 0.532 a	27.18 ± 0.606 b	26.93 ± 0.665 b

Data are represented as means of six samples ± SE.

SE= standard error of mean.

Row with different letters for each parameter is significantly different at P ≤ 0.05.

Clinical signs after challenge with *Strept.* Sp:

No apparent clinical signs were observed in both fish groups received phytobiotics and the control group after *Strept.* Sp infection revealed hemorrhages on the skin, Ulceration on the gill cover and slightly protruded reddish vent, skin darkness and tail rot. Mortalities were observed in control group, group I and the group II. These are shown in (Table 4).

Table (6): Mortality percent of *O. niloticus* challenged with *Strept.* Sp.

Fish group	Mortality / survivability of o.niloticus challeuged with strept sp.												
	No. of fish/group	1st day	2nd	3rd	4rd	5th	6th	7th	8th	9th	10th	Mortality %	Survivability %
Control	10	1	0	2	3	0	1	0	0	0	0	70%	30%
thyme	10	0	0	1	0	1	0	0	0	0	0	20%	80%
thymol	10	0	0	1	0	2	0	0	0	0	0	30%	70%

DISCUSSION

The recent expansion of intensive aquaculture practices has led to the appearance of several concerns like those regarding biological material welfare or growth performance and immunity if we refer especially to recirculating aquaculture systems. To avoid these concerns, different types of natural stimuli, such as probiotics, prebiotics or phytobiotics were introduced into fish diets **(Cristea *et al*., 2012)**.This study was planned to evaluate the effect of phytobiotics on the blood parameters, and immune response of cultured *O. niloticus*. Concerning the effect of both thyme and thymol on the health status of *O. niloticus*, In this study, we detected that some hematological indicators (WBC, RBC, PCV, Heterophil, neutrophils

and monocyte) in *Oreochromis niloticus* fed with diets supplemented with thyme and thymol, significantly increased compared to control. Previous reports showed that hematological indicators increased in fish that were fed garlic and ginseng **(Shelby *et al*., 2006)**, and allicin **(Nya *et al.*, 2010)**. **(Mostafa *et al*., 2009)** reported an increase of RBC, PCV and Hb in *O. mossambicus* fish after feeding them with thyme and fenugreek. The results of protein profile showed a significant increase in total protein and globulin and the results didn't show any significant differences between the different fish groups in the values of albumin. These results were in agreement with **Yilmaz *et al*., (2015)** who reported that the serum total protein, globulin and triglyceride levels of 3g of carvacrol treated groups was significantly higher on the 60th day in *Rainbow Trout*. The results of serum transaminases showed no significant difference in thyme and thymol treated groups. The highest AST and ALT were recorded in the control group before and after infection. These indicated a normal, positive, beneficial effect of these feed additives on the maintenance of the hepatocytes integrity. Herbs additives did not change serum urea, uric acid and creatinine in present study. Similar results were obtained when the fenugreek and thyme was included at 1%in tilapia diet **(Mostafa *et al*., 2009).** Another study has shown that, the stinging nettle decreased serum urea and creatinine in rainbow trout **(Awad 2010).** Immune systems can be activated by the immunostimulants in several ways enhancing the number of phagocytes, activating phagocytes or increasing the synthesis of the involved molecules. The Phagocytic activity and Phagocytic index of treated groups in this study were significantly increased in comparison with the control group which make activation to phagocytic cells to engulf *streptococcus bacteria* .These findings supported by **(Nejdet *et al* ., 2014)** when studied that Feeding the fish with 1% of thyme significantly increased the phagocytic activity in the blood of the *O. mossambicus* by activation of the inflammatory response before antibody production and playsan important rolein antibacterialdefenses.Lysozymeisconstitutivelyexpressed, synthesized and secreted by neutrophils, monocytes and macrophages; the greatest concentration of lysozyme is directly proportional with the leukocytic count **(Yano,1996).** As the supplementation of thyme and thymol in our fish diet increased the leukocytic count, the lysozyme concentration and activity were increased. Previous studies showed that tilapias fed a diet including Cinnamomum verum, Andrographis paniculata **(Rattanachaikunsopon and Phumkhachorn 2009);** Rosmarinus **(Yılmaz *et al*., 2012)** increased the survival rate against *streptococcal* challenge. Similarly, in the present study, we detected reduced mortality in fish groups supplemented with herbs that

were challenged with *Strept*. Sp. In conclusion, the results of this study indicated that supplementation of fish with thyme and thymol could improve the hematological and immunological properties in fish experimentally challenged with *Strept.* sp. These phytobiotics can be used as feed additives immunostimulants to enhance the immune responses and resistance of *O. Niloticus*.

REFERENCES

Ahmedifar, E., Falahatkar, B. and Akrami, R. (2011): Effects of Dietary Thymol-Carvacrol on Growth Performance, Hematological Parameters and Tissue Composition of Juvenile Rainbow Trout, Oncorhynchus mykiss. Journal of Applied Ichthyology, 27, 1057-1060. http://dx.doi.org/10.1111/j.1439- 0426.2011.01763.x.

Ahmad, M. H., and Abdel-Tawwab, M, (2011): The use of caraway seed meal as a feed additive in fish diets: growth performance, feed utilization, and whole body composition of Nile Tilapia, *Oreochromis niloticus* (L.) fingerlings.Aquaculture 314:110 -114**.**

Awad, E.S. (2010): Studies on plant based dietary supplements for control of *Areomonas hydrophila* infections in rainbow trout .PHD .Thesis, University of Heriot Watt.

Cristea, V., Alina, A., Iulia, G., Angela, D., Lorena, D. and Mirela (Creţu) Mocanu. (2012): The use of phytobiotics in aquaculture. Lucrări Ştiinţifice, Seria Zootehnie, Iaşi. Vol. 57 (17).

Claviez, M. (1981): Vascular Specialization in Fish, but No Evidence for Lymphatics. Zeitschrift Fur Naturforschung C-a Journal of Biosciences 36, 490 - 492.

Citarasu, T. (2010): Herbal Biomedicines: A New Opportunity for Aquaculture İndustry. Aquaculture International, 18,403 - 414. http://dx.doi.org/10.1007/s10499-009 - 9253-7.

Conner, D.E. (1993): Naturally Occurring Compounds. In: Davidson, P. and Branen, A.L., Eds., Antimicrobials in Foods, Marcel Dekker, Inc., New York, 441-468.

Chakraborty, S.B. and Hancz, C. (2011): Application of Phytochemicals as İmmunostimulant, Antipathogenic and Antistress Agents in Finfish Culture. Reviews in Aquaculture, 3, 103 -119.

Dytham, C. (1999): Choosing and Using Statistics: A Biologist's Guide. Blackwell Science Ltd., London, UK.

Esteban, M.A., Mulero, A. and Meseguer, J. (2001): Effects of injecting chitin particles on the innate immune of sea bream (*spareus aurata* L) innate immune system. Fish and Shellfish immunology, 11:303 -315.

Feldman, B.F.,J. G. Zinkl and N. C.Jain. (2000): Schalm's veterinary hematology 5th ed., Lippincott Williams and Wilkins, New York.

Goth,L.(1991): Asimple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta 196.143-151.

Henry, R.J., cannon, D.C. and Winkleman, J.W. (1974): Clinical chemistry, principles and techniques, second Ed .Harper and Row, London, UK.

Kirubakaran, C.J.W., Alexander, C.P., and Michael, R.D. (2010): Enhancement of non-specific immune responses and disease resistance on oral administration of Nyctanthes arbortristis seed extract in Oreochromis mossambicus (Peters).Aquac Res 41: 1630 -1639. .

Kumari,J.andSahoo,P.K.,(2006): Non-specific immune responseof healthyand immunocompromised Asian catfish to several immunostimulant. Aquaculture 255,133 - 141.

Mostafa ,A., Ahmad, M.H., Mousallamy ,A. and Samir ,A .(2009): Effect of using dried fenugreek seeds as natural feed additives on growth performance, feed utilization,whole-body composition and enteropathogenic *Aeromonas hydrophila*-challenge of monsex Nile tilapia O. niloticus (L) fingerlings. Aust J BS App Sci 3: 1234 -1245

Nejdet, G., Soner, B., Sevedan, Y., Derya, G. and Seyit, A. (2014): Effects of herbs and spice on health status of tilapia (Oreochromis mossambicus) challenged with *Streptococcus iniae* ACTA VET. BRNO, 83: 125-131.

Nya, E.J., Dawood, Z., and Austin, B. (2010): The garlic component, allicin, prevents disease caused by Aeromonas hydrophila in rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Dis 33: 293-300**.**

Rattanachaikunsopon, P.,and Phumkhachorn,P.(2009): Protective effect of clove oil-supplemented fish diets on experimental Lactococcus garvieae infection in tilapia. Biosci Biotech Biochem 73: 2085-2089.

Reitchman, S., Frankel, S., (1957): AColormetric method for determination of transaminase activity .Am.J.Clin.pathol.28, 56.

Javed, H.; Erum, S.H.; Tabassum, S., F. (2013): An overview on medicinal importance of *thymus vulgaris* journal of Asian scientific research, 3 (10):974 -982.

Siddiqui, A.Q, Howloder, M .S .and Adam, A.A., (1988): effect of dietary protein level on growth, feed conversion and protein utilization in fry of young Nile Tilapia (*Oreochromis .niloticus*). Aquaculture 70, 63-73.

Stoskopf, M. K. (1993): Fish medicine Ed., W.B. Sainders Company, London.

Sevdan, Sebahattin, E. and Ekrem, S.C., (2014): Effect of herbal Supplements on growth performance of sea bass and J.biosci.biotech.217-222.Vogel, W. O. P.

Shelby, R.A., Lim, C.E., Aksoy, M. and Delaney, M.A. (2006): Effects of probiotic feed supplements on disease resistance and immune response of young Nile tilapia (*Oreochromis niloticus*). J App Aquac 18: 23-34

Sumayaa and Buvaneswari, k .M, (2014): Assessment of Nutritional and sensory quality of recipes incorporated with thyme powder, International Journal of Current Research and Development, Vol.2 (1):56-66.

Thrall, M. A, (2004): Veterinary Hematology and Clinical Chemistry ED., Lippincott Williams and Wilkins, Maryland, USA.

Weichselbaum, T. E. (1946): Determination of total proteins Am. J. Clin. Pathol, 7: 40.

Yano, T. (1996): The non-specific immune system. Humoral defence in Fish Physiology, Vol.15, Iwama, G.K. and Nakanishi, T. (Eds), pp.106 -158, academic press, N.Y.

Yılmaz, S., Ergün, S., Türk, N. (2012): Effects of Cumin-supplemented diets on growth and disease (*Streptococcus iniae*) resistance of tilapia (Oreochromis mossambicus). Isr J Aquac IJA_64.2012.768.

Yılmaz, S., Ergün, S. and Çelik, E.Ş. (2014): Effect of Dietary Spice Supplementations on Welfare Status of Sea Bass, Dicentrarchus labrax L. Proceedings of the National Academy of Sciences, Biological Sciences, India, 1-9. http://dx.doi.org/10.1007/s40011-014-044-2

Yilmaz , E., Ergün ,S. and Yilmaz, S.(2015): Influence of Carvacrol on the Growth Performance, Hematological, Non-Specific Immune and Serum Biochemistry Parameters in Rainbow Trout (Oncorhynchus mykiss) Food and Nutrition Sciences, 2015, 6, 523 -531.

Zaki, M.A., Labib, E.M., Nour, A.M., Tonsy, H.D. and Mahmoud, S.H., (2012): Effect of some medicinal plants on mono sex *Nile tilapia* .Asia pacific chemical, Biological and Environmental Engineering Society, P.220-227.

Zheng, Z.L., Tan, J.Y.W., Liu, H.Y., Zhou, X.H., Xiang, X. and Wang, K.Y. (2009): Evaluation of Oregano Essential Oil (Origanum heracleoticum L.) on Growth, Antioxidant Effect and Resistanceagainst *Aeromonas hydrophila* in Channel Catfish (*Ictalurus punctatus*): Aquaculture , 292, 214-218. http://dx.doi.org/10.1016/j.aquaculture.