# EVALUATION OF DIETARY GARLIC (Allium sativum) EXTRACT ON GROWTH, SURVIVAL AND STRESS BIOMARKERS ON HETEROCLARIAS FINGERLINGS UNDER LABORATORY CONDITIONS IN MINNA, NIGERIA

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## ABSTRACT

An Eight-week study was carried out on effects of garlic (*Allium sativum*) on some indices of growth performance and stress biomarkers in Heteroclarias fingerlings. Fifteen fingerlings of initial mean weight (1.90±0.21g) were used and randomly separated into four experimental groups (0, 0.5, 1.0 and 1.5 % garlic inclusion) with 2 replicates and fed twice daily till satiation. Survival rates, stress biomarkers, growth and physicochemical parameters were determined based on standard experimental procedures. Phytochemical analyses showed that garlic was richest in tannin (2.52 ± 0.12 g/100g). Feeding fingerlings with 1.5 % inclusion affected total length and body weight during the study period. This inclusion regimen had the highest ($p<0.05$) effects on final body weight (14.40±2.67 g), weight gain (12.5±0.40 g), percentage weight gain (1250.00 %), length gain (8.36±0.21 cm) and survival rate (97.50 %). Superoxide dismutase and Catalase activities in serum were also highest ($p<0.05$) in this inclusion regimen, 1.23±0.03 and 4.67±0.34 U/L, respectively. However, there were no significant differences ($p>0.05$) in physicochemical properties of garlic-supplemented-diets and control water media. The study revealed that garlic can be used as a feed additive in aquaculture to improve fish growth and disease resistance. There is therefore, the need to study the mechanism of activities of the active components of dietary garlic in fish culturing.

**Keywords*:*** Dietary inclusion, Stress Biomarkers, Growth, Survival and Physicochemical.

## INTRODUCTION

The success of any intensive aquaculture dependent solely on the quality of feed and feeding strategies which are the most important factors influencing growth performance, feed utilization and body chemical composition of fish (Okumus and Mazlum, 2002; Latife and Murat, 2016). The major aim of an intensive aquaculture is to gain maximum yields from water resources by providing artificial diets that thrives fish growth and gain maximum weight in the shortest time as possible (Bhosale *et al.,* 2010). Recently, some valuable components stimulating the defence system and stress responses of fish, the so-called immunostimulants, have been isolated from plants, animals or microorganisms (Sakai, 1999). Garlic (*Allium sativum*) is a perennial bulb-forming plant that belongs to the genus *Allium* in the family Liliaceae, which has been used for centuries as a flavouring agent, traditional medicine, and a functional food to enhance physical and mental health (Dong- Hoon *et al.,* 2012). In addition, it was probably known as one of the earliest medicinal plants (Farahi *et al*., 2010), the use of garlic in aquaculture became popular for providing protection against diseases or inducing fish feeds as a growth promoter. Garlic has been studied in different forms of extracts: aqueous, ethanol and dried powder (Shin and Kim, 2004). It contains a variety of organosulfur compounds such as Allicin, Ajoene, S-allylcysteine, Diallyl disulfide, S-methylcysteine sulfoxide and S-allylcysteine (Chi *et al.,* 1982). Studies on garlic as an alternative of growth promoter in livestock production were conducted and its beneficial effects on growth, digestibility and carcass traits have been reported (Bampids *et al.,* 2005; Tatara *et al.,* 2008). Dietary garlic as a growth promoter in Nile tilapia (Oreochromis niloticus) improved body weight gain, feed intake and feed efficiency (Diab *et al.,* 2002; Shalaby *et al.,* 2006). Fishes from the intensive culture systems are continuously exposed to a form of stress, so it can lead to organism significant changes of biochemical and physiological level. Stress factors include: repeated handling, high density, therapeutic treatments, improper water chemistry and temperature changes. Therefore, Garlic in fish feed, can reduce levels of stress (Marwane *et al*., 2015). Soltan and El-Laithy (2008) also reported that the incorporation of 1% garlic into diets improved survival rate of Nile tilapia. The authors also observed that the use of garlic resulted in good survival rates, but feeding with the higher dose of garlic for extended periods gave better results. Therefore, this study was to evaluate the effects of garlic (Allium sativum) extract as a natural feed supplement on some growth parameters, survival rates and stress biomarkers of Heteroclarias fingerlings.

## MATERIALS AND METHODS

**Experimental Site:** The study was conducted at the Animal Biology laboratory of the School of Life Sciences, Bosso Campus, Federal University of Technology, Minna, Niger State.

**Source of Experimental Fish:** Two hundred and twenty fingerlings of Heteroclarias (220) of four (4) weeks old were purchased from the Water resources, Aquaculture and Fisheries Technology (WAFT) Farm, Bosso campus located at Federal University of Technology Minna, Niger State.

**Acclimatization of the Fingerlings:** The fingerlings were acclimatized in rearing plastic aquaria tanks (60cm x 45cm x 50cm3) containing twenty five (25) litres of borehole water for a period of seven days to allow them to recover from transportation stress and fed twice daily to satiation with a conventional feed (Aquafeed) after every 12 hours between 8am and 8pm (Ayanwale *et al*., 2014). Satiation level was considered when fingerlings no longer attacked food particles and refused feeding (Latife and Murat, 2016). Water exchange was done twice in a week and replaced with fresh bore water between 08:00 and 10:00 hours (Ayanwale *et al*., 2018).

**Feed formulation and Composition of Experimental Diet:** The feed was formulated using the Pearson square method and oven dried at a temperature of 35°C for 8 hours to ensure the moisture content is not high to prevent spoilage (Ndong and Fall, 2007).

**Experimental Set-up:** The experiment consisted of four (4) treatments and each with two (2) replicates. Treatments 1, 2, 3 had 0.5, 1.0 and 1.5 % and 4 the control (0%) of garlic diet respectively. The aquarium tanks were filled with 25 litres of borehole water and 15 fingerlings were carefully distributed into each tank, covered with nets to prevent the fish from jumping out (Olufayo, 2009).

The Heteroclarias fingerlings were fed to satiation every morning and evening (Ayanwale *et al.*, 2014). Water exchange was done twice a week and the experiment was carried out in a closed fresh bore hole water system for a period of eight (8) weeks.

**Determination of Physico-Chemical Parameters:** Water temperature was determined weekly with mercury in bulb thermometer (10-110℃ range). Dissolved Oxygen, Biochemical Oxygen Demand (BOD), Hydrogen Ion Concentration (pH) were determined weekly based on standard methods (American Public Health Association, 2000).

**Determination of Phytochemical Constituent of Garlic (Allium sativum):** The raw Garlic (*Allium sativum* ) was sliced, crushed, dried in air, pulverized in to powder and extracted by soaking 100g of the pulverized garlic in 600ml of distilled water for 24 hours. The residue and the filtrate were obtained by filtering the soaked garlic (*Allium sativum*). The residue was dried on a cardboard paper and 380ml filtrate was obtained as extract (Obadoni and Ochuko 2002).

**Determination of Growth Parameters:** Total length, Length gain, Final length, Weight, Final weight, Weight gain and Percentage weight gain were determined according to the method described by Ayanwale *et al.*, 2014.

**Determination of Survival rate:** This was determined daily by counting the number of fingerling left in each experimental tanks (Akinwole *et.,al* 2006).

**Enzyme Assay:** The activities of superoxide dismutase (SOD) in the blood and catalase (CAT) in the serum were determined spectrophotometrically according to the methods of Misra, 1972 and Sinha (1972) respectively.

**Data Analysis**: Data collected were analysed by the analysis of variance (ANOVA) using a Computer Statistical Package for Social Sciences (SPSS) and Duncan Multiple Range Test (Duncan, 1955) method was used to separate the means.

## RESULTS AND DISCUSSION

### Quantitative phytochemical screening of aqueous extract of *Allium sativum* bulb

The results (mean± standard error) of phytochemical constituents of Garlic (*Allium sativum*) are presented in Table 1. The Table revealed that Tannin (2.52 ± 0.12 g/100g) was significantly ($p<0.05$) highest among the constituents. Although, the percentage composition of Saponin Alkaloid and Flavonoid were not significantly ($p>0.05$) different.

**Table 1: Mean± standard error quantitative phytochemical screening of aqueous extract of *Allium sativum* bulb**

| Phytochemicals (g/100g) | Status | percentage composition |
|---|---|---|
| **Tannins** | + | $2.52\pm 0.12^{c}$ |
| **Saponins** | + | $0.24\pm 0.04^{a}$ |
| **Cardiac glycoside** | + | $1.88\pm 0.26^{b}$ |
| **Alkaloid** | + | $0.12\pm 0.02^{a}$ |
| **Flavonoid** | + | $0.05\pm 0.03^{a}$ |

**Values along the same column with different superscripts are significantly different ($p < 0.05$); Key = + present; - Absent.**

### Growth performance indices and survival rates of laboratory reared Heteroclarias fingerlings fed garlic supplemented diets for a period of 8 weeks

The results (mean± standard error) of growth performance indices and survival rates of Heteroclarias fingerlings fed with different levels of garlic diets are presented in Table 2.

Feeding fingerlings with 1.5 % inclusion affected total length and body weight during the study period. This inclusion regimen had the highest ($p<0.05$) effects on final body weight (14.40±2.67 g), weight gain (12.5±0.40 g), percentage weight gain (1250.00 %), length gain (8.36±0.21 cm) and survival rate (97.50 %).

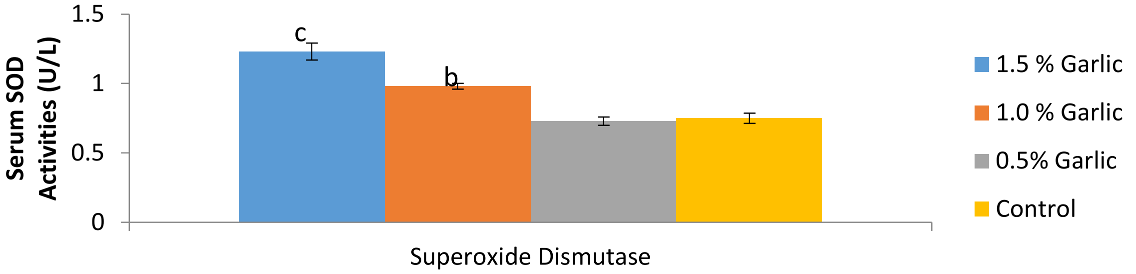
**Table 2: Mean± standard error of growth performance indices and survival rates of laboratory reared Heteroclarias fingerlings fed garlic supplemented diets for a period of 8 weeks**

| | Different Level (%) | | | |
|---|---|---|---|---|
| **Indices of growth performances** | 0.5 | 1.0 | 1.5 | Control |
| **Initial body weight (g)** | $1.90\pm0.0.21^{a}$ | $1.90\pm0.21^{a}$ | $1.90\pm0.21^{a}$ | $1.90\pm0.21^{a}$ |
| **Final body weight (g)** | $9.60\pm0.81^{a}$ | $9.60\pm0.24^{a}$ | $14.40\pm2.67^{b}$ | $9.80\pm0.37^{a}$ |
| **Weight gain (g)** | $7.70\pm0.60^{a}$ | $8.20\pm0.03^{b}$ | $12.50\pm0.40^{c}$ | $8.10\pm0.43^{a}$ |
| **Percentage Weight gain (%)** | $770.00^{a}$ | $820.00^{b}$ | $1250.00^{c}$ | $810.00^{b}$ |
| **Initial total length (g)** | $6.80\pm0.64^{a}$ | $6.50\pm0.19^{a}$ | $6.86\pm0.63^{a}$ | $7.00\pm0.27^{a}$ |
| **Final total length (cm)** | $10.76\pm0.81^{a}$ | $9.54\pm0.17^{a}$ | $15.22\pm2.01^{b}$ | $10.28\pm0.77^{a}$ |
| **Length gain (cm)** | $3.96\pm0.34^{a}$ | $3.64\pm0.43^{a}$ | $8.36\pm0.21^{b}$ | $3.28\pm0.43^{a}$ |
| **Survival rate (%)** | $91.04^{a}$ | $95.97^{b}$ | $97.50^{b}$ | $91.06^{a}$ |

**Values along the same row with different superscripts are significantly different ($p <0.05$)**.

### Serum Superoxide Dismutase (SOD) and Catalase activities of Heteroclarias fingerlings fed with different levels of garlic diets

The result (mean± standard error) of Serum Superoxide Dismutase (SOD) and Catalase activities of Heteroclarias fingerlings fed with different levels of garlic diets are presented in Figure 1.Superoxide dismutase and Catalase activities in serum were also higher ($p<0.05$) in 1.5% Garlic regimen, 1.23±0.03 and 4.67±0.34 U/L, respectively.



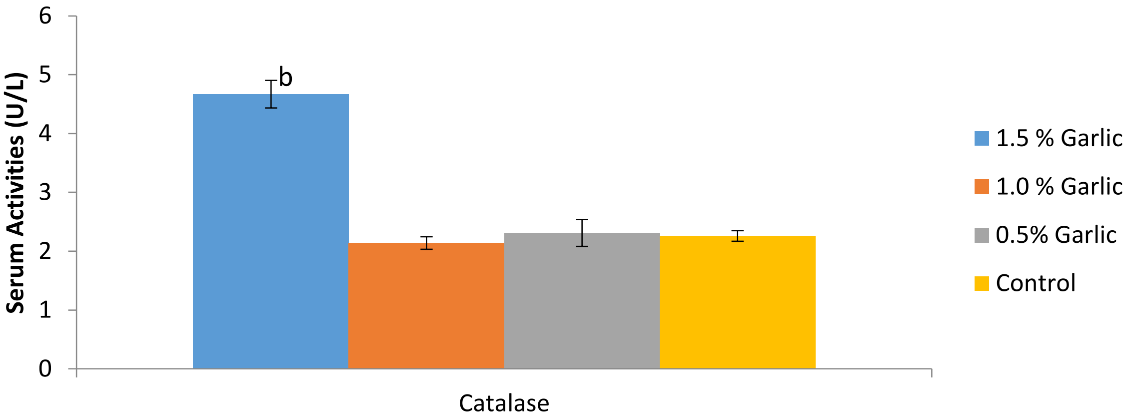




**Figure 1: Effects of garlic supplementation on Serum superoxide dismutase and catalase activities of Heteroclarias fingerlings. Bars with different superscripts are significantly different (p < 0.05).**

**Physicochemical parameters of rearing media of Heteroclarias fingerlings fed with different levels of garlic diets**

The results (mean± standard error) of physicochemical parameters of rearing media of Heteroclarias fingerlings fed with different levels of garlic diets are presented in Table 3. The physicochemical parameters measured were not significantly (p>0.05) affected by the control and inclusion of garlic in the diets.

**Table 3: Mean± standard error of physicochemical parameters of laboratory reared Heteroclarias fingerlings fed different levels of garlic supplemented diets for a period of 8 weeks**

| Garlic% | Temperature ($^0$c) | pH | DO(mg/l) | BOD(mg/l) |
|---|---|---|---|---|
| 0.5 | 27.00±0.42$^a$ | 7.50±0.40$^a$ | 1.10±0.12$^a$ | 1.10±0.21$^a$ |
| 1.0 | 28.00±0.67$^a$ | 7.40±0.37$^a$ | 1.20±0.03$^a$ | 1.00±0.24$^a$ |
| 1.5 | 29.00±0.92$^a$ | 7.60±0.52$^a$ | 1.30±0.16$^a$ | 1.20±0.32$^a$ |
| Control | 26.00±0.20$^a$ | 7.70±0.60$^a$ | 1.00±0.22$^a$ | 1.31±0.37$^a$ |

**Values along the same row with different superscripts are significantly different (p <0.05)**

The findings of this study confirmed the presence of phytochemicals such as alkaloids, flavonoids, cardiac glycosides, saponin, and tannin with highest percentage (2.52%) in the garlic bulb used in this study. These classes of compounds especially alkaloids, saponins, tannins and flavonoids are known to have curative activity against several pathogens (Usman *et al.*, 2009). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action in vertebrates such as humans and fish (Akinmoladun *et al.*, 2007). The fingerlings fed with 1.5% garlic supplemented diet indicated significant improvement in total length, body weight, weight gain and percentage weight gain. These could be attributed to the presence of allicin which promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the utilization of energy for growth (Khalil *et al.*, 2001). These findings were in consonance with the studies conducted on African catfish, *Clarias gariepinus* (Agbebi *et al.*, 2013); Rainbow trout, *Oncorhynchus mykiss*(Gabor *et al.*, 2012; Swordtail, *Xiphophorus helleri* (Kalyankar *et al.*, 2013) and Nile tilapia, *Oreochromis niloticus* (Metwally, 2009; Aly and Mohamed, 2010) where positive effects of administrating garlic in diets on growth and feed utilization of the above cited fish species were also reported . However, contrary to this finding, Abdel- Hakim *etal.* (2010) reported better achievements of dietary garlic on growth performance and feed utilization with low levels of garlic inclusion at 0.5% level were observed in Tilapia. The dissimilarities between the findings of this study and some of the previous works regarding the effects of dietary garlic on growth performance of fish, feed utilization could be attributed to the differences in fish species or fish size, environmental conditions such as water temperature, type or level of the additives accompanying the main ingredients in diet formulation, or type of the garlic source used in the feeds, fish physiology or a combination of these factors together (İrkin and Yiği, 2016) Garlic bulb has been reported to have these classes of compounds especially alkaloids, saponins, tannins and flavonoids and are known to have curative activity against several pathogens (Usman *et al.*, 2009).These attributes confirmed the highest percentage survival rate (97.50%) in the fingerlings fed with 1.5% garlic supplemented diet. To support the above assertion, İrkin and Yiği,( 2016) also observed that garlic incorporation in fish diets improved growth performance, feed utilization, fish health and welfare. Better growth effects have been reported with higher incorporation levels of garlic meal in diets for Nile tilapia by Shalaby *et al.* (2006) who tested garlic incorporation levels from 10 g/kg to 40 g/kg diet, and recommended the incorporation of 3% dietary garlic for an increased growth, reduction of total bacteria, and improvement of fish health and welfare. A complex system of numerous types of antioxidants (such as catalase, glutathione, SOD, and various peroxidases) have been reported to be present in aquatic animals such as fish (Liu *et al.*, 2010). Superoxide dismutase and catalase are among the important biochemical parameters and the first line of antioxidant enzymatic defence. Therefore, measurement of these antlioxidant parameters may provide a hint of the antioxidant status in fish, and these parameters can serve as biomarkers for oxidative stress (Zhang *et al.*, 2013). The increase in SOD and CAT activities of the fingerlings fed 1.5% of garlic diet could be due to the bioactive components of garlic known as allicin. This finding is also similar to the works of Giri *et al*., (2016) on *Labeo rohita* fingerlings who reported that supplementation of 5% Banana (Musa acuminata) peel for 8 weeks resulted in the highest SOD and CAT activities. Similarly, Metwally (2009) also documented that there was significant increase in antioxidant defence enzymes (SOD and catalase) following garlic supplementation in the diet of Tilapia Nilotica (*Oreochromis niloticus*). This therefore indicates that garlic inclusion in the diet of Heteroclarias fingerlings at an appropriate concentration (1.5% and 1.0 %) could stimulate the secretion of antioxidant enzymes as well as antioxidants, which can efficiently eliminate excess free radicals and regulate the balance of free radical in the fingerlings, resulting in improved antioxidant ability (Zhang *et al*., 2013). Bioactive compounds such as phenolic compounds in garlic might also be responsible for their antioxidant activities in the fingerlings (Rebello *et al*., 2014).

## CONCLUSION

The quantitative phytochemical screening of garlic revealed that garlic contain high percentage of tanin. The fingerlings fed with 1.5% of garlic diet had higher total length, body weight, and percentage weight gain and survival rate. The SOD and CAT biomarkers of the Heteroclarias fingerlings fed with 1.5% had higher antioxidant enzymatic defence. Garlic inclusion in fish diet at 1.5% (1.5g/kg) concentration is therefore beneficial for use in aquaculture to enhance the disease resistant status and growth performance of Heteroclarias fingerlings.

## RECOMMENDATIONS

Therefore, we recommend studies to reveal the mechanism of activities of the active components of garlic especially in fish culturing

## REFERENCES


Abdel-Hakim, N.F., Lashin, M.M.E., Al-Azab, A.A.M. and Ashry, A.M. (2010). Effect of fresh or dried garlic as a natural feed supplement on growth performance and nutrients utilization of the Nile tilapia *(Oreochromis niloticus*). *Egyptian Journal of Aquatic Biology and Fisheries*, 14, 19-38.

Agbebi, O.T., Ogunmuyiwa, T.G.and Herbert, S.M., (2013). Effect of dietary garlic source on feed utilization, growth and Histopathology of the African catfish (*Clarias gariepinus*). *J. Agric. Sci.* 5 (5), 26–34. http://dx.doi.org/10.5539/jas.v5n5p26.

Akinmoladun, A.C, Ibukun, E.O, Afor, E, Obuotor, E.M, and Farombi, E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum. Sci. Res. Essay*; 2: 163-166.

Akinwole, A. O. and faturoti, E. O. (2006). Biological performance of African Catfish (*Clarias gariepinus*) cultured in recruiting system in Ibadan. *Aquaculture Engineering* 36:18-32.

Aly, S.M. and Mohamed, M..F.(2010).Echinacea purpurea and Allium sativum as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). *J. Anim. Physiol. Anim. Nutr.* 94 (5), 31–39.

APHA (2005). American Public Health Association. Standard methods for examination of water and waste waters. 18th Edition. Prepared and published by American Public Health Association, American Water Works Association and Water Environment Aquatic Research, 41(2), 211-217.

Ayanwale, A. V., Tsadu, S. M., Kolo, R. J., Lamai, S. L., Falusi, F. M., and Baba, B. M. (2014). *Advance in Agriculture and biology*.1 (3):135-139.

Ayanwale, A. V., Z. Saidu, S. M. Tsadu, M. O. Mustapha and S. A. Aransiola (2018). Influence of Photoperiodic Levels on Skin Melatonization in Heteroclarias Fingerlings in Relation to Water Physico-Chemical Properties in Minna, Nigeria. *Malaya Journal of Biosciences* 5(2):70-78.

Bampidis, V.A., Christodoulou V, Christaki E, Florou-Paneri P, Spais, A.B. (2005).Effect of dietary garlic bulb and garlic husk supplementation on performance and carcass characteristics of growing lambs. *Animal Feed Sci. Technol.* Article in press

Bhosale, S.V., Bhilave, M.P. and Nadaf, S.B. (2010). Formulation of fish feed using ingredients from plant sources. *Research Journal of Agriculture Science* 1, 284-287.

Chi, M.S, Koh, E.T..and Steward, T.J. (1982). Effects of garlic on lipid metabolism in rats fed cholesterol or lard. *J.of Nutr*. 112:241–248.

Diab, A.S., EL-Nagar G.O.Y.M. and, Abd-EI-Hady, Y.M. (2002).Evaluation of Nigella sativa L. (black seeds; baraka),Allium sativum (garlic) and Biogen as feed additives on growth performance and immunostimulants of *O..niloticus* fingerlings. *Suez Canal Veterinary Medicine Journal.* ; 2:745-775.

Dong-Hoon Lee, Seong-Ryul Lim, Jung-Jo Han, Sang-Woo Lee, Chang-Six Ra,and Jeong-Dae Kim (2014). Effects of dietary garlic powder on growth, feed utilization and whole body composition changes in fingerling Sterlet sturgeon, *Acipenser ruthenus*. *Asian-Australian Journal of Animal Sciences*, 27 (9) 1303-1310.

Duncan, D.B. (1955). Multiple Ranges and Mutiple F-test.Biometrics. 11:1-42

Farahi, A., Kasiri, M., Sudagar, M., Iraei, M. and Shahkolaei, M. (2010). Effect of garlic (Allium sativum) on growth factors, some haematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). Aquaculture,Aquarium, Conservation & Legislation *International Journal of the Bioflux Society*,3, 4.

Gabor, E.F., S_ara, A., Bent_ea, M., Creta, C. and Baciu, A.(2012). The effect of phytoadditive combination and growth performances and meat quality in rainbow trout (*Oncorhychus mykiss*).*Anim.Sci.Biotechnology*.45(2),http://www.spasb.ro/index.php/spasb/article/ view/276.

Giri, S. S.. Jai Suda S., Sukumaran, V., and. Park, S. C, (2016).“Dietaryemodin affects the growth performance, immune responses,and disease resistance of *Labeo rohita* against *Aeromonas hydrophila*,” *Aquaculture International*, (24) : 1, 85–99.

Irkin ,L. C. and Yigit, M. (2016). The use of garlic (allium sativum) meal as a natural feed supplement in diets for European seabass (*Dicentrarchus labrax*) juveniles.*Journal of Aquaculture Engineering and Fisheries Research.* 2 (3), 128-141.

Kalyankar, A.D., Gupta, R.K., Bansal, N., Sabhlok, V.P., Singh, D., (2013). Effect of garlic (*Allium sativum*) against *Aeromonas Hydrophila* and health management of Swordtail, *Xiphophorus heller*i . *J. Environ. Sci. Sustainability JESS* 1 (2), 41–48.

Khalil, R.H., Nadia, B. M.and Soliman, M. K., (2001). Effects of Biogen and LevamisolHcl on the immune response of cultured *Oreochromis niloticus* to *Aeromonas hydrophila* vaccine. *Beni-Suef Vetenary Medicine Journal*, 11(2), 381–392,

Latife, C. Irkin and Murat, Yigit (2016). The use of garlic Allium sativum) meal as a natural feed supplement in diets for European seabass (*Dicentrarchus labrax*) juveniles. *Journal of Aquaculture Engineering and Fisheries Research*, 2 (3) 128-141.

Liu, B., Ge, X., & He, Y. (2010). “Effects of anthraquinones extracted from Rheumofficinale Bail on the growth, non-specific immune response of *Macrobrachium rosenbergii*,” *Aquaculture*, 310, (1-2) 13–19.

Marwane Erguig, Ahmed Yahyaoui, Mohamed Fekhaoui and Mohammed Dakki (2015). The use of garlic in *aquaculture*. *European Journal of Biotechnology and Bioscience*, 3, ( 8) 28-33.

Metwally, M.A.A. (2009). Effect of garlic (*Allium sativum*) on some antioxidant activities in Tilapia nilotica *(Oreochromis niloticus*). *World Journal of Fish Marine Science*, 1, 56-64.
Misra, H. P. and Fridovich, J. (1972). The role of superoxide dismutase anion in the antioxidant of epinephrine and a simple assay for superoxide dismutase. *Journal of Biology and Chemistry*, 27(4), 221-226.

Ndong D. and Fall J. (2007). The effect of garlic (Allium sativum) on growth and immune responses of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*).*Document Scientifique du CRODT*, 14:1-22.

Obadoni, B.O. and Ochuko, P.O. (2002).Phytochemical Studies and Comparative Efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences.*8 (2):203-208.

Okumus, I. and Mazlum., M.D. (2002). Evaluation of commercial trout feeds: Feed consumption, growth, feed conversion, carcass composition and bioeconomic analysis. *Turkish Journal of Fisheries and Aquatic Science*, 2, 101-107.

Olufayo, M.O. (2009). Haematological characteristics of Clarias gariepinus (burchell, 1822) juveniles exposed to *Derris elliptica* root powder. *African Journal of Food Agriculture, Nutrition and Development,* 9 (3), 920-933.

Rebello, L. P. G., Ramos, A. M. Pertuzatti,, P. B., M. T. Barcia M. T., Castillo-Mu˜noz,N and Hermos´ın-Guti´errez, I. (2014). “Flour of banana (*Musa acuminata*) peel as a source of antioxidant phenolic compounds,” *Food Research International*, 55, 397–403.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, 172, 63-92.

Shalaby, A.M., Khattab, Y., Abdel-Rahman, A.M. (2006). Effects of garlic, *Allium sativum* and chloram-phenicol on growth performance, physiological parameters and survival of Nile tilapia, *Oreochromis niloticus*. *Journal of Venomous Animal Toxins Including Tropical Diseases*, 12:172-201.

Shin S.H.and Kim, M.K. (2004). Effect of dried powders or ethanol extracts of garlic flesh and peel on lipid metabolism and antithrombiogenic capacity in 16-month-old rats. *J of Nutr. and Health.* 37:515–524

Sinha, A. K. (1972). *Colorimetric assay of catalase*, Analytical biochemistry 47:389-394.

Soltan, M.A. and El-Laithy, S.M. (2008). Effect of probiotics and some spices as feed additives on the performance and behaviour of the Nile tilapia, *Oreochromis niloticus*. *Egyptian Journal of Aquatic Biology and Fisheries*, 12(2), 63-80.

Talpur, A.D. and Ikhwanuddin, M. (2012). Dietary effects of garlic (Allium sativum) on haematoimmunological parameters, survival, growth,and disease resistance against Vibrio harveyi infection in Asian sea bass, Lates calcarifer (Bloch). Aquaculture, 364-365, 6-12.

Tatara, M.R, Sliwa, E, Dudek, K, Gawron, A. and Piersiak, T. (2008). Aged garlic extract and allicin improve performance and gastrointestinal tract development of piglets reared in artificial sow. *Ann. Agric. Environ. Med*., 15:63–69

Usman, H., Abdulrahman, F.I. and Usman, A. (2009). Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *Afr. J. Trad. CAM*. 6 (3): 289 – 295.

Zhang, C.-N, X. Li, X.-F and Xu, W.-N. (2013). “Combined effects of dietary fructooligosaccharide and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*),” *Fish and Shellfish Immunology*, 35(5): 1380–1386.