Sudan University of Science and Technology

College of Animal Production Science and Technology

Department of Fisheries and Wild life Science

The Effects of different *Nigella sativa* meal and oil level on Growth performance ,toxitity and enzymes in Nile Tilapia

تأثير الاختلاف لمستويات مختلفة من بذرة الكمون وزيتها في أداء النمو والسميه والانشطة الانزيمية

A Thesis Submitted in Partial Fulfillment of the Requirement of the B.Sc. Degree in Fisheries and Wildlife Science (Honor)

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القرأن الكريم

قَالَ تَعَالَىٰ:

أَعُوذُ بِٱللَّهِ مِنَ ٱلشَّيۡطَٰنِ ٱلرَّجِيمِ

﴿ وَجَعَلۡنَا مِنَ ٱلۡمَآءِ كُلَّ شَيۡءٍ حَيٍّۚ أَفَلَا يُؤۡمِنُونَ ٣٠ ﴾

سورة الأنبياء: ٣٠

DEDICATION

TO MY LOVELY FAMILY

TO ALL MY

TO MY FRIENDS

WITH ALL OUR DOAA

ACKNOWLEDGMENT

All gratitude is due to Allah who guided us to bring forth to light this project. We feel indebted to our supervisor Dr.Assed H. Widaa for his skilful guidance and invaluable suggestion at various stages of this work, we simply cannot find the right words to express our gratitude to him, patience, advice and unlimited support were our light to find out our way throughout the project period.

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To All We are grateful

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ABSTRACT

Using Statistical Analysis `the obtained data in this study were subjected to one-way (ANOVA) analysis of variance (snedecor,and Cochranl, 1986),(Sokal,and Rohlf,1981). Data were entered and analyzed using Statistical Package for Social Sciences program (SPSS) for Windows.

This study was conducted to investigate the immune modulation effects of *Negilla sativa* meal and oil as feed additives for Nile Tilapia (*Oreochromis niloticus*), influence of such additives on the digestive enzyme activities, to determine the effects of cumin on the feed conversion rate (FCR) in Nile tilapia (O. niloticus).

The results showed that the diet containing 10 and 15% *Negilla sativa* meal improved significantly (P>0.05) growth rate compared with *Negilla sativa* oil 6 and 12% .The effects on serum protein and enzyme levels were investigated. Although the activity of alkaline phosphates increased over experimental period between treatments, the increases were statistically significant.

The results showed the effect of chloride solution for fish fed the *Negilla sativa* meal 15% more resistant to stress and toxicity .

Key Words:

Specific Growth Rate (SGR)- Survival Rate (SR) - Feed Conversion Ratio (FCR) - Protein Efficiency Ratio (PER)

الخلاصة

باستخدام التحليل الاحصائي تعرض البيانات التي تم الحصول عليها في هذه الدراسة الى تحليل (One –Way (ANOVA تحليل التباين وتم ادخال البيانات وتحليلها باستخدام الحزمه الاحصائيه للعلوم الاجتماعية.

أجريت هذه الدراسة للتحقيق في آثار المناعة من مسحوق بذور الكمون(Negilla sativa) وزيت بذور الكمون كإضافات غذائية للبلطي النيلي (OreochromisNiloticus) ، وتأثير هذه الإضافات على الأنشطة الإنزيمية في الجهاز الهضمي، لتحديد آثارالكمون على معدل التحويل الغذائي (FCR) في البلطي النيلي (O. niloticus).

وأظهرت النتائج أن اتباع نظام غذائي يحتوي على مسحوق بذور الكمون 10 و 15٪ تحسن معدل النمو بشكل ملحوظ ($P < 0.05$) مقارنة مع زيت بذور الكمون 6 و 12٪ .وقد تم التحقيق من التأثيرات على البروتين في الدم ومستويات الانزيم. على الرغم من أن نشاط الفوسفات القلوية زاد خلال الفترة التجريبية بين المعاملات ، كانت الزيادات كبيرة إحصائيا.

واظهرت التتائج ايضا ان تأثير محلول الكلور على الأسماك التي تتغذى على مسحوق بذور الكمون 15٪ أكثر مقاومة للإجهاد و السمية .

مفاتيح الكلمات:

معدل النمو – معدل الحياة – معدل التحول الغذائي – معدل البروتين المأخوذ

CHAPTER ONE
INTRODUCTION

1.1. Aquaculture Background

Aquaculture, also known as aqua farming, is the farming of aquatic organisms such as fish, crustaceans, mollusks and aquatic plants.(Klinge,et al,. 2012) Mariculture refers to aquaculture practiced in marine environments and in underwater habitats.

According to the (FAO 2011) aquaculture "is understood to mean the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. (FAO 2011) There are issues about the reliability of the reported figures. *(Watson, et al. 2001)* Further, in current aquaculture practice, products from several pounds of wild fish are used to produce one pound of a piscivorous fish like (SCA, 2005).

1.2. Nile tilapia production in africa

In African environment, the most rare production factor is capital. Yet, the most efficient systems are those making intensive use of the most abounding production inputs, in this case, labor. The chosen systems must therefore rely on productive combinations that take into account the limitations in production (financial, labor, land and marketing constraints).Regarding financial constraints, models requiring an important workingCapital are not suited for rural development (Koffi, et al, 1996).

1.6. *Nigella sativa*

Nigella sativa, the genus name *Nigella* is a diminutive of the Latin Niger (black), referring to the seeds (Hyam and Pankhurst, 1995), an annual herb that belongs to the botanical family of *Ranuculaceae*, showed antibacterial fungicidal effects (Akgul, 1989). *Nigella sativa* seeds have been used as enhancer for performance, growth and immune system of some fish species (Abdel-Ghaffar, et al, 2003; Diab, et al., 2008).

Fish are exposed to some pathogenic microorganisms since they survive in an unfavorable environment. Hence, defense system of fish is very important against microorganisms. The use of antibacterial drugsin aquaculture is risky due to cross resistance against pathogens and residues in tissues (Gyles,et al. 1977). Therefore, using organic, inorganic and synthetic matters as immune stimulant has increased in recent years. For this purpose, levamisole (Findlay and Munday, 2000; Cuesta, et al. 2004), glucan (Jeney and Anderson, 1993; Dalmo and Seljelid, 1995), vitamin C and E (Blazer and Wocke, 1984; Hardie, et al. 1990; Ortuno, et al. 2001), chitin (Sakai, et al. 1992), FK-565 (Lactoyltetrapeptide) (Kitao, et al. 1987) FCA (Olivier, et al. 1985; Kajita, et al. 1992) have been used as immune stimulant and investigations are still being carried out on these substances. The use of plants as medicines began durin9g the earliest years of human evolution. *Nigella sativa* Linn, it has been employed for thousands of years as spice and food preservative, as well as a protective and curative remedy for numerous disorders. It is known to have many medicinal properties in traditional medicine (Salem and Hossain, 2000). Wild growth of this plant is widespread in southern Europe, northern Africa and Asia (Salem, 2005).

The diuretic, antihypertensive, antibacterial and thelmintic effects of black cumin seed in human and animals have been investigated (Agarwal, et al. 1979; Hanafy and Hatem, 1991; Zaoui, et al. 2000 and Mahmoud, et al. 2002). Additionally, as ant diabetic (Kanter, et al. 2003), antioxidant (El Saleh, et al. 2004)andanti-inflammatory (Zedlitz, et al.2002). *Nigella sativa* grows to 20–30 cm (7.9–11.8 in) tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually colored pale blue and white, with five to ten petals.

nigella sativa oil contains conjugated linoleic (18:2) acid, thymoquinone, nigellone (dithymoquinone), (Mohammad and Batool, 2002) melanthin, nigilline, and trans-anethole (Bharat, 2015). Mainly for its seed oil extract, thymoquinone, *Nigella sativa* is under research for its potential to affect human diseases (Ali and Blunden, 2003) such as cancer or medical conditions such as dyspepsia(Banerjee, et al, 2010).

1.4. Objective Of Study

1. To determine the effects of cumin on the feed conversion rate (FCR) and specific growth rate (SGR) in Nile tilapia.

2. To investigate the immune modulation effects of, *Nigella sativa* as feed additives for Nile tilapia.

3. To the influence of such additives on the digestive enzyme activities.

CHAPTER TWO

LITERATURE REVIEW

2.1. Nile Tilapia Biology

Nile tilapia is a deep - bodied fish with cycloid scales. Silver in color with olive/grey/black body bars, the Nile tilapia often flushes red during the breeding season (Picker and Griffiths 2011). It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) (FAO, 2012). The average size (total length) of nile tilapia is 20 cm (Bwanika, et al. 2004). nile tilapia is native to central and North Africa and the Middle East (Boyd, 2004). It is a tropical fresh water and estuarine species. It prefers shallow, still waters on the edge of lakes and wide rivers with sufficient vegetation (Picker and Griffiths, 2011).

2.1.1. Classification And Taxonome Of Nile Tilapias

The name tilapia was an effort by A. Smith its author to spell the bushman word for „fish" which began with a click, rendered (Trewavas, 1982). They represent a large number of freshwater fish within the family Cichilidae. There are three major genuses of tilapia which have been used in aquaculture, namely; Tilapia, Oreochromis and Sarotherodon. The genus Tilapia was first described by Smith (1840). The genus Tilapia was later split based on breeding behavior and feeding habits, into two subgenera: Tilapia (substrate spawners) and Sarotherodon mouth brooders, (El-Sayed2006).The subgenus Sarotherodon was raised to a genus and further subdivided into two genera, Sarotherodon and Oreochromis. This subdivision was based on whether parental females *Oreochromis* or males *Sarotherodon* or both parental sexes *Sarotherodon* perform the mouth brooding behavior (El-Sayed, 2006).

Tilapias of the genus Tilapia guard their eggs and fry. They are generally macropyhte feeders with coarse teeth and less than 12 gill rakers on the lower limb of the first gill arch (Popma and Masser, 1999).The mouth brooding *Sarotherodon* and *Oreochromis* incubate the fertilized eggs and fry in the mouth of the male or female. They are primarily microphagous, feeding on phytoplankton, periphyton and detritus(El-Sayed, 2006). They have fine teeth, more than 14 gill rakers on the lower limb of the first gill arch (Matthew, 1992). The *Sarotherodon* and *Oreochromis* genuses differ in the response of the post larvae to their parents. In *Oreochromis* they respond positively to the retrieving motions of the mother, whereas in *Sarotherodon*neitheir parents or young interact positively and the released young form schools and avoid their parents (FishelsonandYaron, 1983).

the most commonly cultured species of tilapia are *oreochromisniloticus*, *o. mossambicus* and *o. aureus*, and then tilapia rendalli. T. rendalli may be the best candidate for extensive, semi -extensive or polyculture as it feeds on higher plants and has areasonable growth rate when reared in extensive systems and supplemented with plant material (Chikafumbwa, 1996).

2.2.Nile Tilapiaas Desirable Aquaculture Species

In Africa, some 35 million people depend wholly or partly on the fisheries sector for their livelihood (FAO, 1996).The consumption and demand for fish as a source of protein is on the increase in Africa, becausein this region, poverty and lack of basic food nutrients are severe. Since capture species that are presently exploited seem to have reached their natural limits (FAO, 1996), there is considerable need to expand aquaculture in Africa in order to improve food security (Jamu and Ayinla,2003).

Tilapia farming is an old traditional practice in some African countries (Popma and Michael, 1999), where it forms a source of fish protein to many low-income communities (Liti et al., 2005).In these areas protein is the most deficient among nutrients and there has been increased pressure on the exploitation of the wild fishery resources from the natural aquatic ecosystems (Liti et al., 2005). As a result of the decrease in wild fishery, fish farming practices have increased in the rural areas.

Owing to a number of favorable characteristic traits, tilapia species are ideal for fish culture in tropical regions. There are various species of genus tilapia which are commonly farmed in the tropics particularly in Africa. They are normally very hardy and are acclimated to high temperatures and minimal dissolved oxygen content. Most species are omnivorous and are capable of tolerating a high salt content in the water. Up to the size of 4-5 cm Tilapia fry eat natural food, which necessitates pond manuring during this period. On the other hand, plant residues like leaves of cassava *Manihotesculenta* (Crantz), sweet potato *Ipomeabatatus* (L) Lam, and the banana *Musa paradisiacal* (L) are suitable for supplemental feeding of herbivorous species. Feeding material can also be easily obtained from domestic sources, hoteland industrial sources (Martena and Berka, 1982).

2.3. Nutrient Requirement For Nile Tilapia

2.3.1. Protein And Amino Acids

Protein is the most significant component in fish feeds because of its high cost and it is an important constraint to growth (Reay, 1979). The gross digestible protein requirements for a number of fish species have been determined and mostly range from 28-50% of the total dry weight.

An alternative to reducing the protein costs is to utilize cheaper sources of protein, but here the quality of protein in reference to the amino acid composition gains a critical consideration. The minimum dietary level of an amino acid-balanced protein required for optimum growth in absence of natural food is near 50% for tilapia fry and decreases to about 35% as fish increase to 30g in size. Protein requirements for larger fish have been reported to vary from 25% to 35% of the feed; this varies with the size of the fish, amount of natural food in the culture system, and dietary factors such asquality of protein and energy level(Lim, 1989). Tilapias require at least 10 essential amino acids. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine,tryptophan, and valine. Tilapias efficiently digest the fish meal, meat meal and bone meal (Popma, 1982).

2.3.2. Energy

According to(Kubaryk, 1980) tilapia digest gross energy in most commercial feedstuffs. However, limited digestion occurs in highly fibrous feedstuffs, such as alfalfa meal and coffee pulp well for energy needs. They digest carbohydrates in feedstuffs relatively well, much better than salmonids, but as the percentage of starch in the diet increase, digestibility decreases. Fats or proteins are more digestible to tilapia than are carbohydrates.

2.3.3. Essential Fatty Acid

Tilapias appear to have a dietary requirement for fatty acids of the linoleic (n-6) family. Supplementation of tilapia diets with vegetable oils (soya bean or corn oils) rich in 18:2 n-6 has given better performance than those containing fish oils high in 20:5 n-3 fatty acids (Takeuchi et al.,1983a). The optimum dietary levels of n-6 fatty acids have been estimated to be about 0.5% for nile tilapia (Takeuchi et al., 1983b). Deficiency signs in fish fed with diets deficient in n-6 and n-3 fatty acids in tilapia are reduced appetite; retarded growth; and swollen, pale, and fatty livers. Tilapias do not tolerate as high levels of dietary fat assalmonids. A dietary lipid level in excess of 12% depresses growth of juvenile *O.aureus* and *O.niloticus* hybrids (Jauncey and Ross, 1982).

2.3.4. Vitamins

Relatively little information is available on vitamin requirements of tilapias. One reason for this is that most tilapia culture occurs in ponds where the fish consume large amounts of natural foods which probably satisfy their vitamin needs. Vitamin supplements are often minimal in practical feeds for tilapia cultured in semi and extensive ponds. In intensive culture systems where limited or no natural food organisms are present, vitamins must be added to commercial feeds. Due to lack of information on vitamin requirements for tilapia, allowances established for other warm water species are used. Metabolically tilapias probably have similar vitamin requirements as other warm water fishes (Lim chorn, 1989).They show the classical vitamin C deficiency signs when deprived ofnatural foods. Vitamin E deficiency causes reduced growth rate, ceroidsin liver and kidney, failure of mature males to develop sexual coloration, and degenerative changes in the skeletal muscle. (Lovell and Limsuwan, 1982) showed that *O.niloticusproduced* vitamin B12in their long intestinal tract through bacterial synthesis and did not require the vitamin in their diet. Other vitamins may be synthesized by intestinal micro-organisms.

2.3.5. Minerals

Like other fin fishes, tilapia get significant amount of minerals, such as calcium, from water. Although there is lack of information on the mineral requirements for tilapia, it is likely that they require most of the minerals known to be essential for other finfish species. The dietary level of available phosphorus required for maximum growth and normal bone mineralization of *O. niloticus* was estimated to be less than 0.9 % (Watanabe et al., 1980).

2.6. Use Of Plant Material As Feed In Culture Of Nile Tilapia

Fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the world (FAO, 2003).It is one of the fundamental challenges facing the development and growth of aquaculture in the African continent. As a result fish feed development in sub-Saharan Africa has not made a significant progress in aquaculture as expected.

According to (Hetch, 2000), it is observed that the research on inexpensive feed ingredients has not contributed greatly to aquaculture development in Africa and suggested that more research on how best plant protein can be used as fish feed is required.Agricultural by-products form a big proportion of the plant materials currently fed to fish.They are used as supplementary feeds, and may consist of nutritional components absent in the natural aquatic environment. Rice bran, soybean meal, peanut meal, ground-nut oil cake and wheat middling's are some common items added as commercial feeds for fish ponds in Israel, India and Southeast Asia (Reay, 1979). Elsewhere, farmers in the Philippines have become more innovative and resourceful in utilizing whatever agricultural by-products that are available in their particular location. Theprimary objective being to fasten fish growth and increase production while at the same time reducing the feed cost (Pantastico, 1988).

Plant feed sources also generally known as non-conventional plant feed stuff (NCPF) which are many and abundant, almost in every locality in Africa. Their potential and utilization inaquaculture feed have been reviewed (Ugwumba and Ugwumba, 2003).NCPF inclusion levels in fish feeds however varies and largely depends on their availability, nutrient level, processing technique, species of fish and cultural farming pattern prevalent in the locality (Gabriel et al., 2007). The nutritional requirements in different species of fish vary and therefore the nutritional needs of the fish species to be cultured must be known for effective feed formulation to be achieved (Gabriel et al., 2007).

Research has also shown that substitution of fish oils by substantial vegetable oils can be achieved without negatively affecting growth performance of the Nile tilapia (Tortensen et al., 2000).. It has been reported that the use of various palm oil products such as crude palm oil (CPO), refined palm oilor palm fatty acid distillates in the diets of these fish elicited growth and feed utilization efficiency comparable with fish fed equivalent levels of dietary marine fish oils(Ng et al., 2006).

2.7. Cumin As A Feed Ingredient

2.7.1. Morphology Of The Plant

Nigella sativa is anannual flowering plant which grows to 20-90cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike .The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5-10 petals .The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds (Warrier et al, 2004).

2.8.2. *Nigella sativa* Uses To Feed Nile Tilapias

Streptococcal disease causes significant economic losses to the fish culture industry. The disease has been reported in cultured rainbow trout, yellowtail, striped bass, sea bream, and tilapia (Kitao, 2001). Streptococcus iniaeis commonly isolated from diseased tilapia in aquaculture farms in Israel and is controlled mainly by antibiotics (Abutbul, et al., 2004). However, the use of antibiotics is environmentally unacceptable because of the possible establishment of resistant microbial populations (Citarasu, 2010). Medicinal plants and spices are natural alternatives to antibiotics because they have active compounds such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines, and poly peptides (Citarasu, 2010). Herbs and spices can successfully replace antibiotics in fish aquaculture and bacterial disease control (Abutbul, et al., 2005; Bhuvaneswari and Balasundaram, 2006; Zakes, et al., 2008; Immanuel, et al., 2009; Ganguly, etal., 2010; Zilberg, et al., 2010; Harikrishnan et al., 2011; Yılmaz, 2011). *Nigella sativa* (Apiaceae;Cuminumcyminum) has been used as a spice since ancient times (Azeez, 2008).

It is cultivated in Mediterranean countries, Saudi Arabia, Iran, India, Mexico, China, Sicily, and Malta (Amin, 2001).India is the world's largest producer and consumer of *Nigella sativa*, with annual production ranging 0.1-0.2 million tons,and Turkey also cultivates *Nigella sativa* (Azeez, 2008). *Nigella sativa* has high anti-microbial and antioxidant activity with major compounds of 29.1% a-pinene and 17.9% 1.8-cineole (Gachkar et al.,2007; Singh etal.,2002). It issued in medicines as a stimulant of gastrointestinal, immune systems, and tyrosinase inhibitor activity, and has hypoglycemic, hypolipidaemic,andchemo protective effects (Azeez, 2008). Therefore, *Nigella sativa* can possibly be used for disease prevention and growth promotion in fish.

Stress conditions are responsible for fish disease in aquaculture which leads to bacterial disease.Uses of antibacterial drugs in aquaculture are risky due to cross resistance against pathogens,toxicity, residues in tissues and contaminate thenvironment with bioactive product (Hilly et al.,2002). Using of natural plants as immunestimulant in fish is more useful than antibacterial drugs (Aruna et al.,1996)(Blumenthol et al.,2001). Medicinal plant as immunestimulant can be used not only against disease but also as growth promoters (Porucu et al.,2009). From ancient time plants or plant products are used as medicine or therapeutic agent and it is well known (Awad et al.,2007). *Nigella sativa*. Commonly known as the black cumin seed is an annual herb that belongs to the botanical family of Ranuculaceae is a spice and preservative (Olusola et al.,2013).

The seeds of *Nigella sativa* have been used formedicinal purposes as a natural remedy for a number of illnesses and conditions that include bronchial asthma, rheumatism, hypertension,diabetes, inflammation, cough, headache, eczema, fever and influenza (Gabor et al., 2012). Research has been conducted on immune modulatory effect of *Nigella sativa* as an anti-tumor, bactericide, anticestode, antinematode, anti-inflammatory, analgesic, anti-diabetic and on some immunehematological parameters and specific as well as non-specific defence mechanisms of fish and also used as a growth promoter (Blumenthol, et al., 2001; Burits et al., 2000; Mohamed et al., 2010). However, there was no report of this herb as immunestimulant in thai koi or *Anabas testudineas* to prevent diseases.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

In the present study, Nile Tilapia (*Oreochromisniloticus*) fingerlings were obtained from the Collage of Animal Production Science and Technology, Sudan University of Science and Technology, Sudan. Fish were kept for 2 weeks in one 4.5meteric tone capacity tank for acclimation to the experimental conditions.

3.2. Experimental Desing

Fish were fed a mixture of the all tested experimental diets in order to habituate them to locally formulated feed. After the acclimatization period, healthy Nile Tilapia (*Oreochromisniloticus*) fingerlings (n=150) with an average of initial fresh body weight of 20±1.0g fish and were divided into equal five triplicate groups (each replicate contained 10 fish) and the replicate of each fish group was stocked in its corresponding plastic container after start of the experiment and throughout the experimental period (for 40 day) The fish were located in 15 rectangular plastic container (34*22*18cm). All the tanks were supplied with freshwater from water closed system and were continuously aerated by electric air ring compressor. The system was subjected to a photoperiod of 12 h light: 12 h darkness and temperature of 25±6°C and dissolved oxygen was 3.6±1.6mg, while PH was7.9±0.5. At the beginning, during and at the end of experiment, fish in each tank (n=10) and weighed collectively. Fish were fed the tested diets at a rate of 6% from their fresh body weight per day in three equal portions; each portion was given 9:00AM, 1:00PM and 5:00PM. During the experimental period, fish were weighed collectively 10 day to adjust the feeding level for the subsequent period according to the new biomass. The fish were not fed on the weighing day. Dissolved oxygen and water temperature were measured by Oxygen Meter, whereas PH was estimated by using kits. Total ammonia and Nitrite were determined by using Spectrophotometric analysis method and kits.

3.3.Prepation Experimental Feed

Acclimated Nile tilapia (*O .niloticus)* fingerlings were allotted into 5 equal treatments with three replicates (30 fingerlings for each). All treatments were assigned randomly in the aquaria and each aquarium was represented one observation. Fish of the first and second treatments fed on *Negilla sativa* 10% diet and *Negilla sativa* 15% whereas fish of the third and four treatments *Negilla sativa* oil 6% diet and *Negilla sativa* oil12%, fish of fifth treatment fed on basal diet and served as a control (Table3.1).

Table3.1:Formula and proximate analysis of the experimental diets supplemented with various levels of *Nigella sativa,Nigella sativa*oil and control.

Feed Stuffs	**Treatment**				
Treatments	**Control**	**Black seed meal%**		**Black seed oil%**	
Ingredients	**0%**	**10%**	**15%**	**10%**	**15%**
Fish meal	30	30	26.88	30	26.88
Ground nut cake	19	15	15	19	15
Sorghum meal	14.1	10	10	10	10
Wheat bran meal	15	15	15	15	15
Starch	10	10	8.12	10	6.12
Bread floor	5	5	5	5	10
Mineral mix	1	1	1	1	1
Vitamin mix	0.9	0.9	0. 9	0.9	0.9
Sun flower oil	5	3.1	3.1	3.1	3.1
Total	100	100	100	100	100
Proximate analysis					
dry matter	95	95.5	96.5	94.5	94
Crud protein	30.1	28.95	28.9	28.5	28.95
Ether extract	3.1	3.8	3.7	4.9	5.9
Fiber	2.92	2.86	2.92	2.84	2.82
Nitrogen free-extract	46.68	48.69	47.48	46.76	42.84
Ash	12.5	12.5	13.5	11.5	13.5
Moisture	5	4.5	3.5	5.5	6

3.4. *Nigella sativa* Preparation And Diets Formulation

Five diets treatment 1 (T1) treatment 2 (T2) treatment 3 (T3) treatment 4 (T4) treatment 5 (T5) were formulated, to meet the nutrients requirement of Tilapia (*O. niloticus*) fingerlings. All the experimental diets (T1 T2 T3 T4 and T5) were formulated to have almost similar crude protein, CP (29.08±1.08) and were arranged to contain *N. sativa* (10 and 15%) basal diet (T2 and T3), respectively. *N. sativa*oil (6 and 12%) basal diet (T4 and T5), respectively. Diet composition and chemical analysis are shown in (Table3. 1).

Nigella sativa were supplied from a local market of Al-Shagrah city, South Khartoum. The whole seeds and roots were crushed in a blender and mixed well with the treatments before their administration. Ingredients per each diet were grinded and mixed with vitamins and minerals mixture. Sun flower oil was sprayed on the mixture and the control diet. Distilled water was added to each diet until stiff dough resulted and this was pressed through meat grinder machine and the resulting pellets were also were dried air. The ingredients of each diet are illustrated in (Table3.1).

3.5. Chemical Analysis

Ingredients, formulated diets, post experiment carcass samples were analyzed in triplicate using standard methods. Concerning carcass sampling, fish were frozen and were passed through a meat grinder into one composite homogenate per treatment. The diets were analyzed for crude protein (CP), ether extract (EE), crude fiber (CF), ash and moisture while whole body composition of nile tilapia fingerlings samples was also analyzed for the same parameters except for CF. The nitrogen free-extract (NFE %) was calculated as 1000 - (crude protein + crude fat + fiber + ash) g/kg. CP content (total nitrogen×6.25) was determined using a BUCHI digestion unit K-435 and Distillation unit

B-324 and B-324 nitrogen analyzer while crude lipid concentrations were determined by petroleum ether extraction using a BUCHI extraction B-811 Automatic system. In addition, ash content was obtained by incinerating samples in a muffle furnace (VULCANTM) at 550°C for 12 h whereas dry matter was determined by drying the sample in an oven (MEMMERT) at 105°C for 16 h and weighing to the nearest 0.1 mg. Ingredients and diets samples were analyzed for fiber by using VELP SCLENTIFICA unit.

3.5.1. Growth Performance Indices

1. Body Weight Gain = Final body weight - Initial body weight

2. Average Daily Gain = Final body weight - Initial body weight

3. Specific Growth Rate (SGR %)

$$= \frac{\text{LN final weight - LN Initial weight}}{\text{Experimental period (days)}} *100$$

4. Percentage Weight Gain (%) $= \frac{\text{Final BW - Initial BW}}{\text{Initial BW}} *100$

5. Survival Rate (%) $= \frac{\text{Initial number of fish stock-Mortality}}{\text{Initial fish stock}} *100$

FEED UTILIZATION

1. Feed Conversion Ratio (FCR) =Feed fed

   Fish weight gain

2. Protein Efficiency Ratio (PER) =Mean weight gain (g)

   Protein intake

3. Protein Intake (PI) = Feed intake*% of protein in diet

3.6. Toxity test

Acclimated Nile tilapia (O. *niloticus)* was divided into five groups with five fish each. All groups were subjected to immersion in 10 ml of chloride solution in 7 L, as treatments e.g. (T1 T2 T3 T4 and T5), for their response to resist ascidia environment.

3.7. Enzymes Analysis

Similarly, tissues were collected without anticoagulant for serum separation as described by (Leid, et al., 1975). The obtained sera were used for Spectrophotometric determination of thess activities of Alkaline phosphatase (ALP), in addition, serum total protein, albumin values were determined spectrophotometrically as implied by the methods of (Doumas, et al., 1981),(Reinhold, 1953) and (Coles, 1974), respectively.

3.8. Statistical Analysis

`The obtained data in this study were subjected to one-way (ANOVA)analysis of variance (snedecor,and Cochranl, 1986),(Sokal,and Rohlf,1981). Data were entered and analyzed using Statistical Package for

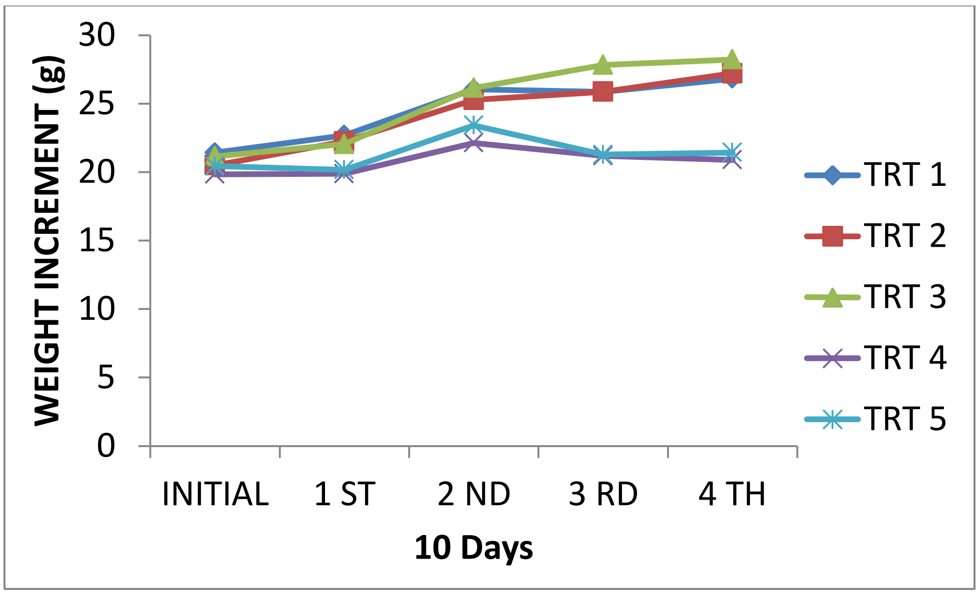
Social Sciences program (SPSS) for Windows, Version 16 (Log Xact8 ,Cross over,USA), was used to compare the proportions and 95% confidence intervals (95% CI) ,differences between proportions were considered statistically significant if 95% CI did not overlap, Results are presented as means ± standard error of the mean (S.E.M).

CHAPTER FOUR

RESULTS

4.1. Growth Parameters

Figure1: The effect of feeding nile tilapia fingerlings diets containing different levels of *N. sativa* meal and oil on their growth performance.



Figur 2:FCR % of O.niloticus fingerlings fed verying levels of *N. sativa* meal and oil.

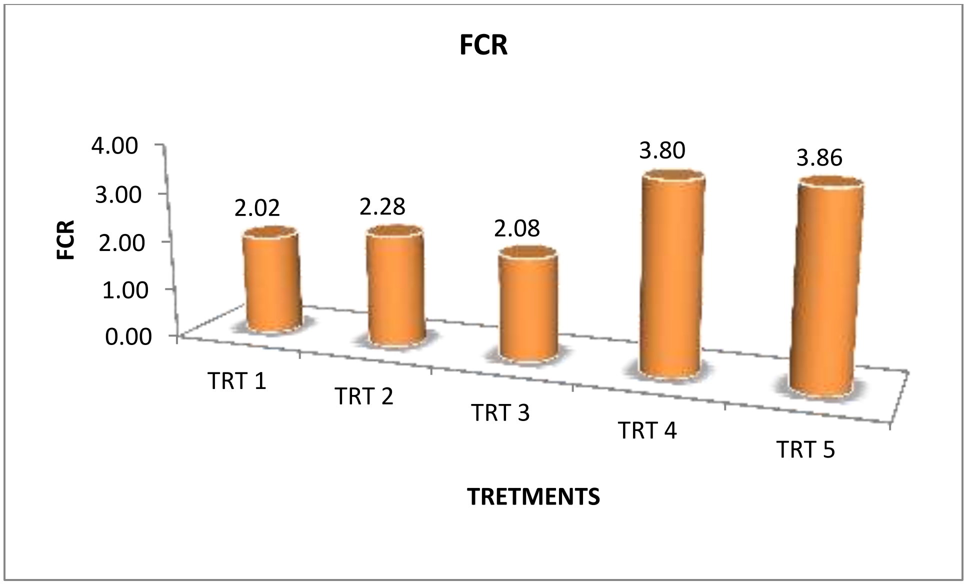
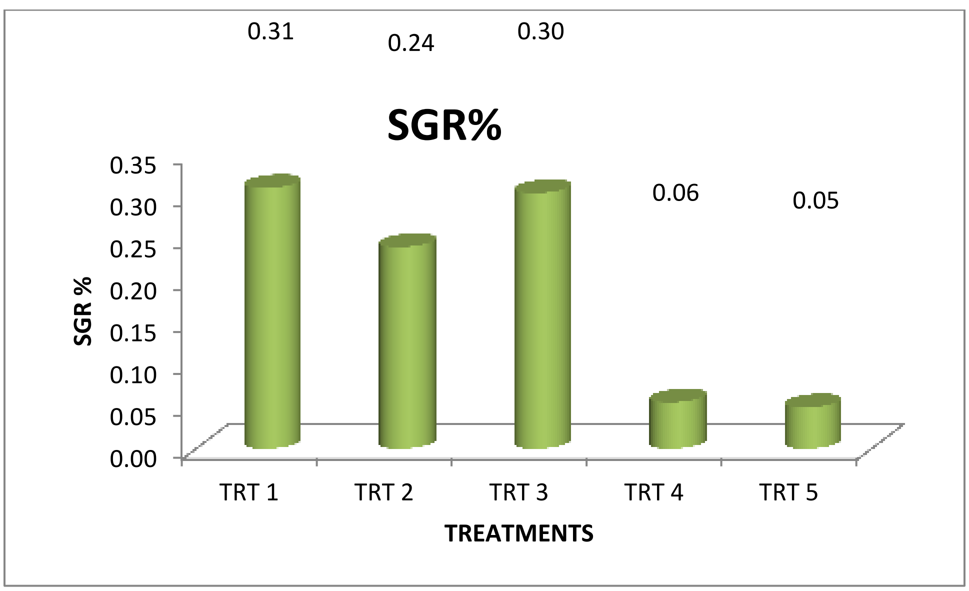
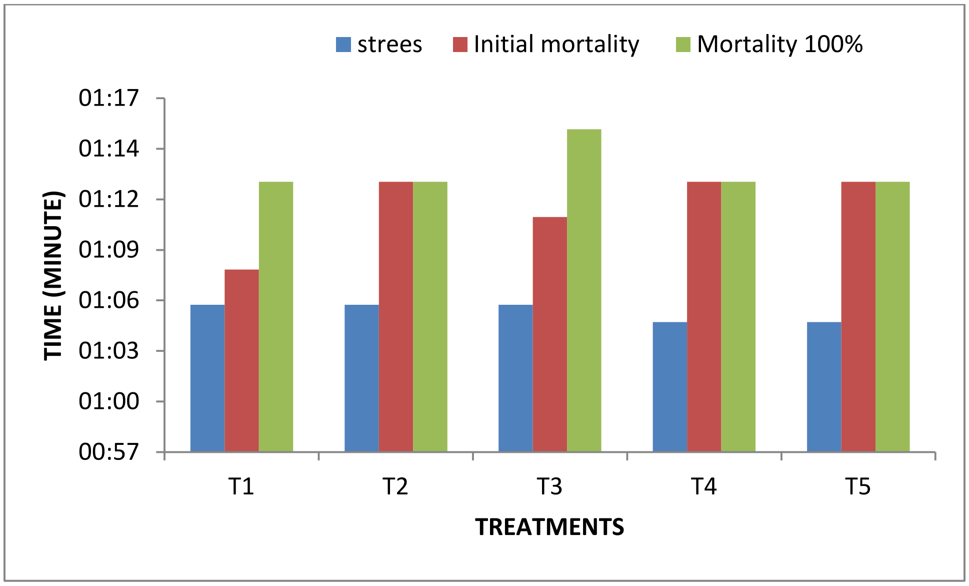


Figure (3).SGR% of O. *niloticus* fingerlings fed varying levels of *N. Sativa* meal and oil.



4.2. Toxitity Test

Figure 4: The effect of Chloride solution in mortality



3.3. Enzymes Analysis

Figure 5: The effect of ALP-Colorimetric enzymes

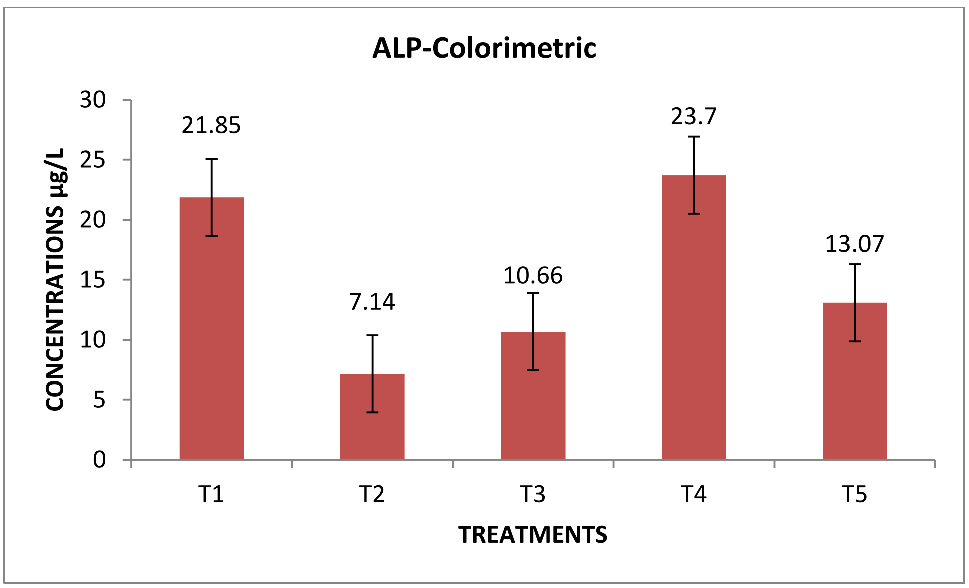


Figure 6: The effect of Total protein

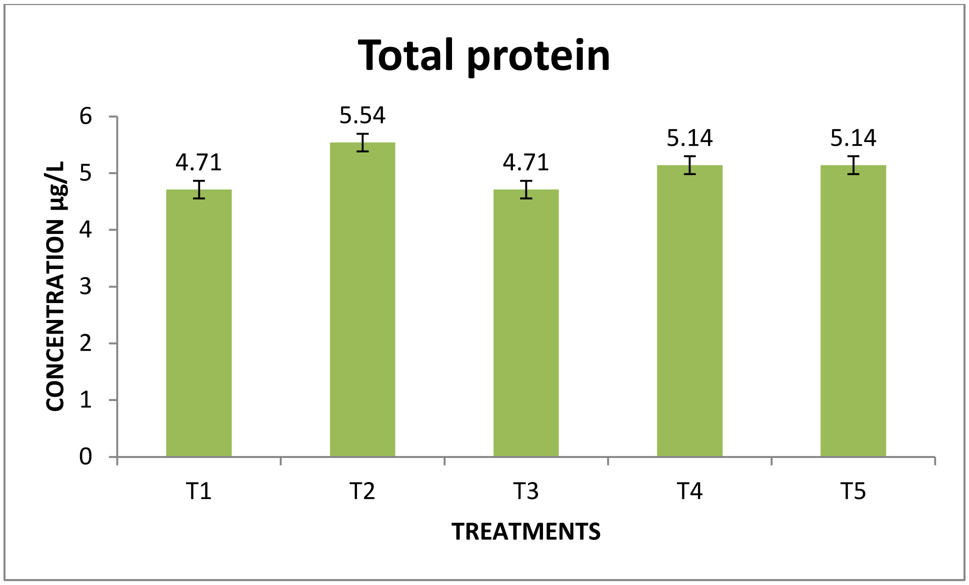
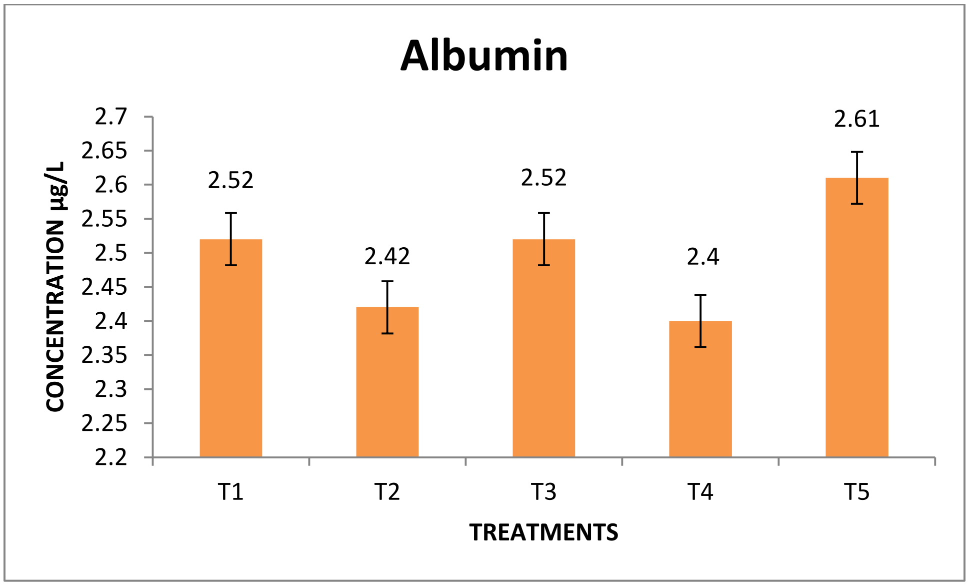


Figure 7: The Effect of Albumin



CHAPTER FIVE

DISCUSSION

Use natural feed additive is becoming useful for fish feeding rather than classic chemical feed additives due to the accumulative effect of the chemical components induced deterrent effects on human health (El-Dakar et al., 2008).

Results showed After 40 days of the feeding trial, nile tilapia fingerlings with diets .In contrast, nile tilapia fed *Nigella sativa* meal exhibited statistically highest mean weight with those offered *Nigella sativa* oil feeding regimes while control group exhibited highest mean weight**.**

The highest final body weight and weight gain (27.207 g and 6.713g, respectively) from *Nigella sativa* meal 15% followed by the fish group fed on diet contained *Nigella sativa* meal 10%, (26.82 and 5.407g, respectively) then the fish group fed on diet *Nigella sativa* oil 6%, (20.887 and 1.047g, respectively) then the fish group fed on diet *Nigella sativa* oil 12% (21.423 and 0.983g) in comparison with control group (28.22 g and 7.07, respectively).

Results showed that protein content was 28.95-30.1 in diets containing *Nigella sativa* 10 and 15% this was in agreement withrequirements for a number of fish species have been determined and mostly range from 28-50% of the total dry weight. The study results indicated that a dietary of black seeds, extraction exhibited good growth performance, health status of Nile tilapia (o.niloticus) fingerlings compared with *Nigella sativa*oil.

Supplementation of *Nigella sativa* at 10 and 15% did not affect growth, feed conversion rate (FCR) and specific growth rate (SGR). Similar to results with *Nigella sativa* in nile tilapia (Sevdan,etal., 2012).Effects of dietary herbal additives on fish growth are contradictory.

Results showed that protein content was almost equal proportionwhitthe control in diets containing *Nigella sativa*10 and 15%, this was the difference with (Khalafalla and Mohsen, 2006) who found that these *Nigella sativa* were rich in protein.

Data indicated that no significant differences in the initial fish weight among different experimental diets, which denoted that there are standardized at the start of the experimental treatments. No significant differences of diet *Nigella sativa* 10 and 15% with control in fish weight were difference with (Abd Elmonem et al., 2002) who found that adding *Nigella sativa* at levels 0, 3, 6 and 9 % gave a positive response of growth performance and SGR at low levels of Red tilapia diets, also that level 5%of *Nigella sativa*was improve growth performance.These results were confirmed the results obtained by (Abou-Zeid1998), who found that *Nigella sativa* promoted growth of nile tilapia because of its digestive stimulating effect through their aromatic substances or essential oils . Survival rate was improved and all treatments showed no mortality all the experimental period.

Results showed all groups were subjected to immersion in chloride stress in fish feed *Nigella sativa* oil appearance after 5 minutes, while fish feeding diet control stress beginning at 6 minutes.Mortality at *Nigella sativa* oil and *Nigella sativa* meal 10% at same time after 13 minutes will *Nigella sativa* meal 15% start mortality at 11 minutes on control diet at 8 minutes. When mortality percentage 100% at *Nigella sativa* oil (6, 12%), *N.*

*sativa*meal (10%) and control on 13 minutes .Will *Nigella sativa* (15%) resistance to 16 minutes.

Results showed obtained are shown on although there were relative differences in the experimental period values for alkaline phosphates (ALP), these were statistically significant. The highest mean value occurred in the *Nigella sativa* oil 6% while the least was in *Nigella sativa* 10%.

Results showed Hematological parameters of fish blood are useful tools that aids in diagnosis of the diseases. It can also be used to study immune potentiates. Such tests are general but not conclusive and must be correlated with biochemical tests of the subject. The present findings indicated that, during the experiment, fish fed *Nigella sativa*meal 10 ,12% and *Nigella sativa* oil 6 , 12% showed an elevation of total protein significantly ($P < 0.05$) than the control.

Results showed revealed also that, albumin was significantly increased ($P< 0.05$) in fish fed of *Nigella sativa* oil 12% during the experiment comparable to *Nigella sativa* oil 6% , the control group and *Nigella sativa* meal.

All values of protein patterns were slight significantly throughout the experimental period (figure6). Similar results were obtained in catfish injected intraperitoneal with *Nigella sativa* oil (Abeer, 2005).The increment of total protein, albumin and globulin perhaps explained either by the fact that, *Nigella sativa* contains high percentage of crude protein (20.5%) and free amino acids (Kudryashova, et al., 1953;Babayan, et al., 1978; Atta, 2003). Such increase perhaps attributed also to the role of

Nigella sativa (Hedaya, 1995) or *Nigella sativa* oil in protein biosynthesis as it is vitally concerned in the growth process.

In conclusion, the present study revealed that, *Nigella sativa* meal at a maximum level of 15 % (dried pellet form) was suitable as a dietary protein supplement for nile tilapia when incorporated 35% replacement for fishmeal protein, without any adverse effect on growth performance, survival rate, feed utilization and economical parameters.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

Increasing demand of food for a growing population, aquaculture is an alternative source of food. Due to its demand, aquaculture has been intensified and diversified, which pose disease problem in aquaculture. Numerous reports have demonstrated the efficiency of *Nigella sativa* in aquaculture. Most of the studies were carried out and to evaluate their potentials under laboratory conditions. Hence, the use of *Nigella sativa* protein under intensive culture conditions is necessary in order to perfectly assess their values.

The results showed that the diet containing 10 and 15% *Nigella sativa* improved significantly ($P>0.05$) growth rate compared with *Nigella sativa* oil 6 and 12% .The effects on serum protein and enzyme levels were investigated. Although the activity of alkaline phosphates increased over experimental period between treatments, thein creases were statistically significant.

The effect of chloride solution for fish fed the *Nigella sativa* 15% more resistant to stress and toxicity.

6.2. Recommendation

- It Recommend further study needed to determine the effects of *Nigella sativa* on growth criteria and fish production and resistance on other fish speces.
- More study of *Nigella sativa* meal and oil an impact in terms of diferent levels in performance on thenile tilapia.
- Further study was needed to determine the effect of *Nigella sativa* meal and oil on fatty acids profile, amino acids and fish fecundity and amino acids .

Reference

AbdElmonem, A., S.M.M.Shalaby and El Dakar, A.Y. (2002).Response of red tilapia to different levels of some medicinal plants by-products black seed and roquette seed meal.

Abdel-Ghaffar FA, El-Feki MA, Faheem HI and Gad MA (2003). Potentiating effects of some natural products on the common carp immune system against *Aeromonashydrophila*. J AquatBiol Fish 7: 23-47.

AbeerEzzat (2005). Effect of black seed (Nigella sativa) on the immune status of catfish (Clariasgariepinus). Ph.D. Thesis, Faculty of Veterinary Medicine, Assuit University, Egypt.

AbouZied, R.M. (1998). Evaluation of some medicinal plants as a feed additive in diets of Nile tilapia (Oreochromisniloticus). M.Sc., Faculty of Agriculture, El-Fayoum, Cairo University**.**

Abutbul S., Golan-Goldhirsh A., Barazani O. and D. Zilberg,(2004). Use of Rosmarinusofficinalisas a treatment against Streptococcus iniaein tilapia (Oreochromissp.). Aquaculture, 238(1-4):97-105.

Abutbul S., Golan-Goldhirsh A.,BarazaniO., Ofir R. and D. Zilberg, (2005).Screening of desert plants for use against bacterial pathogens in fish.Isr. J. Aquacult. -Bamidgeh,57(2):71-80.

Agarwal, R., Kharya, M.D. and Shrivastava, R. (1979).Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn.Indian Journal of Experimental Biology. 17 (11), pp. 1264-1265.

Akgul A (1989). Antimicrobial activity of black cumin (*Nigella sativa* L) essential oil.Gazi Journal of Faculty of Pharmacology 6: 63-68.

Ali BH and Blunden G .(2003). "Pharmacological and toxicological properties of Nigella sativa".Phytother Res 17 (4): 299–305. doi:10.1002/ptr.1309. PMIDmmmmmmmm 12722128.

Amin Gh.,(2001). Cumin. pp. 180-183. In: K.V. Peter (ed.). Handbook of Herbs and Spices.Woodhead Publ., Boca Raton.326 pp.

Aruna BV and Chandran MR.(1996).Efficacy of CIFAX and herbal mixture in the prophylaxis of EUS. Nat Sem Dis Aqu. Kakinada, India.

Atta, M.B. (2003). Some characterisitics of nigella seed (Nigella sativa L.) cultivated in Egypt and its lipid profile. Food chemistry 83:63–68.

Awad SA, Maitland DJ and Moneir S. (2007). New alkaloids from Casimiroaedulis fruits and their pharmacological activity.Chem Nat Com;43:576–580.

Azeez S.,(2008). Cumin. pp. 242-259. In: V.A. Parthasarathy, B. Chempakam, T.J.

Babayan, V.K., Koottungal, D. and Halaby, A.G. (1978). Proximate analysis, fatty acids and amino acid composition of Nigella sativa seeds. J. Food Sci. 43: 1314–1315.

Banerjee S, Padhye S, Azmi A, Wang Z, Philip PA, Kucuk O, Sarkar FH and Mohammad RM (2010). "Review on molecular and therapeutic potential of thymoquinone in cancer". Nutr Cancer 62 (7): 938–46. doi:10.1080/01635581.2010.509832. PMC 4167365.PMID 20924969.

Bharat B and Aggarwal .January (2015).Molecular Targets and Therapeutic Uses of Spices.Google Books.p. 259.ISBN 978-981-4468-95-4. Retrieved 4

Bhuvaneswari R. and C. Balasundaram,(2006).Traditional Indian herbal extracts used in vitroagainst growth of the pathogenic bacteria-Aeromonashydrophila .Isr.J.Aquacult. –Bamidgeh,58(2):89-96.

Blazer, V.S. and Wocke, R.E. (1984).The effects of alpha-tocopherol on the immune response and nonspecific resistance factors of rainbow trout (*Salmogairdneri*Richardson).Aquaculture. 37 (1), pp. 1-9.

Blumenthol M. Goldberg A, Brinckmann J.(2001). Herbal Medicine. Expanded Commission Emonographs. Integrative Medicine Communications, 1029 Chestnut Street, Newton, MA 02464;401-403.

Boskabady.M.H and Mohammadi .B.S (2002)."Effect of Nigella Sativa on Isolated Guinea Pig Trachea" (PDF). Arch. Iran. Med. 5 (2): 103–107.

Boyd, E.C. (2004). Farm-Level Issues in Aquaculture Certification: Tilapia. Report commissioned by WWFUS in 2004. Auburn University, Alabama 36831.

Burits M, BucarF(2000). Antioxidant activity of Nigella sativa essential oil.Phyt Res;14:323-328.

Bwanika, G.N., Makanga, B., Kizito, Y., Chapman, L.J. and Balirwa, J. (2004).Observations on the biology of Nile tilapia, *Oreochromisniloticus*, L., in two Ugandan Crater lakes. African Journal of Ecology 42: 93–101.

Chikafumbwa, F.J.K.T. (1996). The use of napier grass (Pennisetumpurpureum) and maize (Zea mays) bran as low-cost tilapia aquaculture inputs. Aquaculture 146: 101-107.

Citarasu T.,(2010). Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult. Int.,18(3):403-414.

Coles, E.H. (1974). Veterinary Clinical Pathology. W.B. Saunders Company, Philadelphia and London, pp. 211-213.

Dalmo, R.A. and Seljelid, R. (1995).The immunomodulatory effect of LPS, laminaran and sulphatedlaminaran (beta (1,3)-D-glucan) on Atlantic salmon, *Salmosalar*L., macrophages in vitro. Journal of Fish Diseases. 18 (2), pp. 175-185.

Diab AS, Aly SM, John G, Abde-Hadi Y and Mohammed MF (2008). Effect of garlic, black seed and Biogen as immunostimulant on the growth and survival of Nile tilapia, *Oreochromisniloticus*(Teleostei: Cichlidae) and their response to artificial infection with *Pseudomonas fluorescens*. Afr J AquSci 33: 63-68.

Doumas, B.T., Bayson, D.D., Carter, R.J., Peters, T. and Schaffer, R.(1981).Estimation of total serum protein. Clin. Chem. 27:1642–1643.

El Saleh, S.C., Al Sagair, O.A. and Al Khalaf, M.I. (2004).Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats. International Journal of Cardiology. 93 (1), pp. 19-23.

El-Dakar,A. Y., G.D.Hassanien, S.S.Gad and S.E.Sakr (2008).Use of Dried Basil Leaves as a Feeding Attractant for Hybrid Tilapia, Oreochromisniloticus X Oreochromisaureus,

El-sayed, A .-F. M. (2006). Tilapia Culture . CABI Publishers, Oxfordshire , UK, 277 pp .

essential oils: part 10; antibacterial activity of volatile oils of some spices. Phytother.Res., 16(7):680-682.

FAO (2014). The State of World Fisheries and Aquaculture 2014 (SOFIA) $86 thousand million Blumenthal, Les (August 2, 2010)."Company says FDA is nearing decision on genetically engineered Atlantic salmon". Washington Post.Retrieved August 2010.

FAO(2005). Rome. Updated 18 February.[Cited 11 September 2012].

FAO(2011). Global Aquaculture Production Fishery Statistical Collections, Rome. Retrieved 2 October.

FAO(2012). Cultured Aquatic Species Information Programme.*Oreochromisniloticus*.Cultured Aquatic Species Information Programme.Text by Rakocy, J. E. In: FAO Fisheries and Aquaculture Department [online].

Fishelson, L. and Yaron, Z. (1983). (Eds). Proceedings of the International Symposium on Tilapia in Aquaculture. Tel. Aviv University, Tel Aviv, Israel, 11 pp.Fisheries & Aquaculture-FI fact sheet search Www.fao.org. *Retrieved 2015-06-08*

Food and Agriculture Organizations of the United Nations.(2003). Fisheries statistics http://www.fao.org.Accessed 13-01-06.

Food and Agriculture Organizations of the United Nations. (2005). www.FAO.org/News/2000/000704-e.htm

Food and Agriculture Organizations of the United Nations (1996).Aquaculture potential in African Documentation issued on the occasion of the World Food Summit in Rome November 1996. Rome FAO p 20.

Gabor E.F., Ichim O. and Suteu M. (2012). Phytoadditives in rainbow trout (Oncorhynchusmykiss) nutrition.Biharean Biologist, Oradea, Romania;6(2):134-139.

Gabriel U.U., Akinrotimi O. A ., Bekibele D. O. D., N Gachkar L., Yadegari D., Rezaei M.B., Taghizadeh M., Astaneh S.A. and I. Rasooli, (2007). Chemical and biological characteristics of Cuminumcyminum and ***Ganguly S.,PaulI. and S.K. Mukhopadhayay,(2010).*** Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: a review. Isr. J. Aquacult. -Bamidgeh,62(3):130-138.

Hanafy, M.S. and Hatem, M.E. (1991). Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). Journal of Ethnopharmacology. 34 (2-3), pp. 275-278.

Hardie, L.J., Fletcher, T.C. and Secombes, C.J. (1990).The effect of vitamin E on the immune response of the Atlantic salmon (*Salmosalar*L.).Aquaculture. 87 (1), pp. 1-13.

Harikrishnan R., Balasundaram C. and M.S. Heo,(2011). Influence of diet enriched Hetch T (2000). Consideration on African Aquaculture. Journal of World Aquaculture 31: 12-19.

Hedaya, S.A. (1995): Effect of Nigella sativa (black seeds) extract on some haematological and biochemical parameters in rats.Alexandria Journal of Veterinary Science 11: 95–99.

Hetch T (2000). Consideration on African Aquaculture. Journal of World Aquaculture 31: 12-19.

Hilly M, Adams ML and Nelson SC.A.(2002). study ofdigit fusion in the mouse embryo.ClinExpAllergy;32(4):489-98.

Hyam, R. and Pankhurst, R.J. (1995).Plants and their names : a concise dictionary. Oxford: Oxford University Press. ISBN 978-0-19-866189-4.p. 341.

Immanuel G., Uma R.P., Iyapparaj P., Citarasu T., Punitha P.S.M., Michael B.M. and A. Palavesam,(2009). Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia Oreochromismossambicus. J. Fish Biol., 74(7):1462-1475.

in rats. International Journal of Cardiology. 93 (1), pp. 19-23.

Jamu DM and Ayinla O.A. (2003).Potential for the development of aquaculture in Africa NAGA 26:9-13.

Jauncey K. and Ross B., (1982).A guide to Tilapia feeds and feeding.Institute of Aquaculture Stirling.Uk. 111pp.

Jeney, G. and Anderson, D.P. (1993).Glucaninjection of bath exposure given alone or in combination with a bacterin enhance the non-specific defence mechanisms in rainbow trout (*Oncorhynchusmykiss*). Aquaculture. 116 (4), pp. 315-329.

Kajita, Y., Sakai, M., Atsuta, S. and Kobayashi, M. (1992). Immunpotentiation activity of Freund’s complete adjuvant in rainbow trout *Oncorhynchusmykiss*. Nippon Suisan Gakkaishi = Bulletin of the Japanese Society of Scientific Fisheries. 58 (3), pp. 433-437.

Kanter, M., Meral, I., Yener, Z., Ozbek, H. and Demir, H. (2003). Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by *Nigella sativa* L. in streptozotocin-induced diabetic rats.The Tohoku Journal of Experimental Medicine. 201 (4), pp. 213-219.

Khalafalla ,M. M.E. and M. K. Mohsen (2006). Influence of some medicinal plants supplementation as a feed additives on growth and feed utilization in diets of Nile Tilapia (Oreochromisniloticus) fingerlings.

Kitao T.,(2001). Streptococcal infections. pp. 196-210. In: V. Inglis, R.J. Roberts, N.R. Bromage (eds.). Bacterial Diseases of Fish. Blackwell, Oxford. 312 pp.

Kitao, T., Yoshida, T., Anderson, D.P., Dixon, O.W. and Blanch, A. (1987). Immunostimulation of antibodyproducing cells and humoral antibody to fish bacterins by a biological response modifier.Journal of Fish Biology. 31 (Suppl A), pp. 87-91.

Klinger, D. H. et al. (2012). Moving beyond the fished or farmed dichotomy.Marine Policy.
Kubaryk , J.M (1980). Effect of diet, feeding schedule and sex on food consumption, growth and retention of protein and energy by tilapia. PH.D. Diss., Auburn University, Auburn, AL.

Kudryashova, N.A., Kolobkova, E.V., Chernova, E.N. (1953): Content of free amino acids in dormant seeds. DokladyAkademicNauk CCCP 91: 1365–1368 L. Journal of Fish Diseases. 23 (6), pp. 369-378.

Lied E., Gezrde Z. and BraskanO.R. (1975). Simple and rapid technique for repeated blood sampling in rainbow trout. J. Fish. Res. Board of Canada 32: 699-701.

Lim C. (1989). Practical Feeding –Tilapia. In Lovell, R.T. (1989).Nutrition and Feeding of Fish. Van Nostrend Reinhold U.S.A. p11.

Liti D.M., Cherop L., Munguti J.M., and Chhorn L. (2005). Growth and Economic performance of Nile tilapia (Oreochromisniloticus) fed on two formulated diets and two locally available feeds in fertilized ponds. Aquaculture Research36, 746-752.

Lovell, R.T., and Limsumwan T. (1982). Intestinal synthesis and dietrynonessentiality of vitamin b12 for Tilapia nilotica. Trans. A M. Fish.Soc.11:485-490.

Mahmoud, M.R., El Abhar, H.S. and Saleh, S. (2002). The effect of *Nigella sativa* oil against the liver damage induced by Schistosomamansoni infection in mice.Journal of Ethnopharmacology. 79 (1), pp. 1-11.

Martena J. and Berka R. (1982).Fresh-water fish pond management in the world. In:

Matthew, L. (1992). Introduction to aquaculture. John Wiley and Sons, Inc. New York, USA 440 pp.

Michael R.G.(ed) (1982). Managed Aquatic ecosystems. Elsevier Science Publishers B.V. Amsterdam

Mohamed HA, El-Sayed LH and Moawad M. (2010). Protective effect of Nigella sativia seed against imethylaminoazobenzene (DAB) induced liver carcinogenesis. Nat Sci;8(6):80-87.

Ng W.K., Koh C.B. and Din Z.B. (2006). Palm oil-laden spent bleaching clay as a substitute for marine fish oil in the diets of Nile tilapia, Oreochromisniloticus. Aquaculture Nutrition 2006 12:459–468

Olivier, G., Evelyn, T.P. and Lallier, R. (1985).Immunity to Aeromonassalmonicida in coho salmon (*Oncorhynchuskisutch*) induced by modified Freund's complete adjuvant: Its non-specific nature and the probable role of macrophages in the phenomenon. Developmental and Comparative Immunology. 9 (3), pp. 419-432.

Olusola S.E, Emikpe B.O and Olaifa F.E .(2013). The potentials of medicinal plant extracts as bio-antimicrobials in aquaculture. Int J Med Arom Plant.;3:401-412.

Ortuno, J., Cuesta, A., Angeles Esteban, M. and Meseguer, J.(2001). Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparusaurata L.*) innate immune system.Veterinary Immunology and Immunopathology. 79 (3-4), pp. 167-180.

Patastico B.L. (1988). Non-conventional Feed resources in aquaculture: An overview of work done in Phillipines: In ***De Silva S.S. (ed) (1989).*** Finfish nutrition research in Asia: Proceedings of the second fish nutrition network meeting. Heinmann Publishers Asia Pte Ltd. p71.

Picker, M.D. and Griffiths, C.L.(2011). Alien and Invasive Animals – A South African Perspective.Randomhouse/Struik, Cape Town, South Africa.240 pp.

Popma T.J., (1982). Digestibility of selected feedstuffs and naturally occurring algae by tilapia.Ph.D.dissertation.Auburn University, Auburn, Alabama.

Popma, T. and Masser, M.(1999).Tilapia: Life History and Biology, Southern Regional Aquaculture Center (SRAC) Publication No. 283, 4 pp.

Popma, T. and Michael.M.(1999). Tilapia life history and biology.SRAC Publication No.23.Pp.1-5.

Porucu M, Colack SO, Ispir U, Altinterin B and Celayir Y. (2009).The effect of black cumin seeds, Nigella sativa, on the immune response of rainbow trout, Oncorhynchusmykiss. Med Aqu J;2(1):27-33.

Reay P.J. (1979). Aquaculture. The institute of Biology's Studies in Biology no.106. pp 31-40.Reyes, O.S., and ***Fermin A.C.(2003)*** Terrestrial leaf meals or freshwater aquatic fern as potential feed ingredients for farmed abalone Haliotis asinine L. Aquaculture Research, 34,593-599.

Reay P.J. (1979). Aquaculture. The institute of Biology's Studies in Biology no.106. pp 31-40.Reyes, O.S., and ***Fermin A.C. (2003).*** Terrestrial leaf meals or freshwater aquatic fern as potential feed ingredients for farmed abalone HaliotisasinineL. Aquaculture Research, 34,593-599.

Reinhold, R.P. (1953). Determination of serum albumin. Clin. Chem.21: 1370–1372

Salem, M.L. and Hossain, M.S.(2000). Protective effect of black seed oil from *Nigella sativa* against *Murine cytomegalovirus* infection.International Journal of Immunopharmacology. 22 (9), pp. 729-740.

Salem, M.L.(2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed.International Immunopharmacology. 5 (13-14), pp. 1749-1770.

SevdanYılmaz, SebahattinErgün, NeclaTürk .(2012) Effects of Cumin Supplemented Diets on Growth and Disease (Streptococcus iniae) Resistance of Tilapia (Oreochromismossambicus)

Singh G., Kapoor I.P., Pandey S.K., Singh U.K. and Singh R.K., (2002).Studies on Rosmarinusofficinalis essential oils. Food Chem., 102:898-904.

Smith, A. (1840).Illustration of the zoology of South Africa, volume 4, Pisces, Smith, Elder. In*: **EL-SAYED, A.F.M.(2006)**.* Tilapia Culture.CABI Publishers, Oxfordshire, UK, 33 pp.
Snedecor, G.W. and Cochran W.G., (1968).Statistical methods, 6 edn. Iowa State University Press, Ames.

Sokal, R.R. and F.J. Rohlf, (1981).Biometry. W.H. Freeman and Company, New York.

Takeuchi T., Satoh S. and Watanabe W. (1983 a).Dietary lipids suitable for practical feed of Tilapia nilotica.Bulletin of Japanese Society of Fisheries Sciences Fish. 49(9): 1361-1365.

Takeuchi T., Satoh S., and Watanabe W.(1983 b). Requirement of Tilapia nilotica for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 49(7): 1127-1134.

Tortensen, B.E., Lie O. and Froyland, L. (2000). Lipid metabolism and tissue composition in Atlantic salmon salar (L.) –effects of capelin oil, palm oil and oleic-enriched sunflower oil as dietary lipid sources. Lipids, 35:653–664.

Trewavas, E.(1982). Tilapias: taxonomy and specialisation. In: PULLIN, R.S.V. and LOWE - McCONNELL, R.H. (Eds) The biology and culture of tilapias. ICLARM Conference Proceedings No. 7, ICLARM , Manila, Philippines, 3 -13 pp.

Ugwumba A.A.A., Ugwumba A.O. (2003). Aquaculture options and the future of fishsupply in Nigeria. The Zoologist 2: 96-122.

USDA(2016). National Nutrient Database for Standard Reference Release 28 slightly revised May, Basic Report June 24, 2016 22:43 EDT Page 2 of2 Veterinary Immunology and Immunopathology. 101 (3-4), pp. 203-210.

Warrier PK, Nambiar VPK and Ramankutty.(2004). Indian medicinal plants-acompendium of 500species.Chennai: Orient Longman Pvt Ltd; p.139-142.

Watanabe T., Takeuchi T., Murakim A., and Daino C. (1980).The availability to Tilapia of phosphorus in white fish meal with green tea on innate humoral and cellular immune response of kelp grouper

Watson, Reg; Pauly and Daniel (2001)."Systematic distortions in world Fisheries catch trends". Nature 414 (6863): 534. doi:10.1038/35107050**.**

Yılmaz S., (2011). Effects of Dietary Medicinal Herbs on Growth Performance, Feed Utilization and Blood Parameters of Sea Bass (Dicentrarchuslabrax L.,1758). Master Thesis. ÇanakkaleOnsekiz Mart Univ., Turkey Zachariah (eds.). Chemistry of Spices. CAB Int., Oxfordshire. 445 pp.

Zakes Z., Kowalska A., Demska-Zakes K., Jeney G. and Z. Jeney,(2008). Effect of two medicinal herbs (AstragalusradixandLonicera japonica) on the growth performance and body composition of juvenile pikeperch [Sander lucioperca(L.)]. Aquacult. Res., 39:1149-1160**.**

Zaoui, A., Cherrah, Y., Lacaille Dubois, M.A., Settaf, A., Amarouch, H. and Hassar, M.(2000).Effetsdiurétiquesethypotenseurs de *Nigella sativa* chez le rat spontanementhypertendu. [Diuretic and hypertensive effects of *Nigella sativa* in the spontaneously hypertensive rat].Therapies. 55 (3), pp. 379-382.

Zedlitz, S., Kaufmann, R. and Boehncke, W.H. (2002).Allergic contact dermatitis from black cumin (*Nigellasativa*) oil-containing ointment.Contact Dermatitis. 46 (3), pp. 188.

Zilberg D., Tal A., Froyman N., Abutbul S., Dudai N. and A.G. Goldhirsh,(2010). Dried leaves of Rosmarinusofficinalis as a treatment for Streptococcosis in tilapia. J. Fish Dis., 33:361-369.