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Faculty of Veterinary Medicine
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Trials for control of some bacterial infections in cultured Tilapia fish

Thesis

Presented by

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For the degree of Ph.D. of

Vet. Medical science

(Fish Diseases and Management)

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لجنة الاشراف

الأستاذ الدكتور / محمد مصطفى محمد مصطفى

أستاذ أمراض الأسماك و رعايتها- كلية الطب

البيطري- جامعة القاهرة

الأستاذ الدكتور /محمد عبد العزيز احمد

استاذ ورئيس قسم امراض الاسماك ورعايتها- كلية الطب

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رئيس بحوث أمراض الأسماك-معهد بحوث صحة الحيوان

– الدقي

Abstract

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Title of thesis

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This study was conducted to investigate the antibacterial effect of aqueous and ethanol, extracts of *Spirulina platensis* and *Nigella sativa* on some fish pathogenic bacterial Gram-negative bacteria *(Aeromonas hydrophila , A. sobria , Flavobacterium columnare , Vibrio paraheamoliticus,)* Gram-positive bacteria *(Enterococcus fecalis and Streptococcus dysgalactiae).* And evaluate the effect of *Spirulina platensis*, *Nigella sativa* and both of them at rate (0.75%, 0.5% and mix of them) respectively as feed additive for 8 weeks on growth performance, blood picture and immune system in *Oreochromis niloticus* (*O. niloticus*) before and after challenge with *A. hydrophila* .and resistance against ***Aeromonas hydrophila*** and ***Streptococcus dysgalactiae***. The results obtained revealed that aqueous extract of *Spirulina platensis* and *Nigella sativa* has no antibacterial effect on both Gram-positive and negative bacteria. Ethanol extract of Both *Spirulina platensis* and *Nigella sativa* have antibacterial effect *E. faecalis* and *St. dysgalactiae* as well as *V. parahemolyticus, Fl. columnare, A. hydrophila* and *A. sobria).*Besides *E. faecalis* was more sensitive to ethanol extract than other bacteria . and The results also indicated that the addition of *Spirulina* or *Nigella* to diet improve growth performance, health condition, enhancement cellular and humoral immune responses, specifically (phagocytic activity, lysozyme, nitric oxide, total protein and its fraction especially γ globulin) either before and after challenged tests and increase resistance against bacterial infection. The combination of *Spirulina platensis* and *Nigella sativa* showed the highest values of growth performance, immunological parameters and give the lowest mortality percent against bacterial infection.

Key words*: Spirulina platensis, Nigella sativa , immunostimulants, antibacterial effect*

Dedicated to……..

My Dear family

My Dear husband

My Dear son Yousef

My Dear daughter Jana

Every one helps me

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Introduction

Fish is an excellent source of high quality animal protein and essential fatty acids, especially long-chain polyunsaturated fatty acids (LCPUFA) and micronutrients, which are much greater in fishes than in animal-source foods **(FAO–WHO, 2011).**

Aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, and protection from predators, parasites and pathogens. **(FAO, 2013).**

Under the conditions of intensive production, aquatic species are subjected to high-stress condition, increasing the incidence of diseases **(Patricia *et al.*, 2012**). The control of diseases can be achieved by many methods, including the use of drugs and antibiotics, good management practices, genetic selection or stress and disease tolerant strains, and prevention by the use of vaccines. Prevention of diseases is much more desirable than intervention to stop and reverse disease processes once they have begun **(Howes, 1994),** Antibiotic provide a useful means of helping to control many bacterial diseases but there are many problems associated with the development of antibiotic resistance and recurrent outbreaks necessitating further, costly treatment **(Galindo-Villegas and Hosokawa, 2004).**

Also control of diseases can be achieved by using immunostimulants which are promising dietary supplements to potentially aid in disease control of several organisms and increase disease resistance **(Galindo-Villegas and Hosokawa, 2004),** probiotic,

prebiotic, synbiotic, phytobiotic and other functional dietary supplements play role as a dietary supplementation strategies in cultured fish and shell fish all over the world in which various health, immunostimulants and growth promoting. **(Denev, 2008).**

Spirulina platensis (SP) is a photosynthetic, filamentous, blue-green microalgae and is generally regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids and phycocyanin **(Jaime *et al*., 2006).** *SP* is a rich source of protein (60–70%), so Algae gained attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical regions where algae production is high **(El-Hindawy *et al*., 2006).** Also it improved the growth performance, immunity and disease resistance in *Oreochromis niloticus* when used in diet at 10g/kg for 2months **(Mai *et al*., 2013).**

The other immunostimulant in the scope of attention is *Nigella sativa (NS).* It is herbaceous plant belonging to family *Ranunculaceae.* This herb is widely spread in the Mediterranean countries It is known as black cumin or black seed and it cultivated for its seeds, which are used for different medicinal purposes as antimicrobial **(Hanafy and Hatem, 1991).** Some researchers have used black cumin seeds as enhancer for performance, the growth and immune system of some fish species **(Abd Elmonem *et al*., 2002; John *et al*., 2007; Diab *et al*., 2008 and Dorucu *et al*., 2009).**

From the aforementioned facts, this work was planned to evaluate the expected effects of *Spirulina platensis, Nigella sativa*, separately and in combination on *Oreochromis niloticus* through the following:

1. Evaluation of antibacterial effects of *Spirulina platensis, Nigella sativa* against some important bacterial fish pathogens.

2. Studying the using of *Spirulina platensis*, *Nigella sativa* separately and in combination as a feed additive on fish performance

3. Assessment of immunomodulatory effects of *Spirulina platensis, Nigella sativa* separately and in combination on *Oreochromis niloticus*.