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ECONOMIC STUDIES ON IMMUNOSTIMULENTS IN RELATION TO MYCOTOXIN INFECTION IN CULTURED FISH

T.T. SAAD [1], H.A. AHMED [2], M. EL-GOHARY [3] and M.A. ALI [4]

[1]Poultry and Fish Disease Department, Faculty of Veterinary Medicine, Alexandria University, Egypt
[2]Nutrition and Veterinary Clinical Nutrition Department, Faculty of Veterinary Medicine, Damanhour University, Egypt
[3]Fish diseases Dept., Animal health research institute Kafr El-Sheikh branch, Egypt
[4]Anim. Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

*Corresponding Author's E-mail: talaat_fish@yahoo.com; Tel: 01061035503 (Saad, T.T.).

ABSTRACT: *Studies in the past decade confirm that the growth of both gram-positive and gram-negative foodborne bacteria, yeast and mold can be inhibited by garlic, onion, cinnamon, cloves, thyme, sage, and other spices. Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes resulting in death. This study concluded that: When the ration or the fish suffered from fungal infection the addition of black seed, garlic and onion will reduce the infection and improve fish health. In Post mortem lesions the fish suffered from mycotic infection showed severe degenerative changes in internal organs especially in the liver, heart and kidneys. The result cleared that, the blackseed is the best herbs that prevented and improve the aflatoxin effect followed by garlic and onion, respectively. The result also showed that level of RBCs and WBCs, differential leucocytic counts, phagocytosis process, serum protein, biochemical analysis of fish body, body weight and body weight gain improved with addition of blackseed, garlic and onion. The residue of aflatoxin in fish flesh decreased in the groups treated with blackseed, garlic and onion than the control or fish fed on the aflatoxin. The results also showed that, frequent supplementation of fish ration with black seed, garlic and onion can reduce the aflatoxin hazards in the fish. The results also concluded that, the higher economic efficiency measures (total return, total costs, net profit, total returns/total costs and net return to total costs) improved in the groups fed with blackseed, garlic, onion and all of them improved economic efficiency measures than the control groups and when all of them added to the fish treated with aflatoxin diet improved economic efficiency results than the group treated with aflatoxin only.*

ORIGINAL ARTICLE

Key words: *Economic Efficiency, Blackseed, Aflatoxin, Biochemical Analysis.*

INTRODUCTION

The using of synthetic drugs in the therapeutic field may have adverse effects which may be more dangerous than the disease itself (Fluk et al., 1976). From this fact and with the call of "Back to nature", medical plants consider an important target area for several studies to show possibility of its use in a variety of health problems.

The garlic have: Antiviral, antibacterial, antifungal, antiparasitic effect. Garlic is nicknamed Russian penicillin for its widespread use as a topical and systemic antimicrobial agent. Allicin has antimicrobial effects *in vitro* against many viruses, bacteria, fungi and parasites, but dried, powdered and oil preparations of garlic have not been shown to have significant antimicrobial activity. Also, it have antifungal effect thus Garlic enjoys a worldwide reputation as an antifungal folk remedy.

Mona et al. (2011) reported that, Aflatoxine the major toxic metabolites of fungi which are able to induce chronic liver damages. The antioxidant and hepatoprotective effects of Ginseng extract and Nigella sativa Oil 1% on Aflatoxin was investigated. Moreover the liver exhibited some clinicopathological changes and decreased body weight due to the toxic effects of aflatoxin. Both Ginseng extract and Nigella sativa Oil 1% reduced the development of hepatotoxicity by Aflatoxin. Nigella sativa showed more improvement of all enzymes of kidney and liver, and also total lipid and cholesterol were reduced and body weight increased with improvement of economic and productive efficiency of fish production farms (Kim, 2010).

The antifungal activity of green onion on a toxic fungal strain *A. parasiticus*, which can produce aflatoxin, a human carcinogen. In practice, both a solid culture and a liquid culture were attempted because green onion is consumed with various forms of daily food intake. In liquid culture, mycelial growth of experimental group was less

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than that of control group during the incubation period of 15 days. When increasing concentrations of green onion were added to PDA and YES broth, an increasing tendency of inhibitory effect was noted on the growth of the fungal strain (Kim, 2010).

So this study was carried-out to throw the light on using the natural feeds on the improving immunity of the fish *Oreochromis niloticus* and its role in the prevention of aflatoxicosis infection in the fish through study the effect of blackseed (Nagilla sativa), garlic and onion on Body weight and body weight gain of the fish, number of RBCs, WBCs and its differentials, level of serum proteins (Albumin, globulin, total protein and albumin/globulin ratio), Phagocytic activity and phagocytic index as well as the effect of this natural feeds on the constituents of the fish flesh in addition to their effects on the residues of the aflatoxicosis in fish meat.

MATERIALS AND METHODS

A total number of 240 healthy Nile Tilapia (Oreochromis niloticus) were collected from Barseek fish farm at Behera Governorate. Fish were transported alive to the laboratory in plastic bags containing water enriched by air (2/3). Average body weight of fish was about 30 ± 5 gm, of Oreochromis niloticus to aflatoxin B1 and the type of fish that we complete on it the experiment. Fish were kept in prepared glass aquaria (90 x 50 x 35 Cm). These aquaria were used for holding the experimental fish throughout the period of the present study, this aquaria was supplied with cholrine free tap water according to Innes (1966).

The continuous aeration was maintained in each aquarium using an electric air pumping compressors. Water temperature was kept at 22 ± 1 °C. Fish were fed on an ad libitum commercial fish food containing 23-25% crude protein (obtained from Barseek fish feed factory) the diet was daily provided at 3% of body weight as described by Eurell et al. (1978). The daily anmount of food was offered on two occasions over the day (Regular diet), in a ddition to in acute and chronic experiment the feeding design during experiment was as follow:

Aflatoxins: The mycotoxin Afla toxins were kindly provided by Sigma Chemical Co. U. S. A. also by Sigma-Aldrich Chemie Gmbh, Germany.

Yeast strains: The Candida albicans strain was kindly supplied by the Veterinarian Riad H. Khalil. Lecturer, Dept. of poultry and fish diseases, Fac. Vet. Med., Alexandria University.

Experimental design

The Oreochromis niloticus fish used in this study was divided into 8 groups each group of 30 fish for each the design of the experiment was carried-out in the following table.

Table 1 - The design of the experiment

Time	Code	Treatment	Fish No.
4 weeks	1	Black seed	30
	2	Black seed + Aflatoxin	30
	3	Garlic	30
	4	Garlic +Aflatoxin	30
	5	Onion	30
	6	Onion + Aflatoxin	30
	7	Aflatoxin	30
	8	- ve control	30

A total of 240 fish (30±5 gm each) were used in this experiment and divided into 8 groups. Everycontain 30 fish. 4 group of them injected with aflatoxin B1 (10000 ng) (Shehata et al., 1985). All the experimental groups were kept under daily observation for 4 weeks. The clinical signs, mortality and postmortem lesions were recorded. Furthermore, blood parameters, biochemical serum assays, serum electrophoresis, detection of residual level and histopathological examination which were carried-out during acute toxicity were also done in chronic toxicity throughout the 4 week experimental period. Fish weight: During chronic toxicity experiment the fish was weighted weekly and the body weight gain, feed was calculated for every week according to (Osman and El-Barody, 1999). Biochemical Analysis of experimental fish body was performed according to (Shehata et al., 1985). Blood sampling: Blood samples were collected from the caudal artery using disposable tuberculin syringe for the following: a) Haematological picture, b) Determination of phagocytic activity using citrated blood in the ratio of 0.1/1 ml of 3.8% Sod. Citrate solution to 1 ml of blood (Hawk et al., 1965). Serum preparation was done for biochemical determination (Lied et al., 1975). Differential leucocytic count: Blood film was prepared according to the method described by Lucky (1977). Determination of phagocytic activity and phagocytic index: Phagocytic activity was determined according to Kawahara et al. (1991).

Clinico-biochemical analysis

Determination of serum total protein: Serum total protein was determination according to Doumas et al. (1981) using commercial kits produced by Pasteur Lab. Determination of serum albumin: Serum albumin was determined according to Reinhol (1953) using commercially available kits of Chemroy. Determination of serum globulin: Serum globulin was determined by subtracts the total serum albumin from total serum protein according

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to (Coles, 1974). Determination of protein by gell electrophoresis: SDS-PAGE: Sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of serum sample and high molecular weight markers. The electrophoresis was carried out according to ethe procedure of Laemmli (1970). Preparation of 10% separating gel: In a sterial, clean and dry beaker, the separating gel (10%) was prepared according to Laemmli (1970).

Detection of mycotoxine residue in tissue samples: The detection aflatoxine B1 by Aflatest™ method for samples 3 – 100 ppb. Measures of mycotoxins: Add 1.0 ml of dilute mycotoxine™ Developer (made up fresh daily) to elute in the cuvette. Mix well and measure fluorescence in a callbrated fluorometer. Read aflatoxin concentration after 60 seconds. Readout will be in parts per billion total aflatoxins for the 3-100 ppb sample extracted. Economic efficacy measures that include, total returns, total costs, net return, total return/total costs and net return/total costs were calculated according to the methods implied by (Atallah et al., 1999).

Table 2 - Aflatest™ method for measuring residue

Fluorometer calibration	Green	Red	Yellow
Model FX-100 series 3	-1	24	12±2
Model 450 series-2	0	26	13±2
Model 450 series-1	0	20	10±2
Series 4	-1	22	10-2

Statistical analysis

The data of hematological and biochemical examinations of exposed fish were statistically analyzed using t-test, Duncan-test after ANOVA and simple correlation according to (SAS, 1987).

RESULTS

1- Effect of different natural feeds treated with or without aflatoxins on differential leucocytic counts: The results in Table 3, indicated the significant (P<0.01) differences among different treatment in different weeks on lymphocyte, monocyte, and heterophils. The lymphocyte level decreased from the first weeks, to second week, 3rd week and increased in the 4th week from the experiment. While the monocyte level decreased from the 1st to 3rd week of experiment then it increased in the 4th week of the experiment.

Also, the results showed that the heterophils level levels decreased steadily from the 1st week to the 4th weeks of the experiment, and the heterophils level increased in the groups treated with heterophils, followed by onion treated group with aflatoxin and blackseed + aflatoxin. Our results indicated that, the black seed, garlic and onion improve the fish immunity against aflatoxins than the other treated groups and the addition of black seed to the fish diet improves the fish immunity against the aflatoxicosis, followed by garlic and onion.

2- Effect of different natural feeds treated with or without aflatoxins on T.WBCs and T. RBCs: Table 3, indicated that, there is a significant (P<0.01) differences among different treatment in different weeks on T.WBCs and T. RBCs. The level of T. WBCs increased from week 1 to week 4 of the experiment.

The groups treated with blackseed, garlic and onion of higher T. WBCs and T. RBCs a level than the other groups. And the groups treated with black seed and aflatoxin of higher T. WBCs and T. RBCs than the groups infected with aflatoxin with garlic and aflatoxin with onion all over the period of the experiment.

3- Effect of different natural feeds treated with or without aflatoxins on phagocytic activity and phagocytic index: Table 4, explain the significant (P<0.01) differences among different treatment in different weeks on phagocytic activity and phagocytic index in fish. The phagocytic activity increased progressively from the 1st to 4th week of the experiment. The phagocytic index decreased progressively from 1st to 4th week of the experiment. The results showed that the garlic + aflatoxin treated group, onion and blackseed and garlic treated groups of a higher phagocytic activity and phagocytic index than the other treated groups. And the addition of black seed, garlic and onion to the fish diet improve the phagocytic acitivity and index against the aflatoxicosis.

4- Effect of different natural feeds treated with or without aflatoxins on total proteins, albumin, globulin and albumin/globulin ratio: Table 4, explain the significant (P<0.01) differences among different treatment in different weeks on total protein, albumin, globulin and albumin/globulin ratio. The results showed that, the total protein increased progressively from the 1st weeks to the 4th week of the experiment. While the globulin level decreased progressively from the period extended from 1st to 4th week of the experiment. Our results indicated that, the groups treated with blackseed, garlic and onion of a higher total protein, albumin, globulin and albumin globulin ratio than the other treated groups. Also, aflatoxin causes decrease of the serum protein level but by addition of natural feeds as black seed, garlic and onion improve the serum protein level.

5- Effect of different natural feeds treated with or without aflatoxins on body composition of fish:

Table 5 explains the significant (P < 0.01) differences among different treatment on DM content of the fish meat. Crude protein, ether extract and ash content in fish meat showed insignificant difference among different groups. The level of DM content of the fish meat increased in the groups treated with blackseed and garlic. Crude protein content of all treated groups showed numerical increase than those of aflatoxin treated groups only.

6- Effect of different natural feeds treated with or without aflatoxins on body weight of fish at different weeks: Table 6, explain the significant (P<0.01) differences among different treatment in different weeks on body

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weight fish. The body weight showed an increasing level from the 1st week to 4th week of the experiment. The groups feeds aflatoxin achieved the lowest body weight, and the body weight improved when we added blackseed, garlic and onion to them. The higher body weight observed in the groups treated with black seed, garlic and onion.

7- Effect of different natural feeds treated with or without aflatoxins on body weight gain of fish at different weeks: Table 6, explain the significant (P<0.01) differences among different treatment in different weeks on body weight gain fish. The groups feeds aflatoxin achieved the lowest body weight gain, and the body weight gain improved when we added blackseed, garlic and onion to them. The higher body weight gain observed in the groups treated with black seed, garlic and onion.

Table 3 - Effect of different treatments of black seed, garlic and onion with aflatoxicosis on differential eucocytic counts, T.WBcs and T.RBc

Time	Treatment	N	lymph	Mono	Hetero	T.WBCs	T.RBCs
			Mean±Std. Error	Mean±Std. Error	Mean±Std. Error	Mean Std. Error	Mean Std. Error
1st week	1	3	45.00±0.58C	8.67±0.33C	35.67±0.33A	25.00±0.58A	26.33±0.33A
	2	3	43.67±0.33G	9.33±0.88B	35.67±1.20A	23.33±0.33C	25.33±0.33B
	3	3	44.67±0.33D	10.33±0.33A	34.00±0.58B	25.00±0.58A	25.67±0.88B
	4	3	46.67±0.33BD	10.00±0.58A	31.67±1.20D	23.00±0.58C	25.00±0.58B
	5	3	44.33±0.33F	10.33±0.33A	34.67±0.67B	24.67±0.88B	25.67±0.33B
	6	3	47.00±0.58A	8.67±0.33C	33.00±0.58C	25.33±0.33A	25.33±0.33B
	7	3	44.00±0.58E	9.33±0.33B	34.67±0.88B	24.00±0.58B	23.00±0.58C
	8	3	46.67±0.33B	10.33±0.33A	32.33±0.88C	25.00±0.58A	23.33±0.33C
2nd week	1	3	46.33±0.33A	7.67±0.33C	36.00±0.58B	27.33±0.33A	22.33±0.33D
	2	3	46.67±0.33A	8.67±0.33B	33.67±0.33E	27.00±0.58A	22.00±0.58D
	3	3	44.33±2.03C	9.67±0.33A	35.67±2.33C	26.33±0.33B	23.67±0.33C
	4	3	46.33±0.33A	8.33±0.33B	34.67±0.67D	25.00±0.58C	22.33±0.88
	5	3	42.00±0.58D	9.33±0.33A	37.33±1.86A	25.33±0.33C	23.00±0.58C
	6	3	42.33±0.88D	9.67±0.33A	37.00±1.15A	26.33±0.33B	25.67±0.33B
	7	3	42.00±0.58D	9.67±0.88A	37.67±0.67A	27.00±0.58B	26.67±0.33A
	8	3	45.67±0.33B	8.33±0.33B	34.67±0.67D	27.33±0.33B	26.00±1.00A
3rd week	1	3	42.00±0.58C	10.00±0.58A	38.33±1.76B	25.00±0.58A	22.67±0.33C
	2	3	45.67±0.33A	10.33±0.33A	33.00±1.15C	24.00±1.15B	25.33±0.33A
	3	3	41.33±0.33D	8.33±0.33C	38.67±0.88B	22.67±0.33D	25.00±0.58A
	4	3	41.33±0.88D	9.67±0.33B	39.00±2.08A	23.67±1.45C	25.00±0.58A
	5	3	41.33±0.33D	8.67±0.67C	39.33±0.67A	24.00±0.58B	22.00±0.58C
	6	3	44.00±0.58B	8.67±0.33C	38.67±0.67B	22.67±0.88D	22.33±0.88C
	7	3	42.00±0.58C	8.67±0.33C	39.67±1.20A	21.67±0.33E	24.33±0.33B
	8	3	45.00±0.58A	10.67±0.33A	33.00±1.53C	25.00±0.58A	25.33±0.33A
4th week	1	3	38.33±1.45E	10.33±0.33A	41.00±1.15B	30.00±0.58A	21.67±0.33D
	2	3	40.33±0.33D	9.33±0.33B	39.00±0.58C	28.33±0.88C	23.33±0.33B
	3	3	43.00±0.58B	10.33±0.33A	36.00±0.58E	28.33±0.33C	22.33±0.67C
	4	3	44.33±0.88A	10.33±0.33A	34.67±1.45F	29.33±0.33B	23.00±0.58B
	5	3	43.00±0.58B	9.33±0.33B	36.33±0.88E	30.67±0.33A	21.67±0.33D
	6	3	41.33±0.33C	9.67±0.67B	38.33±0.88D	24.33±0.33F	23.00±0.58B
	7	3	41.00±1.15C	10.33±0.33A	38.33±2.33D	26.33±0.33E	22.67±0.33C
	8	3	36.67±0.33F	10.33±0.33A	44.33±0.33A	27.00±0.58D	25.00±0.58A

For each week: Treatments means within the same column of different litters are significantly different at (P < 0.01).

Residue of aflatoxin

Table 6, cleared that, the aflatoxin residue in fish muscle differ significantly (P<0.01) among the different treatments. The lower level of aflatoxin observed in the groups treated with blackseed, garlic and onion. While the groups that we added to them blackseed + aflatoxin, Garlic + aflatoxin and onion + aflatoxin showed also the lower aflatoxin residue while the higher level observed in the groups treated with aflatoxin. Our results indicated that the addition of blackseed, garlic and onion to the ratio of fish decreased the residue of aflatoxin in the fish muscle. According to the results observed in Table 7, the economic efficiency measures differed significantly among different immunostimulants added to the fish diet, the higher economic efficiency results (total return, total costs, net profit , total returns/total costs and net return to total costs) improved in the groups fed with blackseed , garlic, onion and all of them improved economic efficiency measures than the control groups and when all of them added to the fish treated with aflatoxin diet improved economic efficiency results than the group treated with aflatoxin only.

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Table 4 - Effect of different treatments of black seed, garlic and onion with aflatoxicosis on Phagocytic activity, Phagocytic index and serum proteins

Time	Treatment	N	P.A	P.I	Total protein	Albumin	Globulin	Albumin / globulin ratio
			Mean± Std. Error	Mean± Std. Error	Mean± Std. Error	Mean± Std. Error	Mean± Std. Error	Mean± Std. Error
1st week	1	3	24.00±0.58C	20.00±0.58E	5.6±0.6A	3.7±0.3A	1.9±0.3AB	1.95±0.5A
	2	3	29.00±1.15B	21.00±0.58D	5.1±0.1B	3.3±0.3B	1.8±0.4AB	1.83±0.3B
	3	3	32.00±0.58A	19.67±0.88G	5.4±0.4A	3.1±0.2B	2.3±0.3A	1.35±0.3C
	4	3	30.33±0.33B	19.33±0.33G	5.6±0.6A	3.4±0.3B	2.2±0.5A	1.55±0.5C
	5	3	30.33±1.45B	20.67±0.33F	5.1±0.5B	3.6±0.2A	1.5±0.3B	2.4±0.2A
	6	3	31.33±0.33AB	23.00±0.58B	5.4±0.4A	3.3±0.3B	2.1±0.2A	1.57±0.2C
	7	3	30.67±1.76B	22.67±1.20C	5.5±0.5A	3.4±0.4B	2.1±0.4A	1.62±0.2C
	8	3	29.67±0.88B	24.33±0.33A	5.6±0.6A	3.7±0.3A	1.9±0.4AB	1.95±0.5A
2nd week	1	3	25.33±2.03E	21.00±0.58C	4.9±0.4C	3.2±0.3C	1.7±0.4AB	1.88±0.4
	2	3	26.33±0.88D	20.67±0.33D	6.1±0.6A	4.2±0.4A	1.9±0.5A	2.21±0.2C
	3	3	27.00±0.58C	23.33±0.33A	5.6±0.5B	3.6±0.3B	2±0.2A	1.8±0.3D
	4	3	24.67±0.33F	20.67±0.88D	5.4±0.4B	3.6±0.3B	1.8±0.4A	2±0.2C
	5	3	29.67±0.88A	21.00±0.58C	5.3±0.3B	3.4±0.4B	1.9±0.3A	1.79±0.5D
	6	3	30.00±0.58A	20.67±0.33D	5.4±0.4B	3.9±0.3A	1.5±0.2B	2.6±0.2B
	7	3	28.00±1.53B	22.33±0.33B	5.6±0.5B	3.7±0.3B	1.9±0.2A	1.95±0.5B
	8	3	28.67±0.88B	20.33±0.33D	4.9±0.4C	3.8±0.3AB	1.1±0.01B	3.45±0.3A
3rd week	1	3	30.00±0.58C	22.33±0.33B	5.3±0.3B	3.9±0.4A	1.4±0.03B	2.79±0.2A
	2	3	27.67±1.76D	22.33±0.67B	5.1±0.5B	3.4±0.3	1.7±0.3B	2±0.2C
	3	3	33.00±0.58A	21.67±0.33C	5.6±0.6A	3.5±0.3B	2.1±0.1A	1.67±0.1D
	4	3	32.00±2.65B	20.67±0.33D	5.4±0.4AB	3.6±0.4B	1.8±0.2AB	2±0.2C
	5	3	31.67±1.86C	23.00±0.58A	5.3±0.3B	3.7±0.5AB	1.6±0.3B	2.31±0.2B
	6	3	33.33±0.33A	23.33±0.33A	5.1±0.5B	3.8±0.3A	1.3±0.1B	2.92±0.2A
	7	3	30.33±0.33C	21.33±0.33C	5.2±0.2B	3.6±0.4B	1.6±0.2B	2.25±0.2B
	8	3	29.00±1.15C	21.00±0.58C	5.6±0.5A	3.9±0.4A	1.7±0.2B	2.29±0.2B
4th week	1	3	32.00±0.58C	20.67±0.33B	5.4±0.4B	3.9±0.4A	1.5±0.1B	2.6±0.2
	2	3	30.00±0.58D	20.67±0.33B	5.2±0.3C	3.6±0.3B	1.6±0.3B	2.25±0.2C
	3	3	33.00±0.58C	21.33±0.33A	5.1±0.4C	3.4±0.4B	1.7±0.2B	2±0.1D
	4	3	28.33±1.45E	20.33±0.33B	5.6±0.5A	3.3±0.3B	2.3±0.2A	1.43±0.1F
	5	3	32.67±0.88C	19.67±0.33C	5.7±0.7A	3.9±0.3A	1.8±0.3B	2.17±0.2C
	6	3	36.67±0.88A	19.00±0.58C	4.8±0.4C	3.7±0.4AB	1.1±0.1C	3.36±0.3A
	7	3	35.00±0.58B	19.33±0.33C	4.9±0.5C	3.6±0.5B	1.3±0.3C	2.77±0.2B
	8	3	32.33±0.88C	18.67±0.33D	5.3±0.5B	3.4±0.4B	1.9±0.2A	1.79±0.1E

For each week: Treatments means within the same column of different litters are significantly different at ($P < 0.01$).

Table 5 - Effect of different treatments of black seed ,garlic and onion with aflatoxicosis on DM, crude protein ether extract and ash percentage in the last week

Treatments	DM%	CP%	EE%	Ash%
T1	26.55±0.39a	60.00±0.73	20.00±0.01	20.00±0.21
T2	24.36±0.23c	58.40±0.45	19.90±0.26	21.70±0.01
T3	26.00±0.01a	59.40±1.08	19.50±0.96	21.10±0.80
T4	25.04±0.11b	58.40±1.06	19.70±0.36	22.90±0.20
T5	25.55±0.27b	59.00±0.67	20.00±0.45	21.00±0.13
T6	25.84±0.65b	58.10±0.51	19.30±0.30	22.60±0.18
T7	24.61±0.50c	57.58±0.18	19.62±1.10	22.80±0.16
T8	25.68±0.43b	60.15±0.18	19.15±1.00	20.70±0.01

Treatments means within the same column of different litters are significantly different at ($P < 0.01$). DM = dry matter; CP = crud data; EE = ether extract

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Figure 1

Figure 2

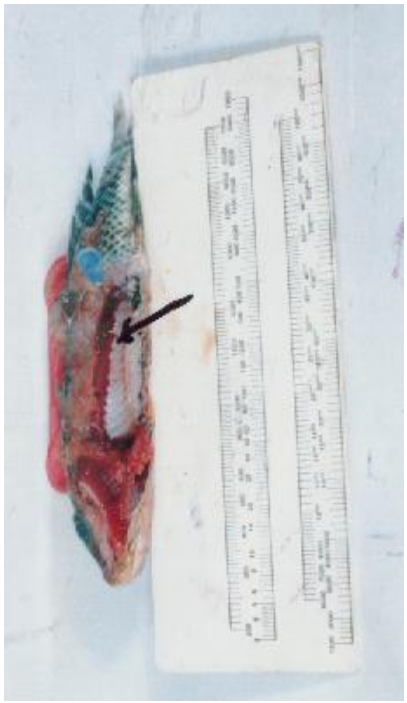

Figure 3

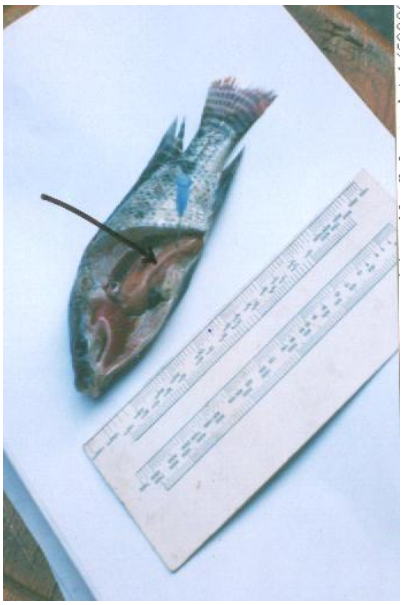

Figure 4

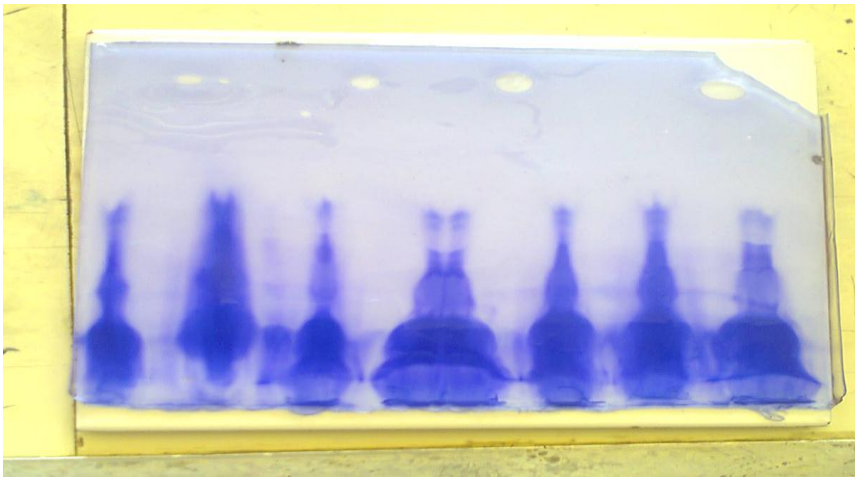

Figure 5

Figure 1. *O. niloticus* exposed to (AFTB1), showing fin erosions, eye cataraca and petechial heamorrhages distributed over the body.

Figure 2. *O. niloticus* exposed to (AFTB1) showing fin erosion and corenal opacity as well as rusty spots formation on belly and dorsal region.

Figure 3. *O. niloticus* exposed to (AFTB1), showing severe congestion of gills and kidney (Arrow).

Figure 4. *O. niloticus* exposed to *(AFTB1),* showing spots of gongested areas in the periphery of the liver. As well as planes of liver (Arrow).

Figure 5. Electrophoretic pattern of different groups exposed to immunostimulents during the experiment in the 4th week.

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Table 6 - Body weight and body weight gain of fish at different treatments among different weeks and aflatoxin residue in the last week

Groups	N	week1	Week2	Week3	Week4	Gain1	Gain2	Gain3	Gain total	AF in Fish Ms. (ppb)
		Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1	30	Ad 21.50±0.52	Ac 25.90±0.46	Ab 28.30±0.42	Aa 33.70±0.83	Ac 4.40±0.81	Ad 2.4±0.50	Ab 5.4±1.03	Aa 12.20±1.04	0.58±0.01F
2	30	A 21.40±0.43	Dc 21.50±0.52	Eb 23.10±0.60	Ea 24.20±0.68	Dd 0.10±0.01	Db 1.60±0.85	Ec 1.10±0.01	Ea 2.80±0.013	22.79±2.33D
3	30	Ad 21.50±0.54	Bc 23.60±0.43	Bb 25.70±0.70	Ba 28.20±0.44	Bc 2.10±0.80	Bc 2.10±0.75	Cb 2.50±0.87	Ba 6.7±0.45	0.66±0.03F
4	30	Ad 21.50±0.31	Dc 21.60±0.40	Fb 22.40±1.28	Fa 23.80±0.36	Dd 0.10±0.005	Fc 0.80±0.07	Db 1.40±0.04	Fa 2.30±0.35	36.45±3.45C
5	30	A 21.30±0.37	C 22.50±0.93	C 24.30±0.47	C 27.00±0.52	Cd 1.20±0.07	Cc 1.80±0.03	Bb 2.70±0.05	Ca 5.70±0.56	0.69±0.03F
6	30	Ad 21.70±0.45	Dc 21.80±0.37	Fb 22.60±0.43	Ga 23.90±0.28	Dd 0.1±0.05	Fc 0.80±0.05	Eb 1.30±0.07	Ga 2.20±0.47	51.55±0.05B
7	30	Aa 22.00±0.42	Eb 21.00±0.82	Gc 20.00±0.75	Hd 18.60±0.27	E b -1.00±0.01	Gb -1.00±0.07	Da 1.40±0.08	Hc -3.40±0.44	89.48±2.44A
8	30	Ad 21.40±0.34	Cc 22.40±0.43	Db 23.80±0.33	Da 24.30±0.40	Dc 1.00±0.04	Eb 1.40±0.54	Ed 0.50±0.43	Da 2.90±0.56	2.89±0.02E

Capital litters: Indicated that means within the same column of different litters are significantly different at ($P < 0.01$)
Small litters: Indicated that means within the same row of different litters are significantly different at ($P < 0.01$)

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Table 7 - Econonomic efficiency measures of different immunostimulants

Groups	N	Total return (L.E)	Costs of immunostimulants	Feed cost	Fixed costs and price of fry	Total costs	Net profit	Total return/ Total costs	Net return/ Total costs
		Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1	30	A 8.08±0.08	A 0.90±0.04	A 1.26±0.02	A 3	A 5.16±0.05	A 2.92±0.02	A 1.57±0.01	A 0.56±0.05
2	30	D 5.80±0.05	A 0.90±0.03	A 1.26±0.02	A 3	A 5.16±0.05	G 0.64±0.04	E 1.13±0.01	E 0.12±0.02
3	30	B 6.76±0.06	C 0.45±0.04	A 1.26±0.02	A 3	C 4.71±0.04	B 2.05±0.02	B 1.44±0.03	B 0.43±0.02
4	30	F 5.52±0.05	C 0.45±0.04	A 1.26±0.02	A 3	C 4.71±0.07	F 0.81±0.02	D 1.17±0.03	E 0.17±0.03
5	30	C 6.48±0.06	D 0.56±0.05	A 1.26±0.02	A 3	B 4.82±0.03	C 1.66±0.04	C 1.35±0.03	C 0.34±0.04
6	30	E 5.73±0.05	D 0.56±0.05	A 1.26±0.02	A 3	B 4.82±0.03	E 0.91±0.03	D 1.18±0.03	D 0.18±0.03
7	30	G 4.46±0.04	-	A 1.26±0.02	A 3	D 4.26±0.04	H 0.20±0.02	E 1.04±0.04	F 0.04±0.01
8	30	D 5.83±0.05	-	A 1.26±0.02	A 3	D 4.26±0.02	D 1.57±0.05	C 1.36±0.03	C 0.36±0.03

Means within the same column of different litters are significantly different at ($P < 0.01$)

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DISCUSSION

Effects of the presence of these spices / herbs can be seen in food products such as pickles, bread, rice, and meat products. The fat, protein, water, and salt contents of food influence microbial resistance. Thus, it is observed that higher levels of spices are necessary to inhibit growth in food than in culture media (Van Houten, 2006).

Our results showed that, the lymphocyte, monocytes and heterophils level increased in the groups treated with black seed, garlic and onion and all of them higher than that of the control group. While, the groups treated with blackseed+Alflatoxin, garlic + aflatoxin and onion + aflatoxin of lower differential leucocyte level.

Our results cleared that, the groups treated with blackseed, garlic and onion of higher T. WBCs and T. RBCs level than the other groups. And the groups treated with black seed and aflatoxin of higher T. WBCs and T. RBCs than the groups infected with aflatoxin with garlic and aflatoxin with onion all over the period of the experiment. Our results indicated that, the black seed , garlic and onion improve the fish immunity against aflatoxins through improving the T. WBCs and T. RBCs level than the other treated groups and the addition of black seed to the fish diet improve the fish immunity against the aflatoxicosis, followed by garlic and onion. Our results indicated that, the black seed , garlic and onion improve the fish immunity against aflatoxins through improving the T. WBCs and T. RBCs level than the other treated groups and the addition of black seed to the fish diet improve the fish immunity against the aflatoxicosis, followed by garlic and onion.

The improvement in the hemaogram may be due to the effects of Blackseed, garlic and onion to over come the necrosis and basophilia of hepatocytes, enlargement of blood sinusoids in the head kidney (congestion, shrinking of glomeruli and melanosis were observed), accumulation of iron pigments in the intestinal mucosaepithelium, and necrosis of gastric glands done by AFB1 (Marzouk et al., 1994).

Our results indicated that, the results showed that the garlic + aflatoxin treated group, onion and blackseed and garlic treated groups of a higher phagocytic activity and phagocytic index than the other treated groups. And the addition of black seed, garlic and onion to the fish diet improve the phagocytic acitivity and index against the aflatoxicosis. Our results also agreed with those of Salem et al. (2010) where they concluded that, from the feeding experiment that aflatoxin contamination of fish diets caused many drastic effects in all tested parameters and it is very dangerous from the view point of fish production and public health. It could be recommended for the use 1% herbs as Piper nigrum L or 1% Coriandrum sativum to alleviate the toxic effects of AFB1 contaminated diets. Moreover, we need a lot of scientific efforts in this trend to use of the natural agents to detoxify of mycotoxins (particularly aflatoxin) in diets of fish.

Our results indicated that, the groups treated with blackseed, garlic and onion of a higher total protein, albumin, globulin and albumin globulin ratio than the other treated groups. Also, aflatoxin causes decrease of the serum protein level but by addition of natural feeds as black seed, garlic and onion improve the serum protein level .

Also, our results cleared that, the moisture content, total solids, fat and total protein content level improved in the groups treated with blackseed, garlic and onion treated groups of a higher moisture, total solids containt, fat, total protein while, the groups treated with aflatoxin of lower moisture and total solids containt and the addition of black seed, garlic or onion improve the moisture, total solid, total protein content, fat level in fish meat . Our results agreed with those of Aly and Mohamed (2010) where they concluded that, garlic (G) supplemented diets has immunostimulant for tilapia (Oreochromis niloticus) and improved the RBCs, WBCs and its differentials, serum proteins, glucose, triglycerides and Hb, PCV, PA and PI, urea, creatinin, in addition to the serum enzymes than the groups fed on diet with aflatoxins . In accordance with the present findings, Abdelhamid et al. (2002b) reported that the aflatoxic diets significantly ($P<0.01$) reduced the fish flesh crude protein content but increased its fat and ash contents proportional to the dietary levels of the aflatoxin . Also, our results cleared that, the ash level increased in the groups treated with aflatoxin group, while the groups treated with aflatoxin in addition to blackseed, garlic and onion the ash containt returned to its normal level during treatment of this group. The level of carbohydrate increased in the groups treated with aflatoxin and decreased in the groups treated with blackseed, garlic and onion.

Our results cleared that, the aflatoxins causes decreasing of body weight, body weight gain and feed conversion but by the addition of blackseed, garlic and onion to them improve of body weight, body weight gain and food conversion of the fish. And the groups treated with blackseed, garlic and onion of a high body weight, body weight gain and feed conversion.

Also, our results agreed with those of Yossef and Ashry (1999) who reported that methanolic extract of N.S. seed given partial protection against aflatoxin B1-induced hepatotoxicosis in rats and this evidenced by decrease serum AST and ALT with improvement of RBCS, WBCs, serum urea, creatinine, glucose, triglycerids and serum protein. Our results agreed with those of Aly and Mohamed (2010) where they concluded that, garlic (G) supplemented diets has immunostimulant for tilapia (Oreochromis niloticus) and improved the feed intake, body weight and body weight gain than the groups fed on diet with aflatoxins.

Our results cleared that, the lower level of aflatoxin observed in the groups treated with blackseed, garlic and onion. While the groups that we added to them blackseed + aflatoxin, Gralic + aflatoxin and onion + aflatoxin showed also the lower aflatoxin residue while the higher level observed in the groups treated with aflatoxin . Thymoquinone, the most abundant constituent of black seed essential oil, has been shown to be the active principle responsible for many of the seed's beneficial effects. In addition *N.sativa* seeds contain fixed oils and

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volatile oils, which are rich sources of quinines, unsaturated fatty acids, amino acids and proteins and contain traces of alkaloids and terpenoids (Gali-Muhtasib et al., 2007).

Chung (2006) Antioxidant properties of garlic compounds representing the four main chemical classes, alliin, allyl cysteine, allyl disulfide, and allicin, prepared by chemical synthesis or purification were reported . The results of onion agreed with those of Kim et al. (2010) where they investigate the inhibitory effect of green onion produced on the growth of *A. parasiticus*, a toxigenic strain. The addition of green onion to the media showed inhibiting the fungal growth after three days of cultivation. The 1.0% concentration of green onion significantly reduced growth with improvement of WBCS, RBCs, serum enzymes, serum protein, urea and creatinine with improvement of body weigh and gain and economic and productive efficiency of fish production farms.

This study concluded that the addition of blackseed, garlic and onion to the fish diet improved the immunity of the fish against different fish diseases especially aflatoxins in addition it will improve the economic returns of the fish production farms.

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