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Role of Some Herbs in Controlling Aflatoxicosis in Cultured *Oreochromis niloticus*

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Abstract: The present study provides that the black seed is the best herbs that decrease the aflatoxin effect in cultured *Oreochromis niloticus* followed by garlic and onion, respectively. The results showed that level of RBCs and WBCs, differential leucocytic counts and phagocytosis process improved with addition of black seed, garlic and onion. The residue of aflatoxin in fish flesh decreased in the groups treated with black seed, garlic and onion than the control or fish fed on the aflatoxin. The results proved that, frequent supplementation of fish ration with black seed, garlic and onion can reduce the aflatoxin hazards in the fish. Also the 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 black seed, garlic and onion and when all of them added to the fish treated with aflatoxin diet improved economic efficiency results than the group treated with aflatoxin only.

Key words: Economic Efficiency · Black Seed · Aflatoxin

INTRODUCTION

The using of synthetic drugs in the therapeutic field may have adverse effects which may be more dangerous than the disease itself by Fluk *et al*. [1], 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 has antiviral, antibacterial, antifungal and 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 has antifungal effect, thus Garlic enjoys a worldwide reputation as an antifungal folk remedy. Mona *et al.* [2], reported that, Aflatoxin 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 clinico-pathological 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. The green onion has antifungal activity on *A. parasiticus* that producing aflatoxin and has carceingenic effect on the human, according to Kim [3]. 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 in the fish through study the effect of black seed (*Nagilla sativa*), garlic and onion on the number of RBCs, WBCs and its differentials, Phagocytic activity and phagocytic index as well as their effect on the residues of the Aflatoxin in fish flesh.

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

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enriched by air (2/3). Average body weight of fish was about 20±5 gm, of *Oreochromis niloticus*. 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, these aquaria was supplied with chlorine free tap water according to Innes [4]. 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 adlibitum commercial fish food containing 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.* [5]. The daily amount of food was offered on two occasions over the day (Regular diet).

Aflatoxins: The mycotoxin Aflatoxins 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 Prof. Dr. Riad H. Khalil., Dept. of Poultry and Fish Diseases, Fac. Vet. Med., Alexandria University.

Feed Additives: Black seed, garlic and onion extract were prepared manually in the laboratory of Poultry and Fish Diseases Department.

Experimental Design: The *Oreochromis niloticus* fish used in this study was divided into 8 groups each group of 30 fish/2 aquaria, Table 1.

A total of 240 fish (20±5 gm each) were used in this experiment and divided into 8 groups. Every group contains 30 fish in 2 aquaria. 4 group of them received ration with Aflatoxin B1 (100 ppb), according to Shehata *et al*. [6]. All the experimental groups were kept under daily observation for 10 weeks. The clinical signs, mortality and postmortem lesions were recorded. Furthermore, blood parameters and detection of residual level which were carried-out during the 10 weeks experimental period. Blood samples were collected from the caudal vein using disposable tuberculin syringe by using citrated blood in the ratio of 0.1/1 ml of 3.8% Sod. Citrate solution to 1 ml of blood according to Hawk *et al.* [7], differential leucocytic count was prepared according to the method described by Lucky [8] and determination of phagocytic activity and phagocytic index was determined according to Kawahara *et al.* [9].

Table 1: Experimental design

Time	Code	Treatment	Fish No.
10 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

Table 2: Aflatest ™ method for measuring residue

Fluorimeter 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

Detection of Aflatoxin residue in tissue samples by Aflatest™ method for samples 3-100 ppb. Measures of mycotoxins: Add 1.0 ml of dilute mycotoxin™ Developer (made up fresh daily) to elute in the cuvette. Mix well and measure fluorescence in a calibrated fluorimeter. Read aflatoxin concentration after 60 seconds. Readout will be in parts per billion total aflatoxins for the 3-100 ppb sample extracted, Table 2.

Statistical Analysis: The data of hematological examinations of exposed fish were statistically analyzed using T-test, Duncan-test after ANOVA and simple correlation according to SAS [10].

RESULTS

The results indicated that the black seed, garlic and onion improve the fish immunity against aflatoxin than the other treated groups and the addition of black seed to the fish diet improved the fish immunity against the Aflatoxicosis followed by garlic and onion, Table 6. The groups treated with black seed, garlic and onion of higher T.WBCs and T.RBCs level than the other groups. The groups treated with black seed and aflatoxin of a higher T.WBCs and T.RBCs than the groups treated with aflatoxin with garlic and aflatoxin with onion all over the period of experiment, Table 5. The results showed that black seed, garlic and onion treated groups of a higher phagocytic activity and phagocytic index than the other treated groups. The addition of black seed, garlic

Fig. 1: *O. niloticus* exposed to (AFTB1), showing pectoral fin hemorrages, erosion on the lateral side and petechial haemorrhages distributed on the abdomen

Fig. 2: *O. niloticus* exposed to (AFTB1) showing haemorrhages on caudal peduncle and fins with congested vent

Fig. 3: *O. niloticus* exposed to *(AFTB1),* showing spots of congested areas in the periphery of the liver. (Arrow)

Table 3: Economic efficiency measures of different immunostimulants

		Total return (LE)	Costs of immune-stimulants	Feed cost	Fixed costs and price of fry	Total costs	Net profit	Total return /Total costs	Net profit /Total costs
Groups	N	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	MeanStd. Error
1	30	A 8.08±0.0.8	A 0.090±0.04	A 1.26±0.02	A 3	A 5.16±0.05	A 2.96±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±0.04	F 0.81±0.02	D 1.17±0.03	E 0.17±0.03
5	30	C 6.48±0.6	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±0.05	-	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)

and onion to the fish diet improved the phagocytic activity and index against Aflatoxicosis, Table 5. The fish showed clinical signs of fin hemorrhages and erosion on the lateral side and petechial hemorrhages distributed on the abdomen and caudal peduncle with congested vent, Fig. 1 and 2 and postmortem changes showing congestion of the liver, Fig. 3.

Table 4: Residue of aflatoxin in fish at different treatments in the last week of the experiment

Treatment	Aflatoxin in fish musculature (ppb)
Black seed	0.58± 0.01F
Black seed + Aflatoxin	12.79± 2.33D
Garlic	0.66± 0.03F
Garlic +Aflatoxin	16.45± 3.45C
Onion	0.69± 0.05B
Onion + Aflatoxin	18.55± 0.05B
Aflatoxin	21.48± 2.44A
- ve control	1.19± 0.02E

Table 5: Effect of different treatments of Black seed, Garlic and Onion with and without aflatoxin on T.WBCs, T.RBCs, phagocytic activity (P.A) and phagocytic index (P.I)

			T.WBCs Cell/mm^3	T.RBCs Cell/mm^3	P.A (%)	P.I
Time	Treatment	N	Mean Std. Erorr	Mean Std. Erorr	Mean Std. Erorr	Mean Std. Erorr
1st week	Black seed	30	27.00±1.5A	2.69±0.019A	24.00±0.58C	21.00±0.58E
	Black seed + Aflatoxin	30	25.85±0.85C	2.52±0.02B	22.33±1.15B	19.33±0.58D
	Garlic	30	26.95±1.4A	2.66±0.015B	23.33±o.58A	20.33±0.88G
	Garlic +Aflatoxin	30	25.70±0.75C	2.51±0.02B	22.00±0.33B	19.00±0.33G
	Onion	30	26.35±1.00B	2.59v0.01B	23.00±1.45B	20.00±0.33F
	Onion + Aflatoxin	30	25.00±0.20A	2.50±0.05B	21.67±0.33AB	18.67±0.58B
	Aflatoxin	30	24.00±0.59B	2.40v0.08C	21.00±1.76B	18.00±1.20C
	- ve control	30	26.00±0.95A	2.55±0.03C	22.67±0.88B	19.67±0.33A
2nd week	Black seed	30	27.33±0.33A	2.35±0.03D	25.67±2.03E	21.00±0.58C
	Black seed + Aflatoxin	30	26.00±0.20A	2.22±0.04D	23.33±0.88D	21.67±0.33D
	Garlic	3	27.00±0.30B	2.34±0.03C	25.33±0.58C	20.67±0.33A
	Garlic +Aflatoxin	30	25.85±0.15C	2.21±0.03D	23.00±0.33F	20.00±0.88D
	Onion	30	26.33±0.25C	2.32±0.02B	24.00±0.88A	20.33±0.58C
	Onion + Aflatoxin	30	25.25±0.09B	2.20±0.03D	22.67±0.30A	19.67±0.33D
	Aflatoxin	30	24.00±0.08B	2.19±0.02A	22.00±1.53B	19.33±0.33B
	- ve control	30	26.20±0.25B	2.25±0.05A	23.67±0.88B	20.00±0.33D
3rd week	Black seed	30	25.00±0.58A	2.26±0.03C	33.00±0.58C	22.33±0.33B
	Black seed + Aflatoxin	30	24.00±0.25B	2.20±0.01A	31.67±1.76D	22.33±0.67B
	Garlic	30	24.95±0.50D	2.25±0.03A	32.67±0.58A	21.67±0.33C
	Garlic +Aflatoxin	30	23.95±0.20C	2.19±0.05A	31.33±2.65B	22.00±0.33D
	Onion	30	24.50±0.45B	2.21±0.02C	32.33±1.86C	23.33±0.58A
	Onion + Aflatoxin	30	23.00±0.15D	2.15±0.03C	31.00±0.33A	21.67±0.33A
	Aflatoxin	30	21.67±0.33E	2.03±0.02B	30.67±0.33C	21.33±0.33C
	- ve control	30	24.20±0.30A	2.20±0.01A	32.00±1.15C	22.67±0.58C
4th week	Black seed	30	30.00±0.58A	2.16±0.03D	32.67±0.58C	20.67±o.33B
	Black seed + Aflatoxin	30	28.85±0.35C	2.10±0.03B	31.33±0.58D	19.33±0.33B
	Garlic	30	29.33±0.50C	2.13±0.03C	32.33±0.58C	20.33±0.33A
	Garlic +Aflatoxin	30	28.33±0.33B	2.09±0.02B	31.00±1.45E	19.00±0.33B
	Onion	30	29.25±0.45A	2.12±0.02D	32.00±0.88C	20.00±0.33C
	Onion + Aflatoxin	30	28.00±0.30F	2.07±0.01B	29.67±0.88A	18.67±0.58C
	Aflatoxin	30	27.33±0.25A	2.00±0.01C	29.33±0.58B	18.33±0.33C
	- ve control	30	29.00±0.40D	2.11±0.02A	31.67±0.88C	19.67±0.33D
5th week	Black seed	30	25.33±0.67	2.16±0.03C	31.33±1.85D	21.00±0.58D
	Black seed + Aflatoxin	30	25.00±0.30B	2.12±0.06C	30.00±0.33D	19.67±0.33A
	Garlic	0	25.30±0.60A	2.15±0.08A	31.00±1.45A	20.67±0.58A
	Garlic +Aflatoxin	30	24.95±0.25B	2.10±0.05C	30.00±1.76D	19.33±0.33A
	Onion	30	25.25±0.33C	2.14±0.07C	30.67±0.58C	20.33±0.33C
	Onion + Aflatoxin	30	24.50±0.88D	2.10± 0.04C	30.00±0.58B	19.00±0.88B
	Aflatoxin	30	24.00±0.50B	2.00±0.30A	30.00±0.58A	18.67±0.33B
	- ve control	30	25.05±0.30C	2.13±0.07B	33.00±1.15B	20.00±0.33B

Table 5: Continued

6th week	Black seed	30	25.33±1.58E	2.46±0.01A	32.33±0.88A	21.00±0.58A
	Black seed + Aflatoxin	30	24.40±1.05D	2.25±0.09B	31.00±0.88B	21.33±0.33B
	Garlic	30	25.20±1.45B	2.40±0.01C	32.00±0.88C	20.67±0.50C
	Garlic +Aflatoxin	30	24.15±1.20C	2.30±0.07D	30.67±0.88B	21.00±0.33D
	Onion	30	25.00±1.30A	2.39±0.01E	31.67±0.88D	20.00±0.44B
	Onion + Aflatoxin	30	24.30±1.00C	2.30±0.09D	30.33±0.67D	20.67±0.58A
	Aflatoxin	30	24.00±0.9C	2.20±o.05A	30.00±0.88C	20.33±0.33B
	- ve control	30	24.85±1.20D	2.35±0.01C	31.33±0.33C	21.67±0.33B
7th week	Black seed	30	24.33±0.95C	2.53±0.01A	32.00±0.58B	21.00±0.58E
	Black seed + Aflatoxin	30	22.75±0.58C	2.40±0.08B	30.00±0.58A	20.00±0.33D
	Garlic	30	24.20±0.70B	2.48±0.13B	31.00±0.58C	20.67±0.33C
	Garlic +Aflatoxin	30	22.40±0.50A	2.38±0.09C	29.67±1.20E	21.67±0.58B
	Onion	30	24.00±0.70A	2.45±0.11D	30.67±0.88D	20.33±0.58A
	Onion + Aflatoxin	30	22.33±0.45C	2.30±0.07C	29.33±0.88F	21.33±0.58B
	Aflatoxin	30	21.00±0.40D	2.29±0.35B	29.00±2.33E	21.00±0.33B
	- ve control	30	23.90±0.65C	2.41±0.01B	30.33±0.88E	20.33±0.33A
8th week	Black seed	30	26.33±1.00C	2.53±0.09C	30.67±0.33B	23.67±0.58B
	Black seed + Aflatoxin	30	25.00±0.58C	2.40±0.05D	29.33±0.58B	22.33±0.33C
	Garlic	30	26.00±0.9B	2.49±0.07E	30.33±0.33A	23.33±0.33A
	Garlic +Aflatoxin	30	24.85±0.50A	2.39±0.04D	29.00±0.58B	22.33±0.88A
	Onion	30	25.80±0.8A	2.47±0.06B	30.00±0.58D	23.00±0.88C
	Onion + Aflatoxin	30	23.67±0.40C	2.32±0.03B	28.67±0.88B	22.00±0.58B
	Aflatoxin	30	23.00±0.58D	2.23±0.03A	28.33±1.76C	21.33±0.33C
	- ve control	30	25.30±0.6C	2.43±0.05C	29.67±0.88B	22.67±0.33C
9th week	Black seed	30	25.67±0.33C	2.40±0.05D	30.67±0.88D	24.00±1.15B
	Black seed + Aflatoxin	30	22.80±0.20D	2.26±0.05D	32.33±1.15A	23.33±0.33D
	Garlic	30	25.00±0.33A	2.39±0.05C	30.33±0.33C	23.67±0.58C
	Garlic +Aflatoxin	30	22.35±0.10B	2.20±0.04C	32.00±1.10A	22.33±0.88C
	Onion	30	24.85±0.30B	2.29±0.04B	33.00±1.00B	23.33±0.58A
	Onion + Aflatoxin	30	22.00±0.05A	2.19±0.03A	31.67±0.58B	22.00±0.33A
	Aflatoxin	30	21.00±0.05B	2.00±0.02B	31.00±1.53A	22.00±0.33B
	- ve control	30	23.67±0.25C	2.29±0.03C	32.67±1.00C	23.00±0.88B
10th week	Black seed	30	25.33±1.67A	2.73±0.013A	30.67±0.88C	20.67±0.33C
	Black seed + Aflatoxin	30	25.33±0.33A	2.63±0.03B	29.33±1.20E	19.33±0.33B
	Garlic	30	24.33±1.30E	2.66±0.12C	30.33±0.33C	20.33±0.33B
	Garlic +Aflatoxin	30	24.33±0.88B	2.40±0.01D	29.00±0.58A	19.00±0.58A
	Onion	30	23.30±1.20F	2.60±0.01B	30.00±0.88B	20.00±0.33B
	Onion + Aflatoxin	30	23.00±0.75D	2.43±0.03D	29.67±0.50C	18.67±0.33A
	Aflatoxin	30	22.33±0.33B	2.40±0.02C	29.33±0.33E	18.33±0.33B
	- ve control	30	23.15±0.9C	2.51±0.09C	29.67±2.08D	19.67±0.58B

For each week: Treatments means within the same column of different letters are significantly different at (p> 0.01)

Economic efficiency in Table 3, 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.* [11]. According to the results observed in Table 3, the economic efficiency measures differed significantly among different immunostimulants added to the fish diet, the economic efficiency results (total return, total costs, net profit , total returns/total costs and net profit to total costs) improved in the groups fed with black seed , garlic and onion and when all of them added to the fish treated with aflatoxin diet improved economic efficiency results than the group treated with aflatoxin only.

Aflatoxin residue in fish musculature differ significantly (P<0.01) among the different treatments, Table 4. The lower level of aflatoxin observed in the groups treated with black seed, garlic and onion. While the groups that we added to them black seed + aflatoxin, Garlic + aflatoxin and onion + aflatoxin showed lower

Table 6: Effect of different treatments of Black seed, Garlic and Onion with and without aflatoxin on differential leucocytic count

			Lymphocytes (%)	Monocytes (%)	Basophils (%)	Neutrophils (%)
Time	Treatments	N	Mean Std. error	Mean Std. error	Mean Std. error	Mean Std. error
1st week	Black seed	30	45.00 ±0.58C	8.67±0.33C	1.33±0.33A	9.33±0.33C
	Black seed + Aflatoxin	30	44.00±0.33G	8.00±0.88B	1.00±0.33B	8.60±0.33B
	Garlic	30	44.67±0.33D	8.40±0.33A	1.20±0.33B	9.20±0.33B
	Garlic +Aflatoxin	30	43.67±0.33BD	7.94±0.58A	0.95±0.33A	8.30±0.25B
	Onion	30	44.33±0.33F	8.33±0.33A	0.85±0.33B	9.00±0.58B
	Onion + Aflatoxin	30	42.00±0.58A	7.30±0.33C	0.80±0.33B	8.00±0.58A
	Aflatoxin	30	42.00±0.58E	8.25±0.33B	0.67±0.33B	11.33±0.33A
	- ve control	30	44.20±0.33B	8.30±0.33A	0.75±0.33A	7.70±0.33C
2nd week	Black seed	30	46.33±0.33A	7.67±0.33C	1.33±0.33A	8.67±0.33D
	Black seed + Aflatoxin	30	42.67±0.33A	7.20±0.33B	0.9±0.33B	7.50±0.33A
	Garlic	30	44.33±2.03C	7.60±0.33A	1.15±0.33B	8.50±0.88B
	Garlic +Aflatoxin	30	41.33±0.33A	7.05±0.33B	0.8±0.33A	7.33±0.33B
	Onion	30	43.00±0.58D	7.25±0.33A	1.05±0.33A	8.20±1.00A
	Onion + Aflatoxin	30	41.30±0.88D	7.00±0.33A	0.6±0.33B	6.95±0.33A
	Aflatoxin	30	40.00±0.58D	6.67±0.88A	0.4±0.33B	6.33±0.33A
	- ve control	30	43.00±0.33B	7.33±0.33B	1.00±0.00A	8.00±0.33A
3rd week	Black seed	30	42.00±0.58C	10.00±0.58A	1.33±0.33A	8.33±0.88
	Black seed + Aflatoxin	30	40.85±0.33A	7.90±0.33A	1.00±0.33B	7.80±0.33A
	Garlic	30	41.33±0.33D	8.33±0.33C	1.33±0.33A	8.30±0.33A
	Garlic +Aflatoxin	30	40.33±0.88D	7.67±0.33B	0.9±0.33B	7.33±0.88BC
	Onion	30	41.25±0.33D	8.15±0.67C	1.25±0.33B	8.20±0.33A
	Onion + Aflatoxin	30	39.90±0.58B	6.67±0.33C	0.8±0.00AB	6.67±0.33D
	Aflatoxin	30	39.00±0.58C	6.40±0.33C	0.33±0.33B	5.33±0.33B
	- ve control	30	41.00±0.58A	8.00±0.33A	1.10±0.33B	8.00±0.33A
4th week	Black seed	30	43.57±0.58B	10.463±0.33A	0.9±0.33B	10.95±0.33A
	Black seed + Aflatoxin	30	40.33±0.33D	9.00±0.33B	0.6±0.00A	8.94±0.33A
	Garlic	30	43.00±0.58B	10.33±0.33A	0.3±0.33B	10.00±0.33A
	Garlic +Aflatoxin	30	40.33±0.20A	8.85±0.33A	0.3±0.33B	8.30±0.33A
	Onion	30	42.00±0.58B	9.33±0.33B	1.00±0.00A	10.33±0.33A
	Onion + Aflatoxin	30	41.33±0.33C	8.20±0.67B	0.98±0.33B	8.00±0.33A
	Aflatoxin	30	41.00±1.15C	8.00±0.33A	0.60±0.00A	7.52±0.88B
	- ve control	30	41.67±0.33F	9.20±0.33A	0.9±0.33B	9.00±0.58C
5th week	Black seed	30	43.00±0.58D	10.00±0.58A	0.00±0.00C	8.67±0.33C
	Black seed + Aflatoxin	30	41.74±0.58B	9.00±0.33A	0.80±0.33B	8.00±0.88C
	Garlic	30	42.33±0.33B	9.67±0.33B	1.00±0.00A	8.33±0.58B
	Garlic +Aflatoxin	30	41.66±0.33A	9.00±0.33B	0.72±0.33B	8.00±0.58B
	Onion	30	42.00±0.30A	9.55±0.33A	0.95±0.33B	8.18±0.33C
	Onion + Aflatoxin	30	41.33±0.33B	8.77±0.33C	0.60±0.00	7.33±0.88B
	Aflatoxin	30	40.33±0.88B	8.54±0.33A	0.47±0.33B	7.00±0.58A
	- ve control	30	42.00±0.58C	9.09±0.33A	0.87±0.33B	8.03±0.33A
6th week	Black seed	30	44.33±0.33B	11.33±0.33A	0.99±0.33A	9.33±0.33B
	Black seed + Aflatoxin	30	42.33±0.58A	10.00±0.33B	0.60±0.33A	8.00±0.33A
	Garlic	30	44.00±0.58A	10.67±0.33C	0.80±0.33A	9.22±0.58A
	Garlic +Aflatoxin	30	42.00±0.58D	9.33±0.33C	0.56±0.33A	7.85±0.33B
	Onion	30	43.75±0.33B	10.33±0.33C	0.73±0.33A	9.00±0.58B
	Onion + Aflatoxin	30	41.45±0.58D	9.00±0.33B	0.50±0.33A	7.23±0.58C
	Aflatoxin	30	41.00±0.58C	8.34±0.33B	0.35±0.33B	7.00±0.33C
	- ve control	30	42.75±0.58D	10.33±0.67B	0.60±0.33B	8.55±0.58B

Table 6: Continued

7th week	Black seed	30	42.00±0.58B	7.00±1.00E	0.95±0.33B	11.33±0.33A
	Black seed + Aflatoxin	30	41.00±0.58D	5.96±0.33D	0.55±0.33B	9.21±0.33C
	Garlic	30	41.67±0.33C	6.90±0.33D	0.80±0.33B	10.81±0.67B
	Garlic +Aflatoxin	30	40.64±0.58A	5.14±0.33C	0.50±0.33A	9.00±0.58C
	Onion	30	41.33±0.88C	6.20±0.33B	0.67±0.33B	9.88±0.33C
	Onion + Aflatoxin	30	40.77±0.33D	5.50±0.33D	0.40±0.33B	9.00±0.88C
	Aflatoxin	30	40.00±0.58D	5.00±0.33B	0.33±0.33B	8.25±0.58B
	- ve control	30	41.22±0.33D	6.00±0.33A	0.60±0.33A	9.33±0.33B
8th week	Black seed	30	38.33±0.33d	11.00±3.51D	1.55±0.33B	11.33±0.33a
	Black seed + Aflatoxin	30	36.85±0.58C	9.00±0.33C	0.9±0.33B	9.33±0.33C
	Garlic	30	38.00±o.33	10.33±0.33A	1.32±0.33B	10.33±0.58B
	Garlic +Aflatoxin	30	36.33±0.33B	9.00±0.58	0.7±0.33B	9.00±0.58C
	Onion	30	37.85±1.15B	10.15±0.58B	1.22±0.33B	10.00±0.33C
	Onion + Aflatoxin	30	36.00±0.33E	8.77±0.33E	0.3±0.33B	8.52±0.88C
	Aflatoxin	30	35.33±0.33C	8.33±0.58B	0.95±0.33B	8.00±0.58B
	- ve control	30	37.00±0.33A	9.55±0.58C	1.00±0.33A	9.75±0.33B
9th week	Black seed	30	43.67±1.20B	10.33±0.33	1.67±0.33	9.67±0.33c
	Black seed + Aflatoxin	30	42.00±0.58A	9.85±0.33A	0.9±0.33A	8.85±0.33D
	Garlic	30	43.33±0.33B	9.67±0.33D	1.33±0.33A	9.60±0.33BC
	Garlic +Aflatoxin	30	42.00±0.33A	9.67±0.33C	0.5±0.33B	8.33±0.33A
	Onion	30	43.00±0.33B	9.33±0.33B	1.05±0.33B	9.34±0.33A
	Onion + Aflatoxin	30	41.33±0.88C	9.33±0.33B	0.2±0.33B	8.00±0.33D
	Aflatoxin	30	41.00±1.45B	9.00±0.33C	0.95±0.33B	7.00±0.58B
	- ve control	30	42.33±0.88C	10.00±0.58B	1.00±0.33B	9.00±0.58B
10th week	Black seed	30	45.67±0.33A	9.00±0.58C	1.67±0.33B	11.33±0.33A
	Black seed + Aflatoxin	30	43.33±0.88D	7.00±0.33B	0.6±0.33B	9.33±0.33A
	Garlic	30	44.67±0.33C	8.33±0.33A	1.33±0.33A	10.33±0.33B
	Garlic +Aflatoxin	30	43.00±0.33A	6.67±0.33D	0.3±0.33B	9.00±0.67A
	Onion	30	44.33±0.33B	8.00±0.33C	1.00±0.33B	10.00±0.33C
	Onion + Aflatoxin	30	42.67±0.33C	6.33±0.33B	0.95±0.33B	8.33±0.33B
	Aflatoxin	30	42.33±0.88D	6.00±0.33B	0.67±0.33B	8.00±0.58B
	- ve control	30	44.00±0.33B	7.33±0.33C	0.9±0.33B	9.67±0.33C

For each week: Treatments means within the same column of different letters are significantly different at (p> 0.01)

aflatoxin residue while the higher level observed in the groups treated with aflatoxin. The results indicated that the addition of black seed, garlic and onion to the ration of fish decrease the residue of aflatoxin in the fish musculature.

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, according to Van Houten [12]. The results showed that, the lymphocyte, monocytes, basophil and neutrophil 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 black seed + Aflatoxin, garlic + aflatoxin and onion + aflatoxin of lower differential leucocyte level.

The results cleared that, the groups treated with black seed, 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 exposed to aflatoxin with garlic and aflatoxin with onion all over the period of the experiment. The results indicated that, the black seed , garlic and onion improve the fish immunity against aflatoxin through improving the T. WBCs and T. RBCs level than the other treated groups. The improvement in the haemaogram may be due to the effects of Black seed, garlic and onion to decrease the necrosis 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 mucosa-epithelium and necrosis of gastric glands done by AFB1, according to Marzouk *et al.* [13].

The results showed that black seed, garlic and onion 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 activity and index against the Aflatoxicosis, that agreed with those of Salem *et al.* [14], 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 the natural agents to detoxify of mycotoxins (particularly aflatoxin) in diets of fish.

The lower level of aflatoxin observed in the groups treated with black seed, garlic and onion. While the groups that we added to them black seed + 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 volatile oils, which are rich sources of quinines, unsaturated fatty acids, amino acids and proteins and contain traces of alkaloids and terpenoids, according to Gali-Muhtasib *et al.* [15], Chung [16]. 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.* [18] 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 weight and gain and economic and productive efficiency of fish production farms.

This study concluded that the addition of black seed, garlic and onion to the fish diet improved the immunity of the fish against aflatoxins in addition it will improve the economic returns of the fish production.

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