

## Role of Oligosaccharides As Biological Additives In Cultured *Oreochromis Niloticus*

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### ABSTRACT

The present study was carried out on 150 *Oreochromis niloticus* randomly collected alive from private freshwater fish farms at Eltal Elkbir- Sharkia governorate. The fish were subjected to clinical, postmortem and parasitological examination. The efficacy of mannan-oligosaccharides (Bio-Mos®) as a biocontrol agent was evaluated on growth performance, haematological parameters, total proteins content and the Serum Lysozyme activity. The isolated parasites were subjected to identification and appeared to be belonging to the protozoa of genera *Trichodina*, *Epistylis* and *Chilodonella*, as well as monogenea of genera *Cichlidogyrus* and *Ancyrocephalus*. The results proved that Bio-Mos® has a growth promoting effect at level of 4 g/Kg of fish diet, with consequent improving the general fish health and increasing the total fish gain.

**Key words:** *Oreochromis niloticus*, serum lysozymes, haematological parameters, total proteins content, growth performance, mannan-oligosaccharides.

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### Introduction

In Egypt, external parasites of freshwater fish may be described as the most important parasitic problems facing cultured freshwater fish. External protozoa and monogenetic trematodes of freshwater fish could be considered as the most prevalent causes of diseases affecting skin and gills. External parasites cause gill inflammation and distortion of normal anatomy which impairing their respiratory foundation (Kuperman and Matey, 2000). Also, they cause skin irritation, inflammation and loss of the surface epithelium which in turn open the way for secondary invaders (Tantawy, 2001). The control of diseases can be achieved by many methods, including the judicious use of drugs and antibiotics either prophylactically or therapeutically, good management practices, genetic selection or stress and disease tolerant strains, as well as 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). There has been heightened research in developing new dietary supplementation strategies in which various health and growth-promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated (Denev, 2008). According to FAO experts "A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (FAO, 2007). There are several recognized functional prebiotic oligosaccharides in use around the world: Fructo-oligosaccharides (FOS), manna-oligosaccharides (MOS), Xylo-oligosaccharides (XOS), Inulin and  $\beta$ -glucan (Qiang *et al.*, 2009). Mannan-oligosaccharides obtained from the cell wall of *Saccharomyces cerevisiae*. In theory, pathogenic, growth-inhibiting bacteria that normally adhere to mannans on the mucosal surface of the intestine may instead bind to the mannan component of Bio-Mos®. Because these pathogens do not attach to the mucosal surface of the intestine, they are flushed from the intestinal tract, so elimination of pathogens would presumably enhance the health and growth. Another possible mode of action of Bio-Mos® is an effect on the immune system by increasing the level of antibody titres, immunoglobulins, and macrophage activity (Davis *et al.*, 2004). So this work was planned to study some common external parasitic diseases in cultured *O. niloticus* and to evaluate the effect of mannan-oligosaccharides on growth and health conditions of *O. niloticus*.

### Material and Methods

#### *Fish:*

A total number of 200 *O. niloticus* were randomly collected from freshwater aquaculture at private fish farms at Eltal Elkbir- Sharkia governorate. The collected fish specimens were with average body weight range  $28.0 \pm 1$  g. The fish were transported alive to the laboratory of fish diseases at Animal Health Research Institute,

Dokki, Giza, Egypt, in large tanks filled with water of the same sources supplied with battery air pumps and examined as soon as possible. Six full glass aquaria 100 x 50 x 50 cm were used for fish holding. Chlorine free tap water was used according to (Innes, 1966), Aquaria were supplied with air pumps. Fish were fed on commercial diet locally prepared according the table referred by the Association of Official Analytical Chemists (AOAC) (1975). Biomoss was added within the ratio of 2 and 4 g/kg.

#### *Bio-Mos®:*

Mannan-oligosaccharides derived from the cell wall of the yeast *Saccharomyces cerevisiae*. It is a product manufactured by Alltech, Inc.UK.

#### *Experimental design:*

one hundred and fifty alive randomly collected *Oreochromis niloticus* were divided according to the parasitological examination into 6 groups. The parasitic positive fish with external parasites were redivided into 3 groups (25 for each). The first group was kept as control (infected, not fed on Bio-Mos®). The second group fed on Bio-Mos® 2 g/kg feed. The third group fed on Bio-Mos® 4 g/kg feed. Both the second and the third groups were treated for 6 weeks. The parasitic negative fish were divided into 3 groups (25 for each). The first group was kept as control (non-infected, not fed on Bio-Mos®). The second group fed on Bio-Mos® 2 g/kg feed. The third group fed on Bio-Mos® 4 g/kg feed. Both the second and the third groups were treated for 6 weeks. The parasitic negative fish after 3 weeks of feeding Bio-Mos® were experimentally infected with external parasites and divided into 2 groups (25 for each). The first group fed on Bio-Mos® 2 g/kg feed. The second group fed on Bio-Mos® 4 g/kg feed for another 3 weeks.

#### *Clinical examination:*

The clinical examination of the collected alive fishes was performed as described by Amlacher (1970). Any clinical abnormalities of naturally infected fishes were recorded.

#### *Parasitological examination:*

The specimens under investigation were grossly examined for detection of any external lesions or visible cysts and microscopically for isolation and identification of external parasites according to Noga (2010).

#### *Growth parameters determination:*

- a. **Body weights:** weighed after 2, 4 and 6 weeks.
- b. **Body weight gain:** Final fish weight (g) – Initial fish weight (g) according to Annet, (1985).
- c. **Specific Growth Rate %:** It was calculated as the percentage increase in weight per fish per day as suggested by Pouomonge and Mbonglang (1993), using the following equation:

$SGR\% = (\ln WT - \ln Wt) / (T-t) \times 100$  Where:

SGR%= percentage increase in body weight per fish per day.

Ln WT= natural log of weight at time T.

Ln Wt= natural log of initial weight.

T= time, t= initial time, Ln= natural logarithm

#### *Haematological examination:*

Blood samples were collected from living fish from the caudal vessel according to Noga (2010). The fish were anesthetized using MS-222 and blood was withdrawn using small caliper needles.

*The collected blood samples were subjected to:*

#### *a-Haematological examinations:*

namely Total and Differential Leucocytic count according to (Kanaev, 1985).

#### *B-Serum Total Protein (g %) examination:*

Assay of total protein was carried by a test kit [Spectrum Diagnostics] according to biuret method described by Gornall *et al.* (1949).

*Lysozymes activity examination:*

Lysozyme activity was measured by agarose gel lysis assay according to the method described by Schultz (1987).

*Statistical analysis:*

Mean value ( $\bar{x}$ ), standard deviation (S.D.), standard error and test of significance (t-test or student's test) were carried out according to the method of Snedecor and Cochran (1976).

**Results and Discussion**

There are several food ingredients mainly carbohydrates used as prebiotic nowadays, but for a food that can be classified as a probiotic, must have some characteristics. Gibson *et al.* (2004) noted a food ingredient that most fulfill the following criteria: resistance to gastric acidity, hydrolysis by digestive enzymes and gastrointestinal absorption, fermentation by intestinal microflora and selective stimulation of the growth and / or the activity of intestinal bacteria associated with health to be classified as prebiotic. Therefore, the use of prebiotic such as mannanoligosaccharide (Bio-Mos), concentrated from the cell wall of *Saccharomyces cerevisiae*, to improve growth and health performance in the aquaculture industry is increasingly important as consumers demand eco-friendly production practices (Dimitroglou *et al.*, 2009).

The results of the examination of 150 fish randomly collected alive from private freshwater fish farms at Eltal Elkbir- Sharkia governorate indicated that 75 *Oreochromis niloticus* were naturally infected with external parasites (*Trichodina*, *Epistylis*, *Chilodonella* and *monogenea*). Fish infected with ciliated protozoa namely *Trichodina*, *Chilodonella* and *Epistylis* showed slimy pale skin with sever blood spots scattered on the body surface especially at the base of fins with detached scales in *Oreochromis niloticus*. Signs of skin irritation manifested as rubbing the bodies against the side of the aquarium were also recorded. Signs of asphyxia manifested as increased opercular movements were also noticed in some fishes photo (1). Monogenea infected fishes showed loss of appetite and sluggish movements. Infected fishes swam near the surface of the water with increased opercular movement, stretched gills covers and expanded pale and sticky gills. Infected *Oreochromis niloticus* were anaemic and exhibited sluggish feeding reflex and detached scales photo (2).

The results of the examination for different parasites isolated from skin, fins, gills and eyes revealed *Trichodina mutabilis* (Noga, 2010), *Epistylis* (Lome,1995), *Chilodonella hexasticha* (Post,1987) and Monogenetic trematodes : *Cichlidogyrus halli* (Lome,1995) and *Ancyrocephalus pellownullae* (Lome,1995) } with high infection rate. Isolated parasites are demonstrated in micrograph 1,2,3,4,5 & 6 successively.

Concerning the results of morphological identification of isolated parasites revealed that *Trichodina mutabilis* was medium to large disc shaped cell. Denticle blade oblong, largely parallel to radial of disc. With squared distal end, central part narrow with oblong to rounded overlapping end. Projection of central part not visible. Central disc without inclusions, nuclear apparatus not observed, this result agree with Noga (2010).

*Epistylis* species were observed as a sessile contractile ciliate, stalk was long and non contractile, often form a branched colony, the distal end of the organism was surrounded by rapidly moving cilia which appear as a blur, this result agree with Lome (1995).

*Chilodonella hexasticha* was typically heart shaped, with broader posterior end. The ventral side was flat with parallel ciliary rows. The dorsal surface was slightly convex and lacked cilia except in the oral groove at the extreme anterior end. 37  $\mu\text{m}$  (35 – 39  $\mu\text{m}$ ) in length and 24  $\mu\text{m}$  (23 – 25  $\mu\text{m}$ ) in width. There were contractile vacuoles spreading all over the cell. The macronucleus was rounded. The morphological characters of these parasites were nearly similar to descriptions given by Meyer (1966), Kabata (1985), and Post (1987).

Concerning the monogenetic trematode *Cichlidogyrus halli* was a small elongated worm of 0.211-0.230 mm. in length. The anterior end had 4 pairs of the head organ, and 1 pair of dark eye spots. At the posterior end opishaptor had 2 pairs of medium hooks and 6-7 pairs of marginal hooks. Copulatory organ and vetilline gland were well developed. The morphological characters of these parasites were nearly similar to description given by Lome (1995).

*Ancyrocephalus pellownullae* was a small elongated monogenetic trematode worm, the anterior end had 4 pairs of the head organ, and 1 pair of dark eye spots. The posterior end opishaptor had 2 pairs of medium hooks and 6 pairs of marginal hooks. Copulatory organ and vetilline gland were well developed. The morphological characters of these parasites were nearly similar to description given by Lome (1995). Concerning the statistical analysis of different growth parameters in *O.niloticus* groups(table,1). Fish infected with external parasites fed on diet supplemented with Bio-Mos® 2 and 4 g/Kg feed revealed significant increase in weight and weight gain in both concentrations. In *O.niloticus* groups non-infected with external parasites the addition of Bio-Mos® 4g/Kg feed in diets showed significant increase in weight and weight gain, but no significance difference among both concentrations in SGR% in all groups was shown. This result may be related with enhanced amino acid

absorption Iji *et al.* (2001), and it is suggested by the improvement in the functional integrity of the enterocyte membrane in MOS fed animals Spring *et al.* (2000) and Shane (2001). Also Gaskins (2001) argued that decreasing the total bacterial population in the intestine would decrease the inflammation of the intestinal tissue, which in turn would decrease the use of energy and amino acids by that tissue and improve productive performance. These findings supported those recorded by Zhou and Li (2004) who stated that juvenile Jian carp Fish fed the diet with addition of 0.24% Bio-Mos® had highest ( $P < 0.05$ ) weight gains and lowest ( $P < 0.05$ ) feed/gain value. Also, with that of Torrecillas *et al.* (2007) who recorded that European sea bass (*Dicentrarchus labrax*) of 35 g were fed during 67 days at 0‰, 2‰ and 4‰ dietary mannanoligosaccharide (Bio-Mos®) level of inclusion in a commercial sea bass diet. Growth significantly increased at both MOS dietary inclusion levels.

Concerning the effect of addition of Bio-Mos® with 2 and 4 g/Kg feed on the hematological parameters of *O. niloticus* (tables 2,3 &4). The results in *O. niloticus* groups infected with external parasites revealed significant increase in WBC<sub>S</sub> count, Neutrophil and Lymphocytes, while Eosinophil and Monocytes revealed significant decrease in both concentrations. In *O. niloticus* groups non-infected with external parasites, Lymphocytes had significant decrease in 2 and 4 g/Kg concentrations. WBC<sub>S</sub> count and Neutrophil showed significant increase in both concentrations. Eosinophil had no significant change, but in the 4 g/Kg concentration there were significant increases. Monocytes had both significant increase and decrease compared to the control in each concentration. In *O. niloticus* groups experimentally infected with external parasites WBC<sub>S</sub> count, Lymphocytes and Monocytes revealed the significant changes only after 14 days post infection. WBC<sub>S</sub> count revealed significant increase in 2 g/Kg concentration and significant decrease in 4 g/Kg concentration. Lymphocytes showed significant increase in 2 g/Kg concentration. Monocytes in concentration 2g/Kg showed significant decrease and significant increase in 4 g/Kg concentration.

These results agree with Andrews *et al.* (2009), who recorded that *Labeo rohita* fingerlings were fed mannanoligosaccharide (MOS) with three graded levels (1%, 2% or 4%) had an increase in leucocyte count. Also, Dimitroglou *et al.* (2010), who observed increased total leucocyte levels when gilthead sea bream (*Sparus aurata*) were fed MOS (0%, 0.2% and 0.4%) in diet. And these findings disagree with Ricardo *et al.* (2008), who reported that Nile tilapia, *Oreochromis niloticus* juveniles (13.62 g) were fed during 45 d with a commercial diet supplemented with 0, 0.2, 0.4, 0.6, 0.8, and 1% dietary MOS, showed no significant differences in hematological parameters between fish fed control and MOS supplementation diets. Also, Razeghi *et al.* (2011), who recorded that there were no significant differences in hematological parameters between control and MOS treatment groups in giant sturgeon juvenile (*Huso huso*).

The increase in the total leukocyte count may be due to that, Bio-Mos® activate the complement system via the alternative pathway Spring (2002), the complement work to trigger the recruitment of inflammatory cells and attract phagocytes, especially neutrophils. Neutrophils then trigger other parts of the immune system by releasing factors that summon other leukocytes and lymphocytes Viera *et al.* (1995). Also, Mannanoligosaccharide was reported to stimulate non specific components of the immune system Dobrescu (2002), for example, the protective effects of MOS have been shown to be associated with the granular cells having the ability to be bound to mannose and to be involved in triggering cellular and humoral defense mechanism in response to a pathogen.

Concerning the effect of Bio-Mos® on serum total proteins, Albumin, Globulin and A/G ratio of *O. niloticus* fish (tables, 5,6 &7). In *O. niloticus* groups infected with external parasites, total protein in 2g/Kg concentration showed significant decrease all over the 6 weeks and in 4g/Kg concentration showed significant decrease after 21, 35 and 42 days. Albumin, revealed significant decrease after 9 days in 2g/Kg concentration and in 4g/Kg concentration showed significant increase after 6 days. Globulin showed significant decrease along the 6 weeks of the experiment in both concentrations. A/G ratio showed significant increase in 2g/Kg concentration except after 9 days and in 4g/Kg concentration showed significant increase after 6, 9, 35 and 42 days. In *O. niloticus* groups non-infected with external parasites total protein showed significant decrease in the 2g/Kg and 4g/Kg concentrations. Albumin, revealed in 2g/Kg concentration significant decrease after 14 days, while in 4g/Kg concentration revealed significant decrease after 42 days and significant increase after 3 days. Globulin showed significant decrease in 2 g/Kg concentration after 3, 9, 14, 21 days and 35 days, while in 4g/Kg concentration revealed significant decrease after 3, 9, 14 and 21 days. A/G ratio had significant increase after 14 and 35 days in the 2g/Kg concentration and in 4g/Kg concentration after 3, 9, 14 and 21 days. In *O. niloticus* experimentally infected with external parasites total proteins and Albumin showed significant decrease after 14 days post infection in the 4g/Kg concentration. Globulin and A/G ratio showed no significance.

These results nearly agree with Razeghi *et al.* (2011), who recorded that the protein remained unaffected between control and MOS treatment groups in giant sturgeon juvenile (*Huso huso*). These results disagree with Yilmaz *et al.* (2007), who recorded that the body protein concentration increased as the level of MOS was increased in the diet from 1.5 to 4.5 g kg<sup>-1</sup> in rainbow trout (*Oncorhynchus mykiss*).

The decrease of total protein, Albumin and globulin may be due to that Bio-Mos® may improve gain by allowing nutrients to be utilized for growth rather than for activation of the specific immune system Miguel *et*

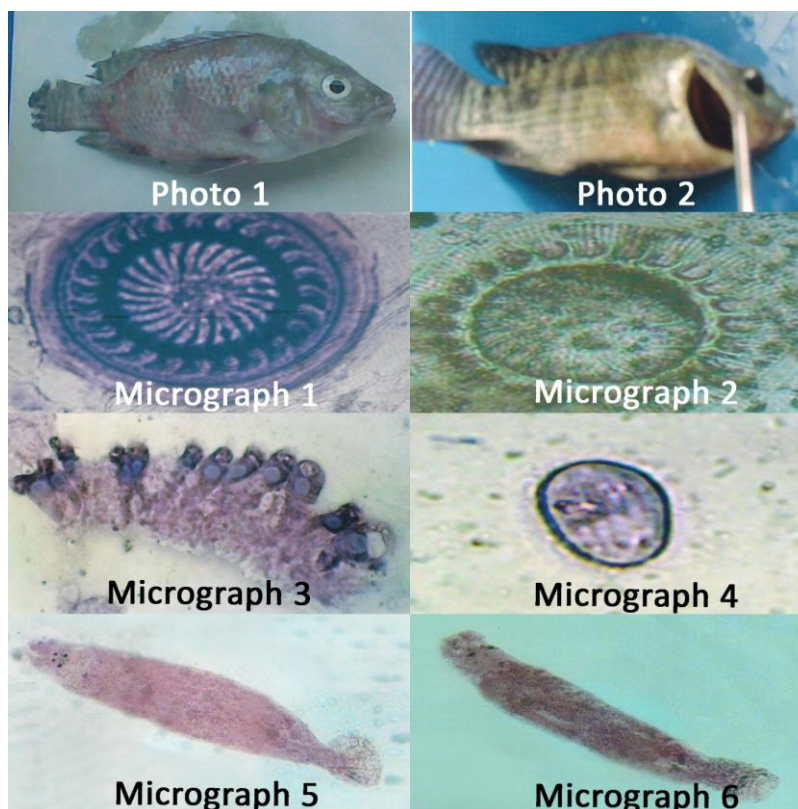
al. (2004). The increase in Albumin concentrations can be due to stronger non-specific responses in fish Andrews *et al.* (2009).

Concerning the effects of Bio-Mos® on Serum Lysozyme activity. Results were demonstrated in tables (8,9&10) and in photo (3). In *O. niloticus* groups infected with external parasites, Serum Lysozyme activity in 4g/Kg concentration showed significant increase after 3, 14, 35 and 42 days. In *O. niloticus* groups non-infected with external parasites Serum Lysozyme activity in 2g/Kg concentration showed significant decrease after 6 and 9 days and at the rest of the 6 weeks the lysozyme was increased but no significant difference was seen, in 4g/Kg concentration showed significant increase after 6 days and significant decrease after 9 and 35 days. These findings agree with Alejandro *et al.* (2010), who recorded that serum lysozyme was significantly ( $P < 0.05$ ) enhanced by mannanoligosaccharide (MOS) in diet of juvenile red drum. Also Zhou *et al.* (2010), who documented that serum lysozyme activity was significantly lower ( $P < 0.05$ ) in juvenile red drum (*Sciaenops ocellatus*) fed the basal diet compared with those fed the diets supplemented with Bio-Mos®.

In *O. niloticus* groups experimentally infected with external parasites Serum Lysozyme activity in 2g/Kg concentration revealed significant decrease after 14 days post infection, and revealed significant increase after 21 days post infection. In 4g/Kg concentration showed no significant change.

Lysozyme is an enzyme present in mucus secretion, blood and other areas of virtually all eukaryotic organisms, and it is an important component of the non-specific immune system in fish.

It is claimed that this enzyme, released from the lysozymic structures, enters plasma and measures of its activity constitute a phagocytic index. Therefore, it is a vital component of humoral immune response and its activity is related mainly to lysis of the affected cells. This increase in lysozyme activity may be evidence of immune system modulation by mannanoligosaccharide, as suggested by work by Ott (2005). Also mannan activates the complement system via the alternative pathway. It is generation of the reaction products of the complement that increases the effectiveness of phagocytic (killer) cells Sisak (1995). MOS enhanced the immune functions (phagocytic activity of leucocytes) Torrecillas *et al.* (2007).



**Photo 1:** Naturally infected *Oreochromis spp.* with *Trichodina* and *Chilodonella* Showing detached scales, haemorrhage and tail erosion.

Photo 2 : Naturally infected *Oreochromis spp.* with *Cichlidogyrus halli* showing gill congestion.

Micrograph 1 : *Trichodina mutabilis* stained with Giemsa stain (X100) .

Micrograph 2 : *Trichodina mutabilis* with silver impregnation (X100) .

Micrograph 3 : *Epistylis articulata* stained with Giemsa stain (X40) .

Micrograph 4 : *Chilodonella hexasticha* stained with Giemsa stain (X100) .

Micrograph(5 : *Cichlidogyrus halli* stained with Carmine stain (X100) .

Micrograph 6 : *Ancyrocephalus pellonulae* stained with Carmine stain (X100) .

**Table 1:** Effect of Bio-Mos® on growth performance of *O.niloticus* during 6 weeks experimental period.

Group / parameters	Mannan – oligosaccharides in ration					
	Infected			Non - infected		
	Control 0 g / kg	2 g / kg	4 g / kg	Control 0 g / kg	2 g / kg	4 g / kg
<b>Weight(g) + SE</b>						
0 time	29.6±0.9	28.1±1.3	29.1±1.4	28.5±1.4	28.9±1.9	29±1.2
2 weeks	31.1±1.1	32.3±0.8	34.5±0.9*	33.8±0.6	34.6±0.8	35.6±0.5*
4 weeks	35.5±0.6	39.9±0.5***	42.1±0.8***	38.1±0.6	40.6±1.1	44.4±0.7***
6 weeks	39.5±1	42.8±0.5*	44.2±0.8**	41.6±0.8	43.7±0.5*	46.6±0.7***
<b>Weight gain(g)</b>						
2 weeks	1.5±0.2	4.2±0.5***	5.4±0.5***	5.3±0.8	5.7±1.1	6.6±0.7
4 weeks	5.9±0.3	11.8±0.8***	13±0.6***	9.6±0.8	11.7±0.8	15.4±0.5***
6 weeks	9.9±0.1	14.7±0.8***	15.1±0.6***	13.1±0.6	14.8±1.4	17.6±0.5***
<b>SGR%</b>						
2 weeks	0.003±0.01	0.009±0.03	0.01±0.03	0.01±0.06	0.01±0.06	0.01±0.06
4 weeks	0.006±0.01	0.01±0.03	0.01±0.02	0.01±0.06	0.01±0.02	0.01±0.02
6 weeks	0.007±0.002	0.01±0.02	0.009±0.01	0.009±0.01	0.01±0.03	0.01±0.01

Data are represented as means of 10 sample ± S.E

\*Slightly significant different from control P<0.05

\*\* Moderately significant different from control P<0.01

\*\*\* Highly significant different from control P<0.001

**Table 2:** Effect of Bio-Mos® on WBCs and differential leukocytic count of *O.niloticus*, infected with external parasites: (Mean ± SE).

Time	WBCs ×10 <sup>3</sup> mm <sup>3</sup> / μl		Neutrophil%		Eosinophil %		Lymphocyte%		monocyte%	
Control + ve	22±0.89		9.8±0.8		22.2±2.35		57.4±0.87		12.2±1.28	
	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg
After 3days	18.3±0.97*	16.8±1**	14.8±1.49*	10.4±0.98	6.4±0.98***	7.2±1.02***	74.4±1.80***	78.8±2.49***	4.4±1.03***	4±1.26***
After 6days	29.6±0.79***	26.3±0.94**	25±1.48***	22±0.89***	8.2±0.49***	7.6±1.21***	59.6±1.29	58.8±1.53	7.2±0.37**	12.4±1.33
After 9days	34.5±0.81***	28.6±0.93***	22.6±0.87***	24.8±0.8***	4.4±0.74***	3.6±1.03***	66.8±1.46***	64.6±1.89**	6.2±0.73**	6.1±0.49***
After 14days	40±1.9***	40.4±1.29***	23.4±1.08***	20.8±0.8***	3.2±0.49***	5.6±0.75***	66.4±0.75***	64.4±1.33**	5.4±0.75***	6.4±0.74**
After 21days	25.8±0.6**	38.2±0.84	21±0.63***	22.8±1.39***	5.8±0.8**	4.8±0.58***	64.2±1.8**	66.6±2.09**	9 ± 0.89	5.8±1.28**
After 35days	29.7±0.99***	36.1±1.54***	14.8±0.73***	22.8±0.86***	2.4±0.51***	1.6±0.75***	78.2±0.49***	70.4±0.51***	4.4±0.24***	5.2±0.49***
After 42days	29.9±1.1***	34.4±0.68***	21.6±0.75***	22.4±1.17***	0.6±0.4***	1.6±0.75***	70.8±1.16***	67.2±1.85***	7±0.89**	8.8±0.49*

N= 10 Control + ve =infected with external parasites

\*Slightly significant different from control P<0.05

\*\* Moderately significant different from control P<0.01

\*\*\* Highly significant different from control P<0.001

**Table 3:** Effect of Bio-Mos® on WBCs and differential leukocytic count of *O.niloticus* non-infected with external parasites: (Mean ± SE).

Time	WBCs ×10 <sup>3</sup> mm <sup>3</sup> / μl		Neutrophil%		Eosinophil %		Lymphocyte%		monocyte%	
Control -ve	23±1.30		13.6±0.75		1.6±0.75		75.6±2.48		7.6±0.75	
	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg
After 3days	22.5±1.66	22.8±1.66	11.6±0.98	9.6±0.75**	3.2±1.36	3±0.45	79±2.28	78.8±0.86	6.2±0.8	8.6±1.08
After 6days	26.5±1.2	21±1.14	23.6±0.75***	19.8±0.8***	3.6±0.75	6±0.89**	63.6±1.94**	65.6±1.21**	9.6±1.17	8.6±0.75
After 9days	37±1.06***	28.7±0.73**	26±1.05***	22.2±0.66***	2.8±0.49	4.2±0.66*	55.2±1.07***	61.8±1.11***	13.6±0.75***	11.8±1.11**
After 14days	36.6±1.86***	26.3±1.86	15.8±1.11	18.2±0.66***	4.8±0.8*	2.4±1.17	74.4±1.12	76.6±2.01	5±0.89*	3.4±1.08**
After 21days	46±1.49***	36.1±1.67***	20.8±1.2***	20.4±2.31*	1.8±0.58	3±1	72.4±2.84	71±1.41	4.2±0.66**	5.6±0.75
After 35days	24.5±1.67	40.8±1.17***	21.4±0.68***	22.2±0.66***	2.2±0.37	1.8±0.58	68.4±1.17*	70.6±1.12	8±0.63	5.4±0.24*
After 42days	35.3±1.44***	38.8±1.16***	22.6±0.75***	18.8±0.73***	1.4±0.68	1±0.45	66.2±1.91*	72±0.89	9.8±2.01	8±0.63

N= 10 Control - ve =infected with external parasites

\*Slightly significant different from control P<0.05

\*\* Moderately significant different from control P<0.01

\*\*\* Highly significant different from control P<0.001

**Table 4:** Effect of Bio-Mos® on WBCs and differential leukocytic count of *O. niloticus*. experimentally infected with external parasites: (Mean ± SE).

Time	WBCs ×10 <sup>3</sup> mm <sup>3</sup> / μl		Neutrophil%		Esinophil %		Lymphocyte%		M onocyte%	
	2g/Kg	4g/Kg	2g/Kg	4g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg
Control	24.5± 1.67	40.8± 1.17	21.4± 0.68	22.2± 0.66	5.4± 0.24	8± 0.63	70.6± 1.12	68.4± 1.15	1.8± 0.58	2.2± 0.37
14 d. post infection	40.5± 0.79***	21.9± 1.38***	20.8± 0.37	19.8± 1.07	8± 1.05*	5.4± 0.51**	69.6± 0.93	72.2± 1.02*	2.6± 0.4	1.6± 0.51
Control	35.3± 1.44	38.8± 1.16	22.6± 0.74	18.8± 0.73	8± 0.63	9.8± 2.01	72± 0.89	66.2± 1.91	1± 0.45	1.4± 0.68
21d. post infection	38.4± 1.11	36.4± 2.87	20.4± 2.4	20.6± 0.6	9.6± 0.98	8± 0.63	70± 1.58	72± 2.19	1.2± 0.58	2.2± 0.66

N= 10 Control = non-infected with external parasites

\*Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

**Table 5:** Effect of Bio-Mos® on total proteins, Albumin, Globulin and A/G ratio of *O. niloticus* infected with external parasites: (Mean ± SE).

Time	Total protein (gm/dl)		Albumin (gm/dl)		Globulin (gm/dl)		A/G ratio	
Control +ve	4.28 ±0.09		1.22±0.11		3.06±0.08		0.4±0.04	
	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg
After 3 d.	2.68±0.26** *	3.26±0.63	1.45±0.07	1.5±0.19	1.23±0.22** *	1.76±0.68	1.25±0.15** *	1.75±0.8
After 6 d.	3.41±0.3*	4.24±0.19	1.49±0.08	1.84±0.07** *	1.91±0.34**	2.44±0.14**	0.85±0.14**	0.76±0.04** *
After 9 d.	1.95±0.33** *	3.24±0.55	0.64±0.12* *	1.25±0.22	1.31±0.26** *	1.99±0.42*	0.52±0.09	0.66±0.1*
After 14d.	3±0.46*	3.27±0.56	1.19±0.27	1.14±0.17	1.81±0.26** *	2.12±0.4*	0.66±0.11*	0.56±0.08
After 21d.	3.29±0.19** *	2.85±0.57*	1.15±0.08	1.11±0.08	2.14±0.16** *	1.75±0.54*	0.55±0.04*	0.82±0.25
After 35 d.	2.26±0.33** *	2.46±0.72*	0.99±0.16	1.4±0.36	1.27±0.18** *	1.06±0.36** *	0.78±0.08**	1.42±0.16** *
After 42d.	1.88±0.25** *	2.09±0.12** *	0.97±0.15	1.35±0.08	0.91±0.19** *	0.73±0.06** *	0.82±0.03** *	1.86±0.13** *

N= 10 Control =infected with external parasites

\*Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

**Table 6:** Effect of Bio-Mos® on total proteins, Albumin, Globulin and A/G ratio of *O. niloticus* non-infected with external parasites: (Mean ± SE).

Time	Total protein (gm/dl)		Albumin (gm/dl)		Globulin (gm/dl)		A/G ratio	
Control -ve	4.28 ±0.09		1.22±0.11		3.06±0.08		0.4±0.04	
	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg
After 3 d.	3.02±0.1***	2.5±0.17***	1.24±0.11	1.65±0.06* *	1.78±0.02**	1.02±0.25** *	0.69±0.07	2.06±0.65*
After 6 d.	3.52±0.24	3.66±0.12	1.31±0.21	1.48±0.18	2.21±0.37	2.19±0.19	0.74±0.3	0.7±0.13
After 9 d.	1.99±0.13** *	1.73±0.23** *	0.81±0.12	0.87±0.12	0.18±0.05** *	0.86±0.12** *	0.69±0.1	1.02±0.07** *
After 14d.	1.47±0.11** *	1.52±0.13** *	0.84±0.04 *	0.92±0.03	0.63±0.07** *	0.6±0.11***	1.38±0.12** *	1.69±0.34**
After 21d.	2.39±0.23** *	1.44±0.1***	1.14±0.05	0.94±0.02	1.24±0.27**	0.5±0.1***	1.1±0.31	2.3±0.69*
After 35 d.	3.21±0.62	3.31±0.67	1.61±0.28	1.33±0.18	1.59±0.34*	1.98±0.62	1.04±0.07** *	0.84±0.19
After 42d.	3.57±0.39	3.14±0.13**	1.19±0.06	0.87±0.02*	2.38±0.35	2.26±0.14	0.52±0.07	0.39±0.03

N= 10 Control - ve =infected with external parasites

\*Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

**Table 7:** Effect of Bio-Mos® on total proteins, Albumin, Globulin and A/G ratio of *O. niloticus* experimentally infected with external parasites: (Mean ± SE).

Time	Total protein (gm/dl)		Albumin (gm/dl)		Globulin (gm/dl)		A/G ratio	
	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg	2g/Kg	4g/Kg
control	3.21±0.62	3.31±0.67	1.61±0.28	1.33±0.18	1.59±0.34	1.98±0.62	1.04±0.07	0.84±0.19
14 d. post infection	2.69±0.68	1.08±0.09**	1.48±0.32	0.43±0.07***	1.2±0.37	0.66±0.06	1.47±0.29	0.67±0.12
control	3.57±0.39	3.14±0.13	1.19±0.06	0.87±0.02	2.38±0.35	2.26±0.14	0.52±0.07	0.39±0.03
21 d. post infection	2.82±0.28	3.44±0.17	1.14±0.04	0.9±0.04	1.67±0.26	2.53±0.15	0.72±0.08	0.36±0.02

N= 10 Control = non -infected with external parasites

\*Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

**Table 8:** Effect of Bio-Mos® on Lysozymes activity of *O. niloticus* infected with external parasites: (Mean ± SE).

Time	Conc. µg / ml	
Control +ve	569±16	
	2g/Kg	4g/Kg
After 3 days	650.2±30.58*	733.8±31.69***
After 6 days	634.2±36.45	634.2±36.45
After 9 days	601±19.59	601±19.59
After 14days	617.6±30.42	650.2±30.58*
After 21days	537.6±15.40	585±19.59
After 35 days	569±16	836.8±21.31***
After 42days	585±19.59	666.8±32.68*

N= 10 Control + ve =infected with external parasites

\* Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

**Table 9:** Effect of Bio-Mos® on Lysozymes activity of *O. niloticus* non-infected with external parasites: (Mean ± SE).

Time	Conc. µg / ml	
Control -ve	585±19.59	
	2g/Kg	4g/Kg
After 3 days	650.2±30.58	585±19.59
After 6 days	506.8±18.86*	649.6±16.60*
After 9 days	522.2±18.86*	506.8±18.86*
After 14days	585±19.59	601±19.59
After 21days	601±19.59	523±30
After 35 days	601±19.59	403.8±22.46***
After 42days	601±19.59	538.2±29.29

N= 10 Control = non-infected with external parasites

\* Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

**Table 10:** Effect of Bio-Mos® on Lysozymes activity of *O. niloticus* experimentally infected with external parasites: (Mean ± SE).

Time	Conc. µg / ml	
	2g/Kg	4g/Kg
control	601±19.59	403.8±22.46
14 days post infection	433±28.50***	375.4±16.90
control	601±19.59	538.2±29.29
21 days post infection	682.8±20.33*	507.6±30.10

N= 10 Control = non-infected with external parasites

\* Slightly significant different from control P&lt;0.05

\*\* Moderately significant different from control P&lt;0.01

\*\*\* Highly significant different from control P&lt;0.001

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