Egypt. J. Vet. Sci. **Vol. 48.** No, 2, pp. 53- 60 (2017)

Trial on Using Some of Herbal Extracts as Promising Immunoprophylaxis Feed Additives in Cultured Oreochromis niloticus

W. S. Soliman *, M. S. Marzouk, M. Abdelaziz**, Nermeen M. Abu-Elala**, H. H. Abbas*, Mona S. Zaki*, and Sahr B.A.***

**Department of Hydrobiology, Veterinary Research Division, National Research Centre and **Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.*

MEDICINAL HERBS and some plant extracts are natural feed additives which have a great impact on fish health. The present study focused on three dietary plant extracts to determine which one could be used as immunostimulant in aqua-feed. A total of 210 apparently healthy *O. niloticus* with an average body weight 30 g were stocked in 21 glass aquaria (10 fish/group/replicate) (3 replicate/group) and fed on pelleted diet supplemented with (0.5mg, 1mg onion leaves extracts {OE}, 0.5mg. 1mg barely seed extracts{BE}, 0.5mg, 1mg rice straw extracts {RE }and 0% additive) twice daily for two months. The blood picture, innate immunological parameters and response toward pathogens were assessed. At the end of experiment there was significant increase ($P < 0.05$) in serum lysozyme activity, phagocytosis in groups fed OE and BE extracts while there is no effect in fish fed RE extract. Globulin level and WBCS count increased in OE and BE groups. The result of this study demonstrated that fish groups fed on OE exhibited the highest immune performance and resistance against experimental infection with *Aeromonas hydrophila*, coming in the second rank the BE, and to lesser extent the RE which was nearly similar to control group. Therefore, we can recommend the use of onion leaves extract in aqua-feed at 0.5mg/kg diet and\or barley seed extract at 1mg/kg diet.

Keywords: Herbal extracts, Phagocytic activity, Lysozyme activity, Challenge, *O. niloticus.*

Introduction

The blue revolution in fish culturing sets Egypt in the second rank after China in tilapia production and involves it within the top fifteen countries in aquaculture sector [1]. However, feeding cost and diseases are still the major limiting factors facing the development, sustainability and profitability of this industry [2]. The cost incurred during disease control, either due to delay in growth performance, medication, mortality and labor is significantly affecting the profit margin of the farms. Lack of efficient vaccination strategy and ban of antibiotic use in fish farms necessitate the development of new alternatives to improve the growth performance, immunity and resistance against diseases [3, 4]. Natural additives as phytochemical herbs and some plant extracts might be used as promising dietary feed additives by both researchers and feed companies. They contain various active compounds like alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils with very potent growth promoting, immunostimulating and anti-microbial properties [5, 6]. Among these plants, Onion (*Allium cepa*) and its flavonoid compounds have been used to accelerate the growth performance of fish beside their potential immunostimulants [7] antibacterial and antioxidant effects [8].The high soluble fiber content of onion could be used as prebiotic feed additive [9] Literature approved onion as one of the most effective immunostimulants that improvesthe innate immunity of juvenile Olive flounder (*Paralichthysolivaceus*) [10] Another plant is Barley (*Hordeumvulgare*), a member of the grass family, Poaceae, is one of the main

Corresponding Author: Waleed Salah El-Din Soliman, 00201141256356. Email: waleed0809@yahoo.com
DOI: 10.21608/ejvs.2017.1446.1018

cereal crops grown around the world [11]. They contain a wide array of phytochemicals, primarily phenolic compounds like flavonols, phenolic acids and procyanidins. Previous studies focused on use of phenolic content of barley as antioxidants [12]. Ferulic, caffeic, and vanillic acids are the major phenolic components in barley seeds [13]. Rice straw (*Oryza sativa*) is one of the abundant lingo-cellulosic waste materials in the world. It is of low cost and its biodegradable favor the growth of bacterial biofilm and prephyton which used as feed for fish [14]. Rice straw extract can be used in fish pond to decrease the turbidity [15]. Therefore the present work was planned for extraction of the most active ingredients in some plant namely onion, barley and rice to assess their effect on tilapia fish immune parameters and resistance against infection by *A. hydrophila* during challenge test.

Material and Methods

Samples

A total of 210 apparently healthy *O. niloticus* with an average body weight 30±5 g, were purchased from a private fish farm at Kafr El-shikh governorate. Fish were randomly dispersed in glass aquaria and acclimated for 2 weeks prior to the experiments, continuous aeration was maintained using electric air pumps and the water temperature was adjusted to 25°C according to Innes [16]. Fish were divided into seven fish groups (10 fish/ group/replicate) (3 replicate / concentration) fed on commercial fish diet supplemented with (0.5mg, 1mg onion extracts, 0.5mg. 1mg barely extracts, 0.5mg, 1mg rice straw extracts and 0% additive for control). Fish groups fed on 3% of the total biomass twice per day for two months. Blood samples (3fish/sample) were collected twice (4th week, 8th week) during the whole experiment from the caudal blood vessels of fish according to Noga [17] on 100IU/ml sodium heparin for measuring of phagocytic activity and lysozyme activity test. Other blood samples were collected for measuring the immunological parameters (Total protein, albumin) at the end of the experiment.

Preparation of Plant extracts and their analysis

Fresh onion leaves, barley seeds and rice straw were dried at room temperature for 2 weeks, ground using a mortar and pestle and soaked in methanol solvent for 1 week then the extracted material was filtered through Whatman No. 1 filter paper then the final extracts were evaporated by rotary evaporator [18]. GC-MS analysis of the plant ethanol extracts were performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) [19]. Standard commercial fish diet was mixed with onion leaves, barley seed and rice straw extracts and6 experimental rations were prepared with two concentrations for each plant (0.5 and 1mg/kg diet).

Determination of the plant extracts concentration

Preliminary test was performed for 2 weeks to determine the suitable concentrations used for these plants.

Clinicopathological examination

Differential leukocytic count

Thin blood smears were prepared and stained with Giemsa stain for 30 minutes. One hundred leukocytes were identified and the percentage values of different white cells were calculated [20].

Determination of serum Total Protein (g/dl)

Assay of total protein was carried by a test kit according to Biuret method described by Gornall et al. [21].

Serum albumin

Thismethod is based on colorimetric end point method according to modified bromcresol green binding assay [22].

Serum globulin:

It was estimated by this equation (total protein- serum albumin) according to the method described by Coles [23].

A/G ratio

It was calculated from albumin present in serum in relation to the amount of globulin.

Evaluation of immune response of experimental fish

Phagocytic activity assay

This test was performed according to Abu-Elala et al. [24]

The phagocytic activity was calculated according to the following equations:

$$\text{Percentage of phagocytosis} = \frac{\text{No. of ingested phagocytes}}{\text{Total no. of phagocytes}}$$

Lysozymes activity

The units of lysozyme activity were calculated by using the hen egg white lysozyme standard curve. According to Abu-Elala et al. [24]

Challenge test

Virulent bacterial strain of *A. hydrophila* was obtained from Hydrobiology Dept., Vet. Res. Division, NRC lab and the LD_{50} of the strain was adjusted to $3x10^8$ CFU/ml [25 , 26]. Fish were firstly anaesthetized by holding them in a separate aquaria containing clove oil (Merck, Germany) at concentration of 50ul L^{-1} [27]. After which they were netted out of the anesthetic bath individually and I/P injected with 0.2 ml of virulent bacterial suspension of *A. hydrophila.* RPS (relative percent survivability) of challenged fish in different groups during 10 days was calculated according to Ngugi et al. [28] by this equation:

$$RPS = \frac{\text{Number of surviving fish after challenge}}{\text{Total number of fish injected with bacteria}} \times 100$$

Statistical analysis

Data were presented as means ± standard error (SE) and the significance of differences was evaluated using analysis of variance (ANOVA) test SPSS 14 [29).

Results

Analysis of Plant extract

Table 1 show the analysis of plant extracts of onion leaves, barely seed and rice straw. Phenolic compounds and flavonoids present in higher amount in onion extract followed by barely extract and in low amount in rice straw extract.

Clinicopathological findings

The clinicopathological results of fish groups treated with onion leaves, barley seeds, and rice straw extracts are represented in Tables (2-6). There was significant increase in WBCs count and lymphocyte % in all treated groups except rice (0.5-1gm) groups compared to control one. Also there was significant increase in total protein levels in all treated groups than control except barley (0.5gm) groups. Albumin levels of all treated groups showed insignificant increase versus control. The globulin levels revealed significant increase in all treated groups except rice (0.5-1gm) respect to control group.

Innate Immunological parameters

Phagocytic activity

The Phagocytic activity and index of fish groups are represented in Table 5. There was significant increase in phagocytic activity and index in all treated groups except rice (0.5-1gm) versus to control group (Fig. 1). The highest significant increment of phagocytosis was recorded in the both groups of onion extract.

TABLE 1. Phenolic and flavonoid contents of plant extracts.

Plants	Total phenolic mg/gm tissue	Total flavonoid mg/gm tissue
Rice straw	0.27 ± 0.32	0.36 ± 0.054
Onion leaves	3.40 ± 0.60	0.75 ± 0.02
Barley	0.71 ± 0.10	0.29 ± 0.019

TABLE 2. WBCs count and differential leukocytes count of different treated O. niloticus groups.

Group	$WBC_S \times 10^3$	Lymphocytes%
Onion 1gm	53.00 ± 1.02^{CD}	80 ± 1.24^{CD}
Onion 0.5gm	51.33 ± 1.85^{CD}	78 ± 1.32^{CD}
Barley 1gm	48.33 ± 0.88^{BD}	75 ± 1.24^{BD}
Barley0.5gm	44.00 ± 2.64^{B}	72 ± 0.89^{B}
Rice 1gm	35.66 ± 4.70^{A}	71 ± 1.21^{A}
Rice 0.5gm	31.10 ± 0.68^{A}	71 ± 0.97^{A}
Control	30.63 ± 0.68^{A}	70 ± 1.02^{A}

Data represented as means ± SE; n= 5

Columns with different letters are significantly difference at $p \leq 0.05$

TABLE 3. Total protein (g/dl) in different fish groups (O. niloticus).

Group	Total protein (g/dl)
Onion 1gm	5.20 ± 0.07^{B}
Onion 0.5gm	4.84 ± 0.05^{BD}
Barley 1gm	4.70 ± 0.08^{D}
Barley 0.5gm	4.00 ± 0.03^{A}
Rice 1gm	2.90 ± 0.10^{E}
Rice 0.5gm	3.00 ± 0.07^{F}
Control	2.99 ± 0.10^{A}

Data represented as means ± SE; n= 5.

Columns with different letters are significantly difference at $p \leq 0.05$.

TABLE 4. Globulin (g/dl) in different fish groups.

Groups	Albumin (g/dl)	Globulin (g/dl)
Onion 1gm	2.09±0.08	3.11 ± 0.03^{EF}
Onion 0.5gm	1.91 ± 0.05	2.93 ± 0.10^{DF}
Barley 1gm	1.92± 0.07	2.77 ± 0.14^{D}
Barley 0.5gm	2.4 ± 0.07	1.60 ± 0.10^{BC}
Rice 1gm	1.85 ± 0.05	1.05 ± 0.08^{A}
Rice 0.5gm	1.97 ± 0.05	1.03 ± 0.06^{AC}
Control	2.00 ± 0.05	0.99 ± 0.11^{A}

Data represented as means ± SE; n= 5

Columns with different letters are significantly difference at $p \leq 0.05$

Lysozyme activity

Table 6 showed the lysozyme activities of different fish groups. Figure 2 showed significant increase in lysozyme activity in all treated groups compared to control except in rice straw (0.5-1gm) groups.

Challenge tests

Challenge test results are represented in Table 7 and Fig. 3, ten days post challenge the onion groups showed the lowest mortality percent followed by barley groups while rice groups was the same as control group.

TABLE 5. Phagocytic activity in different fish group at 4th and 8th week of experiment.

Groups	Phagocytic activity 4th week	Phagocytic activity 8th week
onion 1	42.00±1.20^{E}	50.00±0.25^{C}
onion 0.5	38.00 ±0.88 ^{D}	43.00±0.66 ^{CD}
barley 1	33.50 ±0.88^{C}	35.00±2.60^{BD}
barley 0.5	27.00 ±1.45 ^{B}	30.00±2.08 ^{B}
rice 1	17.50 ±1.20^{A}	19.50±6.26 ^{A}
rice 0.5	17.40 ±0.76^{A}	18.50±0.42^{A}
control	16.00 ±1.73^{A}	16.00±0.88^{A}

Data represented as means ± SE; n= 5
Columns with different letters are significantly difference at p ≤0.05

TABLE 6. Lysozyme activities (µg/ml) of different O. niloticus groups at 4th and 8th week of experiment.

Groups	Lysozyme activity (µg/ml) 4th week	Lysozyme activity (µg/ml) 8th week
Onion 1	43.65 ±2.25^{E}	64.61±3.09^{C}
Onion 0.5	39.89±.333^{D}	60.20±1.05^{C}
Barley 1	32.34±0.75^{C}	46.52±2.61^{B}
Barley 0.5	24.20±0.83^{B}	40.42±2.09^{B}
Rice 1	20.24±1.13^{A}	18.63±2.35^{A}
Rice 0.5	19.30±0.81^{A}	18.33±1.66^{A}
Control	18.61±0.79^{A}	16.34±1.41^{A}

Data represented as means ± SE; n= 5
Columns with different letters are significantly difference at p ≤0.05

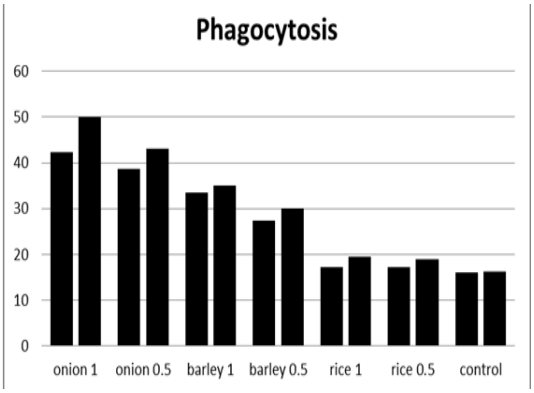

Fig. 1. Phagocytic activity in different fish groupat 4th and 8th week of experiment.

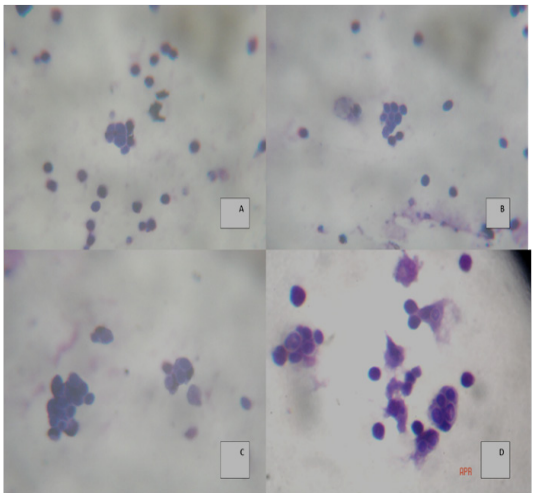

Fig. 2. Phagocytic cells of different experimental O. niloticus groups.

(A), (B) phagocytic cells of fish groups fed on barely 0.5gm and 1gm

(C), (D) Phagocytic cells of fish groups fed on onion extracts 0.5gm and 1gm

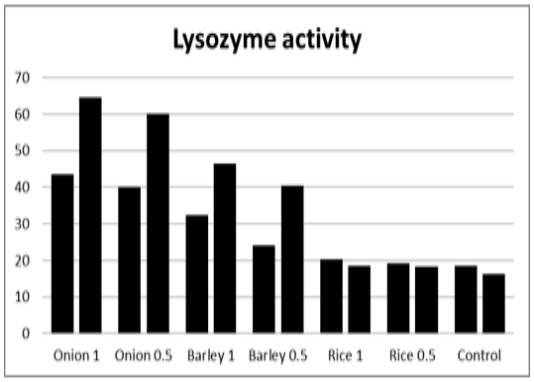

Fig. 3. Lysozyme activity (µg/ml) of different fish groups at 4th and 8th week of experiment.

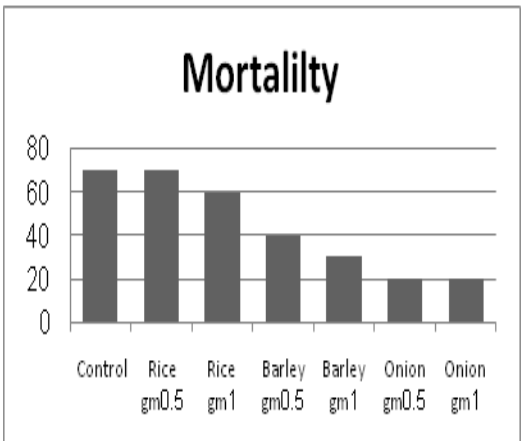

Fig. 4. Mortality % in different fish groups.

TABLE 7. Showing mortality % of all fish groups challenged with A. hydrophilaat the end of experimental period.

Fish groups	Control negative	Control positive	Onion 1gm	Onion 0.5gm	Barley 1gm	Barley 0.5gm	Rice 1gm	Rice 0.5gm
No. of fish	10	10	10	10	10	10	10	10
Day of week								
1st	-	1	-	-	-	2	3	2
2nd	-	2	-	-	1	1	1	2
3rd	-	2	1	1	1	-	1	1
4th	-	1	1	-	-	-	-	-
5th	-	1	-	1	-	-	1	1
6th	-	-	-	-	1	--	-	-
7th	-	-	-	-	-	-	-	-
8th	-	-	-	-	-	1	-	-
9th	-	-	-	-	-	-	-	1
10th	-	-	-	-	-	-	-	-
Mortality %	0	70%	20%	20%	30%	40%	60%	70%
Survival %	100%	30%	80%	80%	70%	60%	40%	30%

Discussion

Boosting the immune system of cultured fish is the area of our concern. Dietary supplementation of natural additives like probiotics, prebiotics, medicinal herbs and plant extracts succeeded to improve the immunity and disease resistance of various fish species against numerous fish pathogens. These additives could play a pivotal role in minimizing the use of chemotherapies in fish farms [30]. Maqsood et al. [31] stated that use of medicinal herbs in aqua-feed is a very important research topic in the health management of fish aquaculture. Enhancement of the non-specific immune responses of fish is considered to be a very important tool in controlling pathogenic infections in fishes**.**

Blood is a mirror of the physiological status of the body, especially the blood leucocytes which considered the first line of body defense against pathogens; their increase is a primary sign to boost the immunity [32]. Result showed a significant increase in WBCs count in both onion and barley extracts treated groups while there were no differences detected in groups fed on rice extract. This may be attributed to the presence of high levels of alkaloids in onion and barely extracts that stimulate the lympho myeloid tissues [33]. These results confirm those of Akrami et al. [32] who indicated that feeding fish on onion supplemented diets have a significant increase in WBCs. The higher levels of serum proteins in fish are supposed to be associated with an increase in the innate immune response. The measurement of albumin, globulin and total protein in serum is considered as diagnostic indicators of the general nutritional status and liver function**.**

The total plasma proteins of both groups fed on onion and barley extracts were higher than other groups. These results proved that the used extracts (onion- barley) enhanced the immunity and improved O. niloticus health. This may be attributed to the active ingredients (flavonoids and antioxidants) found in onion and barley respectively. Akrami et al. [32] and Shalaby et al. [34] indicated an enhancement of total proteins after dietary supplementation of onion and garlic, Allium sativumin fish groups compared to the control groups respectively. Herbs may cause early initiation to the non-specific defense mechanisms of fish and elevating the specific immune response [35]. Phagocytosis is the first attack of monocytes, tissue macrophages and neutrophiles to destroy the pathogenic microbes [36, 37]. The fish groups fed on onion and barley extracts exhibited significant activation of phagocytes, the highest activity was recorded in fish group fed on 1gm onion extract. Dugenci et al. [38] and Sahu et al. [39] reported an increase in phagocytosis of fish groups fed on ginger (Zingiber officinale) extract and garlic (Allium sativum). The flavonoid content of these herbs may increase the proliferation of splenic cells and release of cytokines to enhance the phagocytosis of the peripheral macrophages [40].

Lysozyme activity provides an insight into the immune response of fish [32, 41]. Results indicated an increase in serum lysozyme activity of both fish groups fed on onion and barley extracts. The higher number of phagocytes directly correlated with the lysozyme activity [42]. The higher serum lysozyme activity is caused due to either an increase in the number of phagocytes releasing lysozyme, or to an increase in the amount of lysozyme manufactured per cell. Akrami et al. [32] and Apines-Amar et al. [43] demonstrated an increase in WBCs count, lysozyme activity after dietary supplementation of onion extract in fish diet.

The challenge test showed that onion groups have the lowest mortality rate (20%) followed by fish fed on barley (30-40%) while rice straw extract groups are the highest one (60-70%) after the challenge test with A. hydrophila. Several studies confirmed the antimicrobial activity of medicinal herbs and their potential to increase the aquatic animals resistance against pathogens [44,45]. Nya and Austin [46] showed that adding ginger has the same active ingredient as barley in rainbow trout diet reduced the mortality to zero after the challenge with A. hydrophila. There were also an enhancement in the growth rate, feed conversion and protein efficiency ratio of rainbow trout fed on ginger. Also, the methanolic extract of nettle (flavonoids content) increased the survival rate of rainbow trout after the challenge with A. hydrophila [47]. To conclude our results, herbs and some plant extracts are promising sources of natural and safe therapeutics in fish culture. Adding onion leaves and barley seeds extracts in O. niloticus diet can improve the non-specific immunity of fish. While adding rice straw extract to fish diet has no effect on fish health. So this study recommended the use of onion leaves extract in aqua-feed at 0.5mg/kg diet and barley seed extract at 1mg/kg diet.

References

1. FAO (2014) The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome.

2. Abu-Elala, N., Marzouk, M., and Moustafa, M. (2013) Use of different *Saccharomyces cerevisiae* biotic forms as immune-modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens. *International Journal of Veterinary Science and Medicine*, **1** (1), 21-29.

3. Harikrishnan, R. and Balasundaram, C. (2005) Modern trends in *Aeromonas hydrophila* disease management with fish. *Rev. Fish. Sci.,* **13**, 281-320.

4. Romero Ormazábal, J.M., Feijoó, C.G. and Navarrete Wallace, P.A. (2012) Antibiotics in aquaculture–use, abuse and alternatives. *'Health and Environment in Aquaculture*'. pp. 159-198.

5. Citarasu, T. (2010) Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, **18**, 403-414.

6. Abu-Elala N.M., Galal M. K., Abd-Elsalam R. M., Mohey-Elsaeed O. and Ragaa N.M. (2016) Effects of Dietary Supplementation of *Spirulina platensis* and Garlic on the Growth Performance and Expression Levels of Immune-related Genes in Nile tilapia (*Oreochromis niloticus*). *Journal of Aquaculture Research and Development,* **7**, 433-439.

7. Scalbert, A. and Williamson, G. (2000) Dietary intake and bioavailability of polyphenols, *J. Nutr.* **130**, 2073-2085.

8. Ramos, F.A., Takaishi, Y., Shirotori, M., Kawaguchi, Y., Tsuchiya, K., Shibata, H., Higuti, T., Tadokoro, T. and Takeuchi, M. (2006) Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin, *J. Agric. Food Chem.,* **54,** 3551-3557.

9. Binaii, M., Ghiasi, M., Farabi, S.M.V., Pourgholam, R., Fazli, H., Safari, R. Alavi, S.E., Taghavi, M.J. and Bankehsaz, Z. (2014) Biochemical and hemato-immunological parameters in *juvenile beluga* (Husohuso) following the diet supplemented with nettle (Urticadioica), *Fish Shellfish Immunol*., **36**, 46-51.

10. Cho, H.C. and Lee, S.M. (2012) Onion powder in the diet of the Olive flounder (*Paralichthys olivaceus*): Effects on the growth, body composition and lysozyme activity, *J. World Aquac. Soc.,* **43**, 30-38.

11. Madhujith, T., Izydorczyk, M. and Shahidi, F. (2006) Antioxidant potential of pearled barley (Hordium species). *J. Agric. Food Chem*., **54**, 283-328.

12. Karppinen, S., Myllymaki, O., Forssell, P. and Poutanen, K. (2003) Fructan content of rye and rye products. *Cereal Chem.,* **80**, 168-171.

13. Madhujith, T., Naczk, M. and Shahidi, F. (2004) Antioxidant activity of common beans (*phaseolus vulgaris* L.) *J. Food Lipids.,* **11**, 220-330.

14. Van Dam, A.A, Beveridge, M.C.M., Azim M.E. and Vedegem, M.C.J. (2002). The potential of fish production based on periphyton .*Rev .Fish Biol.Fish.,* **12**, 1-31.

15. Yi, Y., Lin, C.K. and Diana, J.S. (2003) Technique to mitigate clay turbidity problems in fertilized earthen fish ponds. *Aquacult. Eng.,* **27**, 39-51.

16. Innes, W.T. (1966) "*Exotic Aquarium Fishes",* 19th ed., Aquarium incorporated New Jersy.

17. Noga, E.J. (2010) **T**ext Book of Fish diseases, diagnosis and treatment. Blackwell Publishing Co., U.S.A.

18. Arahshahi-Delouee, S. and Urooj, A. (2007) Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chem.* **102**, 1233-1240.

19. Ezhilan, B. P., and Neelamegam, R. (2012) GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. *Pharmacognosy Research*, **4** (1), 11–14. http://doi.org/10.4103/0974-8490.91028

20. Jain, N.C. (1986) "*Schalms Veterinary Hematology*" 4th ed .Lea and Febiger, Philadelphia.

21. Gornall, A.G.; Bardawill, C.J. and David, M.M. (1949) Determination of serum protein by means of the Biuret reagent. *J. Biol. Chem.,* **177**, 751-755

22. Doumas, B.T., Watson, W.A. and Biggs, H.G. (1971) Albumin standards and the measurement of serum albumin with bromcrsol green. *Clin Chim Acta*., **31**, 87-96.

23. Coles, E.H (1986) "*Veterinary Clinical Pathology*", 4th ed. W.B. Saunders, Philadelphia. PP: 11-41, 114-121.

24. Abu-Elala, N.M., Mohamed, S.H., Zaki, M.M., and Eissa, A.E. (2015) Assessment of the immune-modulatory and antimicrobial effects of dietary chitosan on Nile tilapia (*Oreochrmis niloticus*) with special emphasis to its bio-remediating impacts. *Fish & Shellfish Immunology*, **46** (2), 678-685.

25. Soliman, W.S.E. (1999) Bacteriological studies on fresh water fish. Thesis, M.V.Sc., Microbiology, Fac. Vet. Med. Cairo. Univ.

26. Yin, G., Ardo, L., Jeney, Z., Xu, P. and Jeney, G. (2008) Chinese herbs (Lonicera japonica and *Ganoderma lucidum*) enhance non-specific immune response of tilapia, *Oreochromis niloticus*, and protection against *Aeromonas hydrophila*. *Diseases in Asian Aquaculture,* **6**, 269-282.

27. Hamackova, J., Kouril, J., Kozak, P. andStupka, Z. (2006) Clove Oil as an Anaesthetic for Different Freshwater Fish Species. *Bulgarian Journal of Agricultural Science*, **12**, 185-194.

28. Ngugi, C.C., Oyoo-Okoth E., Mugo-Bundi, J., Orina, P.S., Chemoiwa, E.J. and Peninah, A. A. (2015) Effects of dietary administration of stinging nettle (Urticadioica) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. *Fish & Shellfish Immunology,* **44**, 533-541.

29. Meyers, L.S., Gamst, G., and Guarino, A.J. (2006) Applied multivariate research: design and interpretation. Thousand Oaks, CA: Sage Publications, Inc.

30. Ramudu, K.R. and Dash, G. (2013) A Review on Herbal Drugs Against Harmful Pathogens in Aquaculture. *American Journal of Drug Discovery and Development*, **3**, 209-219.

31. Maqsood, S., Singh, P., Samoon, M.H. and Munir, K. (2011)Emerging role of immunostimulants in combating the disease outbreak in aquaculture. *Int. Aquat. Res.,* **3,** 174-164.

32. Akrami, R., Gharaei, A., Mansour, R.M. and Galeshi, A. (2015) Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hematobiochemical parameters of beluga (*Husohuso Linnaeus*, 1754) juvenile., *Fish Shellfish Immunology*, **45**, 828-834.

33. Ates. B., Orun, I., Talas, Z.S., Durmaz, G. and Yilmaz, I. (2008) Effects of sodium selenite on some biochemical and haematological parameters of rainbow trout (*Oncorhynchus mykiss*, Walbaun, 1792) exposed to Pb2+ and Cu2+ . *Fish Physiol. Biochem*., **34** (5), 3-9.

34. Shalaby, A.M., Khattab, Y.A. and Abdel Rahman, A.M. (2006) Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). *J. Venom. Anim. Toxins*, **12**, 172–201.

35. Mukesh, K.B., Jitender, K.J. and Satyanarayana, Y.A. (2012) Animal and plant originated immunostimulants used in aquaculture *J. Nat. Prod. Plant Resour.,* **2** (3), 397-400.

36. Secombes, C.J. and Fletcher, T.C. (1992) The role of phagocytes in the protective mechanisms of fish. *Annual Review of Fish Diseases*, **2**, 53–71.

37. Wiley, J.M., Sherwood, L.M. and Woolverton, C.J. (2011) Rescott's Microbiology. Eight Edition. McGraw-Hill.Hill. 145 p.

38. Dugenci, S.K., Arda, N. and Candan, A. (2003) Some medicinal plants as immunostimulants for fish. *J. Ethnopharmacol.,* **88**, 99–106.

39. Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J. and Sarangi, N. (2007) Effect of Allium sativum on the immunity and survival of Labeorohita infected with *Aeromonas hydrophila*. *J. Appl. Ichthyol.,* **23**, 80–86.

40. Kyo, E., Uda, N., Suzuki, A., Kakimoto, M., Ushijima, M., Kasuga, S. and Itakura, Y. (1998) Immunomodulation and antitumor activities of aged garlic extract. *Phytomedicine,* **5**, 259–267.

41. Fu, G.H., Bai, Z.Y., Xia, J.H., Liu, F., Liu, P. and Yue, G.H. (2013) Analysis of two lysozyme genes and antimicrobial functions of their recombinant proteins in Asian sea bass. *PLoS ONE,* **8** (11), e79743.

42. Engstad, R.E., Robertson, B. and Frivold, E. (1992) Yeast glucan induces increase in activity of lysozyme and complement mediated haemolytic activity in Atlantic salmon blood. *Fish Shellfish Immunol*., **2**, 287–297.

43. Apines-Amar M.J.S., Amar E.C. and Faisan, J.P. (2013) Growth, plasma cortisol, liver and kidney histology, and resistance to vibriosis in brown-marbled grouper, *Epinephelus fuscoguttatus* fed onion and ginger. *AACL Bioflux*., **6** (6),530-538.

44. Nya, E.J. and Austin, B. (2007) Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis.,* **32,** 963–970**.**

45. Aly, S.M. and Mohamed, M.F. (2010) *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). *J. Anim. Physiol. Anim. Nutr. (Berl),* **94**, 31-39.

46. Nya, E.J. and Austin, B. (2009) Use of dietary ginger (*Zingiber officinale Roscoe*) as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout (*Oncorhynchus mykiss*), *J. Fish Dis.,* **32**, 971-977.

47. Bilen, S., Ünal, S. and Güvensoy, H. (2015) Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (Urticadioica) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*)., *Aquaculture,* **454**, 90–94.

(*Received* 6/ 8/ 2017;
accepted 5/10/ 2017)

محاولة استخدام بعض مستخلصات الأعشاب كمحفزات مناعية واعدة باضافتها للأعلاف فى أسماك البلطى النيلى المستزرعة

وليد صلاح الدين سليمان*، محمد سيد مرزوق، محمد عبد العزيز أحمد**، نرمين محمد أبو العلا**، حسام حسن عباس*، منى سعد زكى*، سهر بدراوى عبد العزيز***
*قسم الأحياء المائية - شعبة البحوث البيطرية - المركز القومى للبحوث و **قسم أمراض ورعاية الأسماك – كلية الطب البيطرى - جامعة القاهرة - القاهرة - مصر.

تعتبر الأعشاب الطبية وبعض المستخلصات النباتية من أهم الاضافات الطبيعية لأعلاف الأسماك والتى لها تأثير كبير على صحة الأسماك وقد ركزت هذه الدراسة على ثلاثة مستخلصات نباتية كإضافات لعلائق الأسماك والتى يمكن اختيارها كمحفزات مناعية

تمت الدراسة على ٠١٢ من أسماك البلطى النيلى السليمة ظاهريا والتى تم اختيارها عشوائيا وتم وضعها فى ١٢ حوض زجاجى بكل حوض ٠١ أسماك وزعت على ٧ مجموعات كل مجموعة مكررة ثلاث مرات، المجموعة الأولى والثانية تم تغذيتها على أعلاف مضاف لها مستخلص أوراق البصل (0.5,1mg/kg diet)، المجموعة الثالثة والرابعة تم تغذيتها على أعلاف مضاف لها مستخلص بذور الشعير(0.5,1mg/kg diet) والمجموعة الخامسة والسادسة مضاف لها مستخلص قش الأرز(0.5,1mg/kg diet) أما المجموعة السابعة فكانت مجموعة ضابطة غير مضاف لها اى مستخلص للأعلاف وتم تغذية هذه الأسماك مرتين يومياً لمدة شهرين ويتم تغذيتها بنسبة ٣٪ من وزن الأسماك فى الأحواض.

تم قياس صورة الدم فى مجموعات الأسماك والمحددات المناعية للمناعة الفطرية (الموروثة) وأيضاً الاستجابة المناعية للعدوى البكتيرية تم قياسها باختبار التحدى وفى نهاية التجربة كانت هناك زيادة نوعية فى نشاط الليزوزيم وخلايا البلعمة فى مجموعات الأسماك التى تم تغذيتها على الأعلاف المضاف لها مستخلص أوراق البصل وأيضا بالنسبة للمجموعات التى تغذت على عليقة مضاف لها مستخلص بذور الشعير أيضاً كانت هناك زيادة فى الجلوبيولين وخلايا الدم البيضاء مما يوضح أن هذه المجموعات أظهرت آداء مناعى عالى ومقاومة عالية ضد العدوى الصناعية ببكتريا الأيروموناس هيدروفيلا، بينما مجموعات الأسماك التى تغذت على أعلاف مضاف لها مستخلص قش الأرز لم يكن هناك اى زيادة أو استجابة مناعية والتى لم تختلف كثيرا عن المجموعة الضابطة حيث أظهرت كل منهما نسبة نفوق عالية عند حقنها بالبكتريا فى اختبار التحدى.

لذلك يمكن التوصية باستخدام مستخلص أوراق البصل باضافته لأعلاف الأسماك بتركيز (0.5,1mg/kg diet)، وباضافة مستخلص بذور الشعيربنسبة (1mg/kg diet)

الكلمات الدالة: مستخلصات الأعشاب – نشاط خلايا البلعمة – نشاط الليزوزيم – اختبار التحدى – البلطى النيلى.