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GENERAL INFORMATION

Abbassa International Journal for Aquaculture is Egyptian specific publication in aquaculture of the Egyptian society for water, aquaculture and environment. The journal is published in four volumes per year to include results of research in different aspects of aquaculture sciences. The journal publishes also special issues of advanced topics that reflect applied experiences of importance in aquaculture sector.

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SYNERGISTIC IMMUNOLOGICAL EFFECT OF *ECHINACEA PURPUREA* WITH *HYDRASTIS CANADENSIS* ON *OREOCHROMIS NILOTICUS*

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Abstract

This study investigated the effects of administration of *Echinacea purpurea* with *Hydrastis canadensis* as immunostimulants preparation in the form of a feed supplement on *Oreochromis niloticus*. The results showed effective protection against experimental *Aeromonas hydrophila* infection. The obtained PCV in fish treated with 1:0.5, 1:1 and 1:2 of *Echinacea purpurea* and *Hydrastis canadensis* extract were 26.10, 27.32, 27.62 respectively and 24.8 in the control group. Also, there was significant increasing in monocytes/macrophages and neutrophils in the treated groups. Hemagglutination inhibition test and slide agglutination assay were done for measuring satiability of produced specific antibodies against inoculated challenge bacteria. The obtained cut point by hemagglutination inhibition test was 1/320 at first measuring and 1/160 after 15 days interval in 2nd and 3rd groups while was 1/160 and 1/80 in the 1st group. While in the control was 1/80 and 1/40. The results of slide agglutination assay were confirmed that of hemagglutination inhibition test. The body gain and the specific growth rates in group 2 &3 were significantly higher than those of the group 1 and control group. The results of this study showed that the continuous oral administration combination of goldenseal with echinacea during the rearing period enhanced specific and non specific immune response furthermore increased growth rates.

INTRODUCTION

One of the major issues in intensive finfish aquaculture is loss associated with disease. A number of approaches have been applied in an attempt to address this problem including sanitary prophylaxis,

disinfection, chemotherapy with a particular emphasis on the use of antibiotics, and in recent time vaccination against specific diseases (Chevassus and Dorson, 1990).

Antibiotic therapy is undesirable, as there is the potential for enhanced microbial resistance and the accumulation of residues in the tissues of the fish (Siwicki, 1989). Vaccination, although highly effective in some instances, is time consuming, labour intensive, costly and protection is often for pathogen specific (Robertsen *et al*., 1994). An alternative approach has been the application of various compounds to boost or stimulate the innate immune system of cultured fish. These compounds, termed immunostimulants which include bacteria and bacterial products (Dalmo and Seljelid, 1995), complex carbohydrates (Obach *et al*.,1993), nutritional factors (Clerton *et al*., 2001) animal extracts, cytokines, lectins, plant extracts (Galeotti, 1998) and synthetic drugs (Mulero *et al*., 1998).

Laboratory studies have shown that *Echinacea purpurea* herb and its purified polysaccharides cultures possessed immunostimulatory activity to macrophages and mononuclear cells also, can eliminate bacterial and fungal pathogens in vitro (Diab *et al*., 2006 and Mesalhy *et al*., 2008).

Hydrastis Canadensis also called eye balm, eye root, goldsiegel, ground raspberry and goldenseal was native to the eastern United States. The roots and rootstock, or rhizomes, of the plant are used in herbal remedies (Davis and McCoy, 2008). Two chemicals in the herb (berberine and hydrastine) have been studied for use as medical treatments. goldenseal stimulates the immune system and has been used on the skin to treat wounds. Berberine, a chemical contained in goldenseal, is said to fight off infection caused by some bacteria, fungi, and yeast (Zeiger, 2008).

Thus, the purpose of this work was to expand on these studies and to investigate whether administration of *Echinacea purpurea* with *Hydrastis canadensis* as a feed additive, could modulate innate immune parameters and the growth rates of *O. niloticus*.

MATERIAL AND METHODS

Experimental Design

A total of 600 (14±0.5 g) fish of *O. niloticus* obtained from hatchery of Central Laboratory for Aquaculture Research, Abbassa (CLAR). The trial consisted of four groups of *O. niloticus* were randomly assigned in aquaria.

Prior to the trial, fish were acclimated to the laboratory conditions for 2 weeks supplied with dechlorinzed tap water at 25˚C ± 1˚C and aeration in the wet laboratory of fish diseases department. During acclimatize period fish were allowed fed a commercial food preparation. Following acclimation, three groups used to evaluate the effect of *Echinacea purpurea* with *Hydrastis canadensis* given balanced diet mixed with *Echinacea purpurea* (0.25ppt feed) according to Mesalhy *et al*. (2008) supplemented with *Hydrastis canadensis* by (1:0.5, 1:1 and 1:2). In addition to non treated control group. Fish were fed 3% of their body weight three times per day. *Echinacea purpurea* and *Hydrastis canadensis* extract were procured from MEPACO Company, Egypt. The Experiment was extended to four months (May - August 2010).

Survival and Mortality after Bacterial Challenge

The fish were challenged with virulent *Aeromonas hydrophila* (kindly supplied from Fish Diseases Department of CLAR) was cultured in tryptic soya broth at 25ºC for 3 days. The fish were inoculated with 0.2ml I/P ($6x10^8$ c.f.u./ml), and were observed for the next 21 days to determine survival, and formation of erosion or hemorrhagic lesions on

the body surfaces. Bacteria were isolated by cultivation from gills, eroded lesions, and from visceral organs of dead fish. Similar procedures were performed in surviving fish (Schäperclaus *et al*., 1992).

Blood Parameters

Three fishes from each treated and control groups were anaesthetized with MS-222. Blood samples were collected from the caudal vein to be used as whole blood and for serum separation. Whole blood used for estimation of total and differential leukocytic count according to Stoskoph (1993), as well as determination of packed cell volume (PCV) according to Wintrob, (1967). The blood serum was collected two times with 15 days interval.

Hemagglutination Inhibition Test

For hemagglutination inhibition evaluation blood of fish sources was collected with heparin, washed successive times with phosphate buffered saline (PBS) till obtaining clear fish erythrocytes without fibrin. Then**,** suspension of *A. hydrophila* strain $6x10^8$ c.f.u /ml was serially diluted and mixed with 50µl 1% erythrocytes. The mixture was incubated at 37°C for 1hr. to determined agglutination point concentration of used bacteria. The serum was serial two fold diluted and titerated against suspension of *A. hydrophila* strain at agglutination point concentration, then mixed well and recorded the highest serum dilution inhibit agglutination of erythrocytes. The technique was done according to (Galeotti, 1998). The control was also done.

Slide Agglutination Assay

The slide-agglutination assay was performed by mixing 20 µl of bacterial cell suspension in sterile saline solution (SS, 0.9 % NaCl, pH 7.4) ($6x10^8$ c.f.u./ ml) with 20 µl of serial twofold dilutions of serum. The agglutination assay was performed using fresh cell suspensions antigens. A distinct and immediate agglutination was defined as positive.

Agglutination titer was recorded as the reciprocal of the highest serum dilution giving a positive reaction (Amaro *et al*., 1992).

Growth Parameter

For determined the growth rates, the *O. niloticus* under experiment were weighted and lengthed weekly up to 4 months and the instantaneous growth rate (IGR), specific growth rate (SGR) and condition factor (CF) was calculated as described by Laird and Needham (1988).

IGR= In [final mean body] – In [initial mean body weight (g)] ÷ Time interval

SGR= In [final mean body] – In [initial mean body weight (g)] ÷ Time interval (days) x 100

CF= weight (g) ÷ [length (cm)]^3 x 100

Statistical Analysis

The statistical analysis was performed by one way ANOVA analysis of variance according to (Kachigan, 1991). The multiple tests were carried out to determined difference between treatment means at significance level $P< 0.05$. The stander error was also determined.

RESULTS AND DISCUSSION

Administration of *Echinacea purpurea* and *Hydrastis canadensis* extract to fish diet gave effective protection against experimental *A. hydrophila* infection. The mortality rates of fish treated with 1:0.5, 1:1 and 1:2 of *Echinacea purpurea* and *Hydrastis canadensis* extract were 22.67%, 16.67% and 16% with survival rates 77.33%, 83.33% and 84% respectively at 21 days after challenge (Table 1). In contrast, the mortality rate of non treated fish in the control group following challenge was 50.67% while, the survival rate was 49.33% within the same period. That is better than (76.67%) survival rate recorded by Mesalhy *et al*.,

2008 who used *Echinacea purpurea* alone as feed supplement. This is clearing the role of goldenseal as antibacterial agent which considered a natural antibiotic and when combined with echinacea in preparations designed to strengthen fighting ability of the immune system. The control fish developed erosion and hemorrhages on their body surface and gill cover during 9 days after challenge. Gloss lesions were developed in 87% of the control fish up to 21 days after the challenge. In contrast, development of skin lesions was suppressed significantly in the treated fish by 9%, 5% and 4% in 1st, 2nd and 3rd groups respectively. Only the dead fish in *Echinacea purpurea* and *Hydrastis canadensis* treated groups showed these lesions, but no gloss lesion was observed in any surviving fish. The highly significance different between the treated groups and the control is due to dried root of goldenseal contains several alkaloids including berberine, hydrastine, palmatine and lesser amounts of canadine and hydrastinine (Zeiger, 2008). Berberine can be increasing the flow of healthy mucous in addition of its systemic immune activation role. This giving suggestion that *Echinacea purpurea* and *Hydrastis canadensis* potentate immune system defiance and increase its local and systemic bactericidal activity.

Table 1. Showing Survival and Mortality after Bacterial Challenge.

Group	*Ech.* to *Hyd.*	**M.O**	**Fish No.**	**Mortality**	**Survival**
1	1: 0.5	*A. hydrophila*	150	34 (22.67%)	77.33%
2	1:1		150	25 (16.67%)	83.33%
3	1:2		150	24 (16.00%)	84.00%
Control	----		150	76 (50.67%)	49.33%

Blood packed cell volume used as an estimation of health status of the fish and help in detecting the improving changes through using of immunostimulants as mentioned by Wintrob (1967). The obtained PCV in the 1st, 2nd, 3rd and control were 26.10, 27.32, 27.62 and 24.8 that

indicate the better health state of the fish groups under experiment. The increasing in the leukocyte count (Table 2) appears to be due to sharing the effect of *Echinacea* which having of polysaccharides and echinacocide able to increase the number of leukocytes, and having of cichoric acid and echinacin able to activate macrophages and stimulate reformation of hematopoietic stem cells. In addition to the effect of goldenseal which having berberine that activate whole white blood cells, making them more effective at fighting infection and strengthening the immune system. The significant increase in of monocytes/macrophages and neutrophils is a good indicator of activation of non-specific defense mechanisms in fish (Table 2). This results accepted by (Jeney and Anderson, 1993) who recorded the increasing mechanism of leukocyte count through using goldenseal as immunostimulants in fish.

Table 2. Showing Blood Parameters in the Fish under Experimented.

Group	PCV %	TLC (10^3/µl)	Lymph.	Neut.	Eosin.	Baso.	Mono.
1	26.10^{B} ±0.14	36.84^{B} ±0.5	29.02^{B} ±0.34	5.66^{B} ±0.38	0.61^{B} ±0.02	0.22^{B} ±0.02	1.37^{B} ±0.04
2	27.32^{A} ±0.32	38.27^{A} ±0.35	29.72^{A} ±0.48	5.86^{A} ±0.31	0.65^{A} ±0.02	0.32^{A} ±0.03	1.74^{A} ±.03
3	27.62^{A} ±0.40	38.76^{A} ±0.38	30.92^{A} ±0.48	5.27^{A} ±0.26	0.73^{A} ±0.03	0.36^{A} ±0.03	1.58^{A} ±0.04
Control	24.8^{C} ±0.36	35.48^{C} ±0.27	27.96^{C} ±0.52	4.72^{C} ±0.26	0.57^{C} ±0.02	0.28^{C} ±0.02	1.71^{C} ±0.02

Superscript letters explain degree of significantly at P <0.05. Means having the same superscript letters in the same column are not significantly different

For measuring satiability of produced specific antibodies against inoculated challenge bacteria hemagglutination inhibition test and slide agglutination assay were done, the used *A.hydrophila* strain $6x10^8$ c.f.u/ml was agglutinated blood till forth well (4HA) and so, the collected serum was serial two fold diluted then titrated against suspension of 4HA used bacteria. The obtained cut point by hemagglutination inhibition test was 1/320 at first measuring and 1/160 after 15 days interval in 2nd and

3rd groups while was 1/160 and 1/80 in the 1st group. The control was 1/80 and 1/40. The results of slide agglutination assay were confirmed that of hemagglutination inhibition test. This obtained results clearing the effect of both *Echinacea* and *Hydrastis* in improving of the specific immune response and stability of the produced antibodies. This results were supported by Goel *et al*. (2002) who described the effect of *E. purpurea* in the activation of the immune system through activate of macrophages with the secondary activation of T lymphocytes and memory cells that giving stability for the produced antibodies. In addition, the role of goldenseal which activates white blood cells, making them more effective at fighting infection and strengthening the immune system as descried by (Mills and Kerry, 2000).

At the end of the experiment all fish were weighted and lengthen individually and their growth parameter were calculated. The body gain and the specific growth rates in group 2 &3 were significantly higher than those of the group 1 and control group (Table 3). Also, there was significant change in the condition factor between the experimental groups and control one. This results was disagree with Diab *et al*. (2006) who showed that the average body weight of the treated fish ad that of the control showed no significance different up to three moths from start of the experiment. Also, the results was better than that recorded by Mesalhy *et al*. (2008) (1.54 SGR ad 1.7 CF) who used echinacea alone as feed additive. That was referring to the role of goldenseal in improving the feed conversion that was explained by Parveen *et al.* (2006) who recorded the role of goldenseal in feed digestion and metabolism through up-regulation bile secretion besides reducing plasma cholesterol and conducting bioassay-driven semi-purifications demonstrate that the higher potency of goldenseal achieved through concerted actions of multiple bioactive compound.

Table 3. Showing Growth Parameters in the Fish under Experimented.

Group	Fish No.		Fish No.	Length (cm)	Body Gain(gm)	IGR	SGR	CF
	Initial	Final						
1	150	116	116	17.5^{B} ±0.02	90.24^{B} ±2.09	0.0155^{B} ±0.001	1.55^{B} ±0.01	1.67^{B} ±0.01
2	150	125	125	18.2^{A} ±0.03	100.11^{A} ±3.1	0.0163^{A} ±0.001	1.63^{A} ±0.01	1.71^{A} ±0.01
3	150	126	126	18.4^{A} ±0.03	105.31^{A} ±2.8	0.0167^{A} ±0.002	1.67^{A} ±0.02	1.74^{A} ±0.02
Control	150	74	74	17.1^{B} ±0.02	80.28^{C} ±3.3	0.0145^{C} ±0.002	1.45^{C} ±0.02	1.62^{C} ±0.02

Superscript letters explain degree of significantly at P <0.05. Means having the same superscript letters in the same column are not significantly different

In conclusion the results of this study show that the continuous oral administration combination of goldenseal with echinacea during the rearing period is beneficial. Specific and non specific immune response was enhanced, thus providing for a potential increase in disease resistance at the time of the fish susceptible to infection. Furthermore increased growth rates. The best level of *Echinacea purpurea* to *Hydrastis canadensis* in the fish diet was 1:1 and more than this level not given significance improving of the results.

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مضاعفة التأثير المناعى للإكنسيا بإضافة الجولدن سيل لأسماك البلطي النيلي

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قسم بحوث أمراض الأسماك- المعمل المركزى لبحوث الثروة السمكية-
مركز البحوث الزراعية – وزارة الزراعة – مصر.

الملخص العربى

تبحث الدراسة المقدمة في تأثير إضافة الجولدن سيل إلي الإكنسيا فى عليقة أسماك البلطي النيلي على النشاط المناعي العام لهذه الأسماك. وظهر من خلال الدراسة وجود حماية واضحة للمجموعات المعاملة عندما تعرضت إلي عدوى اصطناعية بميكروب الإيروموناس هيدروفيلا .أوضحت نتائج اختبار PCV فى المجموعات المعالجة بنسب ١:٠.٥، ١:١، ١:٢ كنسب إضافة الجولدن سيل إلي الإكنسيا هـ ٢٦.١، ٢٧.٣٢، ٢٧.٦٢ على التوالي بينما كانت ٢٤.٨ فى المجموعة الضابطة كما كان هناك ارتفاع ملحوظ فى أعداد كرات الدم البيضاء المختلفة فى المجموعات المعالجة.

ولمعرفة مدى ثبات الأجسام المناعة المتكونة نتيجة العدوى الاصطناعية تم إجراء كلا من اختبار منع تلازن الدم واختبار التجلط على الشريحة. وأظهرت النتائج وجود إيقاف تام لتلازن الدم عند تخفيف ٣٢٠/١ فى القياس الأول ثم ١٦٠/١ فى القياس الثاني (بعد الأول بخمسة عشر يوما) بالنسبة للمجموعة الثانية والثالثة بينما كان ١٦٠/١ ثم ٨٠/١ فى المجموعة الأولى وكانت المجموعة الضابطة ٨٠/١ ثم ٤٠/١ كما أيدت نتائج التجلط على الشريحة هذه النتائج.

وكان من الضروري إجراء القياسات الوزنية لمعرفة الأداء العام للأسماك أثناء فترة المعاملة والتي أوضحت وجود زيادة وزنية ملحوظة فى كلا من المجموعتين الثانية والثالثة عن المجموعة الأولى والضابطة. وترى الدراسة أن الإضافة المستمرة للجولدن سيل إلي الإكنسيا فى عليقة أسماك البلطي النيلي خلال فترة التربية تزيد من الاستجابة المناعية العامة والمتخصصة لهذه الأسماك بالإضافة إلى تحسين الوزن.