Effect of Black Cumin Seed Oil (Nigella sativa) on Enhancement of Immunity in the Climbing Perch, Anabas testudineus

Aisha Khatun¹, M. M. M. Hossain¹*, M. Z. Rahman², M. E. Alam¹, Farzana Yasmin¹, M.S. Islam¹ and M. M. Islam¹

¹Department of Fisheries and Marine Bioscience, Faculty of Biological Science and Technology, Jessore University of Science & Technology, Jessore-7408, Bangladesh.  
²The Income and Nutrition (AIN) Project, The World Fish Centre, Jessore, Bangladesh.

Authors’ contributions

This work was carried out in collaboration between all authors. Author MMMH designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AK managed the literature searches, analyses of the study performed the spectroscopy analysis and author FY managed the experimental process author MZR helps to get fish samples from the different fish farmers and hatchery, author MEA managed laboratory works with different equipments adequately and authors MSI and MMI identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/15330

ABSTRACT

Aims: The study was conducted to examine the efficacy of dietary black cumin seed oil (Nigella sativa) on the immune response of climbing perch, Anabas testudineus against A. hydrophila.  

Place and Duration: This experiment was performed in the Laboratory of Fisheries and Marine Bioscience (FMB), Jessore University of Science and Technology (JUST), on July to December 2013.  

Methodology: Fish husbandry and experimental design, Culture and Aeromonas hydrophila Isolation, Diet Preparations, Serum preparation (immune response assay), Growth performance, bactericidal activity, phagocytic activity and challenge test have been performed in this study.
Results: Climbing perch (Anabas testudineus) of average weight 25±5 g were fed for 1, 2 and 4 weeks with diet supplemented 20 ml (20%), 30 ml (30%) and 40 ml (40%) 100 g⁻¹ of N. sativa oil and with normal diet as control (0%). Immunological parameters including bactericidal activity and phagocytic activity were investigated. Treatment groups recorded enhancement in those parameters compared to the control. Treatment groups fed the dose 30% N. sativa oil showed a significant enhancement in bactericidal activity and phagocytic activity. The highest weight gain (WG) 41.7±1.5 was significantly increased with the 30% dose of N. sativa oil but specific growth rate (SGR) and feed conversion ratio (FCR) did not change significantly when compared to the control. Feeding with 30% dose diet to A. testudineus showed lowest cumulative mortality 20% compared to other dose diets and played most effective performance during challenge test.

Conclusion: This result suggests that 30% dose of N. sativa oil enriched diet significantly enhanced the immune response and disease resistance of A. testudineus against A. hydrophila.

Keywords: Anabas testudineus; Nigella sativa; Aeromonas hydrophila; disease resistance; bactericidal activity; phagocytic activity.

1. INTRODUCTION

Stress conditions are responsible for fish disease in aquaculture which leads to bacterial disease. Uses of antibacterial drugs in aquaculture are risky due to cross resistance against pathogens, toxicity, residues in tissues and contaminate the environment with bioactive product [1]. Using of natural plants as immunostimulant in fish is more useful than antibacterial drugs [2,3]. Medicinal plant as immunostimulants can be used not only against disease but also as growth promoters [4, 5]. From ancient time plants or plant products are used as medicine or therapeutic agent and it is well known [5]. Nigella sativa Linn. commonly known as the black cumin seed, is an annual herb that belongs to the botanical family of Ranunculaceae is a spice and preservative [6]. The seeds of Nigella sativa have been used for medicinal purposes as a natural remedy for a number of illnesses and conditions that include bronchial asthma, rheumatism, hypertension, diabetes, inflammation, cough, headache, eczema, fever and influenza [7]. Research has been conducted on immunomodulatory effect of Nigella sativa as an anti-tumor, bactericide, anticestode, antinematode, anti-inflammatory, analgesic, anti-diabetic and on some immunohematological parameters and specific as well as non-specific defence mechanisms of fish and also used as a growth promoter [3,8-13]. However, there was no report of this herb as immunostimulants in thai koi or Anabas testudineas to prevent diseases. Therefore, the aim of the present study was to examine if black cumin seeds, Nigella sativa, extract would influence some immunological parameters and immune response of A. testudineas.

2. MATERIALS AND METHODS

2.1 Fish, Experimental Design

Climbing perch or Thai koi of average weight 25 ± 5 g were collected from commercial fish farm and hatchery in Jessore and acclimatized in aerated freshwater (temperature maintained at 25 – 26°C ) in the aquarium environment for 7 days before starting the experiment and during this time fish were fed twice daily with commercial diet. Length and weight were randomly checked initially upon receipt. Fish were randomly distributed into 4 groups each containing 10 fish representing three replicates and fed for 30 days with N. sativa oil enriched diet at 0% (control), 20%, 30% and 40% and oil was obtained by milling. To avoid dryness, evaporation of oil, lowering binder activity 20 to 40% were considered in substitution of 0.0 to 10% of N. sativa oil. Initially in this study the concentrations of N. sativa oil were tested 0 – 50% and bacterial colony against oil were found in the challenge experiment with 20 to 40% and therefore were chosen. The control group was fed with commercial diet to examine the possible mode of action and effect on immune response. Fish were fed at a rate of 3% to 2% body weight for twice in a day until the end of the experiment.

2.2 Culture and Aeromonas hydrophila Isolation

A. hydrophila isolated from diseased Climbing perch or Thai koi, was used in FMB laboratory for the study. Stocks were grown in brain heart infusion (BHI, Hi-media, Indian) and nutrient broth for 24 hrs at 37°C and then kept in −20°C until use. The subculture was taken and
centrifuged (4000 rpm for 15 min), after centrifugation the supernatant was discarded and the pellet was resuspended in sterile phosphate buffer saline (PBS). The culture was adjusted at 3.5x10^7 colony forming units (CFU) ml^-1 by 10 times serial dilution and incubated at 37°C for 24 hours. The bacterium was confirmed by some biochemical test (Table 1).

### 2.3 Diet Preparations

*Nigella sativa* or black cumin seed was collected from local market, Borobazar in Jessore. The seed was dried at 40°C for 4 hours. Black cumin seed oil was extracted by milling the seed. Oil was flamed to make it disinfection and mixed with the commercial diet. The proximate composition of commercial diet and fatty acid analysis [16] of black cumin seed oil were shown in Table 2. Three experimental diets were prepared of the pellet with 20%, 30% and 40% of *N. sativa* oil were spread to the basal diet and mixed properly. To avoid dryness, evaporation of oil, lowering binder activity 20 to 40% were considered in substitution of 0.0 to 10% of *N. sativa* oil. Prepared feed with *N. sativa* was stored in a closed jar at room temperature.

### 2.4 Serum Preparation (Immune Response Assay)

Blood from the randomly selected fish were drawn directly from the caudal vein with the help of a sterilized 1 ml hypodermal syringe containing EDTA (Ethylene-Diamine-Tetra-Acetic Acid) as an anticoagulant using 24 gauge needles. For serum separation blood was collected without anticoagulant in serological tubes and stored in a refrigerator overnight. The serum was then spun down at 4500 g for 10 min. The collected serum was stored in sterile serum tubes at −20°C until used for assays. All the procedures were carried out in the sterilized condition. After drawing blood fishes were given 1% KMnO4 dip treatment and released in to the tank. For each group (0%, 0.5%, 1.0%, 1.5% and 2.0%) three culture plates were prepared. Bacterial stock solution was serial diluted for 10 times and 10^3, 10^4 and 10^5 concentration were selected for further usage. Then 25 µl volume from each (10^3, 10^4 and 10^5) diluted solution was mixed with 25 µl separated serum (followed by disc diffusion method) of five different groups of fishes then spreaded in different culture plates and finally all plates were placed in an incubator at 37°C for 24 hrs. Then bacterial colonies of all plates were counted.

| Identifying characteristics | Aeromonas hydrophila |
|----------------------------|----------------------|
| Colony                     | Yellowish            |
| Morphology                 | Small rods           |
| Gram strain                | -                    |
| Catalase                   | +                    |
| Oxidase                    | +                    |
| Gelatin liquefaction       | +                    |
| Indole production          | +                    |
| OF test                    | F                    |
| Arabinose                  | +                    |
| Manitol                    | +                    |
| Sucrose                    | +                    |
| Inositol                   | +                    |
| Esculin hydrolysis         | +                    |
| Voges-proskauer reaction   | +                    |
| Ammonium production        | -                    |
| Glucose                    | G                    |

Note: + = positive reaction; - = negative reaction; O = oxidation; F = fermentation; G = gas

### 2.5 Growth Performance

All fish were deprived of food for 24 hour before weighing and sampling. Following parameters were measured for growth performance of Thai koi according to Choudhury et al. 2005 [17].

Weight gain (%) = Final body weight (g) – Initial body weight (g)/Initial body weight × 100

Specific growth rate (SGR) = Final weight (g) – Initial weight (g)/Time interval (days)

Feed conversion ratio (FCR) = Feed intake per body weight/Weight gain

### 2.6 Bactericidal Activity

*A. hydrophila* was used to examine the effectiveness of supplements to kill the bacterial infection. To prepare stock solution of experimental bacterial strain in conical flask containing 100 ml distill water, inoculating loop was touched from single bacterial colony of fresh culture. Bacterial suspension was then diluted using disk diffusion method. 15 µl of serum was added with 15 µl of bacterial suspension and mixed properly. The serum-bacterial mixture (15 µl) was plated onto the nutrient agar and BHI agar plates and incubated for 24 hours at 37°C before the numbers of colonies were counted.
2.7 Phagocytic Activity

Phosphate buffer solution (PBS) was fixed with gluteraldehyde and 6% suspension of thai koi blood cells were mixed in it. 20 µl of bacterial suspension was placed on a coverslip incubated for 30 min in a humid chamber. Then it was carefully washed with PBS and 20 µl of blood cells was added and incubated for 40 min. After staining with giamsa the numbers of engulfed blood cell or phagocytic cell were determined by photographic microscope (Axiocam ERc 5s with Axio vixim driver, Carl Zeiss, Germany).

2.8 Challenge Studies

For the challenge test virulent A. hydrophila strain were prepared from maintaining the serial dilution. On 30th day of feeding each group fishes were injected intraperitoneally (i.p.) with 0.5 ml of 24 hours cultured A. hydrophila which contained 3.2 × 10^6 CFU ml^-1 challenge strain. The clinical signs and mortality was recorded up to 30 days of post challenge. The cumulative mortality was calculated by following Amandi, et al. 1982 [18] and Relative Percent Survival (RPS) was calculated as follow

\[ RPS = 1 - \frac{\% \text{ Mortality in treated group}}{\% \text{ Mortality in control group}} \times 100 \]

2.9 Statistical Analysis

Values for each parameter measured were expressed as the arithmetic mean ± standard error (SE). Effects of herbal diets on growth performance, hematological, and immunological parameters were tested using one-way ANOVA and the mean values were compared by using Duncan’s multiple range tests at 5% level of significance [19].

3. RESULTS

3.1 Disease Resistance (Challenge)

The highest cumulative mortality was 80% in groups fed with 0% N. sativa enriched diets. The lowest mortality of 20% was noted in groups fed with 30% N. sativa enriched diets against A. hydrophila infection while 53% and 33% mortalities were observed when fed with 20% and 40% enriched diets. The survivality increased to 80% with 30% dose diet. Very low survivality 20%, 46% and 66% were found in 0%, 20% and 40% diets (Figs. 1 and 2).

3.2 Bactericidal Activity

The lowest number of bacterial colonies represented that the bacteria was resistant to the certain dose of N. sativa oil and efficiency of immune cells in serum to resist the bacteria. With N. sativa the lowest number of colonies (9 × 10^6) was observed with the 30% dose compared to the control (85× 10^6) (Fig. 3).

3.3 Phagocytic Activity

Phagocytic activity did not significantly enhance with 20%, 30% and 40% with enriched diet on first week against A. hydrophila. With 30% dose the activity significantly increased on 2 and 4 weeks, but not in 20% and 40% as compared with the control (Fig. 4).

3.4 Growth Performance

Fish in each aquarium were counted and group weighed every 2 weeks, following 24 h of feed deprivation. When fish were removed for weighing, aquaria were cleaned thoroughly, two-third of the water removed. Fish were not fed on sampling days. In Thai koi fed with all doses (20%, 30% and 40%) of supplementary diet growth rate significantly increased as compared to the control. The highest weight gain 41.5 ± 1.5 was found in 30% dose of N. sativa oil. However, the specific growth rate (SGR) and feed conversion ratio (FCR) did not significantly increased with any supplementation diet (Table 3).

4. DISCUSSION

Use of plant products in aquaculture industry has been reported to be safe as they are highly biodegradable and do not have any side effects such as drug resistance as observed with synthetic antibiotics [2]. Nigella sativa has immunomodulatory effect on fish against bacterial pathogen. Many researches has been conducted on immunomodulatory effect of Nigella sativa as an anti-tumor, bactericide, anticestode, antinematode, anti-inflammatory, analgesic, anti-diabetic and on some immune-hematological parameters and specific as well as non-specific defense mechanisms of fish [3,8-13, 20]. In the present study, the obtained results indicate that final weight and weight gain (WG) are increased in treatment group compared to the control group. Highest weight gain is observed from the dose of 30% black cumin oil.
with supplemented diet. The present results are similar to those reported by [8,21]. Significant increase in body weight and total biomass production as well as growth performance were seen in Oreochromis niloticus treated with 1.00 ppt stand for Echinacea sp [9]. Kelp grouper fed with all doses diet had significantly increased growth rate when compared to the control [22]. Moreover, Feeding rainbow trout with 1% lupin, Lupinus perennis, mango, Mangifera indica, or stinging nettle Urtica dioica, for 14 days led to significant enhancement in weight gain, SGR and FCR compared to the controls [23].

![Cumulative Mortalities of A. testudineus fed supplementary diets with N. sativa oil and challenged with A. hydrophila (3.2 × 10^6 CFU ml^-1) for 30 days. (*) indicates relatively significance (P < 0.05)](image)

**Table 2. Proximate composition of supplementary feed, N. sativa and oil**

| Supplementary fish feed (%) | N. sativa | Fatty acid of N. sativa oil |
|-----------------------------|----------|-----------------------------|
| Protein                     | 34       | Protein 20.85                |
| Crude fibre                 | 6        | Fat 38.20                    |
| Crude ash                   | 11       | Moisture 4.64                |
| Moisture                    | 18       | Ash 4.37                     |
| Lipid                       | 6        |                                |
| Fat                         | 3        |                                |

(Source: Spectra fish feed Co. Ltd., [14,15])

**Table 3. Growth parameters of A. testudineus fed with different doses of N. sativa supplementation diet against A. hydrophila**

| Growth parameter | Doses | Week one | Week two | Week four |
|------------------|-------|----------|----------|-----------|
| WG               | 0%    | 25.2±1.1 | 26.4±1.2 | 27.3±1.4 |
|                  | 20%   | 27.3±1.4 | 29.5±1.3 | 33.4±1.3 |
|                  | 30%   | 32.4±2.0 | 34.2±1.3 | 41.5±1.5 |
|                  | 40%   | 30.4±1.3 | 33.2±1.5 | 37.4±1.6 |
| SGR              | 0%    | 1.2±0.2  | 1.3±0.1  | 1.4±0.2  |
|                  | 20%   | 1.3±0.2  | 1.4±0.1  | 1.5±0.2  |
|                  | 30%   | 1.5±0.3  | 1.6±0.4  | 1.7±0.3  |
|                  | 40%   | 1.4±0.2  | 1.5±0.3  | 1.6±0.1  |
| FCR              | 0%    | 1.5±0.3  | 1.6±0.2  | 1.7±0.4  |
|                  | 20%   | 1.5±0.2  | 1.6±0.1  | 1.6±0.3  |
|                  | 30%   | 1.2±0.2  | 1.3±0.4  | 1.4±0.3  |
|                  | 40%   | 1.4±0.1  | 1.5±0.2  | 1.6±0.3  |

*Data expressed as mean ± SE, (*) showed relatively significance (P < 0.05). Here, WG – Weight gain, SGR – Specific Growth Rate, FCR – Food Conversion Ratio*
Fig. 2. Survivality of *A. testudineus* fed supplementary diets with *N. sativa* oil and challenged with *A. hydrophila* (3.2 × 10^6 CFU ml⁻¹) for 30 days. (*) showed relatively significance (P < 0.05)

Fig. 3. Bactericidal activity (%) of Thai koi fed supplementary diet with 20%, 30% and 40% of *N. sativa* oil. (*) showed relatively significant (P < 0.05)

Serum bactericidal activity is a mechanism that helps to resist the growth of pathogen [24]. The lowest number of bacterial colonies indicated the efficiency of immune cells in serum to kill the pathogen. In the present study, the lowest numbers of bacterial colonies were observed in the treatment group than the control group especially at the dose of 30% of black seed oil. Awad et al. 2013 [13] who found greater serum bactericidal activity in rainbow trout (*Oncorhynchus mykiss*) administered with dietary supplements comprising 1% of Quercetin and 3% of *N. sativa* oil in his research study. Similarly, serum bactericidal activity has enhanced in catla (*Catla catla*) feeding the dietary supplements comprising prickly chaff-flower seed (*Achyranthes aspera*) [25]. Similar result was found in rohu (*Labeo rohita*) administered with prickly chaff-flower seed (*Achyranthes aspera*) [26].
Phagocytic activity is increased by immunostimulant has been documented by many researcher [27-29]. In this study, the phagocytic activity was significantly increased in treatment group fed with 30% dose of N. sativa enriched diets from 1 to 4 week compared to the control group. Rainbow trout fed with 1% of stinging nettle and garlic recorded higher phagocytic activity than in the 0.1% dose, which was greater than the controls [28]. In olive flounder significantly increased phagocytic activity against A. hydrophila after being fed with 0.1% and 1.0% Hericium erinaceum enriched diets from 1 to 4 week [30]. Similar result was found in olive flounder fed with Prunella vulgaris [30]. Yin et al. 2006 [29] found that feeding tilapia with Scutellaria extract with higher doses (0.5 and 1.0%) caused reduction of function in phagocytic cells, while, when fish were fed with low dose of Scutellaria (0.1%) there was no stimulation on phagocytic activities.

In the present study after challenge with A. hydrophila (3.2 × 10^6 CFU ml⁻¹) mortalities were significantly reduced in all groups compared to the control group. The lowest mortality 20% was observed at the dose of 30% of N. sativa oil. But in 40%, mortality rate was increased because of suffocation due to overdose of oil. However, this result is in agreement with previous study conducted in L. rohita fed with Achyranthes aspera diet [31]. O. mossambicus treated with Eclipta alba leaf extract [32] against Aeromonas hydrophila infection.

Black cumin seed (N. sativa) is an immunomodulator and it exhibits an effective moderate activity in this result. It can be concluded that black cumin seed can be used as an antimicrobial drug for immunity enhancement and increasing survivality in farmed fish that are more susceptible to disease. However, because of its availability, low cost and immunostimulatory effect, it could be recommended to be used for fish to decrease mortalities caused by Aeromonas hydrophila.

5. CONCLUSION

The present study showed that 30% dose of Nigella sativa oil could significantly enhance immune response and reduce mortality and increase survival rate after challenge with the Aeromonas hydrophila. Thus, it can be deduced that using N. sativa oil as an immunestimulant in Anabas testudineus showed an immunity enhancement [33,34] which suggests a promising role for supplements as immunomodulatory components in fish feed help to make immune response in cultured fish against A. hydrophila. Further study needs to optimize the concentration of diet dose to prepare the herbal extract to control A. hydrophila infection in fish properly.

CONSENT

It is not applicable.
ETHICAL

It is not applicable.

ACKNOWLEDGEMENT

The author express to thanks to The WorldFish Centre, Jessore for cordial cooperation’s of fish sample collections (diseased and healthy) and we are also grateful to Department of Fisheries and Marine Bioscience for providing a platform for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. Clin Exp Allerg. 2002;32(4):489-98.
2. Aruna BV, Chandran MR. Efficacy of CIFAX and herbal mixture in the prophylaxis of EUS. Nat Sem Dis Aqu. Kakinada, India; 1996.
3. Blumenthol M, Goldberg A, Brinckmann J. Herbal Medicine: Expanded Commission Emonographs. Integrative Medicine Communications, 1029 Chestnut Street, Newton, MA 02464. 2001;401-403.
4. Dorucu M, Colack SO, Celayir Y. The effect of black cumin seeds, Nigella sativa, on the immune response of rainbow trout, Oncorhynchus mykiss. Med Aqu J. 2009;2(1):27-33.
5. Awad SA, Maitland DJ, Monier S. New alkaloids from Casimiroa edulis fruits and their pharmacological activity. Chem Nat Com. 2007;43(4):576-580.
6. Olusola SE, Emikpe BO, Olaifa FE. The potentials of medicinal plant extracts as bio-antimicrobials in aquaculture. Int J Med Arom Plant. 2013;3:401-412.
7. Gabor EF, Ichim O, Suteu M. Phyto-additives in rainbow trout (Oncorhynchus mykiss) nutrition. Biharean Biologist, Oradea, Romania. 2012;6(2):134-139.
8. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phyt Res. 2000;14:323-328.
9. Khattab YA. Effect of substituting black seed cake (Nigella sativa L.) for Soyabean meal in diets of Nile Tilapia (Oreochromis niloticus L.) on growth performance and nutrients utilization. Egypt J Aqu Biol Fish. 2001;5(2):3-46.
10. John G, Mesalhy S, Rezk M, El-Naggar G, Fathi M. Effect of some immunostimulants as feed additives on the survival and growth performance of Nile tilapia Oreochromis niloticus and their response to artificial infection. Egypt J Aqu Biol Fish. 2007;11(3):1299-1308.
11. Salman MT, Khan RA, Shukla I. Antimicrobial activity of Nigella sativa Linn. Seed oil against multi-drug resistant bacteria from clinical isolates. Nat Pro Rad. 2008;7(1):10-14.
12. Zaher KS, Ahmed WM, Zerizer SN. Observations on the biological effects of black cumin Seed (Nigella sativa) and Green Tea (Camellia sinensis). Glob Vet. 2008;2(4):198-204.
13. Mohamed HA, El-Sayed LH, Moawad M. Protective effect of Nigella sativa seed against imethylaminoazobenzene (DAB) induced liver carcinogenesis. Nat Sci. 2010;8(6):80-87.
14. Awad E, Austin D, Lyndon AR. Effect of black cumin seed oil (Nigella sativa) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, Oncorhynchus mykiss (walbaum). Aquaculture. 2013;33:193-197.
15. Al-Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa) seed growing in Saudi Arabia. 1992;45:239–242.
16. Babayann VK, Kootungdal G, Halaby GA. Proximate analysis, fatty acid and amino acid composition of N. sativa L. seeds. J Food Sci. 2006;43(4):1314-1315.
17. Choudhury D, Pal AK, Sahu NP, Kumar S, Das S, Mukherjee SC. 2005. Dietary yeast RNA supplementation reduces mortality by Aeromonas hydrophila in rohu, Labeorohit juveniles. Fish Shellfish Immunol. 2005;19:281-291.
18. Amandi A, Hiu SF, Rohovec JS, Fryer JL. Isolation and characterization of Edwardsiella tarda from fall Chinook salmon (Oncorhynchus tsawytscha). Appl Environ Microbiol. 1982;43:1380–1384.
19. Zar JH. Production: Biostatistical Analysis, practice Hall, Englewood Cliffs, NJ., USA. 1984;293-305.
20. Mohamad S, Abasali H. Effect of plant extracts supplemented diets on immunity and resistance to Aeromonas hydrophila in Common Carp (Cyprinus carpio).Agril J. 2010;5(2):119-127.
21. Harikrishnan R, Balasundaram C, Jawahar S, Heo M. *Solanum nigrum* enhancement of the immune response and disease resistance of tiger shrimp, *Penaeus monodon* against *Vibrio harveyi*. Aquaculture. 2011;318:67-73.

22. Harikrishnan R, Kim J, Kim M, Balasundaram C, Heo M. *Lactuca indica* extracts as feed additive enhances immunological parameters and disease resistance in *Epinephelus bruneus* to *Streptococcus iniae*. Aquaculture. 2011;318:43-47.

23. Awad E, Austin B. Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis. 2010;33:413-420.

24. Ellis AE. Innate host defence mechanism of fish against viruses and bacteria Dev Com Immunol. 2001;25:827-839.

25. Rao VY, Chakrabarti R. Stimulation of immunity in Indian major carp *Catla catla* with herbal feed ingredients. Fish Shellfish Immunol. 2005;18:327-334.

26. Srivastava PK, Chakrabarti R. Effect of dietary supplementation of *A. aspera* seeds on the immune system of *Labeo rohita* fry. Isrl J Aqu-Bamidgheh. 2012;64:779-786

27. Gannam AL, Schrock RM. Immunostimulants in fish diets. J App Aqu. 1999;9:53-89.

28. Dügenci SK, Arda N, Candan A. Some medicinal plants as immunostimulant for fish. J Ethnoph. 2003;88:99-106.

29. Yin G, Jeney G, Racz T, Xu P, Jun X, Jeney Z. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*. Aquaculture. 2006;253:39-47.

30. Harikrishnan R, Kim J, Balasundaram C, Heo M. Protection of *Vibrio harveyi* infection through dietary administration of *Pueraria thunbergiana* in kelp grouper, *Epinephelus bruneus*. Aquaculture. 2011;318:27-32.

31. Rao VY, Das BK, Jyotymayee P, Chakrabarti R. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immunol. 2006;20:263-273.

32. Christybapita D, Divyagnaneswari M, Michael RD. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish Shellfish Immunol. 2007;23:840-852.

33. Harikrishnan R, Kim J, Kim M, Balasundaram C, Heo M. *Hericium erinaceum* enriched diets enhance the immune response in *Paralichthys olivaceus* and protect from *Philasterides dicentrarchi* infection. Aquaculture. 2011;31:48-53.

34. Harikrishnan R, Kim J, Kim M, Balasundaram C, Heo M. *Prunella vulgaris* enhances the non-specific immune response and disease resistance of *Paralichthys olivaceus* against *Uronema marinum*. Aquaculture. 2011;318:61-66.

© 2015 Khatun et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?id=832&id=8&aid=8044