American Journal of Life Sciences
2016; 4(3): 87-92
http://www.sciencepublishinggroup.com/j/ajls
doi: 10.11648/j.ajls.20160403.14
ISSN: 2328-5702 (Print); ISSN: 2328-5737 (Online)

Effect of *Nigella sativa* (Black Cumin Seed) to Enhance the Immunity of Common Carp (*Cyprinus carpio*) Against *Pseudomonas fluorescens*

Shoumo Khondoker[1], Md. Mer Mosharraf Hossain[1], Md. Hasan-Uj-Jaman[1, *], Md. Eftakher Alam[1], Md. Farid Uz Zaman[2], Nazia Tabassum[1]

[1]Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology, Jessore, Bangladesh

[2]Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh

Email address:

shoumo100429@gmail.com (S. Khondoker), mmiron_bau@yahoo.com (Md. M. M. Hossain), hasan100401@gmail.com (Md. Hasan-Uj-Jaman), eshovon.aqua08@gmail.com (Md. E. Alam), farid100422@gmail.com (Md. F. U. Zaman), naziakeka@gmail.com (N. Tabassum)

[*]Corresponding author

To cite this article:

Shoumo Khondoker, Md. Mer Mosharraf Hossain, Md. Hasan-Uj-Jaman, Md. Eftakher Alam, Md. Farid Uz Zaman, Nazia Tabassum. Effect of *Nigella sativa* (Black Cumin Seed) to Enhance the Immunity of Common Carp (*Cyprinus carpio*) Against Pseudomonas *fluorescens*. *American Journal of Life Sciences*. Vol. 4, No. 3, 2016, pp. 87-92. doi: 10.11648/j.ajls.20160403.14

Received: May 12, 2016; **Accepted**: May 23, 2016; **Published**: June 17, 2016

Abstract: The effect of *Nigella sativa* (black cumin) seed extracts supplemented diets at 2%, 4% and 6% doses with common diet as control (0%) fed to common carp, *Cyprinus carpio* against *Pseudomonas fluorescens* was investigated critically on weeks 1, 2 and 4 as immunomodulator. Immunological factors including bactericidal activity and phagocytic action were examined along with the disease resistance. The outcomes of the research recommend 4% dose of *N. sativa* oil enriched diet as an effective immune response and disease resistance agent for *C. carpio* against *P. fluorescens* although further investigation will facilitate to optimize the precise quantity comparing with the weight of fish body.

Keywords: *Nigella sativa*, Common Carp, *Pseudomonas fluorescens*, Immune Response

1. Introduction

The common carp is one of the major aquaculture species in the globe Vandeputte [1]. Common carp occur within the temperature range of 3–35°C Froese and Pauly [2]. The optimum water temperature for growth and propagation is 20–25°C. Physically, common carp live in the middle and lower sections of rivers and in areas where the water is shallow and the bottom is mud-spattered FAO [3]. According to Chowdhury *et al*., [4] *Aeromonas* sp. and *Pseudomonas* sp. are very frequent in fish disease particularly in carp and live fishes in Bangladesh. Cultured fish species suffer from *Aeromonas* sp. and *Pseudomonas* sp. infections with signs like dermal lesion, scale loss, frayed fins, tail and fin rot and Dropsy Khatun *et al*., [5]. Several approaches have been made to control these diseases such as disinfection, sanitary prophylaxis and chemotherapy with particular emphasis on antibiotics. Conversely, use of chemicals and antibiotics is often costly and detrimental because it guides the antibiotic and chemical resistance in pathogen as well as reduces consumer preference Harikrishnan *et al*., [6]. One of the most proficient methods of controlling diseases in fish culture is tightening the defense mechanisms of fish through prophylactic administration immunostimulants from synthetic or natural sources Robertsen, [7].

In recent times, immunostimulants of herbal origin have been shown to have the capacity to boost disease resistance in fish against a number of diseases by improving non-specific and specific defense mechanisms Harikrishnan *et al*., [6]. Many herbs have been reported that develop the immunity of fishes, such as *Achyranthes aspera* Rao *et al.,* [8], *Nigella sativa* and Quercetin Awad *et al*., [9], *U. dioica* Nya and Austin, [10]; Awad and Austin, [11], *Viscum album*, *Urtica dioica* and *Zingiber officinale* Dügenci *et al*.,

[12], *Eclipta alba* Christybapita *et al.*, [13], *Astragalus membranaceus* and *Lonicera japonica* Ardo *et al*., [14], *Rheum officinale* Xie *et al*., [15],, *Allium sativum*, *Lupinus perennis*, *Mangifera indica* and *Radix astragalin* seu Hedysari and *Radix angelicae* sinensis Jian and Wu, [16] [17] etc. against pathogens. The genus *Pseudomonas*, of the *Pseudomonadaceae* family, are motile gram-negative aerobic bacteria, plump-shaped rods with polar flagella which have an key role in pathogenicity Public Health Agency of Canada, [18]. It is eminent that pseudomonads are ubiquitous bacteria in environment. Due to their ability to exploit a wide range of organic compounds, they inhabit a significant ecological arrangement in the carbon cycle Tryfinopoulou *et al*., [19]. The genus *Pseudomonas* is prevalent throughout nature and especially characterized by promoted metabolic adaptability and enzymatic organization Franzetti and Scarpellini, [20]. *P. fluorescens* has ability to form extra cell enzymes such as amylse, protease, chitinase, cellulase and gelatinase Soesanto *et al.,* [21]. Many strains of *Pseudomonas fluorescens* show potentiality for biological control of phytopathogens especially root pathogens Couillerot *et al.*, [22]. *Nigella sativa*, known as the black cumin seed, is a spice and food preservative. It comprises many applauded therapeutic properties such as bronchodilatory, hypotensive, antibacterial, antifungal, analgesic, anti-inflammatory Khan, [23], anticancer, anidiabetic, antiradical, immunomodulator, spasmolytic, bronchodilator, hepatoprotective, antihypertensive and renal protective Ramadan, [24], anti-nociceptive, anti-ulcer, anti-histaminic Tembhurne *et al*., [25]. The present study also intends to evaluate the effect of dietary Black Cumin Seed oil (*Nigella sativa*) on Common carp, *Cyprinus carpio* against *Pseudomonas fluorescens* infection.

2. Materials and Methods

2.1. Fish Collection and Acclimatization

One hundred twenty (120) pieces of *C. carpio*, Common carp (average weight 30±5 g, length 16±2 cm) were collected from Madhumati Matshyo Utpadon Kendro, Chanchra, Jessore, Bangladesh. The collected fishes were taken into the laboratory of Fisheries & Marine Bioscience in Jessore University of Science & Technology, Jessore in November, 2014 and acclimatized for three weeks in rectangular shaped indoor aquarium (100L) with continuous airstone aeration. At the beginning the study, each aquarium was disinfected with chlorinated water. The fishes were fed with commercial supplementary feed (without herbal extract) at the rate of 5% of their body weight twice a day at 09.00 h and 15.00 h while adapting in the experimental environment. The system was subjected to 12 h light: 12 h darkness. Water quality parameters were taken under measurement approach during the entire experiment.

2.2. N. sativa Supplemented Diet Formulation

N. sativa seed was collected from local market of Jessore, Bangladesh. Black cumin seed oil was extracted by milling of the seeds from Begum Mill, Puraton Kosba, Jessore. The oil (100 g) was mixed with 1000 ml of 85% ethanol in a 2000 ml conical flask kept for 7 days at room temperature and agitated twice a day following Cooper and Gunn [26] to ensure complete absorption. The extracts were filtered through Whatman No. 1 filter paper and the filtrate was dried under reduced pressure. The residues obtained after evaporation were stored in sterilized screw cap glass container, externally disinfected by flaming on spirit lamp and stored at −20°C until use. The black cumin seed oil extract was mixed at 2%, 4% and 6% with supplementary commercial fish feed in three treatment groups respectively. Control group was fed with the supplementary fish feed (without oil extract) twice a day as the treatment groups were. The experimental basal diets were in pellet form and dried in sun for 06 hours to avoid any microbial contamination.

2.3. Culture of Pseudomonas fluorescens

The bacterial strain was cultured in Brain Heart Infusion (BHI) Agar and Nutrient Agar media at the rate of 0.61 g and 0.25 g respectively in 50 ml distilled water. The bacterial strain was gone through serial dilution and put into media in different dilution factors. Then the petri plates were kept in the incubator for 24 hours in 30°C to 120°C. But the bacteria growth was prominent in 37°C. During each experiment freshly cultured bacteria was used to acquire errorless result.

2.4. Experimental Design

C. carpio fishes were divided into four groups each containing 30 fishes (Treatment 1, 2, 3 and control) under two feeding regimes (test group and control group). All treatments were assigned randomly in the aquaria and each aquarium represented one observation. Each treatment group was divided into three replicate groups consisting of 10 fishes. Fishes were fed *ad labitum* two times a day diets enriched with *N. sativa* extracts at 0% (control), 2%, 4% and 6%. Uneaten food was removed by a siphon after 1 h of feeding to avoid the reduced quality and opaqueness of the water. The study was carried out for 30 days and experimental diets were fed for 7 days, 7 days and 16 days with 1%, 3% and 5% body weight correspondingly. Partial water exchange was done thrice a week to maintain the water quality.

2.5. Serum Collection

Firstly blood samples were collected from caudal vein of individual fish with syringe of 1 ml only once to avoid the influence on the assays due to multiple bleeding and handling stress. The collected blood samples were then transferred to Eppendrop, which was containing Ethylenediaminetetraacetic acid (EDTA) solution as anticoagulant to avoid blood clotting. The blood was then centrifuged at 5000 rpm for 10 minutes at 4°C. The serum

was separated in the appendrop with the blood cells. The serum was obtained by centrifugation and was stored in sterile serum tubes at -20°C for further use.

2.6. Bactericidal Activity (Specific Immune Response Assay)

P. fluorescens was used to determine the efficacy of both supplements to neutralize the bacterial infection. Stock solution of experimental bacterial strain was prepared to continue the advance study. Broth culture of the bacteria was prepared from the stock. A media was ready to use and 25µl, 50µl and 75µl serum was spread over the nutrient agar and BHI agar media with micro pipette. The bacterial broths were streaked smoothly on the serum after they were mixed in the same amount as the serum to check the bacterial load on the plates even after treating with treatment group. Then the plates were kept in the incubator for 24 h at 37°C. Bacterial load was measured to find out the bactericidal activity of experimental fish's serum.

2.7. Mucus Collection and Bacteria Culture (Nonspecific Immune Response assay)

Mucus was collected by scarping the body surface of fishes with a scalpel from four groups (0%, 2%, 4% and 6%) and collected mucus was kept in four eppendorps separately. Same like as serum and bacteria culture, four culture plates for each group were prepared as followed by disc diffusion method. 25µl mucus from each group was mixed with same volume of three different diluted bacterial solutions (10^{-3}, 10^{-4} and 10^{-5}) and finally all plates were placed in an incubator at 37°C for 24 hours. After 24 hours all plates were observed.

2.8. Challenge Test

The susceptibility of fish fed with *N. sativa* oil extract to a bacterial challenge was examined *in vivo* and *P. fluorescens* was used as challenge strain. Herbal extract was challenged 4 weeks after the start of treatments. *P. fluorescens* strains were prepared from maintaining the serial dilution which LD_{50}: 10^{-6} CFU ML^{-1} was used. Fish from each group were injected intraperitoneally with 2.9 x 10^{-6} cfu ml^{-1} live *P. fluorescens*. Each dilution trial was conducted in triplicates and mortality was recorded daily for 7 days post challenge. The survival rates significantly increased when the dietary *N. sativa* level was 4%. The cumulative mortality was calculated by following Amend [27] and Relative Percentage of Survival (RPS) was calculated as follows:

$$\text{Percentage of Survival (RPS)} = 1 - \frac{\%\text{ of Mortality in treated group}}{\%\text{ of Mortality in control group}} \times 100$$

2.9. Statistical Analysis

Values for each parameter measured were expressed as the arithmetic mean ±standard deviation (SD). Consequences of herbal diets consumption on 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.

3. Results and Discussion

The global market for herbal drug is estimated as US $62 billion and is likely to develop to US $5 trillion by the year 2050. The worldwide pharmaceutical trade was valued US $550 billion in 2004 and has boosted to US $900 billion in 2008 Maggon, [28]. Black cumin seed (*N. sativa*) is an herbaceous plant and their oil are used by many nations for diverse curative functions. Recently, numerous researches have been conducted designating the considerable role of black cumin seed in increasing immunity and upholding good health status of fish El-Kadi *et al*., [29]; Hedaya, [30]; Mandour *et al*., [31]; Abdel-Ghaffar *et al*., [32]; Taha *et al*., [33].

3.1. Non-specific Immune Response (Mucus)

2% experimental diet showed much more resistance against *P. fluorescens* than control diet fed fishes. But the significant ($p < 0.05$) less amount of bacterial load was exhibited in 4% (Fig. 1) *N. sativa* mixed feed. The number of bacterial colony again boosted in 6% trial fed in 1st, 2nd and 4th weeks consequently.

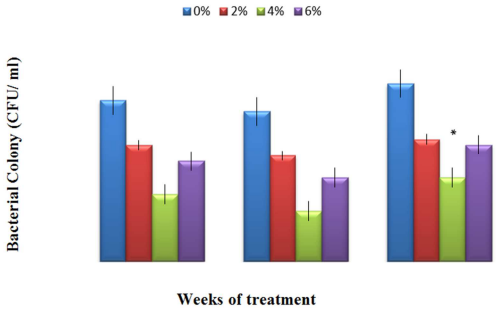

Figure 1. *Bactericidal activity of common carp fed with 0%, 2%, 4% and 6% N. sativa extract supplemented diets against P. fluorescens. [* indicates significant p<0.05].*

3.2. Serum Bactericidal Activity (Specific)

The lowest number of bacterial colonies indicated the efficiency of immune cells in serum to kill the pathogen. With *N. sativa* the lowest number of colonies, 3 × 10^{-5} developing on BHI occurred with the 4% dose (Fig. 2), with highly significant differences to the control, ($p < 0.05$).

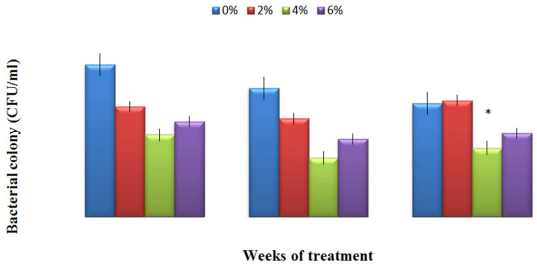

Figure 2. *Bactericidal activity (no. of colony) of C. carpio fed with different doses of N. sativa extract against P. fluorescens. Data are expressed as mean±S.D; (n = 4) and the differences in values (p < 0.05) between groups is indicated by asterisks.*

The results of the present study revealed a significant increase in the bactericidal activity in day 14 with 4% *N. sativa* seed oil supplemented diet compared to the control group. The result was similar to the serum bactericidal activity found in kelp grouper, *Epinephelus bruneus* against *Vibrio harveyi* by Harikrishnan *et al*. [34]. *Aeromonas hydrophila* growth was inhibited by *N. sativa* (3%) oil and Quercetin (1%) fed rainbow trout, *Oncorhynchus mykiss* serum Awad *et al*., [9]. Dip treatment of *C. carpio* with aqueous leaf extract of Azadirachta indica significantly increased serum protein levels and protected the fish from *A. hydrophila* infection Harikrishnan *et al*., [35]. The bath administration of Quil *A. saponin* with *Yersinia ruckeri* enhanced the in vitro bactericidal activities in rainbow trout Grayson *et al*., [36].

3.3. Phagocytic Activity

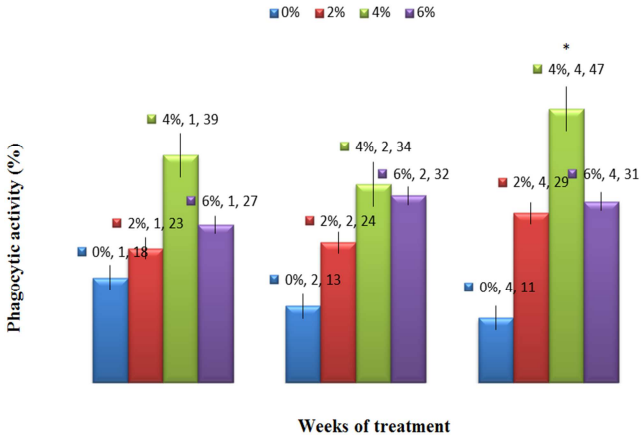

Figure 3. *Phagocytic activity (%) of C. carpio fed with different doses of N. sativa extract against P. fluorescens. Data are expressed as mean±S.D.; (n = 4) and the differences in values (p < 0.05) between groups is indicated by asterisks.*

The phagocytic activity did not significantly enhance with 2.0% and 6.0% enriched diet on first week against *P. fluorescens*. Phagocytic activity of 2% enriched with more pace than previous week in week 4. However with 4.0% doses the activity significantly increased on weeks 4 as compared with the control (Fig. 3).

The highest phagocytic activity was exhibited in week 4 fed with 4% *N. sativa* oil supplemented ration in the current research. Chinese mixed herbal extracts from *Rheum officinale*, *Andrographis paniculata*, *Isatis indigotica*, and *Lonicera japonica* containing diets increased phagocytosis of the white blood cells in on crucian carp, *Carassius auratus gibelio* Chen *et al.*, [37]. In common carp *C. carpio* and large yellow croaker *Papillaria crocea* fed a ration containing a mixture of *Astragalus membranaceus* (root and stem), *Polygonatum multiflorum*, *Isatis tinctoria* and *Glycyrrhiza glabra* containing a mixture diets with 0.5 and 1% observed that significantly increased phagocytic activity Yuan *et al*., [38]. Nile tilapia, *Oreochromis niloticus* fed a mixture of *Astragalus* and *Lonicera* extracts supplementation diets enhanced the phagocytic activity of blood phagocytes and increased survival rate against *Aeromonas hydrophila* Ardo *et al.*, [14]. A similar result was reported in carp *C. carpio* fed a diet combating with *Astragalus* and *Ganoderma* Yin *et al.*, [39].

3.4. Disease Resistance (Challenge Test)

The cumulative mortality was 40%, 20% and 30% in fish fed with 2.0%, 4.0% and 6.0% supplementation diet for 30 days against *P. fluorescens*. 4.0% dose diet showed highest relative percentage survivability (85.7%) compare to other dose diet in the experiment (Table 1). The mortality increased to 20% with 6.0% dose diet. The highest mortality of 70% was observed in fish fed with 0% (control) dose diet (Fig. 4) and the survival rate was highest in 4% dose (Fig. 5).

Table 1. *Treatment challenge of N. sativa against P. fluorescens injected in common carp.*

Treatment	Challenge dose cfu ml^{-1}	Total fish	No. of infected fish	No. of death fish	Mortality (%)	Survivability (%)	RPS (%)
0%	2.9×10^{-6}	10	08	07	70	30	-
2.0%	2.9×10^{-6}	10	06	04	40	60	42.8
4.0%	2.9×10^{-6}	10	2	01	20	80	85.7
6.0%	2.9×10^{-6}	10	03	02	30	70	71.4

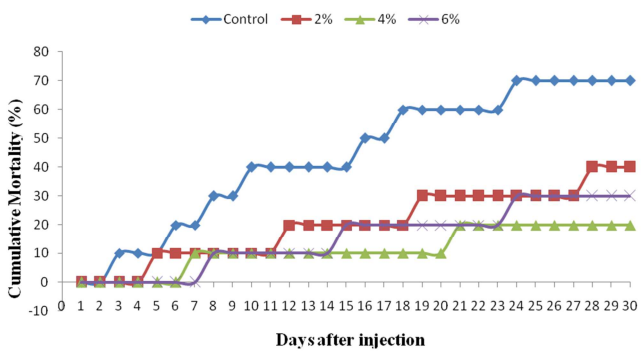

Figure 4. *Cumulative mortalities of common carp, fed diets containing N. sativa and challenged with P. fluorescens for 30 days.*

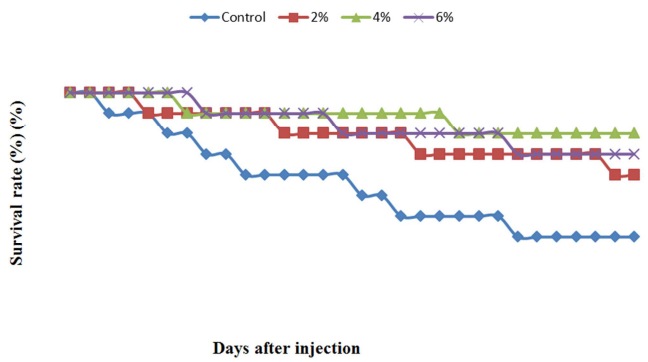

Figure 5. *Survival rate of common carp fed diets containing N. sativa and challenged with P. fluorescens for 30 days.*

The challenge test revealed that, maximum mortality was exhibited in control group whereas lowest amount of mortality of fish was experienced in the treatment group fed 4% black cumin seed where the cumulative mortality was 20%. The next highest mortality was in 6% fed fish group from 24 days to 30 days and the mortality was 30%. Dietary incorporation of *Urtica dioica* at 5% showed significantly higher relative percentage survival (up to 95%) against *A. hydrophila* Ngugi *et al*., [40]. Similar mortality was noted by Mohamad and Abasali [41] in *C. carpio* against *A. hydrophila* injection. In the challenge test of Yilmaz *et al*., [42], a significant increase ($p<0.05$) in the survival rate of *Streptococcus iniae* infected tilapia fry fed the 84%, 72% and 61% diets supplemented with *Cuminum cyminum*, while the survival rates of tilapia fry fed the 45% diets did not significantly change in survival rate compared that of tilapia fry fed the 43% diet. Ngugi *et al*., [40] showed that administration of stinging nettle (*U. dioica)* at 5% on Victoria Labeo (*Labeo victorianus*) reduced mortality significantly. The same results were achieved by Harikrishnan *et al.* [6] for *Pueraria thunbegiana* supplementation in kelp grouper, *Epinephelus bruneus* and Harikrishnan *et al.* [34] for *Solanum nigrum* supplementation in tiger shrimp, *Penaeus monodon* against the same pathogenic bacteria *Vibrio harveyi.* Harikrishnan *et al*., [43] also reported that, cumulative mortality was 50% and 40% in 0.1% and 1.0% *Prunella vulgaris* against *Uronema marinum* infection in *Paralichthys olivaceus*.

4. Conclusion

This study demonstrated that *N. sativa* extract act as immunostimulant and has a positive effect of increasing disease resistance, dominatingly with 4% dose via enhancing the immune response. The dynamic principles of *N. sativa* have growth promoting capacity and perform as appetizer, prompt the immune system, act as broad spectrum antimicrobial characteristics which will be of immense use in common carp and other carp species culture industry. The present study opens up new approach of research to assess the most effective dose under culture conditions, trialing with purified extract of *N. sativa*, degree and duration of the resistance offered, administrative regime for different age group of fish and time of application to ensure improved harvest in culture ponds. Further studies are needed to consider the potential of this plant in other fish and pathogen for disease control strategies.

Acknowledgements

Authors are grateful to Professor Dr. Md. Anisur Rahman, Chairman, Department of Fisheries & Marine Bioscience and all of the respected teachers of Fisheries & Marine Bioscience Department for their advice and valuable technical information during this research work. Authors would also like to thank Md. Iqbal Kabir Zahid, Chairman, and Department of Microbiology for permitting the research team to use the equipment of the departmental laboratory.

References

[1] Vandeputte, M., 2003. Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): a review. *Aquat Living Resour*, 16 (5): 399-407.

[2] Froese, R. and Pauly, D., 2011. FishBase. *World Wide Web electronic publication*, available at: www.fishbase.org/summary/speciessummary.php;

[3] FAO Fisheries and Aquaculture Department, 2015. *Cultured Aquatic Species Information Programme*: *Cyprinus carpio* (Linnaeus, 1758).

[4] Chowdhury, M. B. R. and Baqui, M. A., 1997. Bacterial flora in farmed carp (*Labeo rohita*) in Bangladesh. *In*: Disease in Asian Aquaculture III (eds. T. Flegel and I. H. Mac Rae), *Fish Health Section, Asian Fisheries Society, Manila*. p. 101-105.

[5] Khatun, H., Hossain, M. D., Jahan, S. N. and Khanom, D. A., 2011. Bacterial infestation in different fish at Rajshahi. *J Sci Foundation*, 9 (1 & 2): 77-84.

[6] Harikrishnan, R., Balasundaram, C. and Heo, M. S., 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture*, 317: 1-15.

[7] Robertsen, B., 1999. Modulation of the non-specific defense of fish by structurally conserved microbial polymers. *Fish & Shellfish Immunol*, 9: 269–290.

[8] Rao, Y. V., Das, B. K., Jyotyrmayee, P. and Chakrabarti, R., 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish & Shellfish Immunol*, 20: 263–273.

[9] Awad, E., Austin, D. and Lyndon, A. R., 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture*, 388-391: 193-197.

[10] Nya, E. J. and Austin, B., 2009. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis*, 32: 963–970.

[11] Awad, E. and Austin, B., 2010. 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.* 33 (5): 413–420.

[12] Dügenci, S. K., Arda, N. and Candan, A., 2003. Some medicinal plants as immunostimulant for fish. *J Ethnopharmacol.* 88: 99–106.

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

[14] Ardo, L., Yin, G., Xu, P., Varadi, L., Szigeti, G., Jeney, Z. and Jeney, G., 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the nonspecific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture*, 275 (1-4): 26–33.

[15] Xie, J., Liu, B., Zhou, Q., Su, Y., He, Y., Pan, L., Ge, X. and Xu, P., 2008. Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. *Jian*. *Aquaculture*, 281: 5–11.

[16] Jian, J. and Wu, Z., 2003. Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker, *Pseudosciaena crocea* (Richardson). *Aquaculture*, 218: 1–9.

[17] Jian, J. and Wu, Z., 2004. Influence of traditional Chinese medicine on non specific immunity of Jian Carp (*Cyprinus carpio* var. Jian). *Fish & Shellfish Immunol*, 16: 185–191.

[18] Public Health Agency of Canada, 2011. *Pseudomonas* spp.: *Pathogen safety data sheet Infectious Substances.*

[19] Tryfinopoulou, P., Tsakalidou, E. and Nychas, G. J. E., 2002. Characterization of *Pseudomonas* spp. Associated with spoilage of gilt-head sea bream stored under various conditions. *Appl Environ Microbiol*, 68 (1): 65-72.

[20] Franzetti, L. and Scarpellini, M., 2007. Characterization of *Pseudomonas* spp. isolated from foods. *Ann. Microbiol.*, 57 (1): 39-47.

[21] Soesanto, L., Mugiastuti, E. and Rahayuniati, R. F., 2007. Biochemical Characteristic of *Pseudomonas fluorescens* P60. Dept. of Plant Pests and Diseases, *Faculty of Agcirulture, Jenderal Soedirman University*, P. O. Box 125, Purwokerto 53101.

[22] Couillerot, O., Prigent-Combaret, C., Caballero-Mellado, J. and Moe¨ nne-Loccoz, Y., 2008. *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. The Society for Applied Microbiology*, Lett Appl Microbiol*, 48: 505–512.

[23] Khan M. A., 1999. Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology*, 7 (1): 15-35.

[24] Ramadan, M. F., 2007. Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. *Int J Food Sci Technol*, 42: 1208–1218.

[25] Tembhurne, S. V., Feroz, S., More, B. H. and Sakarkar, D. M., 2014. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. *J Med Plant Res*, 8 (3): 167-177.

[26] Cooper and Gunn, 2005. Tutorial pharmacy. Extraction Methods. *CBS Publishers & Distributors*, New Delhi, India, p. 257-259.

[27] Amend, D., 1981. Potency of testing of fish vaccines. *Dev. Biol. Stand.*, 49: 447-454.

[28] Maggon, K., 2005. Best selling human medicine 2002–2004. *Drug Dis Today*, 11, 739–742.

[29] El-Kadi, A., Kandil, O. and Tabuni, A. M., 1987. *Nigella sativa* and cell mediated immunity. *Archives of AIDS Research.* 1: 232-233.

[30] Hedaya, S. A., 1995. Effect of Nigella sativa (black seeds) extract on some haematological and biochemical parameters in rats. *Alex J Vet Sci*, 11: 95–99.

[31] Mandour, A., Mahmoud, K., Abou El-Wafa, A., El- Agamy, E. and Ragab, O., 1995. Effect of aflatoxin and *Nigella sativa* seeds on serum proteins and its electrophoretic patterns in White Pekin ducklings. *1st Egyptian Hungarian Poultry Conference, Alexandria, Egypt*.

[32] Abdel-Ghaffar, F. A., El-Feki, M. A., Faheem, H. I. and Gad, M. A., 2003. Potentiating effects of some natural products on the common carp immune system against *Pseudomonas florescence. Egypt. Aquat. Biol. Fish.*, 7: 23–47.

[33] Taha, N., Korshom, M., Mandour, A., El-Bahr, S. M. and El-Sirafy, S., 2007. Effect of some immunostimulentant agents on serum proteins and amino acids patterns of Japanese qyils. *Alex J Vet Med*, 25: 47-53.

[34] Harikrishnan, R., Kim, J. S., Balasundaram, C. and Heo, M. S., 2012. Protection of *Vibrio harveyi* infection through dietary administration of *Pueraria thunbergiana* in kelp grouper, *Epinephelus bruneus*. *Aquaculture*, 324-325: 27-32.

[35] Harikrishnan, R., Rani, M. N. and Balasundaram, C., 2003. Hematological and biochemical parameters in common carp, Cyprinus carpio, following herbal treatment for Aeromonas hydrophila infection. *Aquaculture*, 221 (1-4): 41-50.

[36] Grayson, T. H., Williams, R. J., Wrathmell, A. B., Munn, C. B. and Harris, J. E., 1987. Effects of immunopotentiating agents in the immune response of rainbow trout, *Salmo gairdnen* kchardson, to ERM vaccine. *J Fish Biol*, 31: 195–202.

[37] Chen, X., Wu, Z., Yin, J. and Li, L., 2003. Effects of four species of herbs on immune function of *Carassius auratus gibelio*. *J. Fish. Sci. China*, 10: 36–40.

[38] Yuan, C., Li, D., Chen, W., Sun, F., Wu, G., Gong, Y., Tang, J., Shen, M. and Han, X., 2007. Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpuio*). *Fish Physiol Biochem*, 33: 93–101.

[39] Yin, G., Ardo, L., Thompson, K. D., Adams, A., Jeney, Z. and Jeney, G., 2009. Chinese herbs *(Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. *Fish & Shellfish Immunol*, 26: 140–145.

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

[41] Mohamad, S. and Abasali, H., 2010. Effect of plant extracts supplemented diets on immunity and resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio*). *Agri J*, 5 (2): 119-127.

[42] Yımaz, S., Ergün, S. and Soytaş, N., 2013. Dietary supplementation of cumin (*Cuminum cyminum*) preventing streptococcal disease during first-feeding of Mozambique tilapia (*Oreochromis mossambicus*). *J BioSci Biotechnol*, 2 (2): 117-124.

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