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EFFECT OF OCIMUM BASILICUM AND PHYLLANTHUS EMBLICA ON SPECIFIC IMMUNE RESPONSE OF OREOCHROMIS MOSSAMBICUS INFECTED WITH AEROMONAS HYDROPHILA

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ABSTRACT

An investigation was carried out to evaluate the effect of selected plants (*Ocimum basilicum* and *Phyllanthus emblica*) supplemented feed on the relative percent survival rate and the antigen antibody titer. Two experimental feeds were prepared by adding 5 grams of leaf powder to the basal diet and one control feed without leaf powder. The fishes (*Oreochromis mossambicus*) were supplemented with these feeds for 30 days. After 30 days of treatment fishes were infected with *Aeromonas hydrophila* and observed for next 15 days and the immunological parameters such as relative percent survival and antibody production were observed. The data obtained (were analyzed using one way analysis of variance (ANOVA). The fishes fed with 5 grams of *O.basilicum* showed significant effect in terms of survival and antibody production. This study indicated that inclusion of leaf powders in fish feed resulted in better survival and antigen production. The formulation of plant based diet for fish will provide new opportunities.

KEYWORDS: Leaf powder, supplementation, *Oreochromis mossambicus*, *Aeromonas hydrophila*, relative percent survival rate and the antigen antibody titer.

INTRODUCTION

Aquaculture is the fastest growing agricultural industries worldwide (Hishamunda and Ridler, 2006). Aquaculture increases its growing rate of 6% annually and it has been gaining importance over capture fisheries since 1990s (Reverter *et al.,* 2014). A wide range of disease out-breaks and disease causing in aquaculture are due to poor culture practices, overcrowding of fish, poor quality of water etc. Hence it has resulted in the vast loss in production (Gabriel *et al.,* 2015). Fishes are mainly affected by bacterial pathogens interfering with its survival rate. *A. hydrophila* is regarded as an opportunistic pathogen both in the farm and field. The disease is frequently associated with haemorrhagic septicemia (Kuge *et al.,* 1992).*Aeromonas hydrophila* is a ubiquitous gram-negative rod-shaped bacterium which is commonly isolated from fresh water ponds and which is a normal inhabitant of the gastrointestinal tract which causes disease in fish known as "Motile *Aeromonas* Septicemia" (MAS), "Hemorrhagic Septicemia," "Ulcer Disease," or "Red-Sore Disease." (Swann and White, 1989).

Plant extracts with antimicrobial and/or immunostimulant properties have been used as therapeutic and/or prophylactic agents against fish pathogens (Newaj-Fyzul and Austin, 2015). *Ocimum basilicum* is a common herb that is known for its ornamental and therapeutic importance. They are known to have many immuno-stimulant properties. Just like *O.basilicum*, *Phyllanthus emblica* is also rich in many phytoconstituents *viz,* alkaloids, glycosides, reducing sugars, tannins, resins, saponins, sterols, and fixed oils. They are used mainly in the folk medicine because of its antioxidant property (Pandey, 2014). The investigation was carried out with objective of enhancing the immune power of *O.mossambicus* challenged with *A.hydrophila* by using plant leaf powders of *O basilicum* and *P.emblica*.

MATERIALS AND METHOD

An investigation was carried out in our laboratory to evaluate the effect of *O basilicum* and *P.emblica* in enhancing immune parameters of *O.mossambicus* infected with *A.hydrophila*. The materials and methods used for the present study are described under the following headings.

Collection and Acclimation of Experimental Animal
The experimental animal selected for present study was tilapia fish (*O.mossambicus).* The fishes were collected from Tamilnadu Fisheries Corporation Limited, Aliyar dam near Pollachi, Coimbatore, Tamilnadu. Fishes were acclimatized to lab condition for 2 weeks in laboratory condition and care was taken to prevent contamination.

Collection of plant samples
Fresh leaves of *O.basilium* and *P.emblica* were collected in and around Coimbatore.

Preparation of leaf powder
The leaves were washed, shade dried and made into powder using electric pulveriser. The leaf powders were stored in containers and used for further studies.

Preparation of feed
Fish feed was prepared by adding equal proportions of wheat flour and coconut oil cake in the ratio of 1:1 and corn flour as a binder. These substances were mixed thoroughly with hot water and it was steamed for 25-30 minutes and then cooled to room temperature for 30 minutes. Pellets were prepared by using domestic appliances with 0.5mm diameter. It was dried by keeping in the sun. Two experimental feeds were prepared by adding 5 grams of leaf powders separately and the feed without leaf powder was kept as control.

Selection and collection of pathogen
The pathogenic bacteria selected for the present study was *Aeromonas hydrophila.* The bacteria was obtained from the Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India and were maintained on Muller –Hinton agar media (Hi –media) at 4°C. *A. hydrophila* was cultured in a nutrient agar broth for 24 hrs at 37°C in an incubator. The cultured broth was then centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the pelleted bacteria were washed thrice with phosphate buffer saline (PBS) and prepared to 108cfu/ml as determined using Neubaur haemocytometer slide (Rao *et al.*, 2006) This bacterial suspension was used for further experiments.

Determination of LD50 doses
The lethal dose 50% (LD50) of the pathogens was determined using *O.mossambicus* of average weight 5 ± 0.5 g. The fish were divided into 5 groups using random sampling method. Each group containing 8 animals and were injected intraperitoneally (i.p.) with 0.1 ml volumes of 10 fold dilutions of freshly prepared *A.hydrophila* suspensions in saline ranging from 10^2, 10^3, 10^4, 10^5 and 10^6 viable cells/fish, and observed for 7 days. The LD50 value was calculated after the method of Reed and Muench (1938).

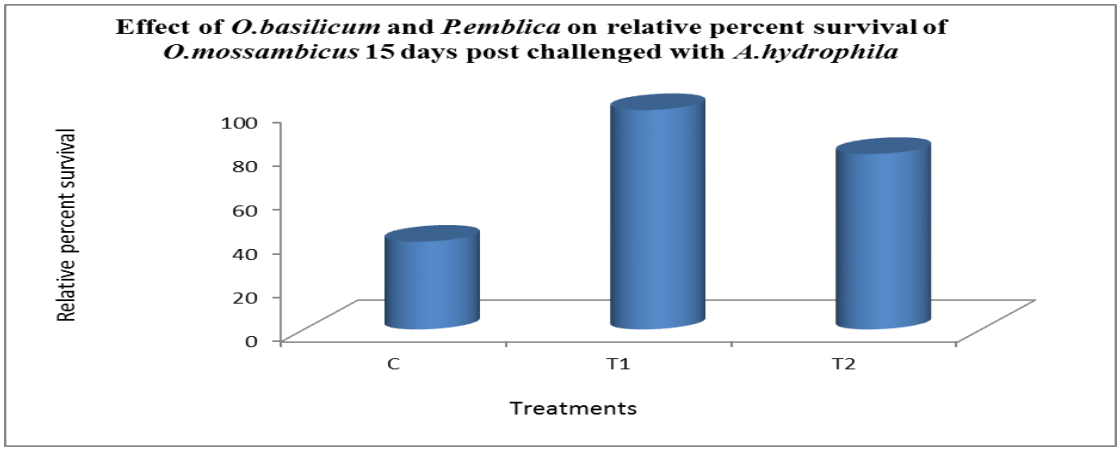
Experimental design
The laboratory experiment was laid in completely randomized design (CRD). Three replications for control(C) and each treatment (T1 and T2) were maintained simultaneously. The experiment was conducted using 15 liter plastic troughs. The troughs were stocked with 10 fishes with mean initial body weight of 5 ± 0.5 grams. The fishes were starved for a night prior to the experiment. The experiment was conducted for 45 days and the fishes were fed with experimental feeds. The medium was changed daily in order to remove the fecal and unconsumed wastes.

Pathogen challenge test
After 30 days of feeding trail, fishes in the control and treatments were injected intraperitoneally with 0.1ml (or) 100 µl of 10^5 cfu/ ml *A.hydrophila* suspension. Mortality was recorded until 15 days after post challenge. Behavioural alterations, feeding response and mortality were observed daily and dead fish were removed. The experiment was conducted in triplicate. Relative percent survival = 100 x (1 - percent mortality in treated group/ percent mortality in control group).

Serum antibody titer (agglutination test)
Serum antibody titer was measured using the agglutination protocol described by Klesius *et al (*2000). After 15 days of infection, antibody titer was assayed in well microtiter plates. Serum (15 µl) was diluted at 1:1 ratio in saline PBS in the first well and was serially diluted in the wells of the first row till the 11th well of the microtitre plate leaving the 12th well as a negative control. Similarly other serum samples were also diluted serially in each row of the microtitre plate. 50ml of the antigen was added to all the wells. Gently shake the microtitre plate for efficiently mixing of the reagents. Incubate the plates at room temperature for an hour. The highest dilution of the sample which shows detectable (macroscopic) agglutination was recorded and expressed as log2 antibody titre of the serum.

RESULTS AND DISCUSSION

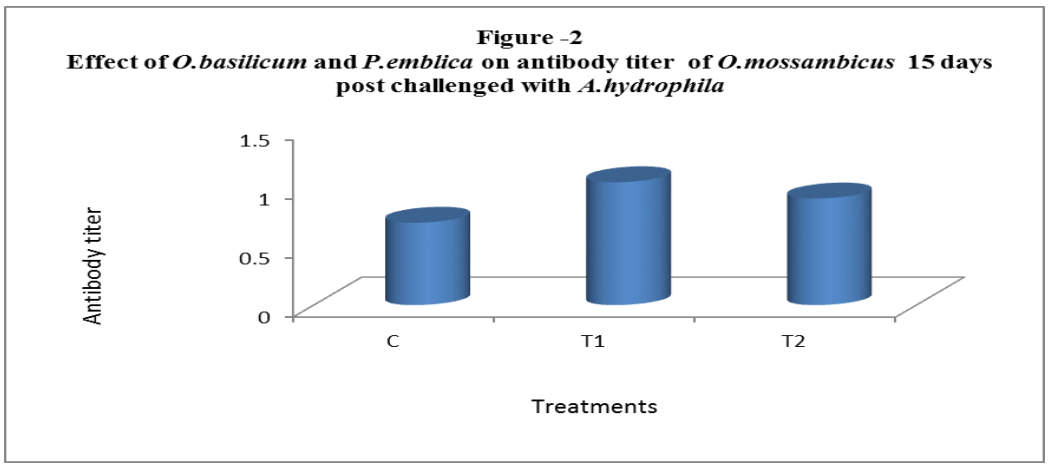
Relative percentage of survival
The results of relative percentage of survival rate of *O.mossambicus* supplemented with leaf powders of *O.basilium* and *P.emblica* and challenged with *A.hydrophila* were shown in figure -1 After challenging fish with *A.hydrophila* there was no mortality of fish up to 12 hours. The group of fishes supplemented with T1 feed showed higher survival rate (100%) as compared to control (40%). The fishes in the experimental groups revealed significant survival rate when compared to control group. When the fishes were injected with *A. hydrophila* the immuno stimulatory effect might be produced by the leaf extracts in the experimental feed. This shows that the leaf extracts were able to stimulate the resistance and inhibit the growth of *A. hydrophila.* Wassom and Kelly, 1990 stated that the resistance level of fish to a particular disease can be determined from the survival rate after bacterial infection.



C – control, T1 – 5 grams *O.basilicum*, T2 – 5 grams *P.emblica.*

The results were supported by Sahu *et al.*, 2007 (a& b) reported that *L. rohita* fed with garlic and mango kernel showed increased resistance and survival against *A. hydrophila*. Abutbul *et al.*, 2004, inferred that *O.mossambicus* fed with *R.officinialis* increased the survival rate against *A. hydrophila*. Yin *et al.*, 2009 reported that common carp fed with *Astragulas* showed enhanced survival rate against *A.hydrophila* when compared to control. Similarly *A.hydrophila* infected fishes such as *O.mossambicus*, *C.carpio* and *L. rohita* were recovered and their survival rate was increased by stimulating the non- specific and specific immune system using *O. sanctum* (Logambal *et al.*, 2000), *E.alba* (Christybapita *et al.*, 2007), and *A. aspera* (Vasudeva *et al.*, 2006) as feed supplement.

Antigen antibody titre: The figure-2 clearly depicts that there was significant effect by the plant leaf powder supplementation on the bacterial agglutination activity. The bacterial agglutination was significantly higher in T1 when compared to control. Antigen antibody titer can be used to measure the humoral immunity an organism. Antibody production can be stimulated by using different types of immunostimulants such as probiotics, antibiotics, chemicals and plant products. In this work the leaf extracts were used as good immuno stimulators to boost the innate immune system of fish. Similar results was observed by Mercy (2006) who stated that a significant haem agglutination antibody titre was observed in *C.mrigala* treated with *P.emblica*. Prathiba and Sukumaran (2014) reported that all the concentrations of leaf extracts of *Euphorbia hirta* enhanced antibody response against *A. hydrophila* in *C.carpio*.



C – control, T1 – 5 grams *O.basilicum* , T2 – 5 grams *P.emblica.*

The leaf extract of *O.sanctum* produced a significant stimulatory effect on both primary and secondary responses against *A.hydrophila* in *O.mossambicus* (Logambal *et al.*, 2000 and Venkatalakshmi and Michael, 2001). Behera *et al.*, 2011 stated that increased bacterial agglutination activities in *A.hydrophila* infected fishes primed with low dose (15mg) curcumin. Kumar *et al.*, 2007 infered that that there was an enhanced antibody response in *L.rohita* fed with polyherbal formulation when challenged against *A.hydrophila*. Lokesh *et al.*, 2012 reported that the herbal extracts and animal originated product have a potential application as an immuno stimulant in fish culture, primarily because they can be easily applied, are not expensive and act against a broad spectrum of pathogens.

CONCLUSION

The results on present study indicated the beneficial role of selected plant leaves of *O. basilium* and *P.emblica* as immunistimulants against the pathogen *A.hydrophila* in *O.mossambicus.* The study revealed that *O.basilicum* at 5grams concentration enhanced the survival rate of the fish against the pathogen. It was also observed to produce antibodies in greater amount against the pathogen. The study thus states the possibilities of using plant powders rich in immunostimulants to enhance the specific immune response of fish against certain pathogens.

REFERENCES

1. Abutbul S, Avi Golan OZ, Baarazani Dina Zilberg. Use of Rosmarinus officinalis as a treatment against Streptococcus iniae in Oreochrombis mossambicus. Aquaculture, 2004; 238(1-4): 97-105.
2. Behera T, Swain P, Sahoo SK, Mohapathra D and Das BK. Immunostimulatory effects of curcumin in fish, Labeo rohita. Indian journal of natural products and resources, 2011; 2(2): 184-188.
3. Christybapita D, Divyagnaneswari and Dinakaran Michael R. Oral administration of Eclipta alba leaf extracts enhances the non-specific immune response and disease resistance of Oreochrombis mossambicus. Fish and shellfish immunology, 2007; 23(4): 840-852.
4. Gabriel UU, Obomanu FG and Edori OS. Haematology, plasma enzymes and organ indices of Clarias gariepinus after intramuscular injection with aqueous leaves extracts of L epidagathis alopecuroides. African Journal of Biochemistry Research, 2009; 3(9): 312-316.
5. Govind Pandey Feed formulation and feeding technology for fishes. International research journal of pharmacy. DOI: 10.7897/2230-8407.04306, 2013.
6. Guojun Y, Ardo L, Thompson, KD, Adams A, Jeney Z, Jeney G. Chinese herbs (Astragalus radix and Gaanoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. Fish and shellfish immunology, 2009; 26: 140-145.
7. Klesius PH, Shoemaker CA, Evans JJ Efficacy of a single and combined Streptococcus iniae isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (Oreochromis niloticus), 2000.
8. Kuge T, Takahashi K, Barcs I, Hayashi F. Aeromonas hydrophila, a causative agent of mass mortality in cultured Japanese catfish larvae (Silurus asotus). Fish Pathol, 1992; 27: 57–62.
9. Kumar R, Mukherjee S, Ranjan R, and Nayak S. Enhanced innate immune parameters in Labeo rohita (Ham.) following oral administration of Bacillus subtilis. Fish Shellfish Immunology, 2008; 24: 168–172. doi: 10.1016/j.fsi.2007.10.008.
10. LaDon Swann M and Randy White DVM. Diagnosis and Treatment of "Aeromonas hydrophila" Infection of Fish. Aquaculture extension. IL-IN-SG-FS-91-2, 1989.
11. Logambal SM, Venkatalaxmi, S and Michael RD Immunostimulatory effect of leaf extracts of Ocimum sanctum Linn. in Oreochrombis mossambicus, 2002.
12. Lokesh J, Fernandes JMO, Korsnes K, Bergh O, Brinchmann MF, KironV Transcriptional regulation of cytokines in the intestine of Atlantic cod fed yeast derived mannan oligosaccharide or β-glucan and challenged with Vibrio anguillarum. Fish Shellfish Immunol, 2012; 33: 629–631.
13. Miriam Reverter, David Laccini, Nathalie Tappisier Bontemps and Bernard Benaigs. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture, 2014; 433: 50–61.
14. Nathanael Hishamunda., Neil B Ridler. Farming fish for profits: A small step towards food security in sub-Saharan Africa. Food policy, 2006; 31(5): 401-414.
15. Newaj-Fyzul A, and Austin B. Probiotics, immune stimulants, plant products and oral vaccines and their role as feed supplements in the control of bacterial fish diseases. Journal of fish diseases, 2015; 38: 973-955.
16. PratibaV and Sukumaran NP. Effect of Euphorbia hirta plant leaf extracts on immune stimulant response of Aeromonas hydrophila infected Cyprinus carpio. Peer J., 2: e671: DO11O. 7717/peerj.671, 2014.
17. Rao SVR., Raju M V L N, Panda AK, Reddy MR. Sunflower seed meal as a substitute for soybean meal in commercial broiler chicken diets. Br. Poult. Sci., 2006; 47(5): 592-598.
18. Reed LJ, and Muench H. A simple method of estimating per cent end points. Am J hyg, 1938; 27: 493-497.
19. Regina Mercy. Influence of leaf extract of Phyllanthus emblica on immunological, haematological and biochemical responses in freshwater fish, Cirrhinus mrigala artificially infeted with bacterial pathogen, Pseudomonas flurescens, 2006.
20. Sahu S, Das BK, Pradhan J, Mohapthra BC, Sarangi N. Effect Of Mangifera indica kernel as a feed additive on immunity and resistance to Aeromonas hydrophila in Labeo rohita fingerlings. Fish And Shellfish Immunology, 2007; 23: 109-118.
21. Venkatalakshmi S and Michael RD. Immunostimulation by leaf extract of O.sanctum in O.mossambics. Journal of Aquaculture in the Tropics, 2001; 16(1): 1-10.
22. Wassom DL, Kelly EAB. Role of the major histocompatibility complex in resistance to parasite infections. Critical reviews in immunology, 1990; 10: 31-52.