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Effect of *Hibiscus rosa-sinensis* and *Cassia auriculata* on immune response of *Cirrhinus mrigala* infected with *Aeromonas hydrophila*

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ABSTRACT

An investigation was carried out to evaluate the effect of Hibiscus rosa-sinensis and Cassia auriculata flower powders as supplemented feed on the relative percent survival and antigen antibody titer. Four experimental feeds were prepared by adding 5 grams and 10 grams of flower powder to the basal diet and one control feed without plant powder. The Cirrhinus mrigala fishes were supplemented with these feeds for 45 days. After 30 days of feeding the fishes were infected with Aeromonas hydrophila and survival rate and antibody titer were noted on 38th and 45th day after treatment. The data obtained were analyzed using one way analysis of variance (ANOVA). The fishes fed with 10 % Hibiscus rosa-sinensis flower powder supplemented feed showed greater effect in terms of survival rate and antigen antibody titer. This study indicated that inclusion of flower powder in fish feed resulted in better survival and antigen production. The formulation of plant based diet for fish will provide new opportunities.

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

INTRODUCTION

Aquaculture ascertained by FAO (1990) as the meadows of aquatic organisms containing crustaceans, fishes and aquatic plants with some species of invasion in the culturing process to reinforce productions such as furnishing, feeding and conservation from predators. From ancient times, the aquaculture has been conducted and it is spread all over the world constantly it is revolutionized from a tradition into science (FAO,1990). It increases its growing rate of 6 % annually and it has been gaining importance over capture fisheries since 1990s (Reverter *et al*., 2014). Disease outbreaks are increasingly being recognized as a potential constraint on aquaculture production and trade and cause massive financial loss either through mortality or reduced profit margins (Plumb and Hanson 2011; Mehana *et al*., 2015). Bacteria, the major group of pathogens, pose one of the most significant threats to successful fish production throughout the world (Roberts, 1989). *Aeromonas hydrophila* is a ubiquitous organism present in the aquatic environment causing diseases in fish under stress (Doukas *et al*.,1998). *A. hydrophila* is a gram negative, motile rod recorded as an opportunistic pathogen in freshwater fish species and it is considered to have

widespread geographical distribution (Davis *et al*., 1978). *A. hydrophila* has been recognized as the causative agent of hemorrhagic septicemia/ motile aeromonas septicemia, skin ulceration, fin/tail rot and sore disease (Haley *et al.*,1967). Often, healthy fish suddenly develop swimming abnormalities, pale gills, bloat (abdominal distension) and dermal/ ocular ulcerations.

Many plant powders and extracts with antimicrobial , antibacterial and immunostimulant properties have been used as therapeutic agents against fish pathogens. Against bacteria, they disrupt the bacterial cell wall, block the synthesis of proteins and DNA, inhibit enzyme secretion and hinder the bacterial signaling mechanism via quorum sensing (Citarasu, 2010). *Hibiscus rosa-sinensis* is a widely grown evergreen ornamental herbs, shrubs and trees of the tropics and sub-tropics and are reported to possess various medicinal properties (Hirunpanich *et al.,* 2006, Chang *et al.*, 2006, Herrara, 2004, Palaniswamy, 2003 and Telefor *et al.*,1998). Just like *Hibiscus rosa-sinensis*, *Cassia auriculata* is also showed antibacterial antioxidants activities (Anushia *et al.*,2009). The investigation aimed at enhancing the immune system of *Cirrhinus mrigala* challenging with *Aeromonas hydrophila* using plant flower powders like *H. rosa-sinensis* and *C. auriculata*.

MATERIALS AND METHODS

Experimental fish collection and maintenance

The experimental animal selected for present study was mrigal fish (*Cirrhinus mrigala*). The fingerlings of *Cirrhinus mrigala* was collected from Aliyar dam, near Pollachi, Coimbatore district, Tamil Nadu. They were acclimatized for 2-3 weeks in the laboratory and was then transferred in to the experimental tanks. They were fed with commercial feed during this period. The tanks were maintained with care and was kept clean. The excess food and fecal matter were removed on a daily basis. The water was changed once in a week keeping 50 % of the habitat water.

Collection of plant samples

The flowers selected for the present study are *Hibiscus rosa-sinensis* and *Cassia auriculata*. The flowers from these plants were collected from in and around Coimbatore, Tamil Nadu.

Processing and preparation of flower powder

Fresh flowers of *H. rosa sinensis* and *C. auriculata* were collected, washed and shade dried. The dried flowers were finely powdered and stored in air tight containers at room temperature for further use.

Preparation of feed

Fish feed was prepared by adding equal proportions of wheat flour and coconut oil cake in the ratio of 1:1:1 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 at room temperature for 30 minutes. Pellets were prepared by using domestic appliances with 0.5 mm diameter. It was dried by keeping in the sun. Four experimental

diets were prepared by adding 5 grams and 10 grams of flower powders separately and the feed without plant powder was kept as control.

TABLE 1: INGREDIENTS USED IN FEED PREPARATION

Composition	Diet				
	C	T1	T2	T3	T4
Wheat flour	50	50	50	50	50
Coconut oil cake	50	50	50	50	50
Corn flour	1	1	1	1	1
C.auricula	-	5g	10g	-	-
H.rosa-sinensis	-	-	-	5g	10g

Selection of Pathogen

The fish pathogen *Aeromonas hydrophila* was provided from the department microbiology, PSG Institute of research and management, Coimbatore. The pathogen was maintained on agar slopes at 4°C and was used for infecting the healthy fish. *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 108 cfu/ml as determined using Neubaur haemocytometer slide (Rao *et al.*, 2006). This bacterial suspension was used for further experiments.

Experimental design

The fishes were randomly distributed into 5 experimental tubs. Each tub consisted of 8 fishes. The fish were fed with experimental feed at the rate of 5 % of body weight once in a day before 9:00 for 45 days. Group C was fed with basal diet and it acts as the control. The remaining groups were fed with 5g of *C. auriculata* (T1), 10g of *C. auriculata* (T2), 5g of *H. rosa-sinensis* (T3), 10g of *H. rosa-sinensis* (T4).

Pathogen challenge test

After 30 days of feeding trail, fishes in the control and treatments were injected intraperitoneally with 0.1 ml (or) 100 µl of 10^{-4} 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. The behavioral changes and survival were observed.

Relative percent survival

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

Antigen-antibody titer (agglutination test)

Serum antibody titer was measured using the agglutination protocol described by Klesius *et al.*, 2000 after 7 days of infection. The agglutination test 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. 50 ml of the antigen was added to all the wells. Gently shake the microtitre plate for efficiently mixing of the reagents. Incubate the titer plates for one hour at room temperature. The highest dilution of the sample which shows detectable (macroscopic) agglutination was recorded and expressed as log2 antibody titre of the serum.

Statistical analysis

The results of the present study were subjected to statistical analysis. The data obtained on immunological parameters were analyzed using one way ANOVA and the level of significance was defined at $p<0.05$.

RESULTS

RELATIVE PERCENT SURVIVAL

The relative percent survival was 100% in T4 fishes on 38th day after treatment when compared to control. On 45th day of treatment, 100% survival was observed in all the treatments whereas control showed minimum relative percent survival of 2% (Figure -1).

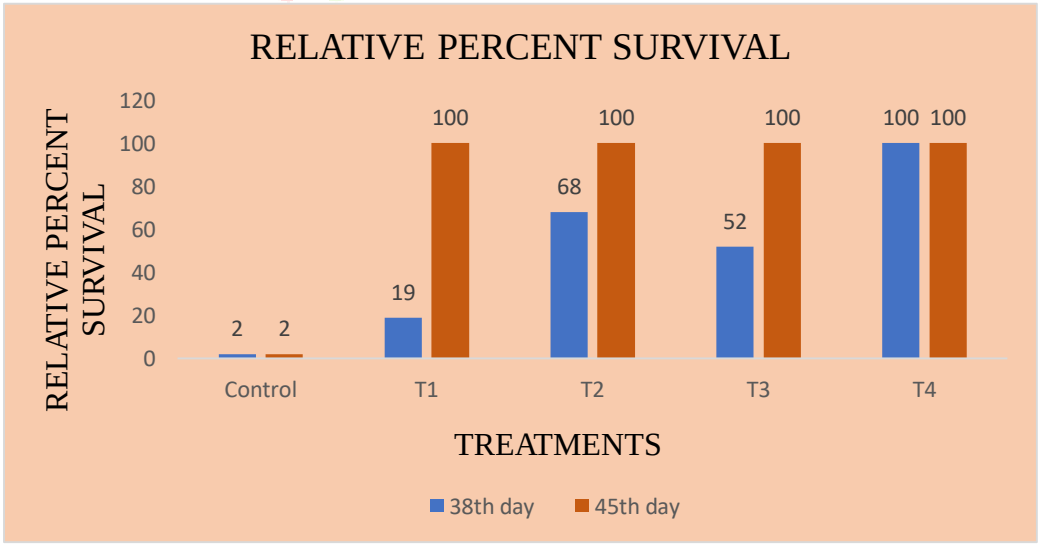

Figure 1- Effect of on the *Hibiscus rosa -sinensis* and *Cassia auriculata* on the relative percentage of survival of *Cirrhinus mrigala*

ANTIGEN-ANTIBODY TITER

The antibody response to *Aeromonas hydrophila* by various experimental groups fed with different concentrations of flower powders of *Hibiscus rosa-sinensis* and *Cassia auriculata* were found out by using antibody titer plate shown in figure- 2.

The antibody response produced by experimental fishes were higher than the control group. The antibody response of T4 fishes was 0.903 whereas control showed a minimum antibody titer value 0.602on 38th day after infection. On 45th day after treatment more antibody response (1) was found in T4 when compared to control.

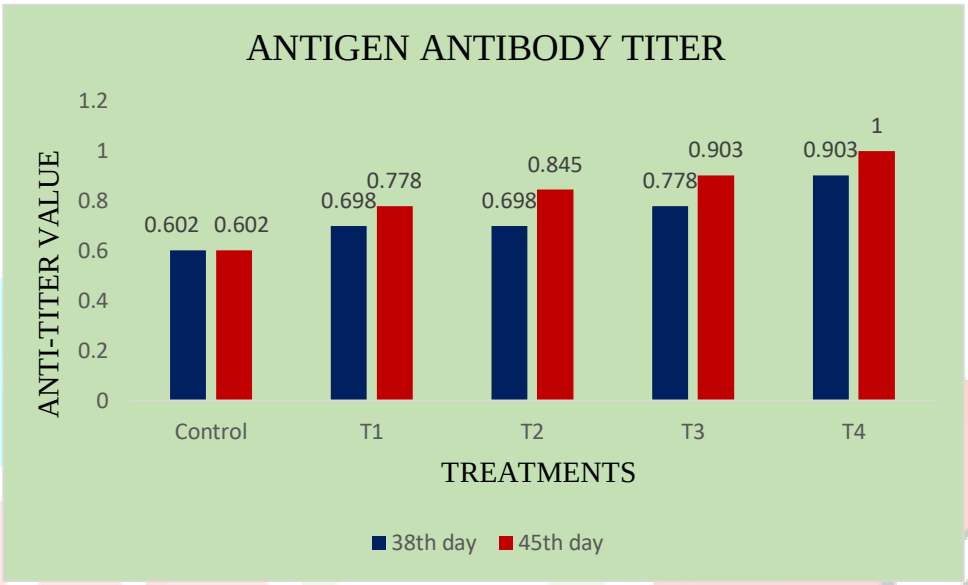

Figure 2- Effect of on the *Hibiscus rosa -sinensis* and *Cassia auriculata* on the antigen antibody titer of *Cirrhinus mrigala*

DISCUSSION

The flower powders used in this study would have enhanced the immune response in all experimental groups. The present study revealed that fishes fed with T4 and T2 showed higher survival rate when compared to control. This might be due to the action of the flower extracts in the fish feed. The secondary metabolites found in the flower extracts might be responsible for significant increase in the survival rate of the fishes in experimental groups.

The results of present study correlate with the findings of Iruthayam *et al.,* 2014 who reported that *Mystus montanus* fingerlings fed with medicinal plants showed increased resistance and survival against *Aeromonas hydrophila*. Sahu *et al.*, (2007 a and b), inferred that *L. rohita* fed with garlic and mango kernel showed increased survival rate against *Aeromonas hydrophila* when compared to control. Similarly, Baba *et al.*, 2016 stated that a positive effect on the survival rate of common carp observed when treated with *Avena sativa* extract against *Aeromonas hydrophila.* Sanjana *et al.*,2019 stated that The fishes fed with 5%

Ocimum basilicum showed greater effect in terms of survival and at 10% it showed better antigen antibody titer.

To *Aeromonas hydrophila* by the experimental fishes fed with *Hibiscus rosa-sinensis* and *Cassia auriculata* extracts at different concentrations showed significant enhancement when compared to control. The antibody titer was significantly higher in experimental groups and this increase in the production of antibody might be due to the immuno stimulatory effect of flower extracts in the experimental feed. The phytochemicals found in the flower extracts were able to resist against bacteria which resulted in enhanced antibody production.

The results of present study were similar to the findings Pratheepa *et al.*,2014 who reported that the *Euphorbia hirta* plant leaf extract enhance antibody response but the extract which are in higher concentration (25 to 50g) were only able to stimulate higher antibody production. Hema Priya *et al.*, (1997) suggested that the leaf extracts of *Phyllanthus niruri* and *Acalypha indica* stimulated the antibody response in Tilapia (*Oreochromis mossambicus*). Similar results were observed by Logambal *et al.*, 2000 who reported that *Ocimum sanctum* positively determined the immunomodulatory effect stimulate the antibody response and disease resistance in *Oreochromis mossambicus* against the infection of *Aeromonas hydrophila.*

CONCLUSION

The results on present study indicated the beneficial role of selected plant flowers of *H. rosa-sinensis* and *C. auriculata* as immunistimulants against the pathogen *A.hydrophila* in *C.mrigala.* The study revealed that *H. rosa-sinensis* at 10 % concentration enhanced the survival rate of the fish against the pathogen. It has also improved the rate of oxygen consumption in flower extract treated groups. In general, the immunostimulant was found to produce antibodies in greater amount against the pathogen. The study thus states that *H. rosa-sinensis* and *C. auriculata* are suitable as supplemented feeds for fishes at farm level as they have been identified as effective immune stimulants against certain pathogens.

REFERENCE

Anushia, C., Sampatkumar, P., and Ramkumar L., (2009). "Antibacterial and antioxidant activities in *Cassia* auriculata", *Global Journal of Pharmacology*, Vol-3, No-3, pp 127-130.

Baba, E., ÜmitAcar, CananÖntaş, Osman Sabri Kesbiç&Sevdan Yilmaz (2016). The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*). *Italian Journal of Animal Science*, 15:2, 325-333.

Chang, Y. C., Haung, K. X., Haung, A. C., HO Y. C. and Wang, C. J. (2006). Hibiscus anthocyanins-rich extract inhibited LDL. Oxidation and oxLDL-mediated macrophages apoptosis. *Food Chem Toxicol*, 44:1015–23.

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

Croux, M.J.P., Loteste, A., and Jimena, C. (2002). Inhibition of plasma cholinesterase and acute toxicity of monocrotophos in a Neotropical fish *Prochilodus lineatus* (Pisces, Curimatidae). *Bull. Environ. Contam. Toxicol.*, 69: 356-363.

Davis, W.A., Kane, J. G and Garaguis, V. G., (1978). Human *Aeromonas* infections: A review of the literature and a case report of endocarditis. *Medicine (Baltimore)* 57,267- 271.

Doukas, V., Athanassopoulou, F., Karagouni, E., and Dotsika, E., (1998). *J. Fish Dis*.21,317–320.

Dube, P. N and Hosetti, B (2010). Behavior survillence and oxygen consumption in the freshwater fish *Labeo rohita* exposed to Sodium cyanide. *Biotechnology in Animal Husbandry*., 26(1-2): 91-103.

FAO, (1990). The definition of Aquaculture and collection of statistics. FAO *Aquaculture*. min., (7):4p.

Haley, R., Davis, S.P. and Hyde, J.M., (1967). Environmental stress and *Aeromonas liquefaciens* in American and thread fin shad mortalities. *Progr.Fish Cult*.29, 193.

Hemapriya, V.S. (1997). Immunostimulatory effect of leaf extracts of few medicinal plants in *Oreochromis mossambicus* (Peters), M.Sc. Thesis. The American College, Madurai, India.

Herrera, A. A., Flores, R. S., Chavez-Soto, M. A. and Tortoriello, J. (2004). Effectiveness and tolerability of a standarized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertention: a controlled and randomized clinical trial. *Phytomedicine*, 11:375–82.

Hirunpanich, V., Utaipat, A., Morales, N. P., Bunyapraphatasara, N., Sato, H., and Herunsalee, A. (2006). Hypocholestremic and antioxidant effect of the aqueous extracts of *Hibiscus sabdariffa* Linn. in hypercholestremic rats. *J Ethanopharmacol*, 103: 252–60.

Iruthayam Vijaya Kumar, Gurusamy Chelladurai, Thangapandi Veni, S. Syed Hussain Peeran, Jayaraj Mohanraj, (2014). "Medicinal plants as immunostimulants for health management in Indian cat fish", *Journal of Coastal Life Medicine*; 2(6): 426-430.

Klesius P.H., Shoemaker C.A. & Evans J.J. (2000). Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). *Aquaculture* 188:237-246.

Logambal, S.M., Venkatalakshmi, S., Micheal R.D (2000). Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus. Hydrobiologia*, 430 (1-3), pp. 113-120.

Mehana, EE., Rahmani, AH., Aly, SM., (2015). Immunostimulants and Fish Culture: An Overview. *Annu Res Rev Biol*. 5 (6):477-489.

Murshigeri, S.B and M. David, (2005). Fenvalerate induced changes in the Ach and associated AChE activity in different tissues of fish, *Cirrhinus mrigala* (Hamilton) under lethal and sublethal exposure period. *Environ. Toxicol. Pharmacol.*, 20: 65-72.

Newaj-Fyzul, A. & Austin B. (2015). 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.* 38, 937-955.

Palaniswamy, U. R. (2003). Purslane-Hibiscus. *The Asian American Studies Institute, School of Allied Health at the University of Connecticut, Storrs.*

Plumb, J. A., and Hanson, LA. (2011). Health Maintenance and Principal Microbial Diseases of Cultured Fishes. Hoboken, NJ: Wiley-Blackwell.

Pratheepa and NatarajaPillai Sukumaran., (2014). "Effect of *Euphorbia hirta* plant leaf extract on immunostimulant response of *Aeromonas hydrophila* infected *Cyprinus carpio". PeerJ,* 2: 671.

Randey, W., (1991). Diagnosis of *Aeromonas hydrophila* infections in fish. Animal Disease diagnostic Laboratory Newsletter. Pp. 1.

Rao, J.V, (2003). Toxicity of chlorpyrifos to the fish, *Oreochromis mossambicus. Bull. Environ. Contam.Toxicol.*, 70: 985-992.

Rao, YV., Das, BK., Jyotyrmayee, P., Chakrabarti, R (2006). Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish Shellfish Immunology* 20:263-273.

Reverter, M. Bontemps,N. Lecchini,D. Banaigs,B. Sasal,P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: *current status and future perspectives Aquaculture.*433:50-61.

Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J., Sarangi N. (2007a & b). Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila. Journal of Applied Ichthyology*, 23 (1), pp. 80-86.

Telefor, P. B. (1998). Effects of an aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Diclipteraverticillata*on some physiological and biochemical parameters of reproduction in immature female rats. *J Ethnopharmacol,* 63:193-200.

Tilak, K.S., K. Veeraiah and V. Janardhana Reddy, (2001). A study on nitrite-nitrogen effect on haemoglobin content of the fish *Puntis sophore* (Hamilton) and *Channa punctatus* (Bloch). *Poll.Res.*, 20(2):179-181.