INFLUENCE OF CERTAIN HERBAL ADDITIVES ON THE GROWTH, SURVIVAL AND DISEASE RESISTANCE OF GOLDFISH, *CARASSIUS AURATUS* (LINNAEUS)

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ABSTRACT

The ornamental fish trade earns foreign exchange, besides serving as a source of employment. Application of medicinal herbs in aquaculture is gaining success. Recently use of medicinal plant is considered as an alternative to antibiotics in fish health management The present investigation was designed to study the influence of two herbal additives viz., Phyllanthus niruri *and* Aloe vera *on the growth and disease resistance in goldfish,* Carassius auratus*. The experiment was conducted with the adult fishes for a period of 60 days. After the 60 days of experiment, fish of each treatment were challenged by pathogenic* Aeromonas hydrophila *which was given by intraperitoneal injection and kept under observation for 10 days to record clinical signs and the daily mortality rates. Phyllanthus, at 1.5 per cent concentration yielded highest mean weight gain (1.769 g) followed by aloe which recorded a mean weight gain of 1.389 g at 1.0 per cent concentration. The highest survival rate (80 %) was observed in aloe fed group at 1.5 per cent concentration followed by phyllanthus (70%) at 1.5 per cent concentration. These results indicate that addition of herbal additives can promote growth and can prevent disease in goldfish farming.*

Key words : Goldfish, Herbal additives, Phyllanthus niruri ,Aloe vera Growth, Disease resistance.

INTRODUCTION

The ornamental fish trade earns foreign exchange, besides serving as a source of employment. It has a significant role in the economy of developed and developing countries. When aquaculture production becomes more intensive, the incidence of diseases including infectious diseases has increased and as a result of it, significant economic losses have been incurred. The use of antibiotics and other chemotherapeutics for controlling diseases has been criticized for their negative impacts. Application of medicinal herbs in disease management is gaining success, because herbal treatment is cost effective, ecofriendly and has minimal side effects. Traditional herbal medicines seem to have the potential immunostimulation.

Use of medicinal plant is an alternative to antibiotics in fish health management (Chakraborty and Chattopadhyay, 1998). These herbs are not only safe for consumers but also but also widely available throughout Asia and they also have a significant role in aquaculture (Direkbusarakom, 2004).Many studies have proved that herbal additives enhanced the growth of fishes and also protected from the diseases (Francis *et al*. (2002), Inamati *et al*., (2003), Jassim and Naji (2003),

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Sasmal *et al*., (2005), Shalaby *et al*., (2006), Sharma *et al*., (2006) and Johnson and Banerji (2007) but studies related to herbal application in ornamental fishes on the growth and disease scanty. Hence, the present study was carried out to determine growth performance and disease resistance of goldfish, *Carassius auratus* fed with herbal additives and post-challenged with *A.hydrophila*

MATERIALS AND METHODS

Herbal Additives

Two herbal additives viz., Phyllanthus (*Phyllanthus niruri*) and Aloe (*Aloe vera*) were selected for this experiment The dried powder of phyllanthus and inner gel of aloe were used for incorporation in the supplementary feed. These were used at three different concentrations viz., 0.5, 1 and 1.5 per cent.

Diet preparation

Seven experimental diets were formulated (37.5% crude protein, 4.5% fat and 13.5% ash) to contain different concentrations of two herbal additives. The feed ingredients and their level used for the preparation of experimental feed is given in Table 1.

Fish culture and feeding regime

Goldfish, *Carassius auratus* were obtained from ornamental fish farm at Thoothukudi. Healthy fish were kept in an indoor fibreglass tank for 15 days for acclimation to the laboratory condition. Goldfish weighing between 3.481 to 3.693 g were stocked at the rate of 10 numbers per 45 l plastic trough. All troughs were connected with aeration facilities. The prepared pelleted feed was fed to the experimental fishes at *ad libitum*. Each diet was fed to triplicate troughs twice daily for a period of 60 days. Fishes in each trough were weighed at every fortnight interval.

Chemical analysis of diet

The experimental diets were analyzed according to the standard methods of AOAC (1990) for moisture, crude protein, crude fat and ash.

Water quality analysis

Water samples were collected at fortnight interval. Temperature, dissolved oxygen, ammonia and pH in all the experimental troughs were estimated according to the standard methods of AOAC (1990).

In all the treatment the temperature varied from 27°C to 29°C. The dissolved oxygen concentration ranged from 4.89 to 4.98 ml/l and pH ranged from 7.2 to 7.5. The ammonia level in water varied from 0.004 to 0.005 ppm.

Determination of median lethal dose (LD_{50})

Aeromonas hydrophila maintained on tryptic soy agar (TSA) slant was streaked onto TSA plate and incubated at 37°C for 24 h to get young culture. One or two young discrete colonies of the bacterium were aseptically picked and transferred to 10 ml tryptic soy broth (TSB) and incubated at 37 °C for 24 h. This young culture was centrifuged at 5000 rpm for 15 minutes. The cell pellets were washed twice with sterile physiological saline and finally re-suspended in 10 ml sterile phosphate buffered saline (PBS, pH 7.4). These re-suspended cells were suitably diluted to the requirements (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, up to 10^{-8}) and spread plating was done on *Aeromonas* isolation medium to find out the colony-farming units.

For determination of LD_{50}, the goldfishes were injected with 100 µl of the serially diluted cell stock (10^{0}, 10^{-1}, 10^{-2}, 10^{-3} and sterile phosphate buffered saline) at the intraperitoneal region. Ten goldfishes were used for each dilution. For calculating LD_{50} value the mortality of the goldfishes were observed for 10 days. The LD_{50} value was calculated by the method of Reed and Muench (1938).

Challenge test

At the end of the study, fishes in all the troughs were challenged with pathogenic *A. hydrophila*. They were injected intraperitoneally with one day old culture of *A. hydrophila* with an LD_{50} dose at 100 µl / fish. The control group was injected with 100 µl saline solution. All the fishes were kept under observation for 10 days to record clinical signs and daily mortality rates.

Statistical analysis

All data were subjected to two-way ANOVA to evaluate the effect of different herbal additives and between the concentrations tried.

RESULTS AND DISCUSSION

The effects of dietary herbal additives on the growth of goldfish are shown in the Table 2. In *Phyllanthus niruri* fed group, the highest mean weight gain (1.769 g) and specific growth rate (0.662) were recorded at 1.5 per cent concentration. The mean weight gain of *Phyllanthus niruri* fed groups was higher than that of control group (1.387 g). In the case of *Aloe vera*, the group fed with 1.0 per cent concentration had highest mean weight gain (1.389 g) and specific growth rate (0.548).

The result of accumulative mortality of goldfish challenged with *A. hydrophila* for the estimation of median lethal dose (LD_{50}) is given in the Table 3. Mortality of fish did not occur at lower doses of *A. hydrophila* (1.90×10^5 cfu / fish). The clinical signs observed in challenged fishes were erosion on the fins, gills and in the caudal region.

At the end of experiment the treated goldfish were challenged with *A. hydrophila* at approximate value of LD50 (2.63 x 106 cfu / fish). The rate of mortality and survival rates of challenged fishes are given in the Table 5. The highest survival rate (80 %) was observed in aloe fed group at 1.5 per cent concentration. In *Phyllanthus niruri* fed group, the highest survival rate (70 %) was noted at 1.5 per cent concentration. There is a significant difference between the different herbal additives at different concentrations on the survival rate of goldfish.

Influence of herbal additives on the growth of goldfish

In *Phyllanthus niruri* fed group, the highest mean weight gain (1.769 g) and specific growth rate (0.662) were observed at 1.5 per cent concentration followed by 1.0 per cent concentration. The mean weight gain of these groups were higher than that of control fed group. The maximum mean weight gain (1.389 g) and specific growth rate (0.548) of *Aloe vera* fed group were recorded at 1.0 per cent concentration followed by 0.5 per cent concentration.

Among the two different herbal additives, the *Phyllanthus niruri* fed group recorded higher specific growth rate followed by *Aloe vera* fed group. Ji *et al*. (2007) observed that the herbs promoted cellular lipid and fatty acid utilization and protein accumulation resulting in good growth performance in *Pagrus major*. The inclusion of herbal additives in diets often provides cooperative action to various physiological functions. This synergistic effect of herbs has also been reported in other fishes including Japanese flounder (Ji *et al*., 2007) and *Clarias gariepinus* (Turan and Akyurt, 2005). Johnson and Banerji (2007) reported that growth increase in *Labeo rohita* fed with herbal supplemented diet was due to improved food utilization and high protein synthesis. Sharma *et al*. (2006) revealed the beneficial utility of herbal growth promoters as an additive in the carp feed. There is a significant difference between the different herbal additives on the effect of growth rate in goldfish.

Table 1

Ingredient composition of feeds used for different experiments

Sl.No.	Ingredients	Percentage of inclusion			
		Control	Experimental diet 1 (0.5 %)	Experimental diet 2 (1.0 %)	Experimental diet 3 (1.5 %)
1.	Fish meal	16.0	16.0	16.0	16.0
2.	Groundnut oil cake	16.0	16.0	16.0	16.0
3.	Sesame oil cake	16.0	16.0	16.0	16.0
4.	Soya flour	16.0	16.0	16.0	16.0
5.	Rice bran	18.0	18.0	17.5	17.0
6.	Tapioca flour	17.5	17.0	17.0	17.0
7.	Vitamin and mineral mixture	00.5	00.5	00.5	00.5

(The herbal additives such as *Phyllanthus niruri* and *Aloe vera* were used at three different concentrations viz, 0.5, 1.0 and

Table 2

Effect of herbal additives on the growth of goldfish, Carassius auratus

Sl. No.	Treatment	Conc. (%)	Mean Weight (g)					Mean weight gain (g)	Weight gain per day (g)	SGR	P value
			1st Day	15th Day	30th Day	45th Day	60th Day				
1.	Aloe	0.5	3.603± 0.30	3.822± 0.20	4.146± 0.10	4.487± 0.20	4.871± 0.20	1.268	0.021	0.503	0.118
2.	Aloe	1.0	3.566± 0.02	3.873± 0.01	4.191± 0.01	4.547± 0.20	4.955± 0.06	1.389	0.023	0.548	
3.	Aloe	1.5	3.481± 0.22	3.778± 0.30	4.090± 0.04	4.407± 0.10	4.743± 0.10	1.262	0.021	0.516	
4.	Phyllathus	0.5	3.691± 0.03	4.076± 0.03	4.510± 0.30	4.902± 0.20	5.289± 0.08	1.598	0.027	0.600	0.358
5.	Phyllathus	1.0	3.693± 0.02	4.076± 0.07	4.435± 0.20	4.889± 0.03	5.390± 0.20	1.697	0.028	0.630	
6.	Phyllathus	1.5	3.628± 0.02	4.008± 0.10	4.468± 0.01	4.908± 0.20	5.397± 0.01	1.769	0.029	0.662	
7.	Control	-	3.584± 0.02	3.839± 0.03	4.209± 0.01	4.574± 0.30	4.971± 0.03	1.387	0.023	0.545	

Influence of herbal additives on disease resistance of goldfish

The non-specific immune system of fish is considered to be the first line of defense against invading pathogens. Neutrophiles and phagocytes, lysozyme and complement are some important indices of non-specific immunity in fishes. Dugenci *et al*. (2003) used medicinal plants viz., ginger, nettle and mistletoe as an adjuvant therapy in rainbow trout through feed. They observed enhanced phagocytosis and cellular and humoral defense mechanisms against fish pathogens in rainbow trout. Jian and Wu (2003) used traditional Chinese medicines in yellow croaker which elevated the non specific defence mechanism and increased the disease resistance of the fish against bacterial pathogens.

The administration of herbal extract through immersion and injection enabled the immunostimulant to be quickly absorbed and functional. Dey and Chandra (1995) produced disease resistant fry of *Catla catla* through immersion herbal treatment (neem, garlic and turmeric) of spawn. Intraperitoneal injection of herbal extract to enhance immunostimulant of fish were carried out by Venkatalakshmi and Michael (2001), Divyagnaneswari *et al*. (2007), Harikrishnan *et al*. (2003) and Logambal and Michael (2001). While in the oral administration, the immunostimulant is slowly absorbed by the fish, but it is the most practical method for immunostimulants in fish farming. This method of administration of herbaladditives to fish was followed by Jian and Wu (2003), Devi and Reddy (1999), Ji *et al*. (2007), Direkbusarakom *et al*. (1998) and Rao *et al*. (2004). In the present study also oral administration of herbs through feed was followed.

The herbal incorporated feed fed fishes were challenged with *A. hydrophila* at the end of experimental period of 60 days. In *Phyllanthus niruri* fed group the highest survival rate of 75 per cent was observed at 1.0 per cent concentration followed by 1.5 per cent concentration (70 %). *Phyllanthus* spp. had some antibacterial activity in tiger shrimp as stated by Direkbusarakom (1998) and Felix *et al*. (2006).

In the case of aloe, higher survival rate of 80 per cent was recorded at 1.5 per cent concentration followed by 1.0 per cent concentration (75 %). Kim *et al* (1999) suggested the usage of aloe as a disease suppressing agent and it may has anti-bacterial effects in juvenile rock fish.

The survival rates of challenged fishes were in the increasing trend when there is an increase in the concentration of all the herbal additives tried in the experiment. There is a significant difference between the different herbal additives at different concentrations on the survival rate of goldfish. The present study indicates that herbal additives positively enhanced the growth performance of goldfish as well as its resistance to A*eromonas .hydrophila* infections.

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Table 3

Accumulative mortality of goldfish challenged with *Aeromonas hydrophila* for median lethal dose (LD_{50}) determination

Dilution	Hours of post - challenge						
	6	12	24	48	72	96	120
10^{0}	*	4/10	6/6	-	-	-	-
10^{-1}	*	4/10	2/6	2/4	*	*	*
10^{-2}	*	*	3/10	2/7	*	*	*
10^{-3}	*	*	*	*	*	*	*
10^{-4}	*	*	*	*	*	*	*
Saline	*	*	*	*	*	*	*

* Mortality Nil

Table 4.

Survival rates of fish fed on diets supplemented with herbal additives after challenged with *Aeromonas hydrophila* (P=5.7E-05)

Sl.No.	Treatment	Conc. %	Mortality	Survival	Survival rate (%)
1.	A1	0.5	4/10	6/10	60 ± 00
2.	A2	1.0	3/10	7/10	70 ± 00
3.	A3	1.5	2/10	8/10	80 ± 00
4.	P1	0.5	4/10	6/10	60 ± 00
5.	P2	1.0	3/10	7/10	70 ± 00
6.	P3	1.5	3/10	7/10	70 ± 00
7.	Control	-	5/10	5/10	50 ± 00

REFERENCES

Chakrabarti, R. and Vasudeva, R.Y., 2006. *Achyranthes aspera* stimulates the immunity and enhances the antigen clearence in *Catla catla*. *International Immunopharmacology*, **6(5)**: 782-790.

Direkbusarakom, S., Ezura, Y., Yashimizu, M. and Herunsalee, A., 1998. Efficacy of Thai Traditional Herb Extracts against Fish and Shrimp Pathogenic Bacteria. *Fish Pathology,* 33(4): 437-441.

Dugenci, S.K., Arda, N. and Candan, A., 2003. Some medicinal plants as immunostimulant for fish. *Journal of Ethnopharmacology*, **88(1)**: 99-106.

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.

Ji, S.C., Jeong, G.S., Im, G.S., Lee, S.W., Yoo, J. H. and Takii, K., 2007. Dietary medicinal herbs improve growth performance, fatty acid utilization and stress recovery of Japanese flounder. *Fisheries Science*, **73**: 70-76.

Ji, S.C., Takaoka, O., Jeong, G.S., Lee, S.W., Ishimaru, K., Seoka, M. and Takii, K., 2007. Dietary medicinal herbs improve growth and some non-specific immunity of red seabream *Pagrus major* . *Fisheries Science*, **73**: 63-69.

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.

Johnson, C. and Banerji, A., 2007. Influence of Extract Isolated from the Plant *Sesuvium portulacastrum* on Growth and Metabolism in Freshwater Teleost, *Labeo rohita* (Rohu). *Fishery Technology,* **44 (2)**: 229-234.

Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J. and Sarangi, N., 2007. Effect of *Allium sativum* on the immunity and survival of *Labeo rahita* infected with *Aeromonas hydrophila*. *J. Appl. Ichthyol*., **23**: 80-86.

Sasmal, D., Babu, C.S. and Abraham, T.J., 2005. Effect of garlic (*Allium sativum*) extract on the growth and disease resistance of *Carassius auratus* (Linnaeus, 1758). *Indian J. Fish*., **52(2)**: 207-214.

Turan, F. and Akyurt, I., 2005. Effects of red clover extract on growth performance and body composition of African catfish, *Clarias gariepinus*. *Fisheries Science*, **71**: 618-620.