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Research Article

Effect of *Melia Azedarach* Extract on Some Selected Physiological Parameters of (*Catla catla*)

Abstract

The present study has been carried out the effect of leaf extract of *Melia azedarach* at different concentration, such as 1.0g, 1.5g and 2.0g formulated diet against 0.1 ml of CFU/ ml 10^5 cells *Aeromonas hydrophila* on *catla catla*. The physiological parameters, such as Survival and Mortality, Antibody response, Phagocytic activity, Oxygen consumption, opercular movement, and Growth rate were studied. Result revealed that control fish showed 60% mortality and 20%, mortality at 1.0g concentration of plant extract, 10% mortality at 1.5g concentration of plant extract 30% mortality at 2.0g concentration of plant extract was observed. The maximum level of physiological activity Survival and Mortality, Antibody response, Phagocytic activity, Oxygen consumption, opercular movement, and Growth rate was recorded at 1.5g of plant extract formulated diet treated fish. When compared with control, However physiological parameters was significantly ($p < 0.05$) increased when compared with control.

Introduction

Aquaculture is one of the important sectors contributing significantly in the Indian economy. Fish culturists are encouraged towards intensification of culture system to increase production and profit. In such practice of fish and shrimp farming, disease becomes major threats. Disease is one of the most important constraints of fish production both in culture system, as well as in wild condition [1]. Fish production is decreased due to the occurrence of disease caused by different pathogens in aquaculture. Viral diseases have posed significant problems in aquaculture for many years. In commercial aquaculture, antibiotics were used for prevention and control the diseases, and hormones were used for growth performance but the cost of antibiotics and hormones are expensive. Several studies have been carried out to find the new compounds from plant sources at cheap and best to prevent the disease causing organisms in aquaculture [2]. Aquaculture has been a tradition in several parts of Asia and according to FAO statistics, over 80% of fish produced by aquaculture come from Asia, where the production was 31.07 million metric tons valued at \$ 38.855 billion [3].

Aeromonas hydrophila is a gram negative motile bacterium. The ulcerative disease is mostly caused by gram negative bacterium. *A. hydrophila* is pathogenic not only to fishes but also to amphibian, reptiles and mammals including man [4]. The Indian major carps, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* are the most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They contribute about 67% of total freshwater fish production [5]. *Melia azedarach* is a well-known ethno medicinal tree used in Ayurveda, its use in the traditional folk medicine. Different parts of *M. azedarach* in traditional system of medicine. Hence, the present study has been carried out the Haematological studies on disease induced Indian major carp; *Catla catla* (L) fed with *Melia azedarach* formulated diet.

Materials and Methods

Alive and activity fishes (12 ± 1 g) were collected from High-tech fish farm, Madurai, Tamil Nadu, India. The fishes were maintained in non-chlorinated at 20 day. The ground nut oil cake, fish meal and rice bran, tapioca, soybean, were mixed and sterilized. And then add a multivitamin tablet. The above mixed foods were added with different concentrations (1.0g, 1.5g and 2.0g) of plant extract prepared using shoxlet apparatus. These extract *Melia azedarach* extract used for experimental fishes and without plant extract diet for control fish. The food was made into small pellets. 0.1 ml of 10 CFU/ml of *Aeromonas hydrophila* was injected intraperitoneally both for control and experimental. In every seven days following physiological studies such as, (Table 1).

Survival and mortality

The survival and mortality rate was calculated by dividing the number of fish died to the total number of fish.

$$\text{Survival rate} = \frac{\text{Number of fish died}}{\text{Total number of fish}}$$

Table 1: The cumulative percentage mortality (%) of fingerlings of, *C. catla* fed with different concentrations of *M. azedarach* and intraperitoneally injected with 0.1 ml of 10^5 CFU /ml of *A. hydrophila*.

	Dose (gm)	Days after treatment						Total mortality %
		0	7	14	21	28	35	
Positive control (Normal fish)	0	0	0	0	0	0	0	0
Negative control (<i>A. hydrophila</i>)	0	0	10	20	30	40	60	60
	1.0	0	10	0	20	0	0	20
Experimental fish (<i>A. hydrophila</i>)	1.5	0	10	0	0	0	0	10
+ <i>M. azedarach</i>)	2	0	10	0	20	20	20	30

Antibody titer

This methods is followed by this (Micheal 2002)

Phagocytic activity

The phagocytic activity assay was performed by the following modified method of Sahoo et al.(2000).

$$\text{Number of WBCs/mm}^3 = \frac{\text{Number of WBC x Dilution}}{\text{Area counted x Depth of the fluid}}$$

Oxygen consumption in fish

The oxygen consumption of the fingerlings of the control and experimental fish was estimated by Winkler's method.

Opercular movement

The fish is taken in a beaker containing water. The number of opercular movements for a minute was recorded with the help of a stop watch in the control and the experimental fish. The triplicate observation was recorded from each sample for the control and the experimental fishes.

Growth rate

The growth of the fish is defined as an increase in the body weight of the fish in definite intervals of time. The weight gain of *Catla catla* was calculated as

$$\text{Weight gain (\%)} = \frac{\text{Bwf} - \text{BWi}}{\text{Bwi}}$$

The statistical significance between control and experimental groups were tested by 't' test.

Results and Discussion

In this study the cumulative percentage of Mortality, Antibody titer, Phagocytic activity, oxygen consumption, Opercular movement and growth rate, were studied in disease induced *Catla catla* using different concentration of melia azedarach formulated diet against *Aeromonas hydrophila*. In control groups showed 60% mortality 1g and 2g fed groups was 20% and 30% mortality in experimental groups. Similar result were observed by [7], reported that Mikania cordata leaf powder significantly increased non-specific immunity and decreased mortality in *C. catla* experimentally infected with *Aphanomyces invadans*. The *M. cordata* leaf powder supplemented diet showed significantly ($p < 0.05$) high disease resistance against *A. invadans* infection when compared with control group. The highest percentage survival was recorded in 20ppm (71.06%) followed by 10ppm (60.95%) and 30ppm (49.84%) groups [8], reported that *A. hydrophila* (10^6 CFU/ml) injected fishes showed 89.47 % mortality and severe lesions and wound were noticed in the infected portions. The injured tails appeared reddish in colour and loss of skin layer was observed.

Antibody titer was increased with increasing concentration of plant extract formulated diet on different day of treatment (7,14,21,28 and 35) the maximum antibody titer was found 1.5g plant extract

formulated diet an 1.104 ± 0.63 the initial day (0day) and 1.605 ± 0.4 (35 day) (Table 2). Similar results were observed by Similar kind of results have been also observed by [9], when they treated the fish with the Immunostimulation herbs which showed increased antibody responses. Herbs, which also act as immuno-stimulants, stimulate non-specific defense mechanisms and provide protection in fish [10].

In the present study, the plant extracts administered experimental groups showed more phagocytic activity when compared with control. During the study period 1.5g /100g of diet treated fishes showed maximum phagocytic when compared to other concentrations. Similar reports are also observed by [11]. They reported that phagocytic activity was significantly increased in fish fed with 2.0% *Azadirachta indica* formulated diet compared to the control, but not with 1.0% and 3.0%. They also suggested that 2.0% supplementation diet significantly influence the growth, haematology, and enhances the innate immune system in silver carp, *Hypophthalmichthys molitrix* against *A. hydrophila* [12], reported that *Basella alba* extract treated tilapia showed significantly higher ($P < 0.05$) phagocytic activity compared to fish fed control diet.

The Oxygen consumption higher in 1.5g plant extract formulated diet treated fishes than control and other experimental groups. Similar reports are also observed by [13], reported that oxygen consumption of fishes in experimental group increased after the treatment of different concentrations (250,500,750mg/kg of food) of extract of Aloe vera were fed to common carp, *Cyprinus carpio* against *Aeromonas hydrophila* [14], observed that experimental group of 250 mg/kg showed more O_2 consumption of fish compared with control. The experimental fish treated with 10 mg of turmeric powder showed increase in the O_2 consumption of fish, when compared to 20mg and 30 mg of turmeric powder [15], reported that presence of toxicants in the medium could bind with globin fraction of haemoglobin of the fish and alter the physiological activity of the body. Thus the decrease oxygen consumption may be due to the damage caused to red blood cells. The concentration of red blood corpuscles can be increased to raise the oxygen carrying capacity of the blood per unit volume [16].

Table 2: Antibody titre (\log_2 values) of *C. catla* fed with different concentrations of *M. azedarach* intraperitoneally injected with 0.1ml of 10^5 CFU/ml of *A. hydrophila*.

	Dose (g)	Days after administration					
		0	7	14	21	28	35
Positive control (Normal fish)	0	0.602	0.802	0.903	0.903	1.00	1.103
		± 0.46	± 0.62	± 0.301	± 0.46	± 0.18	± 0.17
Negative control (<i>A. hydrophila</i>)	0	0.903	0.802	0.702	0.602	0.501	0.401
		± 0.30	± 0.46	± 0.17	± 0.00	± 0.17	± 0.18
Experimental fish (<i>A. hydrophila</i> + <i>M. azedarach</i>)	1.0	1.00	1.204	1.302	1.404	1.505	1.602
		± 0.63	± 0.46	± 0.75*	± 0.75*	± 0.45*	± 0.60*
	1.5	1.104	1.302	1.404	1.503	1.505	1.605
		± 0.63	± 0.46	± 0.60*	± 0.63*	± 0.62*	± 0.42*
	2.0	1.00	1.002	1.103	1.204	1.302	1.404
		± 0.30	± 0.30	± 0.75	± 0.60*	± 0.45*	± 0.75*

Each value (Mean±SD) represents the average of 3 replicates.* statistically significant, < 0.05 , 't' test.

In the present study opercular movement was varied from both experimental and control fishes. The opercular movement in the control fish showed 60.00±0.57 and the plant extract formulated diet found to be maximum number of opercular movement were observed in 1.5g plant extract formulated diet found to be 65.00±1.00 in the initial day(0day) and 86.00±0.57(35 day) Similar result were observed by [17]. They reported that opercular movement of fishes in experimental group increased after the treatment of different concentrations (250,500,750mg/kg of food) of extract of Aloe vera were fed to common carp, *Cyprinus carpio* against *Aeromonas hydrophila* [18], also reported that opercular ventilation of the catfish, (*Pangasius hypophthalmus*) was increased and treated with increasing concentrations of *Azadirachta indica* leaf extract [12]. Observed that increase in opercular movement, mucous secretion, erratic movement etc., were noticed in neem leaf extract exposed fish [19] and also noticed similar behavioural changes in *Channa punctatus* exposed to *Nerium indicum* leaf extract (Tables 3,4).

The 1.5g extract formulated diet treated fishes showed more weight, when compared to other concentrations and control [20], reported that *Basella alba* extract treated tilapia showed significantly higher ($P<0.05$) weight gain compared to fish fed control diet. They also suggest that *B. alba* methanol extract might positively influence the growth and protect the health status of tilapia. Striped catfish, *Pangasianodon hypophthalmus*, fed a diet with 0.15% of *Yucca schidigera* showed the best growth performance and some hematological parameters [21]. A diet with 2% traditional Chinese medicine reduced the mortality of grass carp after *Aeromonas hydrophila* challenge. Arabic coffee at a level of 0.5% diet produced the optimum growth and feed utilization of Nile tilapia; while at a level of 0.2–0.5%, the fish growth and feed utilization were retarded.

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Table 3: Phagocytic activity of *C. catla* fed with different concentrations of *M. azedarach* intraperitoneally Injected with 0.1 ml of 10^6 CFU / ml of *A. hydrophila*.

	Dose (g)	Days after administration					
		0	7	14	21	28	35
Positive control (Normal fish)	0	27.66±0.57	29.66±1.52	31.66±1.52	34.66±1.52	36.66±1.52	39.66±2.08
Negative control (<i>A. hydrophila</i>)	0	19.66±1.52	18.66±1.52	17.66±0.56	16.00±1.00	15.00±1.00	14.00±1.00
Experimental fish (<i>A. hydrophila</i> + <i>M. azedarach</i>)	1.0	33.00±2.60	36.00±1.00*	38.00±1.00*	39.00±1.00*	42.33±1.52*	44.33±1.52*
	1.5	40.33±0.57*	42.00±1.00*	43.66±1.15*	45.33±1.52*	47.33±1.52*	49.33±2.51*
	2.0	34.66±0.57	35.00±2.00	36.00±1.00	37.66±1.15*	39.00±1.00*	40.00±1.00*

Each value (Mean±SD) represents the average of 3 replicates. * statistically significant, <0.05, 't' test.

Table 4: physiological changes of *Catla*, *Catla catla* fed with different concentration of *Melia azedarach* and intraperitoneally injected with 0.1 ml of 10^6 CFU / ml of *Aeromonas hydrophila*.

	Dose (g)	Oxygen consumption						Opercular movement						Growth rate					
		Days after administration																	
		0	7	14	21	28	35	0	7	14	21	28	35	0	7	14	21	28	35
Normal fish	0	1.30	1.40	1.46	1.50	1.53	1.55	62.00	62.33	66.00	70.33	72.00	75.00	11.36	11.63	12.03	12.33	12.80	13.10
		± 0.10	± 0.30	± 0.50	± 0.608	± 0.33	± 0.44	± 1.00	± 0.57	± 1.00	± 0.57	± 1.00	± 1.00	± 0.50	± 0.55	± 0.61	± 0.58	± 0.62	± 0.62
Control (<i>A. hydrophila</i> treated fish)	0	1.43	1.38	1.33	1.27	1.19	1.13	60.00	58.20	51.70	48.33	45.80	43.10	10.83	10.06	9.66	9.36	9.10	8.80
		± 0.13	± 0.57	± 0.60	± 0.33	± 0.42	± 0.53	± 0.57	± 1.00	± 1.15	± 2.08	± 1.00	± 1.00	± 0.30	± 0.20	± 0.15	± 0.15	± 0.20	± 0.10
Exp. fish (<i>A. hydrophila</i> + <i>M. azedarach</i>)	1.0	1.40	1.49	1.56	1.63	1.70	1.83	65.00	66.00	66.90	71.70	75.33	80.00	11.56	12.00	12.30*	12.90	13.16	13.60
		± 0.50	± 0.60	± 0.70*	± 0.40*	± 0.50*	± 0.60*	± 1.00*	± 0.60*	± 0.57*	± 1.00*	± 1.15*	± 1.00*	± 0.50*	± 0.55*	± 0.52	± 0.60*	± 0.46*	± 0.43*
	1.5	1.60	1.68	1.73	1.86	1.93	2.00	66.66	67.00	71.66	78.22	82.33	86.00	12.93	13.03	14.00*	14.50	15.46	16.10
		± 0.33*	± 0.33*	± 0.56*	± 1.00*	± 0.43*	± 0.60*	± 0.57*	± 1.00*	± 0.60*	± 1.00*	± 1.15*	± 0.57*	± 0.65*	± 0.15*	± 0.20	± 0.30*	± 0.51*	± 0.55*
2.0	1.43	1.49	1.53	1.57	1.63	1.66	64.00	66.66	70.33	72.66	77.33	83.66	12.53	12.90	13.36	13.83	14.36	15.03	
	± 0.50*	± 0.60*	± 0.33*	± 0.70*	± 0.52*	± 0.40*	± 1.15	± 1.00	± 1.52*	± 0.57*	± 0.57*	± 0.57*	± 0.55	± 0.56	± 0.47*	± 0.35*	± 0.50*	± 0.55*	

Each value (Mean±SD) represents the average of 3 replication. * statistically significant, <0.05, 't' test.

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