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Effect of fruit extract, *Aegle marmoles* on the physiological parameters of Indian carp *catla catla*

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Abstract

In the medicinal plants of containing diverse chemical active substances of several biological properties**.** So, the aim of the current investigation was to assess evaluate the physiological parametrs effect of *Aegle marmoles. A. marmoles* is an active compound and it contains protein, amino acids, and enzymes. Different concentrations (25, 50, 75mg/g of food) of extract of *Aegle marmoles* fruit extract were fed to *Catla catla*, for 35 days. Fish pathogen, *Aeromonas hydrophila* with 0.1ml of 10^6CFU/ml was injected intraperitonialy. The cumulative percentage mortality, oxygen consumption and opercular movement were calculated and observed. After the treatment, cumulative percentage mortality, increased opercular movement and oxygen consumption were observed. Thus *A. marmoles* plant and fruit acts as an immunostimulant to enhance the activity of fish.

Keywords: *Aeromonas hydrophila*, *Aegle marmoles*, cumulative percentage mortality, opercular movement.

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 (Sivasankar *et al*., 2015).

Fish culture is an important industry and its production is increasing worldwide every year. Some countries have sought to improve productivity and profitability by intensification of

fish production methods, which can adversely affect fish health and, poor environment will lead to an increasing susceptibility to infection (Sakai, 1999)

India can gain much by diversification of aquaculture, recruiting more species from her rich aquatic fauna and flora and also by developing eco-friendly and sustainable aquaculture systems by sharing of experience and technology with our Asian neighbours through cooperative efforts (Kutty ,1999)

Aeromonas hydrophila is a gram negative motile bacterium. The ulcerative disease is mostly caused by gram negative bacterium. *Aeromonas hydrophila* is pathogenic not only to fishes but also to amphibian, reptiles and mammals including man (Llobrera and Gacutan, 1987)
For the last twenty years, the problem of microbial diseases has emerged as a major constraint to aquaculture industry. Increased disease occurrences have resulted due to the transfer of pathogenic organisms among cultured species of fish and shrimp, between different countries without proper quarantine measures. Due to this, the fish industry in India as well as other Southeast Asian countries has suffered significant economic losses (Mastan, 2015)

Catla catla and *Labeo rohita* contribute a major portion to the freshwater fish production in South India. The Indian major carp *Catla catla* mainly inhabits in rivers. It can also be easily cultured in ponds and lakes *Catla* is non-predatory and its feeding is restricted of the *Catla* enables its safe introduction into fish community including *Rohu, Mrigal*, etc. despite its reputations as a vegetarian species (Daniels, 2002)

Herbs and medicinal plants are promising to be an important source of therapeutics in fish culture since these products provide a cheaper source for treatment and greater accuracy without causing toxicity (Madhuri *et al*., 2012)

In analysis of these favourable characteristics the following immunostimulant characters as such as, opercular movement and oxygen consumption were studied.

Material and Methods

Collection and maintenance of *Catla catla*: Fingerlings of *C. catla* were collected from Meenachi fish farm at Madurai district, Tamil Nadu, India. The collected fishes (15±1g) were acclimated to laboratory conditions for 20 days in non chlorinated bore well water. Fishes were reared in the experimental tanks for 35 days in treatment with plant extract supplemented food of different concentrations.

Feed for the fish: The fish feed was prepared from groundnut oil cake, wheat bran and soya bean which were purchased from the local market and were made into the fine granules mechanically. The fine granules were then, made into powder by using a mixie. The powdered groundnut oil cake, soya bean and wheat bran were mixed in the ratio of 5:2:1 and sterilized. A multivitamin tablet was also added. It was made into small pellets (Balasubramanian .V).

Preparation of experimental feed: The experimental fish were fed with a prepared feed as described earlier with little modification; 25, 50, 75 mg/g *Aegle marmoles* fruit extract were mixed with sterilized dry diet and fed to healthy normal fish. After 40 days of feeding a challenge test was conducted by injecting the fish pathogen *A. hydrophila*, 0.1 ml of 10^5 CFU/ml of *A. hydrophila* was intraperitonally injected for control and experimental fishes. Every seven days, the Cumulative percentile mortality, oxygen consumption and opercular movement were studied.

Cumulative percentage mortality: It was estimated by observing the number of fish survived throughout the experiment. The survival rate of common carp, *C.catla* was obtained by dividing the number of the fish survived to the total number of fishes.

$$\text{Survival rate} = \frac{\text{Number of fishes survived}}{\text{Total number of fishes}}$$

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.

Result and Discussion

Table: 1 The cumulative percentage mortality (%) of fingerlings of, *C. catla* fed with different concentrations of *Aegle marmoles* fruit extract and intraperitoneally injected with 0.1 ml of 10^6 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 (*A.hydrophila*)	0	0	10	20	30	40	40	40
Experimental fish	25	0	10	0	20	0	0	20
	50	0	10	0	0	0	0	10
	75	0	10	0	10	10	10	10

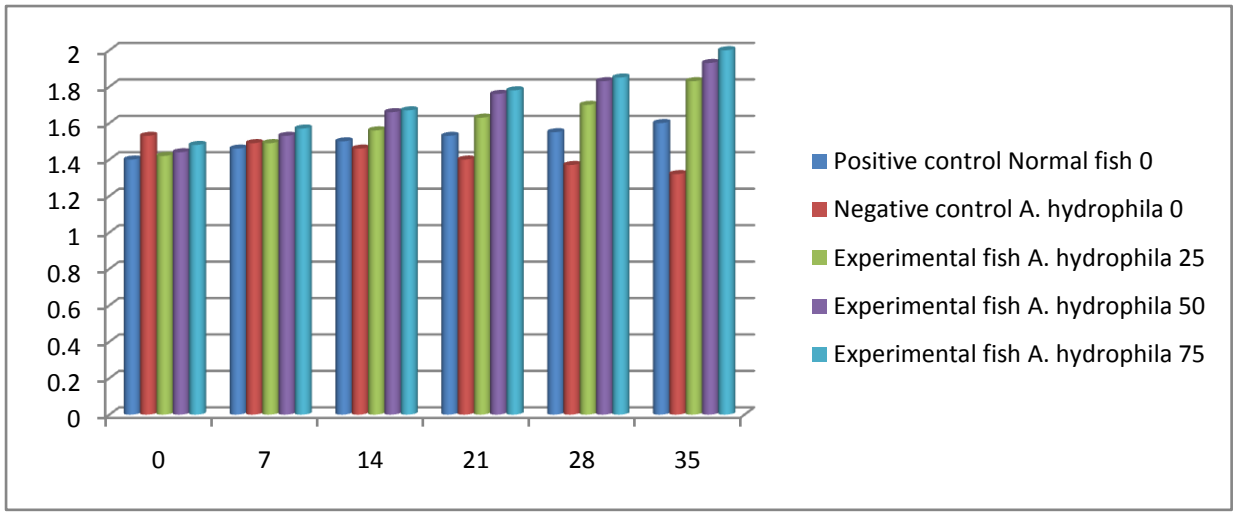

Figure 1: Oxygen consumption (mg/g/h) of *C. catla* fed with different concentration of *Aegle marmoles* fruit extract and intraperitoneally injected with 0.1ml of 10^5 CFU/ml of *Aeromonas hydrophila.*

Table 2: Oxygen consumption (mg/g/h) of *C. catla* fed with different concentration of *Aegle marmoles* fruit extract and intraperitoneally injected with 0.1ml of 10^6 CFU/ml of *Aeromonas hydrophila.*

	Dose (g)	Days after administration					
		0	7	14	21	28	32
Positive control (Normal fish)	0	1.40 ± 0.10	1.46 ± 0.30	1.50 ± 0.50	1.53 ± 0.608	1.55 ± 0.33	1.60 ± 0.44
Negative control (*A.hydrophila*)	0	1.53 ± 0.13	1.49 ± 0.57	1.46 ± 0.60	1.40 ± 0.33	1.37 ± 0.42	1.32 ± 0.53
Experimental fish	25	1.42 ± 0.50	1.49 ± 0.60	1.56 ± 0.66*	1.63 ± 0.40*	1.70 ± 0.50*	1.83 ± 0.57*
	50	1.44 ± 0.33*	1.53 ± 0.33*	1.66 ± 0.56*	1.76 ± 1.00*	1.83 ± 0.43*	1.93 ± 0.60*
	75	1.48 ± 0.50*	1.57 ± 0.60*	1.67 ± 0.33*	1.78 ± 0.70*	1.85 ± 0.52*	2.00 ± 0.60*

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

Table 3: The Opercular movement (Number/Minute) of *C . catla* fed with different concentration of *Aegle marmoles* fruit extract and intraperitoneally injected with 0.1ml of 10^5 CFU/ml of *A. hydrophila*.

	Dose (g)	Days after administration					
		0	7	14	21	28	32
Positive control (Normal fish)	0	62.00 ± 0.57	62.33 ± 1.00	66.00 ± 1.10	70.33 ± 0.57	72.00 ± 1.00	75.00 ± 1.00
Negative control (*A.hydrophila*)	0	60.00 ± 0.57	59.20 ± 1.00	57.70 ± 1.15	52.33 ± 1.10	49.20 ± 1.10	47.10 ± 1.15
Experimental fish	25	65.00 ± 1.00*	66.00 ± 0.57*	68.00 ± 0.57*	71.33 ± 1.00*	75.33 ± 0.60*	79.22 ± 1.10*
	50	66.66 ± 0.57*	67.00 ± 1.52*	70.66 ± 0.60*	78.22 ± 1.00*	80.13 ± 0.57*	86.00 ± 0.57*
	75	67.03 ± 1.15	68.66 ± 1.00	71.33 ± 1.52*	79.66 ± 0.57*	83.33 ± 0.57*	88.00 ± 1.52*

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

Survival and Mortality

In the control fish group *Aeromonos hydrophila* caused 40% mortality and 100% survival on 35th day and later. On the contrary, all the experimental groups treated with different doses of plant extract (25, 50 and 75mg/100g of diet) and injected with 0.1 ml of 10^5 CFU/ ml, *Aeromonos hydrophila* showed no mortality and 100% survival was observed. Ramakrishnan *et al.* (2015) 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. Chitra and Balasubramanian (2011) reported that the experimental groups of *C. carpio* administered with different dose of Cannon-ball tree, *Couroupita guianensis* plant extract treated fishes showed no mortality and 100% survival. This is due to the immunostimulant potential of plant extract.

Oxygen Consumption

The experimental group of 75 mg/g showed increased O_2 consumption of fish (table 2 and fig 1). But the control group showed decrease in oxygen consumption level. The experimental fish treated with 10 mg of turmeric powder showed increase in the O_2 consumption of fish, when ompared to 20mg and 30 mg of turmeric powder(Nithya and Balasubramanian 2008). Valsa and Balasubramanian (2014) 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 *Aegle marmoles* were fed to common carp, *C. catla* against *Aeromonas hydrophila*. The concentration of red blood corpuscles can be increased to raise the oxygen carrying capacity of the blood per unit volume (Svobodova, et al., 1993).

Opercular Movement

In the present study, the opercular movement were observed from experimental and control fishes. The formulated diet treated fishes showed more number of opercular movement, when compared to control (diseased) and negative control (normal fish). The opercular movement in the control fish was found to be 60.00±0.57. The fruit extract formulated diet treated fishes showed maximum number of opercular movement In 75mg of fruit extract diet treated fishes showed 67.03 ± 0.57 in the initial day (0day) and 88.00±1.52 (35th day) (Table 3). The results were statistically significant at 0.05 levels, $p< 0.05$ (t-test). Shailender *et al.* (2013) also reported that opercular ventilation of the catfish, (*Pangasius hypophthalmus*) was increased and treated with increasing concentrations of *Azadirachta indica* leaf extract. Similar finding were observed by Valsa and Balasubramanian (2014). 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 *Aegle marmoles* were fed to common carp, *C. catla* against *Aeromonas hydrophila.*

Conclusion

According to this study, the fruit extract treatment of *Aegle marmoles* increased the survival rate of the fish, *C. catla*. The oxygen consumption and opercular movement of the disease induced fish is low, when compared to the fish treated with the plant extract. High dose is found to be more effective in curing the disease. Therefore cumulative percentage mortality, opercular movement and oxygen content of the plant treated fish is correspondingly higher in the experimental fishes. This type of work is useful for further research.

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