



Effect of Garlic *Allium Sativum* (L.) Extract on Disease Induced Common Carp, *Cyprinus Carpio* (L.)

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ABSTRACT

The present investigation was carried out the effect of *Allium sativum* extract in different formulation, such as, 1g/100g, 2g/100g and 3g/100g of feed against *Aeromonas hydrophila* on common carp, *Cyprinus carpio*. The parameters such as, mortality, haemoglobin content, phagocytic activity and antimicrobial activity were studied. Results revealed that control fishes showed 30% mortality and 10% of mortality was observed 1g/100g garlic extract treated fishes remaining experimental groups showed no mortality. Similarly more haemoglobin content as well as phagocytic activity was observed experimental fishes than control fishes. The maximum antimicrobial activity was increased with increasing concentration of garlic extract.

Keywords: Garlic, immunostimulants, *Cyprinus carpio*, *Aeromonas hydrophila* and Haematological parameters

Introduction

Fish culture is an important industry in which the production of fish worldwide increases every year. Intensification of fish adversely affects fish health and tends to produce a poor environment for fish, increasing their susceptibility to infections (Sakai 1999). Aquaculture plays an important role in fishery production in many countries. Fish is considered equivalent to meat in protein high in essential minerals and vitamins and low in saturated fats. It is an important component of total human food consumption and a principle source of animal protein for more than half of the world's population (Shang 1994). Fish is susceptible to any changes that may occur in water environment. It is expected that such changes would be reflected on the physiology of fish and particularly in values of different haematological

parameters. *Aeromonas hydrophila* is gram negative motile bacteria. *A. hydrophila* is not only pathogenic to fish but also to amphibian, reptiles and mammals including man (Liobrera & Gacutan 1987). The medical plants can act as immunostimulants, conferring early activation to the non-specific defence mechanisms of fish and elevating the specific immune response. Chinese herbs have been used as medicine to treat different fish diseases in China for many years. Garlic is one of the edible plants that have strong interest to scientists for medicinal use. A wide range of microorganisms' including bacteria, fungi, protozoa, and viruses have been shown to be sensitive to crushed garlic preparation. Garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Hence the present study has been carried out to study the effect of garlic formulated food against the bacterial infection on common carp *Cyprinus carpio*.

Materials and Methods

A fresh water *Cyprinus carpio* fish (5 ± 1 g) were collected from fish farm, Madurai, Tamil Nadu, India. The fish was maintained in non-chlorinated water in 20 days. The groundnut cake, soya bean and wheat bran were mixed in the ratio of 5:2:1 and sterilized and mixed to multivitamin tablets and different concentrations of garlic extract to be used for experimental fishes while the same mixture without garlic was given for control group. The food was offered to fish as small pellets. Experimental infection of fish by 0.1 ml of 10^5 CFU/ml of *Aeromonas hydrophila* injected intraperitoneally both for garlic extract treated fishes and without garlic extract treated fishes (Control). Fish was observed for mortality, haemoglobin content, phagocytic activity and antimicrobial activity were studied. The survival rate of the Common carp, *Cyprinus carpio* was obtained by dividing the number of the fish survived to that of total number of fish. The pure culture of *Aeromonas hydrophila* is used for antibiotic sensitivity assay. Haemoglobin content of both control and experimental fish were found out using Salih's method, The number of phagocytic and unphagocytic leucocytes were counted under microscope. The statistical significance of difference between control and treated groups were tested by 't' test.

Results and Discussion

In this study the cumulative percentage of mortality, haemoglobin content, phagocytic activity and antimicrobial activity, were studied in disease induced common carp, *C. carpio*

using different concentrations of *Allium sativum*, formulated feed against *Aeromonas hydrophila*. Results revealed that control group has 30% of mortality 2g and 3g garlic extract formulated food showed no mortality. Similar results were observed by Pavaraj (Pavaraj 2007). They reported that *Ocimum sanctum* leaf extract treated fishes showed 100% survival rate when compared to the control fishes. Chitra (Chitra 2011) reported that the experimental group of *C. carpio* administered with different dose of Cannon-ball tree, *Couroupita guianensis* plant extract showed no mortality and survival rate was 100%. Balaji *et al* 2013 reported that probiotics formulated food diets showed 100% survival and reduced mortality in fish compared with non formulated diet treated fishes. Haemoglobin content in disease induced common carp, *Cyprinus carpio* fed with formulated feed with plant extract of *Allium sativum* were studied in different days of treatment (0 day to 28 days). In the initial 0 day, the control fish showed the haemoglobin content of 8.2 ± 0.25 g/dl than decreased with increasing days of treatment (Table 3). The different concentrations of garlic extract formulated diet treated fishes showed gradually increased the haemoglobin content in different days of treatment. The results were significant at 0.05 level, $p < 0.05$ (t test). Similar result was reported by Witseka who reported that haemoglobin was gradually decreased in *A. hydrophila* infected fish and was significantly increased in group of fish fed on the plant extract formulated diet. The present study revealed that the increase in haemoglobin in garlic extract treated fish was due to elevated immunity of fish and the antibacterial activity of garlic.

The control group showed more phagocytic activity in the beginning of infection with subsequent drop. The garlic extracts administered experimental groups showed more phagocytic activity with increasing days of treatment all concentrations (Table 3). More phagocytic activity observed in the garlic extract treated group than the control. The comparison of both control and experimental groups showed significant increase in phagocytic activity ($p < 0.05$) in the group of fish given 3g garlic formulated diet. The *Allium sativum* extract 1g, 2g and 3g showed inhibition zone of 4 mm, 6mm and 8mm respectively the maximum antibacterial activity was found in 3g garlic extract treated group (Table 2 & Plate 1). The minimum antibacterial activity was found in 1g garlic extract treated group. Here, as the concentration of the garlic extract increased, there was an increase in the zone of inhibition. Onyeagba *et al* 2004 observed the highest inhibition zone of 19mm with the combination of extracts (garlic, ginger and lime) on *Staphylococcus aureus*. *Salmonella Spp* was resistant to almost all the extracts except lime. Sahu *et al* 2007 showed *in vitro* antibacterial activity of aqueous extracts of *Emblica officinalis* and *Coriandrum sativum*. The

diameter of inhibitory zone recorded was the size of (6 mm in diameter). Hence the present study concluded that garlic extract treated fishes showed no mortality and more survival rate, Antimicrobial activity, Haemoglobin content, Phagocytic activity than the control fishes. So we recommend use of garlic extract formulated food for better treatment of disease and to enhance the fish production.

Table: 1 The Cumulative percentage mortality rate (%) of the *Cyprinus carpio* intra-peritoneally injected with 0.1 ml of 10^5 CUF/ml of *Aeromonas hydrophila* treated with different dose of *Allium sativum*.

Parameters	Dose (g /kg of feed)	Days after treatment					Total mortality (%)
		0	7	14	21	28	
Control fish	0	0	0	20	20	30	30
Experimental fish	1	0	0	10	10	10	10
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0

Table: 2 Antimicrobial activity (inhibition zone in mm) of *Allium sativum* (garlic) extract of different concentrations against *Aeromonas hydrophila*

Concentrations of <i>A. sativum</i> extract (%)	Zone of inhibition (mm) against <i>A. hydrophila</i>
1	4
2	6
3	8



Table: 3 Haemoglobin content and phagocytic activity of *C. carpio* intraperitoneally injected with 0.1 ml of 10^5 CFU/ml of *Aeromonas hydrophila* and treated with different concentrations of *Allium sativum* formulated feed.

Days after treatment	Haemoglobin (g/dl)				Phagocytes (%)			
	Different concentrations garlic extracts formulated diet							
	Con	1g	2g	3g	Con	1g	2g	3g
0	8.2 ±0.25	8.5 ±0.20	8.9 ±0.15	8.8 ±0.10	39.6 ±1.15	40.6 ±0.06	41.6 ±0.50	43.3 ±5.52
7	8.3 ±0.20	8.53 ±0.06	9.0 ±0.10*	8.83 ±0.06*	39.0 ±1.00	41.3 ±1.15	42.3 ±0.60	44.0 ±1.00
14	8.03 ±0.06	8.6 ±0.10*	9.06 ±0.06*	8.90 ±0.10*	38.0 ±1.00	42.0 ±1.00*	43.3 ±1.52*	45.6 ±1.52*
21	7.9 ±0.10	8.7 ±0.10*	9.2 ±0.06*	9.1 ±0.11*	36.7 ±1.52	42.0 ±1.00*	45.0 ±1.00*	47.0 ±1.00*
28	.83 ±0.15	8.8 ±0.06*	9.3 ±0.10*	9.23 ±0.06*	36.0 ±1.00	44.6 ±1.52*	46.6 ±1.00*	47.6 ±1.52*

*statistically significant at 0.05 level, $p < 0.05$ (t test).

Acknowledgement

The authors thanks the Management, Principal and Head of the Department of Zoology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi for providing facilities to carry out this research work.

References

- Sakai M 1999 Current research status of fish immunostimulants. *Aquacult.* 172: 63-92
- Shang M C 1994 The role of aquaculture world fisheries, Department of Agriculture and Resource Economics, University of Hawaii, Hawaii, USA. pp. 24
- Liobrera A T and Gacutan R Q 1987 *Aeromonas hydrophila* associated with ulcerative disease epizootic in Languna de Bay, Philippines' *Aquacult.* 67: 273-278
- Pavaraj M 2007 Development of immunity by extract of medicinal plant *Ocimum sanctum* to common carp, *Cyprinus carpio* (L) and M.Sc. Project work submitted to ANJA College (Autonomous), Sivakasi.

Chitra T 2011 Evaluation of some chose plant extracts for the development of immunity in the common *C. carpio* (c) M.Sc., Project Report ANJA College (Autonomous), Sivakasi

Balaji S; Balasubramanian V; Baskarn S; Pavaraj and Thangapandian V 2013 Evaluation immunomodulatory effect of dietary probiotics of the common carp, *Cyprinus carpio* (L.). Res. Immunol. 6(1): 1-6

Witeska M; Kozinska A; Wolnicki J; Sikorska J; Kaminski R and Pruska A 2007 Diet-Influenced performance of juvenile common carp (*Cyprinus carpio*) after experimental *Aeromonas Infection*. Israeli J. of Aquaculture. 59(3) 146-154

Onyeaba R A; Ugbogu O C; Okelu C U and Iroakasi O 2004 Studies on the antimicrobial effect of garlic (*Allium sativum*) ginger (*Zingiber officinale*.R.) and Lime (*Citrus aurantifolia*). Afr. J. Biotech. 3(10): 552-554

Sahu S; Das B K; Pradhan J; Mohapatra B C; Mishear B K and Sarangi N 2007 Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish & Shellfish Immunology. 23: 109-118