



## Immunological Studies of Disease induced common carp *Cyprinus Carpio* fed with Neem extract added Feed

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### Abstract

Low concentration of plant extracts can act as an immunostimulant. It is biodegradable and environmental friendly. *Azadirachta indica* (neem) extract was used to prepare artificial feeds at the concentrations of 250, 500, 750 mg/kg of dry diet. The prepared diets were fed to experimental fishes common carp, *Cyprinus carpio* for 30 days and then injected with 0.1 ml of  $10^3$  CFU/ml of *Aeromonas hydrophila* and every seven days intervals the following immunological aspects, such as, antibody titre, phagocytic activity and hepatosomatic index were studied. The plant extract treated groups no mortality was seen. Low dose of plant extract (250 mg/kg) showed maximum antibody titre, phagocytic activity and hepatosomatic index than the control and other experimental groups. 250 mg/kg feed was found to be more effective than the control and other groups of fish.

**Keywords:** *Aeromonas hydrophila*, *Cyprinus carpio*, Antibody titre, Phagocytic activity, Hepato Somatic Index (HSI), Neem extract.

### Introduction

In the world, medicinal plants are used for the treatment against the various infectious diseases. Aromatic and medicinal plants can produce some active substances which inhibit bacterial or fungal growth<sup>1</sup>. In India various medicinal plants have been used for cure fish diseases, such as, Epizootic ulcerative syndrome, myxobolosis, gyrodactylosis, argulosis etc<sup>2</sup>. And also the medicinal plants are act as immunostimulants for aquaculture<sup>3</sup>. Plants are the richest source of drugs of traditional systems of medicine. Plant immunostimulants have no residual in the environment and traditional medicine for prevent the diseases in man and animals<sup>4</sup>. At the beginning of human civilization the plant and plant products are used to treating bacterial diseases in aquaculture<sup>5,6</sup>. Fish is one of the most important animal protein sources in the tropics and its nutritional value is well suited to human dietary needs and competes favourably with other sources of animal protein<sup>7</sup>.

Age and growth studies are the most important aspects of fish biology because such studies can give information on stock composition, age and maturity, life span, mortality, growth, reduction, management and conservation etc<sup>8</sup>. The gram negative bacteria, *Aeromonas hydrophila*, the most common bacterial pathogen in fresh water fishes and its cause various diseases like tail rot and epizootic ulcerative syndrome. It is a primary pathogen for fish diseases and also reduced the fish production<sup>9</sup>. Immunostimulants enhance the non-specific cellular and humoral defense mechanism in animals. Immunostimulants such as, levamisole, glucan, peptidoglycan, chitin, chitosan are derived from plants and animals are

effective in prevention of diseases<sup>10</sup>. Immunostimulants stimulate the natural killer cells, complement system, lysozyme activity, phagocytic activity and antibody responses in fish and shell fishes<sup>11</sup>. Enhanced respiratory burst activities, leucocyte count and the primary and secondary antibody response were observed *Azadirachta indica* extract treated *Tilapia* fishes<sup>3</sup>. Hence, the present study deals with the immunological studies, such as, antibody response, phagocytic activity and hepatosomatic index (HSI), with the neem extract added feed in *Cyprinus carpio* were studied.

### Material and Methods

Fingerlings of *Cyprinus carpio* were collected from Kallidaikurichi Manju fish farm at Tirunelveli district, Tamilnadu, India. The collected fishes reared for 30 days in laboratory. The artificial feed was prepared from groundnut oil cake, wheat bran and soyabean were mixed in the ratio of 5:2:1. It was made into small pellets. Different concentrations of (250,500,750 mg/kg) leaf extract (*Azadirachta indica*) were mixed with dry diet and fed to healthy normal fish. After 30 days of feeding inject the fish pathogen *Aeromonas hydrophila*. Every seven days intervals phagocytic activity, antibody response and hepatosomatic index were studied.

**Antibody response:** 50  $\mu$ l of physiological saline and heat killed *Aeromonas hydrophila* was added in the wells of clean micro titre plate and also add 50  $\mu$ l of antiserum mixed well. Incubate the plates at room temperature for an hour. The sample which observe to agglutinate the serum and the values are expressed as  $\log_2$ <sup>12</sup>.

**Phagocyte count:** 100 µl of blood obtained from the caudal veins, was added into heparinized centrifuge tubes and the same volume of a bacterial suspension of *A. hydrophila* was added. The number of phagocytic and unphagocytic leucocytes was counted under microscope. The phagocytic ratio was calculated by the formula:

$$\text{Phagocytic index (\%)} = \frac{\text{Phagocytic leucocyte number}}{\text{Observed total leucocyte number}} \times 100$$

**Hepatosomatic Index:** The fish is taken and wired it to dry with a dry cloth. The weight of fish was recorded. The fish is anaesthetized and cut open the ventral side. The liver is carefully removed and the weight is noted. The HSI was calculated by the formula

$$\text{HSI} = \frac{\text{Liver Weight}}{\text{Fish Weight}} \times 100$$

## Results and Discussion

**Antibody titre:** In the present study, different concentrations of the plant extract of *Azadirachta indica* is produced significant immune response than the control. The control group showed peak antibody response on 7<sup>th</sup> day. In the plant extract administered group, the peak antibody response also occurs on 21<sup>st</sup> day .however, the peak antibody titre in the plant extract treated group showed more peak antibody response than the high dose and control. Similar observation was recorded in *O.mosambicus* treated with *Azadirachtin*<sup>13</sup>. The highest dose did not produce any effect, when compared to the other dose<sup>13</sup>. Low dose (10ppm) of plant extract treated groups showed more

antibody response than the high dose (30ppm) and the control. Similar observations were observed in *Cyprinus carpio* treated with *Albizia lebbeck* and *Gymnema sylvestre*<sup>14</sup>.

**Phagocytic activity:** In the present study, the plant extract administered groups showed more phagocytic activity ie, the plant extracts enhanced the phagocytic activity during the course 45 days of experiments. The fish treated with immuno stimulants like levamisole also enhanced phagocytic activity<sup>15</sup> Increase phagocytic activity in the *Cyprinus carpio*, administered with β-1,3glucos from polysaccharides, schizophyllan, scleroglucon and leutin<sup>16</sup>. Phagocytic activity was increased by the activities of immuno stimulants. In the present study, the increased phagocytosis observed on 21<sup>st</sup> day after injection of antigen. The comparison between the control and experimental groups showed significant difference in phagocytic index.

**Hepatosomatic index:** The control group showed maximum HSI on 7<sup>th</sup> day. The HIS of control group increased as the days were increased. On the plant extract administered experimental groups showed the decreased HSI as the days increased. The control group showed increased liver weight. Similar result were observed with experimental fishes were injected intraperitoneally with one ml of *Vibrio anguillarum* culture containing 10<sup>8</sup>cells/ml. The liver tissue was analyzed for histopathological changes and from the analysis it was clear that as the post injection days proceeded. Bacteria caused severe damage to the tissue. Slight focal necrosis and vacolation of hepatocytosis were occurred.<sup>17</sup>

Table-1

Anti body titre (log2 values) of different experimental groups of *Cyprinus carpio* fed with different concentrations of *Azadirachta indica* intra peritonally injected with 0.1 ml of 10<sup>5</sup> cells heat killed *Aeromonas hydrophila*

Dosemg/kg of plant extract	Days after administration					
	0	7	14	21	28	35
control	00	0.90±0.174	0.70±0.1	0.60±0.174	0.60±0.174	0.60±0.301
250	00	0.60±0.301	0.70±0.1	0.80±0.201	0.80±0.2	0.70±0.1
500	00	0.70±0.265	0.60±0.301	0.80±0.201	0.60±0.174	0.50±0.1
750	00	0.80±0.201	0.70±0.265	0.60±0.301	0.60±0.174	0.60±0.301

Table-2

Phagocytic activity of *Cyprinus carpio* fed with *Azadirachta indica* and injected with 0.1ml of 10<sup>5</sup>CFU/ml *Aeromonas hydrophila*

Dose mg/kg of plant extract	Days after administration					
	0	7	14	21	28	35
control	00	4.93±0.306	5.43±0.457	5.85±0.222	5.99±0.393	6.23±0.139
250	00	4.35±0.188	4.63±0.213	5.56±0.524	5.26±0.133	5.40±0.721
500	00	3.64±0.325	2.73±0.177	2.85±0.117	1.79±0.165	1.69±0.197
750	00	4.36±0.181	4.39±0.277	4.04±0.223	2.92±0.553	2.87±0.071

**Table-3**

**Hepatosomatic index of *Cyprinus carpio* after administration of different concentration of feed *Azadiracta indica***

Dose mg/kg of plant extract	Days after administration					
	0	7	14	21	28	35
control	0	5.23	5.67	5.82	5.89	6.08
250	0	5.23	5.47	4.69	4.76	4.89
500	0	5.87	5.90	5.10	4.86	4.59
750	0	5.62	3.44	3.88	3.72	3.22

**Conclusion**

Hence the present study, the low concentration of plant extract treated fishes shows more phagocytic activity, antibody titre, and Hepatosomatic index than the other experimental groups. So this type of works are recommended for fish industries for the better treatment of disease and to enhance the fish production.

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