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Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*

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Abstract

Achyranthes aspera seed was incorporated in the diets (at 0.01%, 0.1% and 0.5%) of *Labeo rohita*, rohu fingerlings (3.0 ± 0.4 g). After 2 weeks, the fish were immunized with heat-killed *Aeromonas hydrophila*, and after a further 2 weeks the rohu were experimentally infected with *Aeromonas hydrophila* (ATCC 49140). After 7 days blood and serum were sampled to determine superoxide anion production, bactericidal activity, lysozyme, serum protein, albumin, globulin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP). Superoxide anion production, serum bactericidal activity, lysozyme, ALP, serum protein, albumin:globulin ratio (A/G) were enhanced in *Achyranthes* treated groups compared to the control group. SGOT and SGPT levels were elevated in control group, but in *Achyranthes* treated groups the levels were similar to the uninfected-control group. Higher cumulative mortalities were observed in the control group (77%) up to day-9 after infection. This gradually decreased with increasing dose of *Achyranthes*, 66% mortality in 0.01% group, 57% mortality in 0.1% group and 28% mortality in 0.5% group. These results indicate that *Achyranthes aspera* stimulates immunity and increases resistance to infection in *L. rohita*.

Keywords: *Labeo rohita*; *Achyranthes aspera*; *Aeromonas hydrophila*; Immunity; Disease resistance

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1. Introduction

Immunostimulation is one of the useful tools in aquaculture where vaccination and/or treatment by injections are difficult and laborious processes, and where repeated chemotherapy poses a problem of developing drug resistant strains of pathogens. Immunostimulants potentiate the immunity of the host itself, enabling it to defend more strongly against pathogens. The modulation of the immune response by various substances has been reported, including synthetic, bacterial, animal and plant products. Several reports are available in which treating animals with immunostimulants increases the resistance and reduces the mortality rates after experimental infections with pathogens. The macrophage is the key regulator of most of these processes due to its production of various cytokines upon recognition of conserved microbial structures. Fungal β-glucans and peptidoglycan have been shown to enhance the resistance of fish against bacterial infections. Injection of microbial wall polymers into fish appear to result in production of acute phase proteins and activation of macrophages. dsRNA has been shown to enhance the resistance of fish against infections with viral pathogens which can be explained by induction of type I interferons, which in turn induce the production of antiviral proteins. In salmonids dsRNA has been shown to induce the production of Mx proteins which in man and mouse are known to have potent antiviral activity against certain negative-stranded RNA viruses [1]. The immunostimulatory effects of glucan, chitin, lactoferrin and levamisole for fish and shrimp have been reported. These immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Several immunostimulants also stimulate the natural killer cells, complement, lysozyme and antibody responses of fish. The effects of immunostimulants depends on various factors like time, dosage, method of administration and the physiological condition of the fish [2]. Indian major carp, *Labeo rohita*, is an important commercial fish in India. Disease outbreaks have been causing severe losses and hence farmers are frequently using chemotherapy to control and prevent diseases, which has several drawbacks such as development of resistant pathogens and bioaccumulation. *Achyranthes aspera* L., a herb belonging to the family Amaranthaceae, is an indigenous medicinal plant of Asia, South America and Africa. It is commonly used by traditional healers for the treatment of fever, especially malarial fever, dysentery, asthma, hypertension and diabetes [3–5]. The roots of *Achyranthes aspera* are reported to have application in infantile diarrhoea and cold [6], while dry leaves are used against asthma [7]. The seeds are regarded as having emetic and hydrophobic properties [8]. The whole plant of *Achyranthes bidentata*, and particularly the roots, contains various saponins, sterols, polysaccharides and alkaloids [9]. Various triterpenoid saponins have been identified and are linked to the effectiveness of *Achyranthes aspera* as a cardiac stimulant and diuretic (www.herbal-provider.com/lean-care.html). Immunostimulatory properties of *Achyranthes aspera* on mice have been reported [10]. To overcome the disease problem in fish culture systems, the objective of the present study was to develop a diet using this indigenous medicinal plant *Achyranthes aspera* as an ingredient to increase the immunity and disease resistance of the fish.

2. Materials and methods

2.1. Preparation of fish feed

Achyranthes aspera plants were grown at the Department of Zoology, University of Delhi. The ripe seeds were collected, washed with tap water, air-dried and ground. Artificial diet (30% protein) was prepared using fishmeal, wheat flour, cod-liver oil and vitamin–mineral premix. Three experimental diets were prepared using 0.01, 0.1 and 0.5% of *Achyranthes aspera* seed along with other ingredients. Control diet was prepared using the same composition of ingredients, except the seed of *Achyranthes* (Table 1).