EFFECT OF UV-B RADIATION ON THE DEFENCE SYSTEM OF Labeo Rohita
(ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE) LARVAE AND ITS MODULATION
BY SEED OF DEVIL’S HORSEWHIP, Achyranthes aspera

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Background. Ultraviolet (UV-B) radiation affects the immune system of fish. Dietary supplementation of natural immunostimulants may enhance the immunity of fish. The presently reported investigation evaluates the UV-B protective property of Achyranthes aspera seed in larvae of rohu, Labeo rohita (Hamilton, 1822).

Materials and methods. Larvae (1.19 ± 0.03 g) were fed four formulas of diet containing 0.0% (control), 0.1%, 0.5%, and 1.0% Achyranthes aspera seeds. After 51 days, larvae of each feeding treatment were divided into two groups. One group was exposed to UV-B radiation (80 μW · cm⁻²) and the other one remained unexposed.

Results. Average weight of fish was significantly (P < 0.05) higher in fish fed 0.5%-seed-supplemented diet (compared to other treatments). UV-B radiation affected the growth of fish fed 0.1%-seed-supplemented and control diets; other two treatments remained unaffected. Total serum protein-, albumin-, and globulin levels were significantly (P < 0.05) higher in exposed fish compared to the unexposed ones. Among the exposed groups, serum glutamic oxaloacetic transaminase and serum glutamate pyruvate transaminase levels were minimum in fish fed 1.0%-seed-supplemented diet, whereas the highest levels of myeloperoxidase, hemagglutination titre, and white blood cells were found in fish fed 0.5%-seed-supplemented diet.

Conclusion. Dietary supplementation of A. aspera seed at 0.5% level enhanced the growth and immunity of UV-B exposed fish.

Keywords: rohu, UV-B radiation, Achyranthes aspera seed, immunostimulation

INTRODUCTION
Solar ultraviolet B (UV-B, 280–320 nm) is a potent environmental stressor to aquatic organisms. UV-B radiation affects both wild and cultured species. The effect of UV-B on aquatic organisms depends on the capacity of the radiation to penetrate into the aquatic environment, which is determined by the depth of the water column, presence of dissolved organic carbon, and the quantity of organic and inorganic particulate matter (Häder et al. 1998, 2007, Bancroft et al. 2007). The harmful effects of UV-B include damage that compromises the physiology, biochemistry, reproduction, and growth of the exposed animals (Lesser et al. 2001, Armstrong et al. 2002, Van Uitregt et al. 2007, Nahon et al. 2009).

In fishes, UV-B radiation can induce injury to the skin, including sunburn and appearance of sunburn cells, epidermal hyperplasia, depletion of the mucus layer, or even sloughing of the epidermis solar elastosis with wrinkling, melanomata (Bullock 1988, Berghahn et al. 1993, Little and Fabacher 1994, Blazer et al. 1997, de Oliveira Miguel et al. 2003, Sharma et al. 2005). These changes in the skin can be accompanied by infections. The skin lesions of Atlantic salmon, Salmo salar L., contained Vibrio spp., and mycobacteria (McArdle and Bullock 1987). UV-B irradiated rainbow trout, Oncorhynchus mykiss (Walbaum, 1792), had skin fungal pathogens (Saprolegnia) (see Fabacher et al. 1994). Thus the primary barrier of the defence system becomes damaged and the normal physiology of fish is affected due to the radiation. The immune system of fish can be strongly modulated by UV-B radiation (Salo et al. 2000). UV-B exposure induces pronounced immunomodulation in cyprinids (Markkula et al. 2006). The digestive physiology and immune system of catla, Catla catla (Hamilton, 1822), were affected by UV-B radiation (Sharma et al. 2010). Outbreak of diseases seriously affects the freshwater aquaculture industry, especially in the developing countries. The majority of the freshwater species are vulnera-
ble to the UV-B exposure for the following reasons: animals are cultured in clear water, so UV-B radiation can easily reach them. The majority of the species breed during summer months, when highest UV indices are recorded. Moreover, the larvae are poorly developed, the skin is less pigmented and the scales are absent. Sharma et al. (2005) reported severe damage of the skin and eye of UV-B radiated larvae of ayu, *Plecoglossus altivelis* (Temminck et Schlegel, 1846). UV-B radiation harshly damaged the gills of catla larvae (Sharma and Chakrabarti 2006). The early developmental stage is more prone to UV-B damage as these larvae are unable to recognize the harmful radiation. In a study with the orientation behaviour of larvae of red seabream, *Pagrus major* (Temminck et Schlegel, 1843), it was found that the sensitivity of the larvae towards the UV-B developed during ontogenetic development (Sharma et al. 2007).

Immunostimulants enhance immunocompetence and disease resistance in cultured fish. Fish rely more on non-specific defence mechanisms than mammals do (Anderson 1992). Microbial levan served as immunostimulant for common carp, *Cyprinus carpio* L. (see Rairakhwada et al. 2007) and juveniles of rohu, *Labeo rohita* (Hamilton, 1822) (see Gupta et al. 2008). Increased levels of lysozyme, nitroblue tetrazolium, serum protein, and albumin/globulin were found in fish fed microbial-levan supplemented diet. Kumar et al. (2007) showed that gelatinized and non-gelatinized starch served as immunomodulator for rohu. Used as a dietary supplement, some immunostimulants can increase disease resistance in fish by improving the non-specific/innate ‘arm’ of the immune system (Kamiya et al. 2008). This may be induced by an increase of known defensive proteins such as complement (zymosan induced) or interferon or the activation of cellular defences such as macrophages (Sakai 1999). The use of immunostimulants, as dietary supplements, can improve the innate defence of animals providing resistance to pathogens during periods of high stress (Bricknell and Dalmo 2005). In Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852), intraperitoneal administration of hot water extract of *Toona sinensis* (Plantae: Sapindales) resulted in higher survival rate of fish challenged with bacteria *Aeromonas hydrophila* compared to the control diet fed fish (Wu et al. 2010). Sheikhzadeh et al. (2011) found that decaffeinated green tea enhanced innate and specific immune responses of rainbow trout, *Oncorhynchus mykiss*. Complement and respiratory burst activity were increased by administration of inulin and *Bacillus subtilis* in gilthead seabream, *Sparus aurata* L. Higher IgM level was also recorded in treated fish compared to control ones (Cerezuela et al. 2012).

Haematological parameters are good indicators of health status of fish and therefore are important in diagnosing the structural and functional status of fish exposed to toxicant (Adhikari et al. 2004). Serum protein, albumin and globulin help to understand the nutritional status and health condition of the fish. The amino transferases, aspartate aminotransferase (SGOT), and alanine aminotransferase (SGPT) are usually found in a variety of tissues viz. liver, muscle, kidney, etc. These are released into the serum in case of tissue damage. Elevated amount of these amino transferases are indicators of tissue damage; SGPT is more specific for liver. Myeloperoxidase is most abundantly expressed lysosomal protein and it is stored in azurophilic granules in neutrophils. It produces hypochlorous acid from hydrogen peroxide and chloride ion during the neutrophil’s respiratory burst. It oxidizes tyrosine to tyrosyl radical using hydrogen peroxide as an oxidizing agent. Hypochlorous acid and tyrosyl radical are cytotoxic. These are used by the neutrophil to kill bacteria and other pathogens. Release of myeloperoxidase by neutrophils and monocytes during inflammation plays an important role in the innate immune response (Chakrabarti et al. 2012). White blood cells (granulocytes, monocytes, lymphocytes, and thrombocytes) play a major role in the defence mechanism of the fish. Granulocytes and monocytes act as phagocytes to salvage debris from injured tissue and lymphocytes produces antibodies (Wedemeyer and Mcleay 1981, Maheshwaran et al. 2008).

The devil’s horseship, *Achyranthes aspera*, an herb belonging to the family Amaranthaceae is widely available in India. This plant has showed immunostimulatory effect in carps (Rao and Chakrabarti 2005a, Chakrabarti 2006). The early developmental stage is also reported in this important species. This investigation was aimed to study the impact of UV-B radiation on immune system of rohu, *Labeo rohita* larvae and to assess the UV-B remedial measures of the *Achyranthes aspera* seed.

**MATERIALS AND METHODS**

**Culture of fish and exposure to UV-B radiation.** Larvae of one of the Indian major carps—rohu, *Labeo rohita*—were obtained from the Chatterjee Brother’s fish farm, Mogra, West Bengal. The larvae weighed 1.19 ± 0.03 g and were produced by induced breeding. Larvae were acclimatized in tank (500 L), maintained in the wet laboratory for 48 h, and then introduced into glass aquaria (each 15 L). The stocking density was 15 larvae per aquarium. Larvae were fed four different types of diets for 51 days; then divided into two groups, one group was exposed to UV-B radiation (80 µW · cm⁻²) and the other remained unexposed. Three replicates were used for each treatment. We measured the ambient UV-B level in Delhi, India (28°38′ N, 77°13′ E) as 80 µW · cm⁻² in October 2012 using Radiometer PMA 2100 (Version 1.21, Solar Light Company, Glenside PA 19038, USA). The intensity used to be much higher during April–June. Therefore, we have selected the lower dose for the presently reported study. The duration of UV-B exposure was 24 days and the total duration of experiment was 75 days.

Dechlorinated, transparent water was used and the depth of water in the aquarium was 20 cm. Water temper-
ature and pH ranged from 30 to 31°C and 7.5 to 8.1, respectively throughout the study period. Dissolved oxygen level was maintained above 5 mg · L⁻¹ with the help of aerator. The source of UV-B (280–320 nm) was a Philips tube light TL 20/12 RS made in Holland, suspended above each aquarium. Aquaria were covered with black plastic sheets to shield outside light. All tubes were pre-burned for 100 h to give a stable output. The spectral output of the tubes, as defined by the manufacturer has maximum emission at 313 nm, with negligible energy above 320 (Bertoni and Callieri 1999). UV-B tubes were covered with cellulose acetate, which absorbs wavelength < 280 nm. Fish were exposed everyday at a fixed time (1220 h) for 10 min. In our earlier study, we have found the harmful effect of UV-B radiation in carp, Catla catla, after 5, 10, and 15 min of exposure (Sharma and Chakrabarti 2006). We have selected the moderate exposure duration 10 min. Both these treated and control groups were kept under full-spectrum bulb (Philips 20 W) without UV components from 6000 h to 1800 h (photoperiod of 12 h : 12 h).

Preparation of diet and feeding of fish. Experimental diets (40% protein) were prepared using 0.1%, 0.5%, and 1.0% Achyranthes aspera seed along with other feed ingredients: dry fish powder, wheat flour, cod liver oil, and vitamin-mineral premix. Control diet was prepared using the same ingredients, except the seed (Table 1). Three replicates were used for each feeding regime. Feed was given at the rate of 5% of body weight daily twice at 9000 h and 1700 h throughout the study period.

Sampling. Fish were anaesthetized with MS-222 (Sigma, USA) and blood sample was collected from the caudal vein of individual fish using syringe containing ethylene diamine tetraacetic acid (EDTA). Blood samples collected from 4 fish of each aquarium were pooled. There were 3 pooled samples for each feeding regime. Samples were allowed to clot and stored in a refrigerator at 4°C overnight. The clot was then spun down at 2000 rpm for 10 min; then the serum was stored in sterile Eppendorf tube at −20°C until used for assay. Weight of individual fish was recorded.

Biochemical assay. Total serum protein, albumin, and globulin fraction were measured following the method of Lowry et al. (1951) and the absorbance was recorded at 750 nm using Microplate Reader (BioTek, Synergy HT, New York, USA).

Hemagglutination assay was conducted to determine the antigen-specific antibody response. The chicken blood (c-RBC) was collected in Alsever’s solution (1 : 3) and stored overnight at 4°C. The cells were washed in PBS (phosphate buffer saline, pH 7.5) and resuspended in 20% (v/v) PBS. Fifty µL serum of control and test fish of each group was serially diluted in PBS in 96-well round-bottomed microplate. Equal volume of c-RBC (2%) was added to all wells and kept for 1 h at room temperature; then overnight at 4°C. The reciprocal of the highest dilution that gave agglutination was measured as the hemagglutination antibody titre.

<table>
<thead>
<tr>
<th>Ingredient [g kg⁻¹]</th>
<th>Control diet</th>
<th>Experimental diet 0.1%</th>
<th>0.5%</th>
<th>1.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry fish powder</td>
<td>583.3</td>
<td>583.3</td>
<td>583.3</td>
<td>583.3</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>402.7</td>
<td>401.7</td>
<td>397.7</td>
<td>392.7</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Achyranthes aspera seed</td>
<td>0.0</td>
<td>1.0</td>
<td>5.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Both serum glutamic oxaloacetic transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were determined using diagnostic kits (Siemens Healthcare Diagnostics Ltd., Baroda, India). Absorbance was recorded at 340 nm. Myeloperoxidase activity was measured according to Quade and Roth (1997). The optical density was measured at 450 nm in Microplate Reader.

Total white blood cells (WBC) were counted using an improved Neubauer-ruled hemocytometer (Tripathi et al. 2004). The blood sample was diluted (1 : 20) in Turk’s fluid. The fluid was allowed to stand in the pipette for 8–10 min before charging into the Neubauer’s chamber. Total WBC count [µL⁻¹] was performed by counting all WBCs in the 4 corners of primary squares.

\[
WBC = n \times 20 \times 0.4^{-1}
\]

where: \( n \) = number of WBCs observed in the 4 primary squares, 20 = dilution factor, and 0.4 = volume of fluid in 4 WBC squares.

Cells were counted in both chambers of the hemocytometer (×40 objective) and the number was averaged to produce the raw WBC count to reduce analytical variation.

Specific growth rate (SGR). The specific growth rate was calculated using the formula:

\[
SGR = 100 \left[ \ln W_f - \ln W_i \right] \cdot t^{-1}
\]

where: \( W_i \) and \( W_f \) were the initial and final body weight [g] and \( t \), the time in days.

Food conversion ratio (FCR). The food conversion ratio was calculated according to the following formula:

\[
FCR = FC \cdot WG^{-1}
\]

where: \( WG = \) wet weight gain, \( FC = \) dry feed consumed [g].

In a pilot study, the feed consumption rate of individual fish (5% of body weight) was determined and the same feeding scheme was followed throughout the study period.

Statistical analysis. Data were compiled as mean ± standard error (SE). All data were analyzed by using one-way analysis of variance (ANOVA) and Duncan’s multiple range test, DMR (Montgomery 1984). Statistical significance was accepted at \( P < 0.05 \) level.

Ethical issues. The presently reported study has been carried out in accordance with the country’s regulations on experiments on animals.
RESULTS

**Growth performance of fish.** Average weight was significantly ($P < 0.05$) higher in both UV-B exposed (3.86 ± 0.13 g) and unexposed rohu (3.78 ± 0.3 g) fed 0.5%-seed-supplemented diet than in fish of other treatments. This was followed by unexposed and exposed fish fed 1.0%-seed-supplemented diet. There was no significant ($P > 0.05$) difference between the exposed and unexposed fish fed 0.5%-seed-supplemented diet. Similar trend was found in fish fed 1.0%-seed-supplemented diet (Table 2). The specific growth rate was significantly ($P < 0.05$) higher in both UV-B exposed and unexposed rohu groups fed 0.5%-seed-supplemented diet then in fish of other treatments (Table 2). Significantly ($P < 0.05$) lower FCR was observed in fish fed 0.5%-seed-supplemented diet compared to others (Table 2). There was, however, no significant ($P > 0.05$) difference between exposed and unexposed fish in this treatment.

**Biochemical assay.** Total serum protein level was significantly ($P < 0.05$) higher in UV-B exposed group compared to its counterpart of UV-B unexposed group regardless of feeding scheme, except for 1.0%-seed-supplemented diet fed fish (Table 2). In this treatment, total serum protein level (91.23 ± 4.1 mg · mL$^{-1}$) was significantly ($P < 0.05$) higher in UV-B unexposed group compared to the UV-B irradiated one (85.19 ± 0.06 mg · mL$^{-1}$). Serum protein level was minimum in unexposed control group (75.94 ± 5.63 mg · mL$^{-1}$).

Serum albumin level was significantly ($P < 0.05$) higher in UV-B irradiated group fed 1.0%-seed-supplemented diet compared to others. Albumin level was minimum in UV-B unexposed and exposed groups fed control diet. Serum globulin level was significantly ($P < 0.05$) higher in UV-B unexposed fish fed 0.5%- and 1.0%-seed-supplementated diets compared to others. Like albumin, the globulin level was also minimum in control diet fed fish (Table 2).

Significantly ($P < 0.05$) higher SGOT level was found in UV-B exposed fish fed control diet (217 ± 33.37 U · L$^{-1}$) compared to the other groups. Among the exposed groups, minimum SGOT was found in rohu fed 1.0%-seed-supplemented diet. Though the SGOT level was significantly ($P < 0.05$) higher in each feeding scheme of exposed group compared to the respective feeding scheme of unexposed group, but there was no significant ($P > 0.05$) difference in SGOT level between exposed (123.7 ± 1.05 U · L$^{-1}$) and unexposed (122.80 ± 10.66 U · L$^{-1}$) groups of rohu fed 1.0%-seed-supplemented diet (Fig. 1a).

Similar trend was also found with SGPT. Significantly ($P < 0.05$) higher SGPT level was found in UV-B exposed fish fed control diet (217 ± 33.37 U · L$^{-1}$) compared to the other groups. Among the exposed groups, minimum SGPT was found in rohu fed 1.0%-seed-supplemented diet. Though the SGPT level was significantly ($P < 0.05$) higher in each feeding scheme of exposed group compared to the respective feeding scheme of unexposed group, but there was no significant ($P > 0.05$) difference in SGPT level between exposed (123.7 ± 1.05 U · L$^{-1}$) and unexposed (122.80 ± 10.66 U · L$^{-1}$) groups of rohu fed 1.0%-seed-supplemented diet (Fig. 1a).

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental diet</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1%</td>
<td>0.5%</td>
</tr>
<tr>
<td>W[g]</td>
<td>2.36 ± 0.07d</td>
<td>2.95 ± 0.11c</td>
</tr>
<tr>
<td>SGR [%]</td>
<td>0.91 ± 0.04a</td>
<td>1.21 ± 0.05b</td>
</tr>
<tr>
<td>FCR</td>
<td>3.84 ± 0.22a</td>
<td>2.55 ± 0.17b</td>
</tr>
<tr>
<td>T [mg · mL$^{-1}$]</td>
<td>83.38 ± 1.34b</td>
<td>77.33 ± 1.12d</td>
</tr>
<tr>
<td>A [mg · mL$^{-1}$]</td>
<td>66.34 ± 4.83b</td>
<td>62.65 ± 6.32c</td>
</tr>
<tr>
<td>G [mg · mL$^{-1}$]</td>
<td>16.86 ± 1.79a</td>
<td>15.31 ± 0.66b</td>
</tr>
</tbody>
</table>

$W$ = mean weight (± SE); T = total serum protein; A = albumin; G = globulin; Each replicate composed of four fish. Three replicates were used for each treatment; Means sharing different letters in the same row are significantly ($P < 0.05$) different.
A significantly higher SGPT level was observed in exposed rohu fed control diet (179.92 ± 6.85 U·L⁻¹) compared to others (Fig. 1b). Among the exposed groups, minimum SGPT was found in 1.0%-seed-supplemented diet fed fish. Though the SGPT level was significantly (P < 0.05) higher in UV-B irradiated fish of each feeding scheme compared to their unexposed counterparts, but there was no significant (P > 0.05) difference between the UV-B exposed (139.12 ± 6 U·L⁻¹) and unexposed (138.88 ± 4 U·L⁻¹) groups fed 1.0%-seed-supplemented diet.

Significantly (P < 0.05) higher myeloperoxidase level was found in UV-B unexposed fish compared to UV-B exposed fish regardless of feeding regime (Fig. 2). Highest myeloperoxidase level was found in unexposed fish fed 0.5%-seed-supplemented diet (3.137 ± 0.0783, λ 450 nm). Among the exposed fish, the highest level was found in fish fed 0.5%-seed-supplemented diet (2.127 ± 0.0127, λ 450 nm), but there was no significant (P > 0.05) difference between fish fed 0.5% and 1.0%-seed-supplemented diets.

The hemagglutination antibody titre level was significantly (P < 0.05) higher in UV-B unexposed fish of each feeding scheme compared to their UV-B exposed counterparts (Fig. 3). Highest hemagglutination antibody titre level was observed in unexposed rohu fed 1.0%-seed-supplemented diet (256 ± 128). Among the exposed groups, the highest level was found in fish fed 0.5%-seed-supplemented diet. The level was minimum in exposed (7 ± 1) groups fed control diet.

WBC count was significantly (P < 0.05) higher in UV-B unexposed fish of each feeding scheme compared to their UV-B exposed counterparts. The highest value was recorded in unexposed fish fed 1.0%-seed-supplemented diet (675 267 ± 8577 µL⁻¹). Among the UV-B irradiated fish, the highest number of WBC was found in fish fed 0.5%-seed-supplemented diet (451 533 ± 4628 µL⁻¹). The number of WBC was the lowest in exposed group fed control diet (Fig. 4).

**DISCUSSION**

UV-B radiation affected the growth of rohu fed 0.1%-seed-supplemented- and control diets, but the supplementation of *Achyranthes aspera* seed at 0.5% and 1.0% levels helped the fish to overcome the harmful effect of UV-B radiation. This is clear from the presently reported study as there is no significant difference in the average weight of exposed and unexposed rohu of these two feeding schemes. Supplementation of seed enhanced the growth of even UV-B irradiated rohu. Food was also efficiently utilized in seed-supplemented diet fed fish compared to control group. This is evident from the lower values of FCR in the majority of treatment groups. A similar result was also reported by Rao et al. (2006). UV-B irradiation affected the FCR and consequently resulted into poor growth. The nutritional value of *Achyranthes aspera* seed plays a significant role. Previous studies showed that a number of oleanolic acids, bisdesmosidic-triterpenoid-based saponins, ecdysterone, and various amino acids were present in the seed (Hariharan and
in affected tissues such as gills, liver and kidneys which were damaged by the foreign substances (Gey Van Pittius unpublished; Van der Merwe unpublished; Wepener unpublished). The white blood cells leave the circulating blood to protect the body by moving to the infected tissues. Seeds of Achyrantas aspera promoted the increased number of WBC in the fish. Chakrabarti et al. (2012) reported the presence of two essential fatty acids linolenic acid and oleic acid in the seeds of Achyrantas aspera which probably stimulated the immune system of carp.

CONCLUSIONS

Exposure of fish to UV-B radiation resulted into elevated protein-, SGOT- and SGPT levels in fish. Simultaneously it resulted into reduced levels of myeloperoxidase and hemagglutination titre and white blood cells count. Poor physiological and immunological systems make the fish more prone to disease. Supplementation of seed of Achyrantas aspera at 0.5% level in diet of larvae showed promising results to overcome the problem of UV-B radiation in aquatic system. This may serve as natural immunostimulant for fish.

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