Effect of an *Andrographis paniculata* Enriched Diet on Immunomodulation of *Macrobrachium malcolmsonii* against *Lactococcus garviae*

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**Abstract**

This investigation studied the innate immune parameters of *Macrobrachium malcolmsonii* fed diets enriched with 0, 0.01%, 0.1%, or 1.0% *Andrographis paniculata* extract and infected with *Lactococcus garviae*. The total hemocyte count did not significantly differ between treatments during the first week but was significantly higher in prawns fed the 0.1% or 1.0% diets during weeks 2 and 4. Phenoloxidase activity, respiratory burst activity, superoxide dismutase activity, and phagocytic activity were significantly higher in prawns fed the 0.1% or 1.0% diets than in those fed the control or 0.01% diet. Mortality was lower in prawns fed the enriched diets than in those fed the control. Our results suggest that diets enriched with *A. paniculata* have a positive effect on the modulation of the immune system in *M. malcolmsonii* against *L. garviae*.

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Introduction

*Macrobrachium malcolmsonii* is an omnivorous bottom-dwelling prawn that naturally feeds on decomposing plants and animals, small worms, insects, and insect larvae. It is a fast growing species among freshwater prawns and has good taste and potential for culture in freshwater ponds and tanks. It can withstand daily fluctuations of temperatures ranging 20-35°C. However, successful semi-intensive *M. malcolmsonii* farming requires supplementary feeding for good health and growth. In monoculture, *M. malcolmsonii* juveniles can be stocked at 30,000-50,000/ha whereas in polyculture with fast-growing compatible carps such as *Catla catla*, *Hypophthalmichthys molitrix*, *Labeo rohita*, and *Ctenopharyngodon idella*, they should be stocked at 15,000-20,000/ha (Radheyshyam, 2009).

The prawn culture industry suffers from viral and bacterial diseases (Sahoo, 2008; Lio-Po et al., 2009). *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp., and *Lactococcus garviae* cause high mortalities in *Macrobrachium rosenbergii* hatcheries (Delves-Broughton and Poupard, 1976; Tonguthai, 1995; Sung et al., 2000; Chen et al., 2001; Phatarpekar et al., 2002). *Lactococcus garviae* is an important bacterial disease in *M. malcolmsonii* culture in India.

A number of natural and herbal-based immunostimulants enhance the innate and adaptive immune resistance to disease in fish (Ganguly et al., 2010; Harikrishnan et al., 2011). *Andrographis paniculata*, a species in the Acanthaceae family, is used as a latent-heat clearing, anti-inflammatory, anti-inflammatory, defervescent, febrifugal, antiphlogistic, and analgesic agent for treating acute infections of the gastrointestinal tract, respiratory organs, and urinary system (Kirtikar and Basu, 1975; Nazimudeen et al., 1978; Chopra et al., 1980; Choudhury and Poddar, 1985; Sheeja and Kuttan, 2008). It has good immunostimulatory properties (Puri et al., 1993; Iruretagoyena et al., 2005). The aim of the present study is to investigate the effect of *A. paniculata* enriched diets on immunomodulation in *M. malcolmsonii* against *L. garviae*.

Materials and Methods

**Diets.** *Andrographis paniculata* was collected locally and identified by the Plant Science Department. The herb was washed, dried, ground, and extracted with 85% ethanol by the cold maceration process (Singh et al., 2007). The formulated basal diet (control) contained 25% fishmeal, 20% rice bran, 13% soy flour, 16% wheat flour, 15% ground nut cake, 9% tapioca powder, 1.5 aminovit, and 0.5 vitamin mix. Diet ingredients were well mixed and extruded by a pellet extruder (EX 920, Matador, Denmark). Four diets were prepared by spraying 0%, 0.01%, 0.1%, and 1.0% *A. paniculata* extract onto the basal diet and slow even mixing in a drum mixer. The control diet included the basal diet sprayed with the same volume of solvent but without the extract. The diets were air dried under sterile conditions for 12 h. The pellets were dried in an oven at 30°C for 18 h, packed, and stored in a freezer at -20 °C until use. The proximate composition of the diets were quantified following AOAC methods and comprised 47.2% crude protein, 7.9% crude lipid, 18.9% crude ash, and 12% crude carbohydrate.

**Bacterial culture.** *Lactococcus garviae* was isolated from diseased prawns and cultured in MRA (Oxoid Ltd.) agar containing 10 ppm sodium azide and 10 ppm cycloheximide. Colonies were maintained in MRS (Oxoid) broth containing 0.5% CaCO₃. The strain was putatively identified as *L. garviae* based on Gram staining, biochemical analysis, and polymerase chain reaction with specific primers as described by Zlotkin et al. (1998).

**Animals and experimental design.** *Macrobrachium malcolmsonii* were obtained from a commercial hatchery and reared in 500-l round holding tanks for two weeks of acclimatization. After acclimatization, the prawns were divided into four triplicate groups of 25 fish, stocked in 50-l tanks, and fed the experimental diets twice a day at a daily rate of 10% body weight. Water temperature ranged 26-32°C, transparency 30-60 cm, and pH 7.0-8.5. Dissolved oxygen was above 5 mg/l, free CO₂ below 8 mg/l, hardness 50-100 mg/l, total alkalinity 80-150 mg/l, NH₄⁺-N 0.02-0.20 mg/l, calcium 30-80 mg/l, phosphorus 0.01-0.90 mg/l, and nitrogen 0.05-90.5 mg/l. After 30 days of feeding, all
prawns were injected intraperitoneally in the ventral sinus of the cephalothorax with 50 µl PBS containing *L. garviae* at 3.7 x 10^7 cfu/ml.

**Sample collection.** One, two, and four weeks post-infection, six prawns were randomly collected from each tank and 100 µl hemolymph was extracted from the ventral sinus into a 1-ml sterile syringe (25 gauge) containing 0.9 ml anticoagulant solution (trisodium citrate 30 mM, sodium chloride 0.34 M, EDTA 10 mM, pH 7.55; osmolality was adjusted with glucose to 780 mOsm/kg). A drop of the anticoagulant-hemolymph mixture (100 µl) was placed on a hemocytometer, and total hemocyte was counted under an inverted phase-contrast microscope (Leica DML, Leica Microsystems, Wetzlar GmbH, Germany). The remainder of the mixture was used for subsequent tests.

**Immunological assays.** Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from dihydroxyphenylalanine following the procedures of Hernandez-Lopez et al. (1996). Respiratory burst of the hemocytes was quantified by reduction of nitroblue tetrazolium to formazan as a measure of superoxide anion (O2⁻) as described by Liu and Chen (2004). Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical dependent reactions using a Ransod Kit (Randox, Crumlin, UK) following Biagini et al. (1995). Phagocytic activity was measured following Liu and Chen (2004).

**Statistical analysis.** Data were compared using SAS computer software (SAS Institute Inc., Cary, NC, USA). Differences were considered statistically significant at the 5% level.

**Results**

Mortality, total hemocyte count, phenoloxidase activity, respiratory burst activity, superoxide dismutase activity, and phagocytic activity during weeks 1, 2, and 4 are given in Figure 1.

![Figure 1](image-url)

Fig. 1. (a) Cumulative mortality, (b) hemocyte count, (c) phenoloxidase activity, (d) respiratory burst activity, (e) superoxide dismutase activity (SOD), and (f) phagocytic activity of *Macrobrachium malcolmsoni* (means±SE, n = 6) fed diets enriched by 0, 0.01%, 0.10%, or 1.0% *Andrographis paniculata* extract and challenged with *Lactococcus garviae*. Data at a single exposure time with asterisks significantly differ between treatments (p<0.05).

**Discussion**

The hemocyte count was significantly higher in weeks 2 and 4 in the 0.1% and 1.0% groups. Likewise, phenoloxidase activity, respiratory burst activity, SOD activity, and phagocytic activity were significantly enhanced in the 0.1% and 1.0% groups. The higher hemocyte count, phenoloxidase activity, and respiratory burst activity of the herbal diets
indicate that the activity of SOD responsible for the scavenging of superoxide anion increased, and that the activity of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase is responsible for the reduction of $O_2$. The increase of superoxide anion in prawns that received the herbal diets is probably due to the increase in hyaline cells and total hemocytes. Superoxide anion enhancement of hemocytes strengthens the immune system (Munoz et al., 2000).

The enhanced activity of SOD that catalyses superoxide anion to hydrogen peroxide indicates that the herbal extract affected immune stimulation. Phagocytic activity was also significantly enhanced in prawns fed the 0.1% and 1.0% herbal diets and mortality was lower. Thus, further research is recommended to evaluate the immunostimulatory effect of *Andrographis paniculata* extract on the resistance of prawns against other bacterial diseases.

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**References**


