IMMUNOMODULATORY EFFECT OF *RICINUS COMMUIS* ENRICHED DIET ON FRESH WATER FISH (*CATLA CATLA*) AGAINST PATHOGEN INFECTION

Salini. R* & Rajakumari. M

Department of Biochemistry, V.V.Vanniaperumal College for Women, Affiliated to Madurai Kamaraj University, Virudhunagar-626001, Tamil Nadu, India

ABSTRACT

Intensive aquaculture is the rapid-growing sector of an animal food production industry and the growth reached USD 211450 million in 2021. However, world aquaculture production is susceptible, and culture intensification could result in fractional or total loss of production because of an upsurge in disease outbreaks, including infections through various pathogens. Hence, the present study intends to develop immune response in fish through feed using the natural plant extract *Ricinus communis.* Three different extracts were prepared using leaves and seeds. All the extracts were tested for phytochemicals qualitatively as well as quantitatively and the results revealed the presence of diverse secondary metabolites. Further, the fish feed prepared using these extracts and after feeding with the prepared immunomodulant feed, the fish growth was stimulated. Totally 10 morphologically different pathogens were isolated from a dead diseased fish gut and subjected to several biochemical tests to identify it. The Grams staining reaction showed 5 of them are Gram positive and rest of them are Gram negative. The isolated pathogen 1 (potent pathogen) was identified as Pseudomonas Sp., Further infection with pathogen 1 to fishes revealed that the fish fed with the feed containing *R. communis* leaf and seed extract has an immunomodulatory effect and it reduced the mortality and morbidity but the fish fed with normal feed showed several signs of infection along with mortality. *R. communis* extracts significantly act on the immune system of the fish against the invasion of pathogen further it increased the growth rate and weight of the tested fish.

Keywords: aquaculture, Feed, immunomodulant, infection and *R. communis*.

1. INTRODUCTION

Aquaculture is a fast emerging sector. However, unmanaged fish culture practices and adverse environmental conditions affect the fish health leading to production losses. Thus, fish farmers have to carry out careful husbandry practices[1]. In order to achieve optimal fish production, better prophylactic, diagnostic and therapeutic measures are warranted during fish farming operations. The recent expansion of aquaculture has led to a growing interest in understanding fish diseases, so that they can be treated or prevented and has at least partial success. However, the emergence of antibiotic-resistant microorganisms is an important obstacle to their extensive use [2].

The use of expensive chemotherapeutants and antibiotics for controlling disease has been widely criticized for their negative impact like residual accumulation in organs and tissues, antimicrobial resistance and immunosuppression, consequently causing reduced consumer preference for fish treated with antimicrobials[3].Prevention of disease is much more desirable than intervention to stop and reverse the disease process once it begins. Hence, as an alternative to chemotherapeutic agent, upward attention is being given to the use of immunostimulants for infection control methods in aquaculture. *Catla catla* deserve higher economic reputation due to consumer preference and it is the chief cultured fish species in India, Pakistan, Burma and some other countries.

Medicinal plants have a vibrant role in the human healthy life. The *Ricinus communis* or castor plant has great traditional medicinal value in the prevention and cure of the diseases and to maintain healthy life. Conventionally the plant is used as fertilizer, purgative, fungicide and laxative, etc. However the plant possesses innumerable beneficial properties such as antihistamic, anti inflammatory, anti-oxidant, antiasthmatic, immunomodulatory, antiulcer, lipolytic, wound healing, Antidiabetic, Antifertility, antimicrobial, central nervous system stimulant, and many other therapeutic properties[4].

The present work was carried out to evaluate the immunostimulant potential of *R. communis* by incorporating its extracts with formulated diet and feeding to the fish *C. catla,* followed by infection with *Pseudomonas sp.*

2. MATERIAL AND METHODS

2.1 Collection and Preparation of the sample

Ricinus communis leaves and seeds were collected from nearby Virudhunagar (January –March 2021). The plant material was shade dried at room temperature. The dried plant material was powdered using a mixer grinder.

2.2 Extraction and sample preparation

2.2.1 Aqueous extraction

For aqueous extraction, 5 g of powdered leaves and seeds of *R. communis* were mixed with 50 mL of distilled water and boiled for 5 hrs. Then it was filtered through muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and crude aqueous-leaf and seed extract has been obtained. Then it was transferred in to screw cap bottles, labelled and stored.

2.2.2 Solvent extraction

5g of *R. communis* leaves and seed powder were weighed, and extracted with 95% ethanol and 95% methanol separately. The crude preparation was left for 72 hours in a shaker at room temperature. The extract obtained by cold extraction was concentrated using vacuum evaporator. A greasy final material obtained was transferred to screw cap bottles, labelled and stored.

2.3 Phytochemical Analysis

Methanolic, Ethanolic and Aqueous extractions of *R. communis* leaves and seeds were assessed qualitatively and quantitatively for the existence of the phytochemicals using standard methods[5].

2.4 Preparation of feed:

The Control diet was prepared by mixing Soya flour 23g, dry fish meal 24g, Rice bran 10g, Ground nut oilcake 23g, Tapioca flour 10g, Wheat bran 10g and made as a dove, sterilized in pressure cooker for 30 minutes, cooled, 2% of vitamins & minerals and probiotics premix were added and finished in the form of noodles which are then shade dried, fragmented into small required sized pieces. Test 1 and Test 2 diet were prepared by adding 2% of aqueous extract of *R. communis* leaves and 2% of aqueous extract of *R. communis* seeds to the control diet respectively.

2.5 Pathogen isolation and identification

The infected dead fish was collected from an aquaculture farm (January 2021) and stored in a sterile container with PBS. Using sterile scalpel the digestive tract was removed and the obtained sample had serially diluted and further it was plated then incubated at 37^0C for 24 hours. After that the pure culture was isolated and used for further assays (Biochemical characterization and Immunomodulatory Effect).

2.6 Immunomodulatory Analysis

The experimental fish *C. catla* (weight 3±0.5g) were purchased from local fish farm and allowed to acclimate to laboratory conditions for 10 days. During acclimatization they were fed with the control feed. During the experimental period the water quality variables: temperature (28±1°C), pH (7.2±0.3). The water was changed daily in order to maintain the fishes in healthy state.

The fishes were grouped into 6 categories and each group carries 5 fishes. Group 1 (healthy fish fed with control feed), Group 2 (healthy fish fed with controlfeed and infected with Pathogen), Group 3 (healthy fish fed with Test 1 formulated feed), Group 4 (healthy fish fed with Test 1 formulated feed and infected with Pathogen), Group 5 (healthy fish fed with Test 2 formulated feed), Group 6 (healthy fish fed with Test 2 formulated feed and infected with Pathogen) and mortality and other infection signs were analysed.

3. RESULTS & DISCUSSION

3.1 Phytochemical Screening

The qualitative and quantitative phytochemical analysis of three extracts of *R. communis* leaves showed the presence of various phytochemicals (Table1, Table 2 and Table 3). Ethanol, Methanol and aqueous extract showed positive results for carbohydrate, phenol, terpenoids, saponins, flavonoids, alkaloids, glycosides, coumarins and Tannins.

Table: 1. Qualitative phytochemical analysis of *R. communis* leaves and seed extracts

S.No	Leaf extract			Seed extract		
	aqueous	methanol	ethanol	aqueous	methanol	ethanol
Carbohydrate	+	+	+	+	+	+
Phenol	+	+	-	-	-	-
Terpenoids	+	+	+	-	-	-

Saponins	+	+	+	+	+	+
Flavonoids	+	-	+	-	-	-
Alkaloids	-	+	+	+	+	-
Glycosides	+	+	+	+	+	+
Coumarins	+	-	+	-	-	-
Tannins	+	+	-	-	-	-

'+' indicate presence and '–'indicates absence

Table: 2. Quantitative phytochemical analysis of *R. communis* leaves extracts

S.No	Phytochemical	Ethanol	Methanol	Aqueous
1.	Flavonoids	0.05g	0.04g	0.06g
2.	Phenols	0.01g	0.2g	0.4g

Table: 3. Quantitative phytochemical analysis of *R. communis* seed extracts

S.No	Phytochemical	Ethanol	Methanol	Aqueous
1.	Flavonoids	0.07g	0.05g	0.06g
2.	Phenols	0.07g	0.05g	0.08g

R. communis is rich in varied diversity of phytochemicals, our results well correlated with the findings of Alugah and Ibraheem[6]. They have reported the flavonoids and tannins content in the castor plantDifferent phytochemicals have been found possess a wide range of activities, which may help in protection against chronic diseases [7,8]. Saponins protect against hypercholesterolemia and antibiotic properties. The importance of alkaloids, saponins and tannins in various studies as alternative to antibiotics in treating common pathogenic strains has recently been reported [9].

3.2 Pathogen isolation and Biochemical characterization

From the dead diseased fish totally 10 morphologically different bacterial cultures were isolated. In order to identify the biochemical nature of the isolated pathogens several biochemical test has been done and the rests revealed that the isolated pathogens were different and have varying biochemical mechanisms (Table 4).

Table: 4. Biochemical analysis of isolated fish pathogens

Isolated Pathogens	Catalase	Oxidase	Indole	MR-VP Test	Gram Staining
1	+	+	-	+	-
2	+	+	-	+	-
3	+	+	+	+	-
4	-	-	-	-	+
5	+	+	+	+	-
6	-	-	-	+	+
7	-	+	-	-	+
8	-	+	-	-	+
9	+	+	+	+	-
10	-	-	+	-	+

'+' indicate presence and '–'indicates absence

All the isolated pathogens infecting ability was tested and found that the isolate 1 was a potent pathogen and it showed the similar infection signs (diseased dead fish from the farm). Hence, this pathogen alone used for further assays and based on the biochemical and molecular analysis it was identified as *Pseudomonas Sp*.

Pseudomonas is a part of common fish micro biota, conversely under stressful conditions the bacteria have converted into a highly opportunistic pathogen, causing serious illness including, gill necrosis, splenomegaly, haemorrhagic septicemia, abdominal distension, congested kidney and friable liver[10].

3.3 Growth and Development

The specific growth rate exhibited an increasing trend in all the 6 experimental groups; however it was significantly higher in group 5 and 6, test 2 fed fishes. Thus it is evident that dietary incorporation of *R. commnis* act as growth promoter. Aly and Mohamed (2010) observed that Nile Tilapia fed with *Echinacea purpurea* and *Allium sativum* fortified diet exhibited significant increase in specific growth rate[11]. Rodrigues et al., (2020) proposed that β-Glucans act as an immunomodulatory food supplement and have been involved in provoking immunity in commercial aquaculture[12]. Incorporation of *R. communis* in the diet influences the fish and may have enhanced palatability, assimilation and absorption of nutrients.

3.4 Immunomodulatory Effect

The fishes were grouped into six. Upon infection the mortality rate has been reduced significantly in the tested groups. This indicates the immunomodulatory effect of the prepared feed. The infected fish showed signs of infection in their body as well as redness near eyes but it was absent in the fishes fed with immunomodulant feed (Fig. 1). The duration of time that fish are fed probiotics seemed to be also an important factor on influencing the immunological parameters in fish.

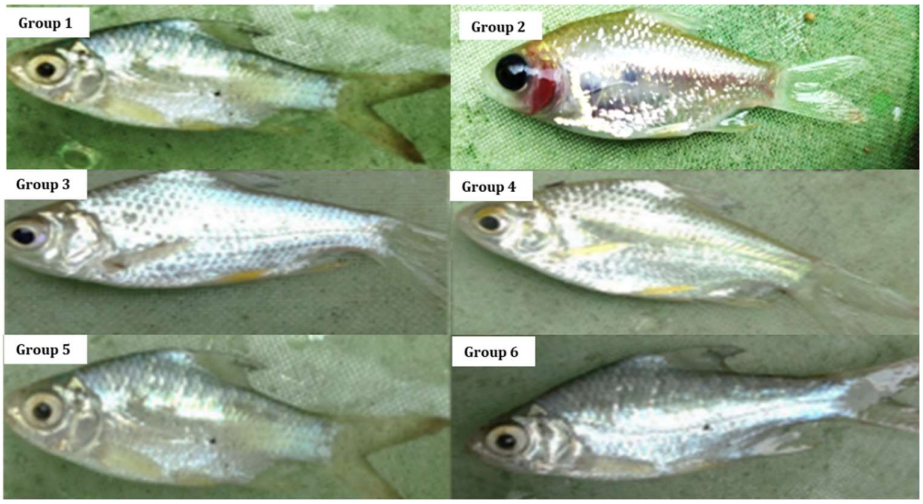

Fig. 1. Immunomodulatory effect of formulated feed on the growth and survival rate of fish

Similar to our results the efficacy of microalgae as green algae (*Chlorella vulgari)* or blue green algae (*Spirulina platensis)* were revealed in *Nile tilapia* (*O. niloticus*)[13]. In addition, the favourable protective efficacy of microencapsulated seaweed extracts was revealed against *A. salmonicida* in *O. mossambicus*[14]. Algae treatments were administered

orally as a feed supplement except the polysaccharide fraction of a marine macroalga (Caulerpascalpelliformi), which was injected intraperitoneally[15] and all treatments demonstrated significant differences in survival rate and protection effect between algae groups and control groups against *Aeromonas* infection. In addition, a significant increase of non-specific immune responses has been showed in *Aeromonas* challenge due to algal alternatives [16].

4. CONCLUSION

In the present study it was observed that the Specific Growth Rate of fishes fed with formulated feed using *R. communis* were significantly higher. The mortality and infection sign of the fishes upon infection with *Pseudomonas* Sp. (Isolated pathogen 1) was greatly reduced due to immunomodulation. Thus from the current study it was perceived that incorporation of *R. commnis* in fish feed formulations not merely acts as Immunostimulator however it also acted as Growth Promoter.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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