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RESEARCH PAPER

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Immunomodulatory effect of Panchagavya and *Lactobacillus* probiotics on *Oreochromis mossambicus* (W. K. H. Peters)

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ABSTRACT

In the current investigation, the immune stimulatory effect of panchagavya and probiotics *Lactobacillus* on the growth, immunity and disease resistance of *Oreochromis mossambicus* and improve the disease resistance against *Edwardsiella tarda* and *Aeromonas hydrophila*. The efficacy of basal diet (BD) of the following experimental diets was treated with *O. mossambicus*: Control diet, T1 (Basal diet + Panchagavya-0.1%); T2 (Basal diet + Panchagavya-0.5%); T3 (Basal diet + Panchagavya-1%); T4 (Basal diet + Panchagavya-2%); T5 (Basal diet + Panchagavya-4%); and T6 (PC - Positive Control with *Lactobacillus*). Various haematological and immunological parameters were examined at 7, 14, 21 and 28 days post-feeding. Fishes were challenged with *A. hydrophila* and *E. tarda*, 30 days post-feeding and mortalities were recorded over 14 days post infection. Results showed that administration of panchagavya for 28 days had significant effects on the specific and non-specific immune response in *O. mossambicus*.

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INTRODUCTION

In intensive aquaculture, fish experience stress conditions that compromise their immune systems, resulting in the onset of diseases (Janak *et al.*, 2024). These diseases have led to significant production losses and continue to be a major concern for fish farms (Magnadottir, 2010). To sustain fish health and enhance performance, immune stimulants have been incorporated as dietary supplements, leading to improvements in weight gain, feed efficiency and disease resistance in farmed fish. Throughout the years, intensive aquaculture has broadened its scope and is becoming one of the most viable and promising sectors for ensuring nutritional and food security for humanity. Historically, vaccines, antibiotics and chemotherapeutics have been utilized for the management of diseases. Nevertheless, the use of antibiotics presents numerous challenges, including the emergence of drug-resistant pathogens, environmental risks and issues related to food safety (Giri *et al.*, 2014).

Traditional, Agriculture is widely recognized as a collaborative endeavor between humans and cattle. Recently, significant emphasis has been placed on the individual products derived from animals and their formulations. Panchagavya is a formulation described in Ayurveda, consisting of five components obtained from cows: milk, curd, ghee, urine and dung (Mathivanan *et al.*, 2006). Liquid organic fertilizers, including panchagavya and cow urine, are frequently utilized in organic agriculture to ensure that crops receive a well-rounded nutritional supply (Devakumar *et al.*, 2008). The presence of naturally occurring beneficial microorganisms, primarily bacteria, yeast, actinomycetes and certain fungi has been observed in organic liquid manures. The extensive use of antibiotics in aquaculture to prevent and manage bacterial diseases has resulted in a rise in antibiotic-resistant bacteria (Alderman and Hastings, 1998; Teuber, 2001).

Consequently, various alternative approaches to the application of antimicrobials have been suggested, including the utilization of probiotics as biological control agents (Irianto and Austin, 2002). Probiotics, which are live microorganisms that can act as dietary supplements to enhance fish growth and immune responses, have garnered some interest in the field of aquaculture (Gatesoupe, 1999; Kesarcodi *et al.*, 2008). *Lactobacillus* is utilized in the baking industry and comprises a range of immune-stimulating compounds, including β-glucans, nucleic acids, and mannan oligosaccharides. It possesses the ability to improve immune responses (Siwicki *et al.*, 1994; Anderson *et al.*, 1995) and promote growth (Ortuno *et al.*, 2002; Oliva and Goncalves, 2001) in various fish species. Nevertheless, the use of yeast has been acknowledged to significantly influence immune stimulant functions (Lara *et al.*, 2003). The preservation of fish health and welfare, significantly affected by the feed provided and the surrounding environmental conditions, is currently the primary focus in aquaculture (Li and Gatlin, 2003; Sakai, 1999).

The obvious decline in fish mortalities among the three treated groups could be results of immune system activation against all kinds of pathogenic as well as opportunistic bacterial invaders. Antibiotics can be replaced by alternatives such as prebiotics, probiotics and botanicals. Recently, Council for Scientific Industrial Research (CSIR), India has identified cow urine distillate for its antimicrobial and antifungal properties. Panchagavya is one such formulation mentioned in Ayurveda, which is prepared with five components derived from cow viz. milk, curd, ghee, urine and dung and can be used as growth promoters in animals (Dhama *et al.*, 2014).

Commercial additives of plant origin like herbs, species and various plant extracts are also considered to be natural products that consumers would have received an increased attention.

Andrographis paniculata is one of such plant having antimicrobial and growth promoting activity and hence may be used as alternative to antibiotics and tonic. The effect of panchagavya and *Andrographis* *paniculata* on haematological, serum biochemical parameters and immune status of broilers was investigated. However, role of these alternatives on health indicating parameters like haematological, serum biochemical characteristics and immunity in broilers (Mathivanan and Kalaiarasi, 2007). Even though already some works has been carried out in rats and other species (Girish *et al.*, 2004). The green synthesis of Copper nanoparticles using Panchagavya was studied for antibacterial, anticancer and environmental applications (Samuthirapandi *et al.*, 2025).

At present, there exists considerable scientific and commercial interest in the modulation of gut microbiota via dietary supplements that are enriched with advantageous microbes, commonly referred to as probiotics. These beneficial microorganisms proliferate and establish themselves within the host's gut, resulting in various positive effects on both the host and the surrounding environmental conditions (El-Bab *et al.*, 2022). This study was conducted to investigate the impact of panchagavya and probiotics (*Lactobacillus)* administered to *Oreochromis mossambicus* over a brief period, on the immune response to *E. tarda* and *A. hydrophila*.

MATERIALS AND METHODS

Experimental animal and their maintenance

Healthy *Oreochromis mossambicus* weighted of 12.5±0.10g and a length of 8.3±0.5cm, sourced from a local fish farm in Kumbakonam, Tamil nadu, India and were maintained in dechlorinated water. Prior to the treatment, all experimental fish were acclimatized to a pH of 7.0 ± 0.2, at a stable temperature of 22 ± 1˚C, subjected to a photoperiod of 16 hours of light and 8 hours of darkness for a duration of two weeks, during which they were fed control feed once daily.

Diet preparation

A commercially available basal diet was formulated for *Oreochromis mossambicus*, containing 13% moisture, 47% protein, 12% oil, 16% crude ash and providing 3,500 kcal/g of energy. This basal diet acted as the control diet, which did not include *Lactobacillus* or Panchagavya. The panchagavya was prepared by following the methods outlined (Natarajan, 2003). Meanwhile the *Lactobacillus* sporogenes spores, which are commercially available as the pharmaceutical product SPORLAC, were utilized in the current study (Venkatalakshmi and Ebanasar, 2015).

The feed preparation was done by adding panchagavya in various concentrations (0.1%, 0.5%, 1%, 2% and 4%) and *Lactobacillus* with basal diet. The prepared feeds were air-dried at room temperature (25°C) for a minimum of 48 hours and subsequently stored at 4°C until it was needed for further use (Sattanathan *et al.*, 2020).

Experimental design

Oreochromis mossambicus fishes were allocated into 15 tanks, with each tank housing 25 fishes. The fishes were treated with a basal diet that did not include panchagavya serving as the control diet, followed by T1 (Basal diet + Panchagavya-0.1%); T2 (Basal diet + Panchagavya-0.5%); T3 (Basal diet + Panchagavya-1%); T4 (Basal diet + Panchagavya-2%); T5 (Basal diet + Panchagavya-4%) and T6 (PC - Positive Control with *Lactobacillus).* The fishes were fed with the experimental diet at a rate of 4% of their body weight, twice daily at 09:00 and 17:00 hours, for a total of 30 days. From each experimental group, six fishes were sampled and blood was collected on the 7th, 14th, 21st and 28th days to assess various immunological parameters. The remaining fishes were subsequently challenged with virulent *A. hydrophila* and *E. tarda* at 30th days after feeding and the relative percentage survival (%) was recorded 14 days post-treatment (Rahman and Arifuzzaman, 2021).

Blood sample collection

The fishes were sampled randomly from each tank and were anaesthetized; about 2 ml of blood was drawn from the caudal vein, using a non-heparinized syringe, after they were starved for 24 h. One half of each blood sample was transferred to microtube containing heparin anti-coagulant and the other half was transferred to non-heparinizes microtube, placed at room temperature and allowed to clot for 2 hrs. Sera were separated by centrifugation at 1500g for 20 min and stored at refrigerator for future use (Dagur and Mccoy, 2015).

Hematological parameters

The total erythrocyte count and total leucocyte count were manually counted by haemocytometer. Hematocrit (Hct%) was determined by micro centrifuge technique, using standards heparinized micro haematocrit capillary tubes (75mm at 7000g for 10 min). Thin blood smears slides were prepared and stained with Wright-Giemsa stain. A total of at least 100 leucocytes were counted under a light microscope and the percentages of leukocyte types were calculated. The Heamoglobin level of blood was analyzed spectrophotometrically at 540nm by the cyanomethemoglobin method (Girish *et al.*, 2004).

Immunological parameters

Serum Lysozyme activity was assessed using the turbidimetric assay described in (Parry *et al.*, 1965), along with the microplate optimization detailed in (Hutchinson and Manning, 1996). The overall myeloperoxidase activity in the serum was detailed by (Quade and Roth, 1997) and partially adjusted by (Sahoo *et al.*, 2005). The serum antiprotease assay was conducted (Rao and Chakrabarti, 2004).

Challenge study

Fishes were challenged with *A. hydrophila* and *E. tarda*, 30 days post-feeding and mortalities were recorded over 14 days post infection. The Survival rate (SR) was calculated to evaluate the efficacy of panchagavya and *Lactobacillus* in fishes (57).

Statistical analysis

The data underwent statistical analysis utilizing the statistical software SPSS version 21, where a two-way ANOVA was performed; Duncan's multiple range test (DMRT) was employed.

RESULTS AND DISCUSSION

Fish culture is increasing to compensate the shortage of animal protein all over the world. Fish under intensive culture conditions will be badly affected and often fall prey to different microbial pathogens that have been treated with chemotherapeutic substances of which antibiotics were intensively used. These curative substances produce the problem of bacterial drug fastness on one hand and the public health hazards on the other hand (Deepika *et al.*, 2016).

In the current investigation, the immune stimulatory effect of panchagavya and probiotics *Lactobacillus* on the growth, immunity and disease resistance of O. *mossambicus* was examined.

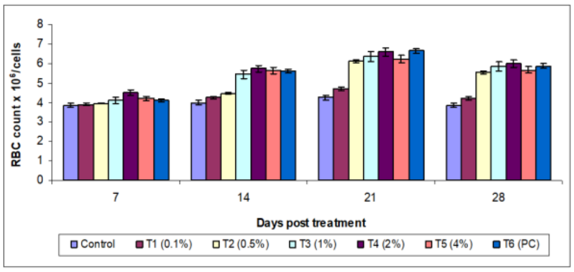

Fig. 1. Effect of dietary supplement of panchagavya and *Lactobacillus* on total Erythrocyte count in *O. mossambicus*

The total erythrocyte count in *O. mossambicus* with different treatment groups was shown in the Fig. 1. The post feeding trails on fishes showed higher total erythrocyte count on 21st day was T6 (6.64±0.04) followed by T4 (6.59±0.02), T3 (6.35±0.05), T5 (6.23±0.01), T2 (6.12±0.02), T1 (4.69±0.04) and control diet (4.25±0.03). On 28th day, the peak value were found on T4 (5.99±0.03) were the feed diet contains 2% of panchagavya concentration.

It is possible that the mode of action of MP plant extract on hematological indices could be due to the effect of its vitamin C on the increase of iron absorption from intestine of fish and other vitamins and minerals help to improve the hematopoiesis (Schnurer and Magnusson, 2005). The results of this study reinforce the growing view that some herbal plants are beneficial to rainbow trout by conferring protection against disease and stimulating the immune response (Lim *et al.*, 2000).

In present study, the total Leucocyte count were found to be (8.74±0.53) in T4 treatment group which was higher than T6 (8.69±0.35) and control (7.33±0.54) on 28th day post treatment in *O. mossambicus* which was depicted in the Fig. 2. The WBC levels are well considered as one of the important factors of body defense and many investigations were shown that the herbal plant could be act as immunostimulants and increased the total WBC of rainbow trout (Jessus *et al.*, 2002). The obtained results agree with those obtained in common carp, Asian seabass, Caspian white fish and Caspian brown trout after receiving *Mentha piperita* (Ribeiro *et al.*, 2016).

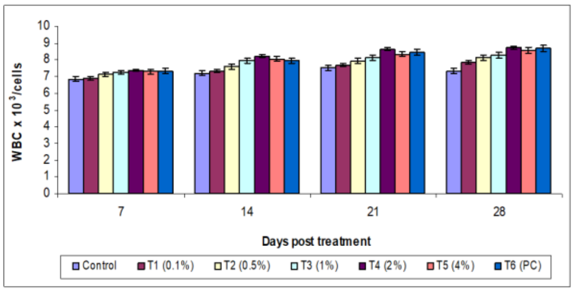

Fig. 2. Effect of dietary supplement of panchagavya and *Lactobacillus* on total Leucocyte count in *O. mossambicus*

The effect of different feed treatment on *O. mossambicus* was carried out for 28 days. The average increase in Heamoglobin was manifested in the Fig. 3. On the 7th and 28th days, the treatment groups which shows results as follows: control (4.95±0.23 and 5.71±0.4), T1 (5.12±0.11 and 6.35±0.24), T2 (5.25±0.53 and 6.68±0.11), T3 (5.35±0.54 and 6.84±0.5), T4 (5.39±0.24 and 7.02±0.3), T5 (5.23±0.12 and 6.44±0.53) and T6 (5.22±0.64 and 6.52±0.34) respectively.

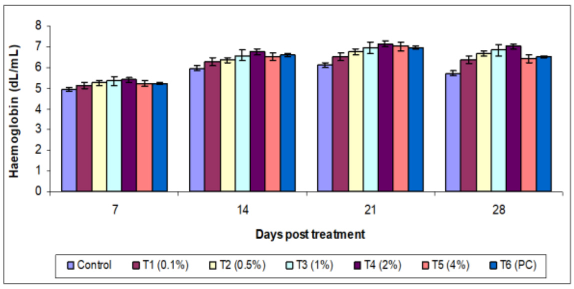

Fig. 3. Effect of dietary supplement of panchagavya and *Lactobacillus* on Haemoglobin content in *O. mossambicus*

The changes in immunological parameters such as 7serum lysozyme activity, myeloperoxidase activity and serum antiprotease activity were analyzed during 7 to 28 days feeding treatment.

Based on the results of serum lysozyme activity, fish feed diets with 0.1%, 0.5%, 1%, 2% and 4% levels of supplemented panchagavya feed showed significantly higher levels of the digestive enzyme than fish fed with control diet. The blood serum sample from the T4 group on the 21st day had greater total serum lysozyme activity than previous post-treatment groups (Fig. 4). On the 7th day after treatment, T4 (2%) had serum lysozyme that reached a maximum of 300.47µg/ml and a minimum of 212.24µg/ml in the control. On the 21st post-treatment day, the highest peak was found to be on T4 (395.74 µg/ml) followed by T6 (390.33 µg/ml).

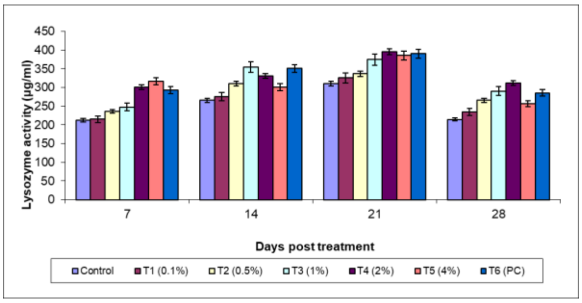

Fig. 4. Effect of dietary supplement of panchagavya and *Lactobacillus* on lysozyme activity in *O. mossambicus*

Lysozyme is considered as one of the important bactericidal enzymes and an indispensable tool of fish to fight infectious agents. *Lactobacillus* is found to trigger the serum lysozyme level in teleost. Our research showed that the use of such biotic forms have a significant increase on the lysozyme activity in all treated fish. Lysozyme is constitutively expressed, synthesized and secreted by neutrophils, monocytes and macrophages. The greatest concentration of lysozyme was directly proportional to the leukocytic count. In the current research, the addition of *Lactobacillus* in fish diet has remarkably increased the leukocytic count, which in turn elevated lysozyme concentration and activity (Engstad *et al.*, 1992). In the present study pachagavya with *O. mossambicus* treated group found in lysozyme activity.

The similar result was observed in *Labeo rohita*, *Cirrhinus mrigala* (Esteban *et al.*, 2001; Rao and Chakrabarti, 2005).

Phagocytic activity is responsible for early activation of the inflammatory response before antibody production and plays an important role in antibacterial defenses (Rao *et al.*, 2006; Nya and Austin, 2009). The highest Phagocytic activity and index were recorded in synbiotic and probiotic treated fish groups followed by prebiotic group. These findings supported those of (Salnur *et al.*, 2009; Tewary and Bidhan, 2011).

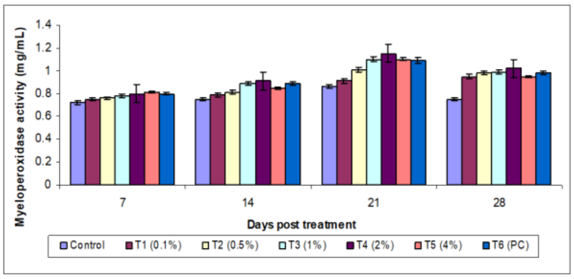

Fig. 5. Effect of dietary supplement of panchagavya and *Lactobacillus* on myeloperoxidase activity in *O. mossambicus*

The Myeloperoxidase activity results for the *O. mossambicus* fishes are shown in Fig. 5. The maximum Myeloperoxidase activity was noted at concentration in T4 (2%) on the 21st day after treatment was 1.15±0.02 mg/ml, whereas the minimum level was 0.72±0.02 mg/ml in the control at 7th day. On days 7 and 14, the peak gradually increased, remained constant or high on day 21 and began to decrease on day 28 (Fig. 5).

The ability of macrophages to kill pathogenic microbes is probably one of the most important mechanisms of protection against diseases among fish. The oxygen radicals and nitric oxide are the most destructive products produced by activated macrophages (Adel *et al.*, 2015; Sirbu *et al.*, 2022). The three used biotic forms showed significant enhancement of theneutrophils/macrophages activity than in control group, which coincides with those previous publications presented by (El-Boshy *et al.*, 2010; Bryan and Grisham, 2007).

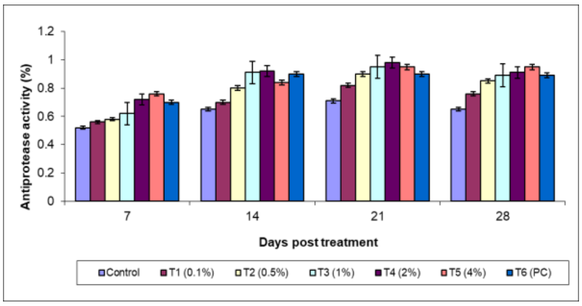

Fig. 6. Effect of dietary supplement of panchagavya and *Lactobacillus* on antiprotease activity in *O. mossambicus*

In the present study, the effect of *O. mossambicus* fish species on the serum antiprotease activity is depicted in (Fig. 6). The blood serum sample from all the post treatment groups was studied on 28th day. The T5 treatment group had the highest significant value of 0.95±0.02%, while compare with T6 (0.89±0.01%) and control (0.65±0.01%).

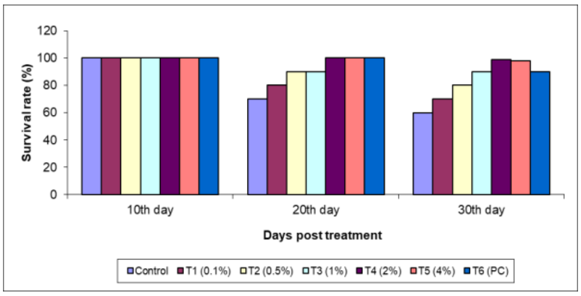

Fig. 7. Effect of dietary supplement of panchagavya and *Lactobacillus* on the survival rate in *O. mossambicus*

The stimulatory effect of yeast cell wall components mainly b-glucan, mannoprotein; chitin and yeast RNA on the circulating and tissue macrophages. The presence of glucan and mannose are not only stimulating the phagocytosis but also increasing their destructive and killing ability (Esteban *et al.*, 2004).

In the investigation, the effects of panchagavya and probiotics *Lactobacillus* administrated to *O. mossambicus* enhance the immune mechanism against *E. tarda* and *A. hydrophila*. The recorded cumulative mortality rates were 25% and 16.6% in the groups fed a diet supplemented with T5 for 14 days, specifically against *A. hydrophila* and *E. tarda* respectively (Fig. 7-9).

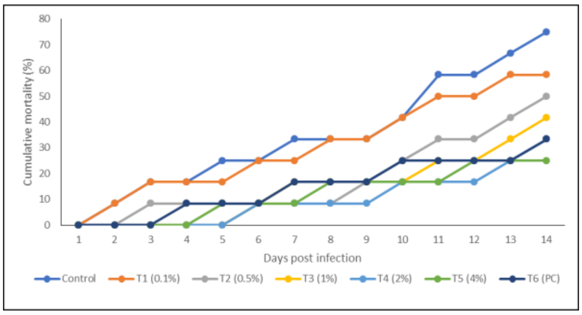

Fig. 8. Effect of dietary supplement of panchagavya and *Lactobacillus* on the cumulative mortality rate in *O. mossambicus* against *A. hydrophila* infection (n=10)

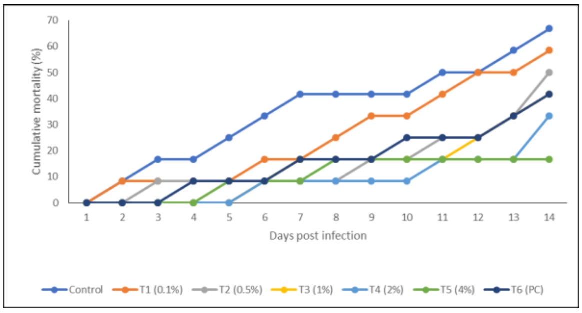

Fig. 9. Effect of dietary supplement of panchagavya and *Lactobacillus* on the cumulative mortality rate in *O. mossambicus* against *E. tarda* infection (n=10)

In contrast, mortality rates in the groups that received T4 and those in the positive control group were 33.3% and 41.6%. The control group experienced. Significantly higher mortality rates of 75% and 66.6% compared to the other groups. Notably, there were no recorded deaths during the first three days of bacterial infection in the T6 positive control group.

Although a few studies have pointed to the *in vitro* antimicrobial activity of either the Panchgavya mixture (Gajbhiye *et al.*, 2015) or its individual components (Chauhan, 2004; Selvaraj *et al.*, 2005), this study stands out as the first to demonstrate the in vivo anti-infective efficacy of the Panchgavya mixture. The protective effect of Panchgavya against bacterial infections may partly arise from its immunomodulatory properties. This brief investigation reinforces the therapeutic potential of Panchgavya as outlined in Ayurvedic texts (Saxena *et al.*, 2004). Future research aimed at characterizing this ancient formulation, such as generating a metagenomic analysis to identify beneficial microbes and chemical profiles, can provide valuable insights into the mechanisms behind its anti-infective capabilities (Li *et al.*, 2006).

Natural immune stimulants are biocompatible, biodegradable and safe for both the environment and human health. Moreover, they possess an added nutritional value (Abou *et al.*, 2025; Gajbhiye *et al.*, 2018). The results of this study reinforce the administration of panchagavya are beneficial to O. mossambicus by conferring protection against disease and stimulating the immune response.

CONCLUSION

In conclusion, the results of this study revealed that panchagavya and *Lactobacillus* frequently used probiotics supplements to *O. mossambicus* for preventing the fish pathogens. Also the diets supplemented with panchagavya and *Lactobacillus* improve the specific and non-specific immune responses in fish *O. mossambicus*. Finally, these probiotics could provide healthy and safe fish production from aquaculture replacing the Xenobiotics (antibiotics) for both fish and fish consumers.

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