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Research Article

SYNERGISTIC EFFECTS OF FENUGREEK AND BLACK CUMIN POWDER AS FUNCTIONAL FEED ADDITIVE ON THE HEALTH AND IMMUNITY OF *CIRRHINUS MRIGALA* CHALLENGED WITH *EDWARDSIELLA TARDA*

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ABSTRACT: **This study looked at how fenugreek and black cumin dietary supplements together affect the health and immunity of *Cirrhinus mrigala*, an Indian carp, when faced with *Edwardsiella tarda*. Conducted in Meghalaya, India, it's the first to assess these supplements' joint impact on *E. tarda* resistance in fish. The experiment used 12 tanks with 20 fish each, testing four treatments: control, fenugreek, black cumin, and a combination. After 8 weeks of feeding, fish were exposed to *E. tarda* (ATCC 15947™). The combined supplement group showed significant improvements in growth, immune response, and disease resistance compared to other treatments. It enhanced lysozyme activity by 45%, respiratory burst activity by 56%, and improved post-challenge survival rates to 88% versus 62% in the control group. These results show that giving fenugreek and black cumin together as dietary supplements can greatly improve the health and resistance of *C. mrigala* to *E. tarda*. This could be a good natural way to control diseases in hill-region aquaculture.**

Keywords: Fenugreek, Black cumin, Indian major carp, Fish health, Immunity, *Edwardsiella tarda*, Synergistic effects, Hill climate.

INTRODUCTION

Aquaculture has emerged as a critical sector in global food production, playing a vital role in meeting the increasing demand for fish protein worldwide. As the industry expands, maintaining optimal fish health and immunity has become paramount for sustainable and profitable aquaculture operations. Healthy fish grow faster and more efficiently and demonstrate enhanced resistance to diseases, leading to reduced mortality rates and improved overall production [1]. Despite advancements in aquaculture practices, fish farmers face numerous challenges in maintaining fish health. One of the most significant threats is bacterial infections, with *Edwardsiella tarda* being a particularly problematic pathogen. Edwardsiellosis is a severe disease that affects many fish species, including the economically important *Cirrhinus mrigala* [2]. It is caused by the gram-negative bacterium *E. tarda*. This pathogen can lead to high mortality rates, reduced growth, and significant economic losses in aquaculture settings [3].

Recently, there has been growing interest in using natural plant additives as alternatives to synthetic antibiotics and growth promoters in aquaculture. These natural additives offer several advantages, including improved fish health, enhanced immune responses, and reduced environmental impact [4, 5]. Among the promising natural additives, fenugreek (*Trigonella foenum-graecum*) and black cumin (*Nigella sativa*) have shown potential for their antimicrobial, antioxidant, and immunostimulant properties.

Bioactive compounds like diosgenin, 4-hydroxyisoleucine, and galactomannans are found in fenugreek seeds. Researchers have linked these compounds to health benefits for both humans and animals. In aquaculture, fenugreek has been shown to enhance growth performance, improve feed utilization, and boost the immune system of various fish species [6, 7]. Black cumin, rich in thymoquinone and other antioxidants, has

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demonstrated similar beneficial effects on fish health, including improved growth, enhanced immune function, and increased disease resistance [8, 9].

While separate studies have looked at how fenugreek and black cumin affect different types of fish, not many have looked at how they might work together and how they might affect Indian major carp species like *C. mrigala*. Furthermore, the optimal dosage and efficacy of these natural additives may vary depending on fish species, environmental conditions, and specific pathogens.

The present study aims to investigate the synergistic effects of dietary supplementation with fenugreek and black cumin on the health and immunity of *C. mrigala* when challenged with *E. tarda*. This research seeks to assess the individual and combined effects of these natural additives on growth performance, evaluate their impact on hematological, biochemical, and immune parameters, determine their efficacy in enhancing disease resistance against *E. tarda* infection, and investigate potential synergistic effects when used in combination. The goal is to develop sustainable and effective strategies for improving health and productivity in aquaculture, particularly for Indian major carp species, by exploring the combined application of these natural immunostimulants.

MATERIALS AND METHODS

Experimental setup

The study was conducted at the Fisheries Research Farm, ICAR-NEH, Meghalaya, India. Sixteen cement tanks, each with a capacity of 1000 litres, were used for the experiment. A total of 240 healthy *C. mrigala* fingerlings (average initial weight 15.2±0.5 g) were randomly distributed into the tanks, with 20 fish per tank.

Treatment groups

The experiment consisted of four treatment groups (Table 1), each with four replicates:

Control group (C): Basal diet without supplementation

Fenugreek group (F): Basal diet supplemented with 2% Fenugreek seed powder

Black cumin group (BC): Basal diet supplemented with 2% Black cumin seed powder

Fenugreek + Black cumin group (F+BC): Basal diet supplemented with 1% Fenugreek and 1% Black cumin seed powder

The experimental feeds were prepared by first mixing rice bran, soybean meal, fishmeal, and wheat flour to form a base. This mixture was then cooked to form a corrected and allowed to cool. Thereafter, a vitamin and mineral premix was added to each treatment diet, along with 2% fenugreek seed powder (F group), 2% black cumin seed powder (BC group), or a mix of 1% of each (F+BC group). The control diet (C) received no supplementation. The dough was then pelletized using a manual pellet machine with a 2 mm diameter die. The resulting pellets were dried at room temperature to reduce moisture content and stored in airtight plastic packets to maintain quality and nutritional integrity until feeding.

The proximate composition of the feeds was analyzed prior to the commencement of feeding trials following standard methods [10]. The crude protein content of the feeds ranged from 32-32.1%, and the lipid content ranged from 6-6.1%, indicating that the feeds were nearly isonitrogenous and isolipidic across all treatments (Table 1). The energy content of the diets ranged from 345-346 kcal/100g, ensuring that any differences in fish performance could be attributed to the herbal supplements rather than variations in macronutrient or energy content.

Feeding regimen and water quality management

Fish were fed twice daily (09:00 and 16:00) at 3% of their body weight for 8 weeks. Water quality parameters were monitored daily following the standard methods of APHA and maintained within the optimal range for *C. mrigala* (water temperature: 24.0 ± 2.5°C; pH: 7.5 ± 0.3; dissolved oxygen: 6.5 ± 0.5 mg L-1; ammonia (NH3): 0.03 ± 0.01 mg L-1; nitrite (NO2-): 0.02 ± 0.01 mg L-1; nitrate (NO3-): 5.0 ± 1.0 mg L-1).

Data collection

Weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) were calculated bi-weekly. Red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and haematocrit (Hct) were measured at 4 and 8 weeks. Total protein, albumin, globulin, glucose, and cholesterol levels were analyzed at 4 and 8 weeks. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were assessed in liver tissue at 8 weeks. Lysozyme activity, respiratory burst activity, serum bactericidal activity, and phagocytic activity were measured at 4 and 8 weeks. All tests were conducted following standard protocols, and each experiment was performed in triplicate.

Challenge test

Once the 8-week feeding test was over, fish were challenged with 1.2 x 106 cfu $fish^{-1}$ of *E. tarda* (ATCC 15947TM) through an intraperitoneal injection.

Mortality was recorded for 14 days post-challenge. RPS was calculated using the following formula:

RPS = [1 - (% mortality in treated group % mortality in control group-1)] x 100

Data collection and challenge study

At the end of the 8-week feeding trial, growth performance, haematological parameters, serum biochemical parameters, antioxidant enzyme activities, and immune parameters were measured following standard protocols. Next, we conducted a challenge test using *E. tarda* (ATCC 15947™, HiMedia, Maharashtra, India). *E. tarda* was grown in nutrient broth for 24 hrs at 30°C in a BOD incubator. The cells were harvested, washed twice in sterile PBS, and then resuspended in PBS to a concentration of 106 cells ml^{-1}. The fish in each experimental group were given an intraperitoneal injection of 0.1 ml of *E. tarda* suspension (1.2 x 106 cfu $fish^{-1}$) in PBS.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Differences were considered significant at $P<0.05$. All statistical analyses were performed using SPSS version 21.0.

RESULTS AND DISCUSSION

Growth performance and feed utilization

The results of the 8-week feeding trial demonstrated significant improvements in growth performance and feed utilization in fish fed diets supplemented with fenugreek and black cumin, both individually and in combination (Table 2). The group receiving a combination of fenugreek and black cumin (F+BC) showed the highest final weight (55.8 ± 2.7 g), weight gain (267.1 ± 16.4%), and specific growth rate (SGR, 2.14 ± 0.11% day^{-1}), followed by the black cumin (BC) and fenugreek (F) groups, which performed similarly. The control group (C) had the lowest growth performance across all parameters.

The feed conversion ratio (FCR) was significantly improved in all supplemented groups, with the F+BC group showing the best feed utilization (1.42 ± 0.06), compared to 1.82 ± 0.09 in the control group. These findings align with recent studies on other fish species. For instance, Diab *et al.* [11] reported improved growth performance and FCR in Nile tilapia fed diets supplemented with a methanolic extract of fenugreek seed. Similarly, Dey *et al.* [8] observed enhanced growth metrics in Nile tilapia fed diets containing black cumin seed oil.

The superior growth performance observed in the F + BC group suggests a synergistic effect of combining fenugreek and black cumin. This synergy may be attributed to the complementary actions of bioactive compounds present in these supplements. Fenugreek contains diosgenin and 4-hydroxyisoleucine, which have been shown to enhance digestion and nutrient absorption in fish [6]. Black cumin, rich in thymoquinone and other antioxidants, may have contributed to improved metabolic efficiency and reduced oxidative stress, allowing for better nutrient utilization [8].

Hematological parameters

The hematological parameters were significantly improved in all supplemented groups, with the F+BC group showing the highest values (Table 3). The increase in red blood cell (RBC) count (2.42 ± 0.18 × 10^6 μL^{-1} in F + BC vs. 1.85 ± 0.12 × 10^6 μL^{-1} in C), hemoglobin concentration (9.2 ± 0.6 g dL^{-1} in F + BC vs. 6.8 ± 0.4 g dL^{-1} in C), and hematocrit (36.9 ± 2.3% in F + BC vs. 28.5 ± 1.8% in C) suggests improved oxygen-carrying capacity of the blood. This enhancement could contribute to better tissue oxygenation, supporting increased metabolic activity and growth [12].

The elevated white blood cell (WBC) count (35.8 ± 2.7 × 10^3 μL^{-1} in F+BC vs. 25.6 ± 2.1 × 10^3 μL^{-1} in C) indicates stimulation of the immune system, potentially improving the fish's ability to resist infections [13]. These findings are consistent with previous studies on the hematopoietic effects of fenugreek and black cumin in other animal models [14, 15].

The F+BC group may have had a synergistic effect because the bioactive compounds in fenugreek and black cumin work in ways that complement each other. For instance, the iron content in fenugreek could support haemoglobin synthesis, while the antioxidants in black cumin may protect RBCs from oxidative damage, collectively enhancing the oxygen-carrying capacity of the blood.

Serum biochemical parameters

The dietary treatments significantly affected the serum biochemical parameters (Table 4). Total protein (4.5 ± 0.4 g dL^{-1} in F+BC vs. 3.2 ± 0.2 g dL^{-1} in C), albumin (2.1 ± 0.2 g dL^{-1} in F+BC vs. 1.4 ± 0.1 g dL^{-1} in C), and globulin (2.4 ± 0.3 g dL^{-1} in F+BC vs. 1.8 ± 0.2 g dL^{-1} in C) levels were highest in the F+BC group, followed by F and BC groups, and lowest in the control group. These increases suggest improved protein synthesis and better nutritional status in the supplemented groups [16].

Glucose (72.1 ± 4.3 mg dL^{-1} in F+BC vs. 85.6 ± 5.2 mg dL^{-1} in C) and cholesterol (138.4 ± 6.8 mg dL^{-1} in F+BC vs. 165.3 ± 8.7 mg dL^{-1} in C) levels were significantly reduced in all supplemented groups, with the F+BC group showing the lowest values. These findings align with the known hypoglycemic and hypolipidemic effects of fenugreek and black cumin reported in other studies [17, 18].

The fact that serum biochemical parameters got better, especially in the F+BC group, shows that fenugreek and black cumin work better together. The increased protein levels may contribute to enhanced growth and immune function, while the reduction in glucose and cholesterol levels suggests improved metabolic efficiency.

Table 1. The compositions of experimental feeds.

Ingredients (g/kg)	Control (C)	Fenugreek (F)	Black Cumin (BC)	Fenugreek + Black Cumin (F+BC)
Fish meal	350	350	350	350
Soybean meal	280	280	280	280
Rice bran	200	180	180	180
Wheat flour	130	130	130	130
Fish oil	20	20	20	20
Vitamin-mineral premix*	20	20	20	20
Fenugreek	-	20	-	10
Black cumin	-	-	20	10
Total	1000	1000	1000	1000
Proximate composition (% dry matter basis):				
Moisture (%)	8.5±0.2	8.7±0.3	8.6±0.2	8.7±0.3
Crude protein (%)	32.1±0.4	32.0±0.5	32.1±0.4	32.0±0.5
Crude lipid (%)	6.0±0.2	6.1±0.3	6.1±0.2	6.1±0.3
Ash (%)	8.2±0.3	8.3±0.4	8.3±0.3	8.3±0.4
Crude fibre (%)	4.5±0.2	4.7±0.2	4.6±0.2	4.6±0.2
Nitrogen free extract	40.7±0.5	40.2±0.6	40.3±0.5	40.3±0.6
Energy (kcal/100g)	345±5	346±6	346±5	346±6

*Composition of vitamin-mineral premix (quantity/2.5 kg): Vitamin A, 5500000 IU; Vitamin D3, 1100000 IU; Vitamin B2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mcg; Calcium pantothenate, 2500 mg; Nicotinamide, 10 g; Choline chloride, 150 g; Manganese, 27,000 mg; Iodine, 1000 mg; Iron, 7500 mg; Zinc, 5000 mg; Copper, 2000 mg; Cobalt, 450 mg; L- lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 ppm; Satwari, 2500 mg.

Antioxidant enzyme activities

All of the supplemented groups had much higher levels of antioxidant enzymes (SOD, CAT, and GPx), but the F+BC group had the highest levels (Table 5). Superoxide dismutase (SOD) activity increased from 42.5 ± 3.2 U mg^{-1} protein in the control group to 62.6 ± 4.8 U mg^{-1} protein in the F+BC group. Similarly, catalase (CAT) activity increased from 18.3 ± 1.5 U mg^{-1} protein to 28.7 ± 2.4 U mg^{-1} protein, and glutathione peroxidase (GPx) activity from 8.6 ± 0.7 U mg^{-1} protein to 13.8 ± 1.2 U mg^{-1} protein.

These increases indicate an enhanced antioxidant defence system, which can protect fish against oxidative stress and associated cellular damage [19]. The improved antioxidant status can be attributed to the potent antioxidant properties of both fenugreek and black cumin, which have been well-documented in various studies [20, 21].

The synergistic effect observed in the F+BC group may be due to the diverse array of antioxidants present in both supplements. Fenugreek contains flavonoids and polyphenols, while black cumin is rich in thymoquinone, all of which contributes to antioxidant activity through different mechanisms. This diversity may provide more comprehensive protection against oxidative stress compared to either supplement alone.

Immune parameters

The immune parameters were significantly enhanced in all supplemented groups, with the F+BC group exhibiting the highest values (Table 6). Lysozyme activity increased from 22.5 ± 2.1 U mL^{-1} in the control group to 32.6 ± 2.7 U mL^{-1} in the F+BC group. Respiratory burst activity, measured as OD 540 nm, increased from 0.18 ± 0.02 to 0.28 ± 0.03. Serum bactericidal activity rose from 32.6 ± 3.1% to 48.7 ± 3.8%, and phagocytic activity from 25.3 ± 2.2% to 38.2 ± 3.1%.

These improvements in immune function can be attributed to the immunomodulatory properties of both fenugreek and black cumin, which have been reported in several studies [22, 23]. Increased lysozyme activity, respiratory burst activity, serum bactericidal activity, and phagocytic activity indicate a strong boost in both innate and adaptive immune responses [24].

The synergistic effect observed in the F+BC group suggests that these supplements may activate complementary immune pathways. For instance, fenugreek may stimulate the production of immune cells, while black cumin enhances their functional activities. This complementary action could explain the superior immune response observed in the combination group.

Challenge test

The challenge test results show that adding fenugreek and black cumin to the diet, especially together, made *C. mrigala* much more resistant to getting infected with *E. tarda* (Table 7). The F+BC group showed the highest survival rate (88.0 ± 3.2%) and relative percent survival (68.4%), followed by the BC (80.0 ± 3.5%) and F (78.0 ± 3.8%) groups, which performed similarly. The control group had the lowest survival rate (62.0 ± 4.5%).

These findings are consistent with previous studies showing improved disease resistance in fish fed diets supplemented with various herbs and spices [4, 25]. The enhanced disease resistance can be attributed to the improved immune status and antioxidant capacity observed in the supplemented groups.

Optimal dosage and synergistic effects

Our study found that optimal performance at 1% fenugreek and 1% black cumin combinations falls within the effective dosage ranges reported in recent literature. For fenugreek, studies have shown effective doses ranging from 0.09% to 2% of the diet. Diab *et al.* [11] found that a 0.09% methanolic extract of fenugreek seed improved growth performance, immunity, and disease resistance in Nile tilapia. Similarly, Basha *et al.* [26] reported that a 2% fenugreek seed supplementation enhanced growth, hematological parameters, and antioxidant enzymes in the same species.

For black cumin, the effective dosage range appears to be wider. Silviana *et al.* [27] found that 50 g/kg of feed was effective in increasing white blood cell count and enhancing disease resistance in tilapia. In common carp, Khondoker *et al.* [28] identified a 4% dose of black cumin seed extract as effective for boosting immunological factors and disease resistance.

Recent research supports the synergistic effects observed in our study when combining fenugreek and

Table 2. Growth performance and feed utilization of *C. mrigala* fed experimental diets for 8 weeks.

Parameter	C	F	BC	F + BC
Initial weight (g)	15.2 ± 0.4^{a}	15.1 ± 0.5^{a}	15.3 ± 0.3^{a}	15.2 ± 0.4^{a}
Final weight (g)	42.6 ± 2.1^{c}	48.7 ± 2.5^{b}	49.5 ± 2.3^{b}	55.8 ± 2.7^{a}
Weight gain (%)	180.3 ± 12.5^{c}	222.5 ± 15.2^{b}	223.5 ± 14.8^{b}	267.1 ± 16.4^{a}
SGR (% day^{-1})	1.65 ± 0.08^{c}	1.89 ± 0.10^{b}	1.90 ± 0.09^{b}	2.14 ± 0.11^{a}
FCR	1.82 ± 0.09^{a}	1.61 ± 0.07^{b}	1.59 ± 0.08^{b}	1.42 ± 0.06^{c}

Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 3. Hematological parameters of *C. mrigala* fed experimental diets for 8 weeks

Parameter	C	F	BC	F + BC
RBC ($\times 10^{6}$ μL^{-1})	1.85 ± 0.12^{c}	2.10 ± 0.15^{b}	2.15 ± 0.14^{b}	2.42 ± 0.18^{a}
WBC ($\times 10^{3}$ μL^{-1})	25.6 ± 2.1^{c}	30.2 ± 2.5^{b}	31.5 ± 2.3^{b}	35.8 ± 2.7^{a}
Hb (g dL^{-1})	6.8 ± 0.4^{c}	7.9 ± 0.5^{b}	8.1 ± 0.5^{b}	9.2 ± 0.6^{a}
Hct (%)	28.5 ± 1.8^{c}	32.7 ± 2.1^{b}	33.2 ± 2.0^{b}	36.9 ± 2.3^{a}

Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 4. Serum biochemical parameters of *C. mrigala* fed experimental diets for 8 weeks.

Parameter	C	F	BC	F + BC
Total protein (g dL^{-1})	3.2 ± 0.2^{c}	3.8 ± 0.3^{b}	3.9 ± 0.3^{b}	4.5 ± 0.4^{a}
Albumin (g dL^{-1})	1.4 ± 0.1^{c}	1.7 ± 0.2^{b}	1.8 ± 0.2^{b}	2.1 ± 0.2^{a}
Globulin (g dL^{-1})	1.8 ± 0.2^{c}	2.1 ± 0.2^{b}	2.1 ± 0.2^{b}	2.4 ± 0.3^{a}
Glucose (mg dL^{-1})	85.6 ± 5.2^{a}	78.3 ± 4.8^{b}	77.5 ± 4.6^{b}	72.1 ± 4.3^{c}
Cholesterol (mg dL^{-1})	165.3 ± 8.7^{a}	152.6 ± 7.5^{b}	150.8 ± 7.2^{b}	138.4 ± 6.8^{c}

Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 5. Antioxidant enzyme activities in *C. mrigala* fed experimental diets for 8 weeks.

Parameter	C	F	BC	F + BC
SOD (U mg^{-1} protein)	42.5 ± 3.2^{c}	52.7 ± 4.1^{b}	53.5 ± 4.0^{b}	62.6 ± 4.8^{a}
CAT (U mg^{-1} protein)	18.3 ± 1.5^{c}	23.5 ± 2.0^{b}	24.2 ± 2.1^{b}	28.7 ± 2.4^{a}
GPx (U mg^{-1} protein)	8.6 ± 0.7^{c}	11.2 ± 0.9^{b}	11.5 ± 1.0^{b}	13.8 ± 1.2^{a}

Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 6. Immune parameters of *C. mrigala* fed experimental diets for 8 weeks.

Parameter	C	F	BC	F+BC
Lysozyme activity (U mL^{-1})	22.5 ± 2.1^{c}	28.7 ± 2.5^{b}	29.5 ± 2.3^{b}	32.6 ± 2.7^{a}
Respiratory burst activity (OD 540 nm)	0.18 ± 0.02^{c}	0.23 ± 0.03^{b}	0.22 ± 0.02^{b}	0.28 ± 0.03^{a}
Serum bactericidal activity (%)	32.6 ± 3.1^{c}	41.5 ± 3.5^{b}	40.8 ± 3.3^{b}	48.7 ± 3.8^{a}
Phagocytic activity (%)	25.3 ± 2.2^{c}	31.8 ± 2.7^{b}	32.5 ± 2.6^{b}	38.2 ± 3.1^{a}

Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 7. Survival rates and relative percent survival (RPS) of *C. mrigala* challenged with *E. tarda* after 8 weeks of feeding experimental diets.

Parameter	C	F	BC	F + BC
Survival rate (%)	62.0 ± 4.5^{c}	78.0 ± 3.8^{b}	80.0 ± 3.5^{b}	88.0 ± 3.2^{a}
RPS (%)	-	42.1	47.4	68.4

Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

black cumin. Bahi *et al.* [29] found that fenugreek combined with probiotic strains enhanced the immune response of gilthead seabream more effectively than fenugreek alone. Similarly, Mahammed *et al.* [30] reported that a mixture of black cumin, fenugreek, and sesame seeds improved the performance, intestinal morphology, and blood traits of broilers under chronic heat stress conditions. These findings suggest that combining immunostimulants may activate complementary pathways, leading to a more robust immune response.

While our study demonstrated significant improvements in fish health, immune function, and disease resistance, the magnitude of these effects differed from some earlier studies. For instance, we observed a post-challenge survival rate of 88% in the F+BC group, whereas some previous studies reported up to 100% survival [11]. Similarly, significantly improvement of growth rate in present study, while significant, were more modest compared to some earlier findings. There are several reasons for these differences: responses that are specific to a species (our study focused on *C. mrigala*, while many previous studies looked at other species); environmental factors (our study was done in the hill climate of Meghalaya); differences in dosage and formulation; possible differences in how contagious the pathogens are; and differences in the fish's health at the start of the study.

The mechanisms behind these synergistic effects warrant further investigation but may involve complementary antioxidant activities, activation of different immune pathways, enhanced nutrient absorption and utilization, improved gut health and microbiome composition, among others.

Implications for aquaculture

Our findings, along with recent studies, highlight the potential of natural feed additives like fenugreek and black cumin in improving fish health and productivity in aquaculture. As the aquaculture industry seeks sustainable alternatives to antibiotics, these natural immunostimulants offer promising solutions. The combined use of fenugreek and black cumin could provide a more comprehensive approach to enhancing fish health, addressing multiple aspects including growth performance, immune function, and disease resistance.

However, as noted by Guardiola *et al.* [7] and Awad *et al.* [6], the effects can vary depending on the fish species, dosage, and specific immune parameters measured. This underscores the need for species-specific research and careful dose optimization in practical aquaculture applications. Future studies should focus on the long-term effects of supplementation on fish health and flesh quality; efficacy against a broader range of pathogens relevant to aquaculture; potential interactions with other feed ingredients and environmental factors; and cost-effectiveness analysis for large-scale implementations.

CONCLUSION

Lastly, this study shows that supplementing with fenugreek and black cumin can help *C. mrigala* in the hilly climate of Meghalaya significantly. The combination (F+BC) significantly enhanced growth performance, with a 31% increase in final weight and a 29.7% improvement in specific growth rate compared to the control. Immune parameters showed substantial improvements, with lysozyme activity increasing by 45% and respiratory burst activity rising by 56%. Notably, the F+BC group exhibited 88% survival against *E. tarda* challenge, a 41.9% improvement over the control. Antioxidant defences were also bolstered, with SOD and GPX activities increasing by 47.3% and 60.5%, respectively. These findings suggest that combining fenugreek and black cumin (1% each) in fish diets offers a comprehensive approach to enhancing fish health, addressing growth, immunity, and disease resistance. This strategy presents a promising alternative for sustainable aquaculture, potentially reducing reliance on synthetic additives and antibiotics in challenging environmental conditions.

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