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EDITED BY
Angel Isidro Campa,
Centro de Investigación Biológica del Noroeste (CIBNOR), Mexico

REVIEWED BY
Neelesh Kumar,
Rani Lakshmi Bai Central Agricultural University, India
Prasanta Jana,
Birsa Agricultural University, India

*CORRESPONDENCE
Rohitash Yadav
✉ rohitashyadav093@gmail.com;
✉ draquayadav093@gmail.com
Narinder Kumar Chadha
✉ narinderkchadha99@gmail.com

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Khejri seed extract (KSE) enhances the growth, digestive, metabolic, and immunological responses of *Labeo rohita* fingerlings

Rohitash Yadav [1]*, Manish Jayant[1], Narinder Kumar Chadha [1]*, Ved Prakash Saini[2], Paramita Banerjee Sawant[1], Manohar Lal Ojha[3], Bhanu Kumar Sharma[3] and Dharmendra Kumar Meena[4]

[1]ICAR – Central Institute of Fisheries Education, Mumbai, Maharashtra, India, [2]College of Fisheries, Bihar Animal Sciences University, Bihar, India, [3]College of Fisheries, Maharana Pratap University of Agriculture & Technology, Udaipur Rajasthan, India, [4]ICAR – Central Inland Fisheries Research Institute, Barrackpore, Kolkata, West Bengal, India

The onset of diseases, combined with the reliance on therapeutics such as chemicals and antibiotics, as well as the alarming rise of antibiotic-resistant pathogen strains, postulates the urgent exploration of alternatives to antibiotics in semi-intensive and intensive aquaculture systems. In response to this critical need, a sixty-day feeding trial was conducted to evaluate the dietary effect of khejri seed extract (KSE) on the growth performance and immunological responses of rohu (*Labeo rohita*) fingerlings. Following this trial, a fifteen-day challenge study assessed the rohu fingerlings' resistance against *Aeromonas hydrophilia*. Four experimental diets were supplemented with different levels of khejri seed extract (KSE) *viz.* 0.0, 2.5, 5.0, and 7.5 g KSE kg^{-1} of diet and designated as C, KSE0.25, KSE0.50, and KSE0.75, respectively. The supplementation of dietary KSE led to significant improvements in growth performance and protein utilization in rohu fingerlings ($P < 0.05$). The activity of digestive (except amylase) and metabolic enzymes (except lactate dehydrogenase in muscle) activities varied significantly ($P < 0.05$). For the enzymes superoxide dismutase (SOD) and catalase (CAT), the observed trend was control > KSE0.25 > KSE0.50 > KSE0.75 in both pre- and post-challenge studies. Total serum protein showed significant variations ($P < 0.05$) in both pre- and post-challenge studies, with albumin and globulin concentrations being significantly higher in the KSE0.50 group compared to control during the post-challenge phase. Moreover, KSE supplementation significantly enhanced the respiratory burst activity and haematological parameters (except total leukocyte count in the pre-challenge and serum glucose levels in pre- and post-challenge study) of rohu fingerlings ($P < 0.05$). Following *A. hydrophilia* challenge, fish mortality significantly decreased with higher dietary KSE levels ($P < 0.05$), reaching the lowest mortality in the KSE0.50 group. These results suggest that supplementing the diet with 5.0 g kg^{-1} khejri seed extract can effectively enhance growth, improve feed conversion, boost immunity, and increase survival against *A. hydrophila* infection in rohu fingerlings.

KEYWORDS

khejri seed extract, growth indices, digestive-metabolic responses, rohu, *Labeo rohita*, *Aeromonas hydrophilia*

1 Introduction

Aquaculture, one of the fastest-growing sectors in animal food-producing industries, plays a pivotal role in ensuring global nutritional and livelihood security. Fish production increased significantly, rising from 125 to 179 million tons during 2001–2020, while per capita fish consumption ascended from 16 to 20.5 kg during the same period (Food and Agriculture Organization, 2022). In recent years, there has been a shift toward intensified feed-based aquaculture and the diversification of fish species to enhance production. Meanwhile, there has been a decrease in extensive aquaculture practices, declining from 43.9 to 30.5% from 2000 to 2020, respectively (Food and Agriculture Organization, 2022).

The intensive aquaculture practices expose fish to environmental stress, and increases their vulnerability to various pathogens, including viruses, bacteria, fungi, and parasites (Wang et al., 2015). These disease outbreaks can lead to high mortality rates and reduced meat quality, causing significant economic losses for producers. In fact, pathogens can results in hatchery losses reaching up to 20% (Shinn et al., 2015). While, bacterial infections are a major concern in fish farming, accounting for nearly 50% of production losses (Ibrahim et al., 2014; Leung and Bates, 2013).

Carps are the predominantly cultured fish species in Asia, especially in India (Tejaswini et al., 2024) where they represent the largest share (51.60%) of global finfish production (Food and Agriculture Organization, 2024). Rohu (*Labeo rohita*) is among the most widely cultured species, with production steadily increasing (Food and Agriculture Organization, 2024). In 2020-2021, rohu and catla accounted for a substantial 6.03 million tons, constituting 12.30% of global production (Food and Agriculture Organization, 2022). Among freshwater aquaculture, *Aeromonas hydrophilia* is a prevalent bacterial pathogen, causing significant harm to carp production (Giri et al., 2013). The rampant use of antibiotics and chemotherapeutics in intensive aquaculture has rightfully drawn criticism, due to concern over antibiotic-resistant bacterial strains and harmful accumulation of antibiotic residues in fish tissues. Following the European Union's ban on sub-therapeutic antibiotics in 2003, research has aggressively pursued alternative, environmentally sustainable methods to improve fish health without jeopardizing safety for consumers and the environment (Ringø and Song, 2016; Hoseinifar et al., 2020). Researchers are now actively exploring a variety of substitutes, including probiotics, immuno-stimulants, and herbal products (Giri et al., 2015; Reverter et al., 2017; Shiu et al., 2016).

Moreover, there is a growing commitment to leveraging indigenous technological knowledge for effective fish health management, as highlighted by the Food and Agriculture Organization (FAO) (Food and Agriculture Organization, 2002). Herbal immunostimulants offer a wide range of beneficial effects and serve as viable alternatives to chemicals or antibiotics in aquaculture practices (Van Doan et al., 2019) and are being recognized as eco-friendly and cost-effective (Elumalai et al., 2020). Several studies have documented the immunostimulatory effects of herbal extracts from plants like *Catharanthus roseus, Rosmarinus officinale*, and *Eclipta alba* in fishes (Christybapita et al., 2007; Divyagnaneswari et al., 2007; Xie et al., 2008). Similarly, it had been reported that dietary supplementation of lemon peel extract positively impacted feed intake, growth performance, and digestive-metabolic enzyme activities in *L. rohita* fingerlings at low temperatures (Tejaswini et al., 2024). Likewise, Sundararajan et al. (2024) also observed the growth-promoting and immunostimulant properties of dietary papaya peel extract (PPE) in *L. rohita* fingerlings.

Prosopis cineraria, commonly referred to as the golden tree or wonder tree. It belongs to the Fabaceae family, is widely distributed in arid and semi-arid regions globally (Rai et al., 2021; Yadav et al., 2022). In India, it holds special significance as the state tree in Rajasthan and Telangana, and it is also the national tree of the United Arab Emirates (Afifi, 2018; Krishnan and Jindal, 2015). While *P. cineraria* is the only native species in the Indian subcontinent, along with other species such as *P. julifora*, *P. pallida*, *P. glandulosa*, *P. chilensis*, *P. tamarugo*, and *P. alba* are cultivated (Bhansali, 2010; Kalia et al., 2014). Beyond its nutritional value, *Prosopis* spp. is well-known for its medicinal properties like antiviral, antibacterial, antifungal, anthelmintic, and anticancer properties, effectively treating various diseases without causing adverse side effects (Preeti et al., 2017; Kuchana et al., 2014; Vaza and Bhalerao, 2018). The wide-ranging benefits of *Prosopis spp.* such as analgesic, antipyretic, hypoglycaemic, antioxidant, and hypocholesterolemic stem from various phytochemicals found throughout its pods, stems, roots, leaves, and seeds. These include alkaloids, saponins, tannins, flavonoids, flavonols, total phenols, vitamins, fatty acids, and free amino acids (Garg and Mittal, 2013; Garg et al., 2020; Malik et al., 2013; Yadav et al., 2018). Furthermore, the *Prosopis* extract have demonstrated antibiotic properties comparable to broad-spectrum antibiotics, effectively combating the issue of multidrug resistance (Khan et al., 2010). The focus of contemporary aquaculture nutrition research is decisively aimed at developing innovative products that enhance fish immune systems without compromising the health of fish, environment, or human safety. Despite the known benefits of Prosopis spp., limited research has explored the potential of *P. cineraria* seed extract in aquaculture nutrition. In this context, this study aims to evaluate the effects of dietary khejri (*P. cineraria*) seed extract (KSE) on growth, immune responses, and disease resistance in *L. rohita* fingerlings.

2 Materials and methods

2.1 Site, collection, and preparation of khejri seed flour

A feeding trial, hematological analysis, and challenge study against *A. hydrophilia* were conducted at the wet laboratory, ARSU-Aquaculture Research and Seed Unit, under the MPUAT-Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India-313001. Biochemical and enzymatic analysis were performed at the laboratories of the Divisions of Aquaculture and FNBP-Fish Nutrition, Biochemistry & Physiology, ICAR-CIFE, Mumbai, Maharashtra, India-400061. Khejri (*P. cineraria*) seeds were obtained from Jaipur (Latitude: 26.8400°N and Longitude: 75.7964°E), Rajasthan, India. They were cleaned, oven-dried at 45°C, ground, and sieved (mesh size 60; 250 μm particle size). The

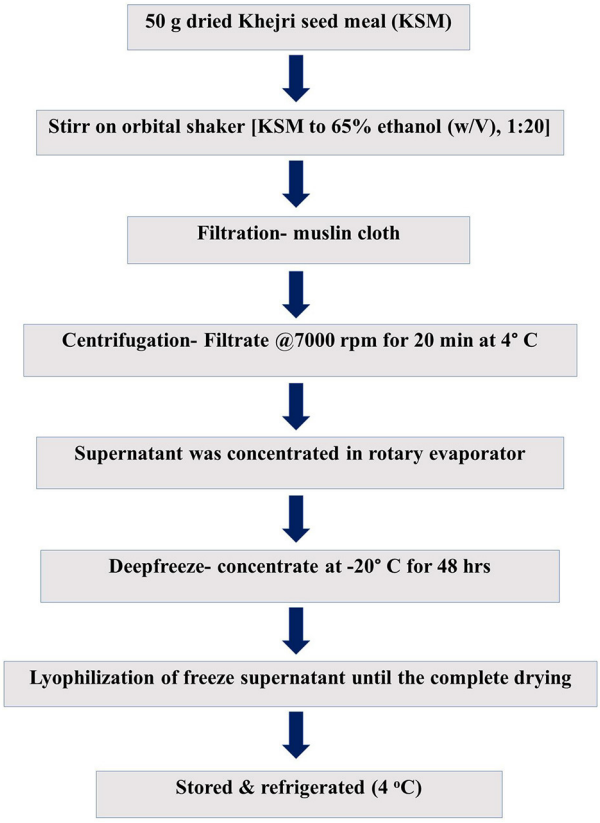

FIGURE 1
Preparation of crude ethanolic extract from khejri seed meal.

powdered seeds were then stored in labeled airtight polythene bags at room temperature.

2.2 Preparation of khejri seed extract

Ethanolic extract from khejri seed meal was prepared by following the methodology of Sundararajan et al. (2024) and illustrated in Figure 1.

2.3 Experimental design and fish rearing

Three hundred fingerlings of rohu (*Labeorohita*) were acclimated for 15 days in well-oxygenated 3000 L FRP tanks at the Aquaculture Research and Seed Unit, (MPUAT, Udaipur, India. Twelve fiber reinforced plastic (FRP) rectangular tanks (500 L; 1.0 ∗ 1.0 ∗ 0.50 m) were used as experimental units. FRP tanks were first cleaned and disinfected with potassium permanganate ($KMnO_4$), thoroughly rinsed with water, and then sun-dried. Subsequently, each tank was filled with 350 L of chlorine-free borewell water, and continuous aeration was maintained throughout the trial. Nylon nets were placed over all tanks to prevent fish from escaping. One hundred twenty *L. rohita* fingerlings (mean weight 7.23 ± 0.07 g) were randomly distributed among twelve experimental tanks. The fish were fed to satiation twice daily (at 9:30 am and 5:30 pm). Uneaten feed and fecal matter were siphoned daily, with an equal volume of clean groundwater replacing the removed water. Sampling was conducted every 2 weeks to monitor the growth and health of the fish. The physico-chemical parameters, including water temperature, pH, alkalinity, dissolved oxygen, ammonia, nitrite, and nitrate, were routinely monitored following APHA (American Public Health Association, 2005) guidelines. The pH ranged from 7.71 to 8.09, and the water temperature varied between 23.25 and 26.10°C. Alkalinity and dissolved oxygen levels ranged from 93.75 to 104.05 mg L^{-1} and 5.78 to 6.53 mg L^{-1}, respectively. Ammonia, nitrite, and nitrate levels were between 0.01 and 0.04 mg L^{-1}, 0.01 and 0.06 mg L^{-1}, and 0.14 and 0.35 mg L^{-1}, respectively. No traces of free carbon dioxide were detected in any experimental tank.

2.4 Formulation and preparation of experimental diets

Four iso-nitrogenous (303.07 g crude protein kg^{-1}) and iso-caloric (17.63 MJ gross energy kg^{-1}) experimental diets were supplemented with graded levels of khejri seed extract (KSE) at 0, 2.5, 5, and 7.5 g kg^{-1} of diet, denoted as C, KSE0.25, KSE0.50, and KSE0.75, respectively (Table 1). We weighed the dietary ingredients, mixed thoroughly, and moist-steamed for 30 min. The mixer was cooled and mixed thoroughly with oils & feed additives (butylated hydroxyl toluene, choline chloride, vitamin-mineral premix, and extract. The mixed was pressurized through a 2- 2.5 mm die in mechanical pelletizer, air-dried and stored in well-labeled container at room temperature.

2.5 Proximate composition

Method of Association of Official Analytical Chemists (A. O. A. C. Animal feed, 1995) followed to analyze composition of fish body and experimental diets. For moisture content, pre-weighed samples were subjected to oven-drying at 105°C. The crude protein content was assessed by measuring crude nitrogen through the Kjeldahl method, which involved digestion with concentrated sulphuric acid (H_2SO_4), distillation using 40% (w/V) sodium hydroxide (NaOH) in a semi-automatic distillation unit (KES 08L E, Pelican, Chennai, India), followed by titration with N/10 hydrochloric acid using a SI Analytics TitroLine 5000 titrator (Xylem Analytics, Weilheim, Germany). The ether extract was determined via solvent extraction, employing diethyl ether at temperature between 80–180°C using a Socsplus-SCS-08-AS apparatus (Pelican Equipment, Chennai, India). The ash content of experimental diets and carcass tissue was quantified by incinerating moisture-free samples at 550°C for 6 h in a muffle furnace (Muffle Furnace 1400c, Faridabad, Haryana, India). To determine crude fiber in fish feed, the method begins with an initial acid digestion using 1.25% H_2SO_4 (v/V) followed by alkali digestion with 1.25% NaOH (w/V) and incineration in a muffle furnace. The nitrogen-free extract of diet was calculated by subtracting the

TABLE 1 Feed composition of experimental diets with different KSE levels (g kg^{-1}).

Ingredients	C	$KSE_{0.25}$	$KSE_{0.5}$	$KSE_{0.75}$
Fish meal[b]	50.00	50.00	50.00	50.00
Soybean meal[b]	300.00	300.00	300.00	300.00
GNOC[b]	150.00	150.00	150.00	150.00
DORB[b]	300.00	300.00	300.00	300.00
Wheat flour[b]	70.00	70.00	70.00	70.00
Corn flour[b]	33.80	33.80	33.80	33.80
Fish oil[b]	20.00	20.00	20.00	20.00
Veg oil[b]	20.00	20.00	20.00	20.00
Cellulose[c]	20.00	17.50	15.00	12.50
Extract KSE[a]	0.00	2.50	5.00	7.50
BHT[c]	0.20	0.20	0.20	0.20
CMC[c]	15.00	15.00	15.00	15.00
Choline chloride[c]	1.00	1.00	1.00	1.00
*Vit-min mix	20.00	20.00	20.00	20.00
Total	1000.00	1000.00	1000.00	1000.00
Proximate compositions of the experimental diets (g kg^{-1}, dry weight basis)				
Dry matter	920.40 ± 3.33	921.69 ± 2.34	918.50 ± 1.67	921.05 ± 3.41
Crude protein	303.90 ± 7.81	302.48 ± 1.52	302.62 ± 3.67	303.29 ± 1.38
Crude fiber	82.89 ± 0.53	79.85 ± 2.89	76.98 ± 0.57	74.12 ± 0.77
Ether extract	61.66 ± 2.39	61.27 ± 1.25	60.93 ± 0.33	61.15 ± 1.16
$NFE	457.65 ± 6.68	463.00 ± 0.44	465.57 ± 1.69	466.63 ± 0.83
Total ash	93.91 ± 1.79	93.40 ± 0.56	93.90 ± 1.74	94.80 ± 1.38
#Gross energy (MJ kg^{-1})	17.70	17.65	17.61	17.57

Data expressed as mean ± SE $n = 3$[a]; KSE, khejri seed extract; prepared in Division of Aquaculture, ICAR-CIFE, Mumbai, India.
[b]Purchased from local animal feed ingredient dealer, Mumbai, India.
[c]Himedia Pvt, Mumbai, India.
*Composition of vitamin mineral mix (Agrimin forte) (quantity kg^{-1}): Vitamin A, 55,00,000 IU; vitamin D3, 11,00,000 IU; vitamin B2, 2,000 mg; vitamin E, 750 mg; vitamin K, 1,000 mg; vitamin B6, 1,000 mg; vitamin B12, 6 mcg; calcium pantothenate, 2,500 mg; nicotinamide, 10 g; choline chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 mg; L, lysine 10 g; DL, methionine 10 g; selenium, 125 mg.
#Gross energy (GE) contents were calculated according to gross caloric values of using the values of 23.6, 39.5, and 17.2 kJ g^{-1} for crude protein, crude fat, and total carbohydrate, respectively (Brett, 1973).
$Nitrogen free extract (g kg^{-1}) = 1,000 − [CP + EE + CF + Total ash].

other components of proximate composition (crude protein, ether extract, crude fire and ash) from 100. Lastly, the gross energy (GE) content of the feed was assessed using a Parr bomb calorimeter (Model 1241, Fifty-Third Street, Moline, IL).

2.6 Growth and nutrient utilization parameters

Nutrient utilization, growth, conversion, survival and body indices were calculated by using the following formula (Jayant et al., 2020; Yadav et al., 2022).

i. Weight gain = FW - IW
ii. Weight gain percentage = FW - IW*100/IW
iii. Specific growth rate (%/day) = Ln (FW) – Ln (IW)*100/Experiment days
iv. Feed conversion ratio = FC (DW)/BWG (WW)
v. Feed efficiency ratio = BWG (WW)/FC (DW)
vi. Protein efficiency = NWG (WW)/Protein diet
vii. Hepatosomatic index (%) = LW(g)*100/WF(g)
viii. Viscero-somatic index (%) = VW(g) (g)*100/WF(g)
ix. Survival (%) = Total no. of fishes catch*100/Total no. of fishes stocked.

Where, FW: Final weight; IW: Initial weight; DW: Dry weight; WW: Wet weight; FC: Feed consumed; BWG: Body weight gain; NWG: Net weight gain; LW: Liver weight; VW: Viscera weight; WF: Weight of Fish.

2.7 Biochemical and enzymes assay of fish tissue samples

2.7.1 Sampling

The overall biomass of each tankor triplicate was recorded to calculate the growth parameters. Fish were starved for 24 h for the gut emptying. Three fish from each treatment group were randomly selected to assess the whole-body composition. They were then sedated with clove oil (50–60 $\mu l\ L^{-1}$). Further, blood was withdrawn, transferred to 1 ml Eppendorf tube coated with a thin layer of EDTA and shaken to avoid haemolysis. Similarly, blood was collected from the sedated fish, transferred to 1 ml Eppendorf tubes, and allowed to clot at room temperature. The samples were then centrifuged at 3,500 rpm for 10 min at 4°C. The resulting yellow straw-colored serum was collected and stored at −20°C. Three fish from each treatment were dissected out to collect intestine, gill and liver. The tissue was ground in a 0.25 M sucrose solution (1:20 w/v) and centrifuged to obtain 0.5% tissue homogenates, which were then stored at −20°C for enzyme assay. The protein content in the tissue was measured utilizing the Bradford method (Bradford, 1976).

2.7.2 Digestive enzymes assays

Amylase, Lipase and protease activities were assayed as per Rick and Stegbauer (1974), Cherry and Crandall (1932) and Drapeau (1974), respectively.

2.7.3 Metabolic enzymes assays

Malate dehydrogenase (MDH) activity was evaluated following the protocol outlined by Ochoa (1955) and lactate dehydrogenase (LDH) as detailed by Wróblewski and Ladue (1955), respectively. Alanine aminotransferase (ALT) activity in various tissues of *L. rohita* was determined following the procedure specified by Wootton (1964). Similarly, aspartate aminotransferase (AST) activity was assessed using the same methodology as for ALT activity. Superoxide dismutase (SOD) and Catalase (CAT) activities were measured following the protocol detailed by Misra and Fridovich (1972) and Takahara et al. (1960), respectively.

2.7.4 Biochemical, and haemato-immunological parameters

Red blood cell (Nos. × 10^6 mm^{-3}), White blood cell (Nos. × 10^3 mm^{-3}) counts and hemoglobin (g dL^{-1}) were measured as described by Blaxhall and Daisley (1973) and Drabkin and Austin (1932), respectively. Serum protein levels and albumin levels were determined using the biuret and bromocresol green binding method by Reinhold (1953) and Doumas et al. (1971), respectively. Nitro blue tetrazolium (NBT) assay and serum glucose levels were determined using the method described by Stasiack and Bauman (1996) and Nelson (1944), respectively.

2.8 Challenge study

In the study, four fish from each replicate (twelve fish each treatment) were randomly selected at the conclusion of a 60-day feeding trial. A virulent strain of *A. hydrophilia* (MTCC-12301) was sourced from the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH) in Chandigarh, India. The bacteria were grown on nutrient agar and incubated at 37°C for 24 h. After incubation, the bacterial cells were collected by centrifugation at 4,000 rpm for 10 min. The resulting pellet was washed twice with phosphate-buffered saline (PBS) and then resuspended in PBS to achieve a turbidity equivalent to a 5 McFarland standard (10^8 CFU ml^{-1}).

The pathogenic bacterial strain was grown in Brain Heart Infusion (BHI) broth and incubated at 30°C for 28–30 h in a BOD incubator. Following incubation, the bacterial cells were collected by centrifugation at 3,000 rpm for 10 min, washed twice with sterile PBS, and diluted to a concentration of 10^8 cells ml^{-1}. Serial dilutions were performed to achieve a final concentration of 10^7 cells ml^{-1}. Each fish was intraperitoneally injected with 100 μL of the bacterial suspension and challenged for 15 days. During this period, mortality and pathological symptoms were closely monitored. Three fish from each replicate were sampled, anesthetized, and sacrificed for blood and tissue collection. The exact sampling and tissue homogenization procedures described previously were followed. Enzyme activities, along with biochemical and hematological analyses, were performed on the blood/serum as well as liver and gill tissue samples using the same methods. Mortality was recorded daily for 15 days, and relative percentage survival (RPS) was calculated following the methods of Amend (1981). The RPS was determined using the following formula:

$$RPS(\%) = [1 - \{MT(\%)/MC(\%)\}] \times 100$$

Where, MT (%), mortality of KSE fed group (%); M (%), mortality of control (%).

2.9 Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) in IBM-SPSS Statistics 22.0. Duncan's multiple range test (DMRT) was applied to identify significant differences between means, with a significance level set at $p < 0.05$. All data are presented as mean ± standard error of the mean. To assess significant changes between pre- and post-challenge conditions in *L. rohita* fingerlings, a paired *t*-test was utilized at a 5% level of significance.

3 Results

3.1 Proximate analysis of the experimental diets

The proximate composition of the experimental diets included dry matter (918.50 ± 1.67 to 921.69 ± 2.34 g kg^{-1}), crude protein

TABLE 2 Growth performance, nutrient utilization, body indices, and survival (%) of *L. rohita* fingerlings fed with different experimental diets.

Treatments	C	$KSE_{0.25}$	$KSE_{0.50}$	$KSE_{0.75}$	P-value
WG	$7.86^{a} \pm 0.05$	$7.95^{a} \pm 0.06$	$8.30^{b} \pm 0.06$	$7.88^{a} \pm 0.15$	0.030
WG (%)	$100.43^{a} \pm 0.59$	$101.70^{a} \pm 0.72$	$106.15^{b} \pm 0.93$	$100.60^{a} \pm 1.87$	0.024
SGR	$1.16^{a} \pm 0.01$	$1.17^{a} \pm 0.01$	$1.21^{b} \pm 0.01$	$1.16^{a} \pm 0.02$	0.025
FCR	$2.40^{b} \pm 0.01$	$2.38^{ab} \pm 0.01$	$2.30^{a} \pm 0.01$	$2.40^{b} \pm 0.04$	0.043
FER	$0.42^{a} \pm 0.002$	$0.42^{a} \pm 0.002$	$0.43^{b} \pm 0.003$	$0.42^{a} \pm 0.007$	0.036
PER	$1.39^{a} \pm 0.01$	$1.40^{a} \pm 0.01$	$1.45^{b} \pm 0.01$	$1.39^{a} \pm 0.02$	0.028
ANPU (%)	$24.55^{a} \pm 0.37$	$25.28^{ab} \pm 0.06$	$26.12^{b} \pm 0.28$	$24.38^{a} \pm 0.52$	0.036
HSI (%)	$1.39^{d} \pm 0.03$	$1.31^{c} \pm 0.01$	$1.18^{b} \pm 0.01$	$1.03^{a} \pm 0.03$	<0.001
VSI (%)	3.42 ± 0.02	3.46 ± 0.03	3.36 ± 0.07	3.38 ± 0.05	0.482
Survival (%)	100.00	100.00	100.00	100.00	-

Data expressed as Mean ± SE ($n = 3$); Mean values in the same row with different superscripts differ significantly ($P < 0.05$).
WG, weight gain (g); WG (%),weight gain (%); SGR, specific growth rate (% day^{-1}); FCR, feed conversion ratio; FER, feed efficiency ratio; PER, protein efficiency ratio; ANPU, apparent net protein utilization; HIS, hepatosomatic index; VSI, viscerosomatic index.

(302.48 ± 1.52 to 303.90 ± 7.81 g kg^{-1}), ether extract (74.12 ± 0.77 to 82.89 ± 0.53 g kg^{-1}), crude fiber (60.93 ± 0.33 to 61.66 ± 2.39 g kg^{-1}), nitrogen-free extract (457.65 ± 6.68 to 466.63 ± 0.83 g kg^{-1}), and total ash contents (93.40 ± 0.56 to 94.80 ± 1.38 g kg^{-1}), respectively (Table 1).

3.2 Growth parameters

Significant differences ($P < 0.05$) were observed in body weight gain (WG), weight gain (%), and specific growth rate (SGR) among the various treatment groups (Table 2). The highest growth rates were recorded in the $KSE_{0.50}$ fed group ($P < 0.05$), while the growth rates in the C, $KSE_{0.25}$, and $KSE_{0.75}$ groups were similar ($P > 0.05$). The mean values of FCR, FER, and PER differed significantly ($P < 0.05$) among the various dietary treatments. The highest FER was observed in KSE0.50, while it was significantly lower in C, KSE0.25, and KSE0.75 ($P < 0.05$) whereas, FCR followed a negative trend with FER, and growth rates ($P < 0.05$). Highest PER and ANPU was observed in $KSE_{0.25}$ and$KSE_{0.50}$ fed groups whereas the lowest ANPU was found in C and $KSE_{0.75}$ fed groups, ($P < 0.05$).

3.2.1 Body indices and survival (%)

The Hepatosomatic Index (HSI) values of rohu fingerlings were significantly different ($P < 0.05$) among the various treatment groups (Table 2). HSI was decreased linearly with dietary KPE inclusion ($P < 0.05$). Dietary KSE supplementation did not alter the VSI values in rohu, *L. rohita* fingerlings ($P > 0.05$). There was no mortality observed between control and dietary KSE fed groups ($P > 0.05$).

3.3 Digestive enzymes activities

There was no significant difference ($P > 0.05$) in the amylase activities observed in rohu fingerlings fed with control and KSE-based experimental diets (Table 3). However, significant variations were noted in protease and lipase activities in *L. rohita* fingerlings in response to dietary KSE ($P < 0.05$). The higher protease activities were found in $KSE_{0.50}$ whereas, the lowest value was found in C. KSE fed fish exhibited significantly higher lipase activities ($P < 0.05$) than control. However, lipase activities in KSE fed groups did not differ ($P > 0.05$).

TABLE 3 Digestive enzymes activities of *L. rohita*fingerlings fed with fed with different experimental diets.

Variables	Amylase	Protease	Lipase
C	9.73 ± 0.30	$0.30^{a} \pm 0.017$	$0.64^{a} \pm 0.018$
$KSE_{0.25}$	8.82 ± 0.64	$0.33^{a} \pm 0.007$	$0.78^{b} \pm 0.049$
$KSE_{0.50}$	7.18 ± 0.32	$0.38^{b} \pm 0.014$	$0.88^{b} \pm 0.058$
$KSE_{0.75}$	8.64 ± 0.75	$0.32^{a} \pm 0.018$	$0.83^{b} \pm 0.025$
P-value	0.057	0.024	0.015

Data expressed as Mean ± SE ($n = 3$): Mean values in the same column with different superscript differ significantly ($P < 0.05$).
Protease as micromole of tyrosine released min^{-1} mg^{-1} protein.
Amylase as micromole of maltose released min^{-1} mg^{-1} protein.
Lipase as U mg^{-1} protein.

3.4 Whole body composition

The moisture, crude protein, and total ash contents did not show significant differences ($P > 0.05$) among the various experimental groups (Table 4). The crude lipid value was significantly higher ($P < 0.05$) in the C (4.39 ± 0.03), and a lower value was found in the $KSE_{0.75}$ (3.97 ± 0.01). The crude lipid contents were found in decreasing trend from C (control) to $KSE_{0.75}$ ($P < 0.05$).

TABLE 4 Whole body composition of *L. rohita*fingerlings fed with fed with different experimental diets (% wet weight basis).

Variables (%)	C	$KSE_{0.25}$	$KSE_{0.50}$	$KSE_{0.75}$	*P*-value
Moisture	74.27 ± 0.01	74.30 ± 0.02	74.24 ± 0.14	74.49 ± 0.09	0.221
Crude protein	16.42 ± 0.18	16.58 ± 0.05	16.63 ± 0.13	16.36 ± 0.16	0.486
Crude lipid	4.39^{c} ± 0.03	4.24^{bc} ± 0.08	4.12^{ab} ± 0.03	3.97^{a} ± 0.01	0.001
Total ash	3.99 ± 0.07	4.06 ± 0.12	4.04 ± 0.014	4.04 ± 0.01	0.965

Data expressed as Mean ± SE ($n = 3$); Mean values in the same row with different superscript differ significantly ($P < 0.05$).

3.5 Metabolic enzymes activities

3.5.1 Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH)

Dietary KSE inclusion significantly lowered ($P < 0.05$) the LDH activity in the liver of rohu, *L. rohita* fingerlings (Table 5). Maximum and minimum hepatic LDH activities were observed in C and $KSE_{0.75}$ fed groups, respectively. However, LDH activity in muscle did not differ ($P > 0.05$) among the different treatments. The MDH activity in the liver and muscle of *L. rohita* fingerlings was significantly influenced by dietary KSE inclusion ($P < 0.05$). MDH activities in these tissues exhibited a negative linear relationship with the level of dietary KSE inclusion ($P < 0.05$).

3.5.2 Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The AST and ALT activities in the liver and muscle differed significantly ($P < 0.05$) among the various treatment groups (Table 5). The highest AST and ALT activities were observed in the $KSE_{0.50}$ fed groups, showing a linear relationship with dietary KSE inclusion ($P < 0.05$).

3.6 Challenge study

3.6.1 Survival

The challenge study results demonstrated that including KSE in the diet offers improved protection for the fish against *A. hydrophila*. The relative percentage survival (RPS) and cumulative survival percentage of rohu fingerlings of the different experimental groups were presented in Figures 2, 3. Mortalities significantly decreased ($P < 0.05$) in fish fed a diet containing extract ($KSE_{0.50}$) compared to the control. The highest RPS (%) was observed in fish fed with 5 g kg^{-1} khejri seed extract (KSE).

3.7 Anti-oxidative enzymes activities

3.7.1 Superoxide dismutase (SOD) and catalase (CAT) activities

SOD and CAT activities in the liver and gills of fish showed significant differences ($P < 0.05$) between the control (C) and KSE-fed groups in both pre- and post-challenge studies (Table 6). The SOD and CAT activities in the liver and gills of *L. rohita* fingerlings exhibited the following pattern: C > KSE0.25 > KSE0.50 > KSE0.75. Statistical analysis (paired *t*-test) indicated a significant increase in SOD and CAT activities in the liver and gills of rohu fingerlings during post-challenge study. But, KSE fed groups exhibited lower SOD and CAT activities than control group.

3.7.2 Serum protein biochemistry

The serum protein indices, including total protein, albumin, globulin contents, and the A/G ratio of *L. rohita* fingerlings, are presented in Table 7. Dietary KSE inclusions significantly enhanced ($P < 0.05$) the serum total protein contents in rohu fingerlings compared to control (C) during the pre-challenge study. It was maximum in $KSE_{0.50}$ (3.43 ± 0.06) fed groups while minimum in C and $KSE_{0.50}$ fed groups. Albumin, globulin contents, and A/G ratio were found to be non-significant in a pre-challenge study ($P > 0.05$). Statistical analysis using paired *t*-tests revealed a significant increase in total protein and globulin contents, along with a decrease in the A/G ratio of fish during the post-challenge study ($P < 0.05$). The highest levels of total protein, albumin, and globulin were observed in the $KSE_{0.50}$ and $KSE_{0.75}$ fed groups ($P < 0.05$). However, the A/G ratio was higher in the treatment groups compared to the control during the post-challenge study.

3.8 Haemato-immunological parameters

3.8.1 Nitro blue tetrazolium (NBT) and glucose contents

The NBT activity was significantly increased ($P < 0.05$) in the groups fed with KSE, both before and after the challenge study (Table 8). Statistical analysis of paired *t*-test revealed that NBT activity was significantly enhanced during the post-challenge study. Maximum NBT activity was observed in $KSE_{0.50}$ and $KSE_{0.75}$ fed groups during the pre- and post-challenge study. Dietary inclusion of KSE significantly lowered the serum glucose content in *L. rohita* fingerlings ($P < 0.05$) in both pre-and post-challenge studies. Statistical analysis of paired *t*-test revealed that serum glucose content was significantly increased during the post-challenge study. Maximum glucose content was noticed in C (control) during pre- and post-challenge studies, and minimum in $KSE_{0.50}$ &$KSE_{0.75}$ during pre-challenge studies, and$KSE_{0.75}$ fed groups during post-challenge studies, respectively.

3.8.2 Hematological parameters

Dietary inclusion of KSE significantly affected the hemoglobin (Hb) content and total erythrocyte count (TEC) in *L. rohita* fingerlings in a pre-challenge study (Table 9). Maximum Hb

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TABLE 5 Metabolic enzymes activities of *L. rohita* fingerlings fed with different experimental diets.

Variables	LDH		MDH		AST		ALT	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
C	$4.22^{d} \pm 0.14$	8.32 ± 0.45	$0.71^{c} \pm 0.03$	$2.40^{c} \pm 0.11$	$15.08^{a} \pm 0.58$	$5.46^{a} \pm 0.21$	$8.62^{b} \pm 0.18$	$2.73^{a} \pm 0.08$
$KSE_{0.25}$	$3.16^{c} \pm 0.01$	9.15 ± 0.91	$0.70^{c} \pm 0.02$	$1.48^{b} \pm 0.04$	$16.29^{ab} \pm 0.63$	$6.20^{ab} \pm 0.05$	$9.21^{bc} \pm 0.16$	$3.04^{a} \pm 0.16$
$KSE_{0.50}$	$2.68^{b} \pm 0.02$	8.37 ± 0.43	$0.56^{b} \pm 0.04$	$1.28^{ab} \pm 0.05$	$17.66^{b} \pm 0.30$	$6.98^{b} \pm 0.47$	$9.76^{c} \pm 0.27$	$3.66^{b} \pm 0.13$
$KSE_{0.75}$	$2.31^{a} \pm 0.06$	9.06 ± 0.70	$0.44^{a} \pm 0.03$	$1.05^{a} \pm 0.07$	$14.95^{a} \pm 0.51$	$5.83^{a} \pm 0.29$	$7.74^{a} \pm 0.16$	$3.10^{a} \pm 0.10$
***P*-value**	<0.001	0.722	<0.001	<0.001	0.021	0.033	0.001	0.004

Data expressed as Mean ± SE ($n = 6$); Mean values in the same column with different superscripts differ significantly ($P < 0.05$).
LDH: specific activity expressed as Units^{-1} min^{-1} mg protein^{-1} at 37°C.
MDH: specific activity expressed as Units^{-1} min^{-1} mg protein^{-1} at 37°C.
AST specific activities expressed as Nano moles of oxaloacetate released min^{-1} mg protein^{-1} at 37°C.
ALT: specific activities expressed as Nano moles of sodium pyruvate formed min^{-1} mg protein^{-1} at 37°C.

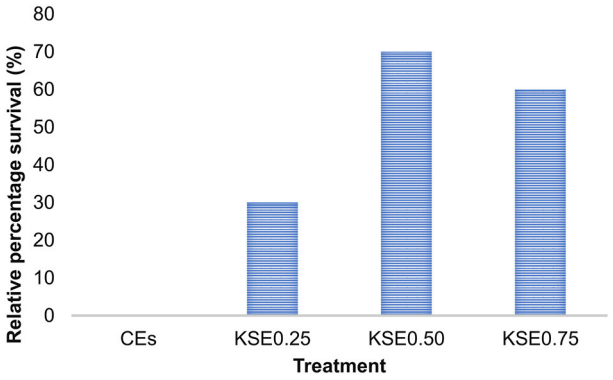

FIGURE 2
Relative percentage survival of rohu, *L. rohita* fingerlings challenged with *A. hydrophila* for 15 days.

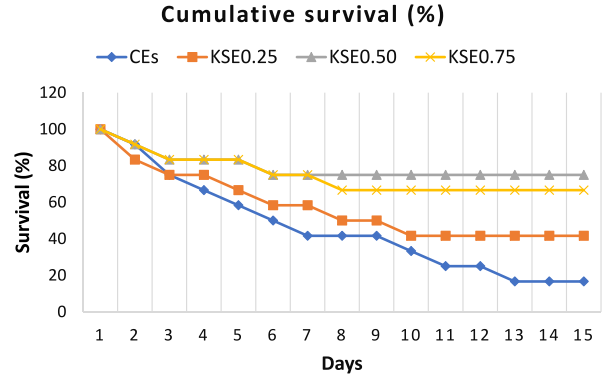

FIGURE 3
Cumulative survival percentage of *L. rohita* fingerlings challenged with *A. hydrophila* (0 to 15 days).

content and TEC count were observed in the $KSE_{0.50}$ fed group and the minimum in the C fed group. Statistical analysis of paired *t*-tests stated an enhancement in Hb content and TEC count during the post-challenge study, with the highest values in $KSE_{0.50}$ and $KSE_{0.75}$ fed group ($P < 0.05$). However, dietary KSE inclusion did not significantly influence TLC ($P > 0.05$). TLC count was significantly higher in treatment groups than control-fed group during the post-challenge study ($P < 0.05$). The highest TLC count was observed in fish fed with 5 g kg^{-1}and 7.5 g kg^{-1} KSE-supplemented diet.

4 Discussion

The use of natural immunostimulants in aquaculture could be an effective approach for preventing and managing fish diseases. As these substances offer environmentally friendly solutions, provide a sustainable option that diverge from chemotherapy, and contribute to a conducive environment within the host organism thereby offering eco-friendly preventive measures. These extracts have demonstrated several benefits including improved growth, enhanced feed utilization, stress reduction, bolstered immune

TABLE 6 Oxidative stress enzymes activities of *L. rohita*fingerlings during pre and post challenges conditions.

Variables	SOD				Catalase			
	Liver		Gill		Liver		Gill	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
C	$4.02^{cA} \pm 0.12$	$8.80^{dB} \pm 0.10$	$4.90^{cA} \pm 0.09$	$9.30^{dB} \pm 0.19$	$6.80^{cA} \pm 0.27$	$13.87^{dB} \pm 0.39$	$3.61^{cA} \pm 0.06$	$9.73^{dB} \pm 0.18$
$KSE_{0.25}$	$3.32^{bA} \pm 0.06$	$8.20^{cB} \pm 0.05$	$4.30^{bA} \pm 0.30$	$7.76^{cB} \pm 0.16$	$5.70^{bA} \pm 0.27$	$9.87^{cB} \pm 0.59$	$2.84^{bA} \pm 0.11$	$7.99^{cB} \pm 0.17$
$KSE_{0.50}$	$2.97^{bA} \pm 0.17$	$7.46^{bB} \pm 0.14$	$3.10^{aA} \pm 0.05$	$6.72^{bB} \pm 0.16$	$5.23^{abA} \pm 0.13$	$7.53^{bB} \pm 0.12$	$2.34^{aA} \pm 0.11$	$6.79^{bB} \pm 0.08$
$KSE_{0.75}$	$2.35^{aA} \pm 0.11$	$6.73^{aB} \pm 0.06$	$3.02^{aA} \pm 0.05$	$6.04^{aB} \pm 0.15$	$4.60^{aA} \pm 0.20$	$5.70^{aB} \pm 0.06$	$2.14^{aA} \pm 0.04$	$5.53^{aB} \pm 0.18$
P-value	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001

Data expressed as Mean ± SE ($n = 6$); Mean values in the same column with different lowercase superscript and same row with different uppercase superscript differ significantly ($P < 0.05$).
SOD (superoxide dismutase) activity is expressed as 50% inhibition of epinephrine auto- oxidation min^{-1} mg $protein^{-1}$.
CAT (catalase) activity expressed as nanomoles H_2O_2 decomposed min^{-1} mg $protein^{-1}$.

TABLE 7 Serum protein biochemistry of rohu, *L. rohita* fingerlings during the pre and post challenged conditions.

Variables	Total protein (g dl^{-1})		Albumin (g dl^{-1})		*Globulin (g dl^{-1})		#A:G ratio	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
C	$3.08^{aA} \pm 0.06$	$3.32^{aB} \pm 0.04$	$1.09^{A} \pm 0.04$	$1.02^{aB} \pm 0.04$	$1.99^{A} \pm 0.09$	$2.30^{aB} \pm 0.04$	0.55 ± 0.04	$0.44^{a} \pm 0.02$
$KSE_{0.25}$	$3.27^{abA} \pm 0.08$	$3.53^{bB} \pm 0.03$	$1.13^{A} \pm 0.03$	$1.22^{bB} \pm 0.03$	$2.14^{A} \pm 0.05$	$2.31^{aB} \pm 0.04$	0.53 ± 0.00	$0.53^{b} \pm 0.02$
$KSE_{0.50}$	$3.43^{bA} \pm 0.06$	$3.75^{cB} \pm 0.05$	$1.16^{A} \pm 0.03$	$1.29^{bcB} \pm 0.04$	$2.27^{A} \pm 0.03$	$2.46^{bB} \pm 0.03$	0.51 ± 0.00	$0.52^{b} \pm 0.01$
$KSE_{0.75}$	$3.18^{aA} \pm 0.06$	$3.83^{cB} \pm 0.06$	$1.15^{A} \pm 0.04$	$1.34^{cB} \pm 0.02$	$2.03^{A} \pm 0.06$	$2.49^{bB} \pm 0.04$	0.57 ± 0.03	$0.54^{b} \pm 0.00$
***P*-value**	0.028	<0.001	0.513	0.001	0.052	0.008	0.440	0.014

Data expressed as Mean ± SE ($n = 6$); Mean values in the same column with different lowercase superscript and same row with different uppercase superscript differ significantly ($P < 0.05$).
*Globulins (g dl^{-1}) = Total protein (g dl^{-1}) - Albumin (g dl^{-1}).
#Albumin-to-globulin ratio (A:G ratio) was determined by dividing the albumin values by the globulin values.

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TABLE 8 Respiratory burst activity (NBT) and glucose content of rohu, *L. rohita* fingerlings during the pre and post challenged conditions.

Variables	*NBT		Glucose (mg dl^{-1})	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
C	$0.23^{aA} \pm 0.01$	$0.25^{aB} \pm 0.01$	$83.48^{cA} \pm 1.18$	$141.56^{dB} \pm 2.61$
KSE$_{0.25}$	$0.27^{bA} \pm 0.01$	$0.28^{bB} \pm 0.01$	$78.54^{bA} \pm 0.50$	$126.29^{cB} \pm 2.36$
KSE$_{0.50}$	$0.29^{bA} \pm 0.00$	$0.31^{cB} \pm 0.01$	$65.87^{aA} \pm 2.11$	$104.56^{bB} \pm 1.92$
KSE$_{0.75}$	$0.28^{bA} \pm 0.01$	$0.32^{cB} \pm 0.01$	$69.68^{aA} \pm 0.37$	$92.89^{aB} \pm 1.73$
***P*-value**	0.003	<0.001	<0.001	<0.001

Data expressed as Mean ± SE ($n = 6$); Mean values in the same column with different lowercase superscript and same row with different uppercase superscript differ significantly ($P < 0.05$).
*NBT: Nitro-blue tetrazolium.

function, and heightened metabolic activity in animals (Tejaswini et al., 2024; Rashidian et al., 2020; Sundararajan et al., 2024). *Prosopis spp.* are recognized for containing bioactive compounds such as alkaloids, flavonoids, terpenes, and phenolic compounds (Gurib-Fakim, 2006). The aim of this study was to investigate the effect of khejri seed extract as an antioxidant on the growth, immunity and disease of *L. rohita* against *A. hydrophilia.*

The supplementation of khejri seed extract (KSE) to the diet of *L. rohita* had a significant impact on various growth rates and protein utilization indices. Dietary KSE supplementation improved the growth (WG, WG%, SGR) and nutrient utilization (FCR, PER, FER, and ANPU) parameters. It suggests that bioactive compounds present in KSE have growth-promoting effects. Earlier study of Mahdavi et al. (2013) suggested that aloe vera extract can be used as a growth promoter, appetite stimulant, tonic, and immune-stimulant in fish. Similarly, growth-promoting effects have been demonstrated in different fishes fed with different plant-based extracts *viz. Apium graveolens* leaf extract in *L. chrysophekadion* (Sutthi et al., 2020), shatavari herb feed in *L. rajasthanics* (Keer et al., 2020), and lemon peel extract in *L. rohita* (Tejaswini et al., 2024). Hepatosomatic index (HSI) showed significant differences in response to diet, consistent with the findings of Syed et al. (2022) showed that Nile tilapia feeding groups fed different levels of aloe vera extract had lower HSI compared to the control group. However, there was no significant difference in visceral body measurements between the control and other treatment groups. Furthermore, no mortality was observed in *L. rohita* fingerlings throughout the entire feeding trial, indicating that there was not any toxicity from KSE and results are in agreement with finding of Adeshina et al. (2019) in *Clarias gariepinus* fed with *Eugenia caryophyllata* bud extract.

Digestive enzymes play a vital role in breaking down the nutrients, which are then metabolized to either assimilate nutrients or meet energy demands (Furne et al., 2005). Nutrients from the feed are broken down in the fish gut with the help of digestive enzymes like lipase, protease, and amylase (Nopitawati and Jusadi, 2015). The inclusion of herbal preparations in feed can enhance the release of digestive fluids and promote intestinal motility, potentially leading to increased feed intake and growth in fish (Pu et al., 2017). In this study, a significant increase in digestive enzyme activity (protease and lipase) was observed in groups fed with KSE compared to the control group, while amylase activity did not differ between the groups. This suggests that supplementation with 5 g kg^{-1} KSE promoted the secretion of protease and lipase, thereby enhancing nutrient digestion and absorption, ultimately leading to improved growth in *L. rohita*. It had been reported that phyto-additives may enhance gut health by promoting the growth of beneficial microbes, which in turn could modulate the activity of digestive enzymes in fish (Hashemi and Davoodi, 2011). These findings are supported by Bilen et al. (2018), who reported a significant improvement in lipase and protease activities in *Cyprinus carpio* fed with *Anethum graveolens* and *Lepidium sativum*.

Moisture, protein (CP) and total ash (TA) contents did not differ in control and KSE-fed groups. It indicates that KSE did not have a significant impact on the biochemical constituents of *L. rohita*. Similar findings have been documented in previous studies. For example, Yousefi et al. (2019) observed that supplementation with rosemary leaf in *Cyprinus carpio* did not significantly affect whole-body composition. Likewise, Keer et al. (2020) reported that feeding *L. rajasthanics* with shatavari extract had no notable impact on whole-body composition. However, a decrease in crude lipid content was observed with dietary KSE levels. It infers that bio-active compounds in KSE might have hypolipidemic properties. Previous studies also witnessed the hypolipidemic and hypercholesterolemic properties of khejri bean in mice (Agarwal and Chauhan, 1988) and pod extract in rabbits (Ram et al., 2020), respectively.

In our study, fish fed with 2.5 and 5.0 g KSE kg^{-1} diet exhibited higher AST and ALT activities compared to the control. This finding aligns with Abalaka et al. (2011), who reported enhanced ALT and AST activities in *Clarias gariepinus* when fed with aqueous and ethanolic pod extracts from *Parkia biglobasa*. Moreover, fish on diets supplemented with KSE extract exhibited reduced LDH activity in both muscle and liver compared to the control group. The reduced LDH activity in the treatment groups indicates a stress-mitigating effect of dietary KSE plant extracts in *L. rohita*. A similar decreasing trend in LDH activity with herbal plant extract supplementation was also observed by Kazeem et al. (2017). Malate dehydrogenase (MDH), an enzyme involved in the TCA cycle that catalyzes the conversion of malate to oxaloacetate and vice versa (Hemre et al., 2002), showed decreased activity in our study with dietary KSE supplementation compared to the control. Likewise, Garg et al. (2019) found lower LDH and MDH activity in *L. rohita* fingerlings fed *Houttuynia cordata* leaf extract, supporting the stress-mitigating role of phytochemicals present in KSE.

TABLE 9 Hematological parameters of *L. rohita* fingerlings during pre and post challenged conditions.

Variables	Hemoglobulin (g dl^{-1})		[1]TEC (10^6 cells mm^{-3})		[2]TLC (10^{3}cells mm^{-3})	
	Pre challenge	Post challenge	Pre challenge	Post challenge	Pre challenge	Post challenge
C	$7.94^{aA} \pm 0.26$	$8.19^{aB} \pm 0.06$	$1.58^{aA} \pm 0.09$	$1.84^{aB} \pm 0.06$	$187.63^{A} \pm 4.16$	$220.09^{aB} \pm 4.46$
KSE$_{0.25}$	$8.78^{bA} \pm 0.17$	$9.01^{bB} \pm 0.12$	$1.85^{bA} \pm 0.07$	$2.24^{bB} \pm 0.07$	$192.63^{A} \pm 4.39$	$238.34^{bB} \pm 4.13$
KSE$_{0.50}$	$9.26^{bA} \pm 0.08$	$9.53^{cB} \pm 0.03$	$2.22^{cA} \pm 0.03$	$2.44^{bcB} \pm 0.08$	$199.40^{A} \pm 2.24$	$264.35^{cB} \pm 4.77$
KSE$_{0.75}$	$8.68^{bA} \pm 0.25$	$9.49^{cB} \pm 0.06$	$1.93^{bA} \pm 0.09$	$2.54^{cB} \pm 0.05$	$190.41^{A} \pm 3.44$	$268.93^{cB} \pm 5.52$
***P*-value**	0.012	<0.001	0.003	<0.001	0.210	<0.001

Data expressed as Mean ± SE ($n = 6$); Mean values in the same column with different lowercase superscript and same row with different uppercase superscript differ significantly ($P < 0.05$).
[1]TEC: Total erythrocyte counts.
[2]TLC: Total leucocyte counts.

In this study, groups fed with khejri seed extract (KSE) had lower activities of antioxidant enzymes (SOD and CAT) compared to the control group during both pre- and post-challenge studies. This suggests the seed extract may effectively scavenge or neutralize free radicals produced by oxidative stress caused by pathogenic organisms. The phenolic compounds found in khejri seed extract likely play a significant role in these scavenging, corroborating the findings of Henciya et al. (2017) and Chaudhary et al. (2018). Additionally, Fawole et al. (2018) also reported similar results indicating that ethanolic leaf extracts from *Psidium guajava* and *Mangifera indica* reduced SOD and CAT activities in rohu.

In this study, it was clearly demonstrated that rohu fingerlings fed dietary KSE exhibited a significant enhancement in serum total protein levels. However, the albumin and globulin contents remained consistent across both control and treatment groups. Notably, infected fish consuming KSE showed substantial increases in serum total protein, albumin, globulin contents, and higher albumin-globulin ratio. This rise in serum total protein and globulin levels indicates an augmentation in immunoglobulin concentration, particularly within the gamma fractions of globulin. These findings support the assertion that bioactive compound in KSE enhance the non-specific immune system and substantially improve the overall health of infected fish. Moreover, similar effects were observed by Das et al. (2015) with extracts from *Ocimum sanctum* (Tulsi), which significantly elevated total immunoglobulin levels in plasma alongside serum total protein, globulin, total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin, superoxide anion production, and lysozyme activity, all in comparison to the control group. Additionally, Sutthi et al. (2020) also observed marked increases in serum total protein, albumin, and globulin levels in *Labeo chrysophekadion* fed with ethanolic leaf extract of *Apium graveolens,* which further aligns with the findings of Fawole et al. (2016).

Dietary supplementation of KSE positively affected NBT activity in both pre- and post-challenge studies. The enhanced NBT activity confirms increased phagocyte activity, which h is essential for effectively eliminating bacterial pathogens (Sahu et al., 2007). Fish fed with 5.0 and 7.5 g KSE kg^{-1} diet exhibited maximum NBT activities, aligning with Musthafa et al. (2018)' findings in Nile tilapia that utilized *Mucuna pruriens* seed meal extract. Furthermore, our study confirmed that KSE supplementation led to a reduction in glucose levels in the fish in both pre and post-challenge studies, indicating a lower stress response (Citarasu et al., 2006). This finding is supported by Heydari et al. (2018), who observed lower glucose levels in diabetic rats fed with hydroalcoholic fruit extract of *Prosopis farcta*. The anti-diabetic properties of *P. farcta* extract are in line with the findings of Soni et al. (2018).

In this study, the dietary KSE supplementation increased the total erythrocyte count (TEC) and hemoglobin content in rohu fingerlings under both pre- and post-challenge conditions. This enhancement in TEC count and Hb content may contribute to improved oxygen supply during oxidative stress induced by pathogenic infections. While the white blood cell count was not changed during pre-challenge conditions. It was increased in infected fish, possibly indicating enhanced non-specific immunity. This finding is consistent with Amirkhani and Firouzbakhsh (2015), who observed an increase in WBC, Hb, and TEC counts in common carp fingerlings fed with basil (*Ocimum basilicum*) ethanolic extract compared to the control. Similarly, Kaleeswaran et al. (2011) reported the significant improvements in hematological and biochemical parameters of *Catla catla* fed with *Cynodondactylon* ethanolic extract. These results align with the findings of other researchers such as Giri et al. (2017) and Chowdhury et al. (2021).

No mortality was observed in rohu fingerlings during the pre-challenge study, indicating the absence of toxicity from khejri pod extract in fish. Our study demonstrated that supplementation of khejri seed extract enhanced the survival of infected *L. rohita* fingerlings, and Fish fed with the 5.0 g kg^{-1} dietary inclusion of khejri seed extract exhibited the highest relative percentage survival. Similarly, various studies have reported improved survival in fish against *A. hydrophila* with dietary supplementation of different herbal extracts (Basha et al., 2013; Palanikani et al., 2020).

Additionally, the findings from the post-challenge study against *A. hydrophila* clearly showed that rohu fingerlings fed KSE exhibited enhanced resistance. This was evident through improved innate immune responses, including significant increases in hematological indices (TEC, WBC, and hemoglobin), NBT activity, and serum proteins (total protein, albumin, globulin, and the albumin-globulin ratio) when compared to the control group. Additionally, KSE-fed groups exhibited reduced stress enzyme activities and lower serum glucose levels compared to the control, firmly asserting the efficacy of khejri seed extract in

improving fish surivival, health and immunity. Therefore, it can be concluded that the 5.0 g kg^{-1} KSE dietary supplementation improved the growth performance, feed conversion, digestive enzymes, metabolic enzyme activities, survival, and immune status of *L. rohita* fingerlings.

5 Conclusion

Dietary khejri seed extract (KSE) significantly influenced the growth performance, nutrient utilization, and body indices of rohu fingerlings. At an inclusion of 5.0 g kg^{-1}, KSE promoted growth without altering digestive enzyme activities. Hematological parameters and certain serum biochemical markers also improve in KSE-fed groups. Post-challenge studies clearly indicate that stress enzyme activities and hematological measures were significantly enhanced compared to the control group. Most importantly, the KSE fed groups consistently achieved the significantly lowest mortality rates in infected *L. rohita* fingerlings. Therefore, it is unequivocal that ethanolic seed extracts of *P. cineraria* could serve as powerful growth promoters and herbal immunostimulants, effectively enhancing growth and strengthening non-specific immune responses in carps.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the Corresponding authors.

Ethics statement

The animal study was approved by the ethical procedures for the Animal Care as guided by the Ethical Committee of ICAR-CIFE, Mumbai, India was strictly adhered to conduct the current study. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RY: Investigation, Software, Writing – original draft, Formal analysis. MJ: Data curation, Methodology, Writing – review & editing. NC: Conceptualization, Writing – review & editing. VS: Methodology, Resources, Writing – review & editing. PS: Formal analysis, Writing – review & editing. MO: Formal analysis, Resources, Writing – review & editing. BS: Supervision, Writing – review & editing. DM: Formal analysis, Writing – review & editing.

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Conflict of interest

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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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