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Original article

Effect of dietary supplementation of Neem, *Azadirachta indica* leaf extracts on enhancing the growth performance, chemical composition and survival of rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

Rainbow trout *Oncorhynchus mykiss* has a great nutritional value and delicious taste. A 90-days experimental trial was conducted to investigate the effect of dietary leaf extract of neem tree *Azadirachta indica* as a feeding supplement on the growth performance and proximate composition of *O. mykiss*. Four experimental diets were designed as T_1 (with 5% *A. indica* leaf extract), T_2 (with 7% of *A. indica* leaf extract), T_3 (with 10% *A. indica* leaf extract), and T_4 (control group feed with a regular diet with 0% *A. indica* leaf extract). The average initial weight of fry 0.4 ± 0.14 g was stocked at 25 fish/tank with two replicates per treatment (4 × 2 = 8). After 90 days of the experimental trial, One-way ANOVA showed significant differences in final body weight, weight gain, specific growth rate, feed conversion ratio, and survival rate among the treatment groups ($p < 0.05$). The highest final body weight (48.10 g) and weight gain (47.70 g) was observed in T2 with 7% *A. indica* leaf extract, which was significantly different from the other treatments ($p < 0.05$). The lowest FCR was recorded in T2 (1.90), which was significantly different compared to other treatment groups ($p < 0.05$). Inclusion of *A. indica* leaf extract in formulated feed for rainbow trout had significant effects in the hepatosomatic index, viscerosomatic index and Fulton's condition factor ($p < 0.05$), but there was no significant difference in the survival rate of rainbow trout fry treated with different experimental diets ($p > 0.05$). The phenomenal regression indicates that 7.5% *A. indica* inclusion is optimum for best growth performance for rainbow trout under a controlled environment. Thus, the present study suggests that the dietary leaf extract has performed an excellent nutritional supplement by enhancing growth performance and health conditions of rainbow trout in the hatchery conditions.

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1. Introduction

The world population is increasing at an alarming rate and a 30% increase is expected by 2050. When all the people at all the times have easy access physically and economically to safe, sufficient and healthy, good nutritionally balanced food to fulfill their requirement needs for healthy and productive life (FAO, 2009; Hassan et al., 2021a). Currently, aquaculture actually plays a crucial role in the culture of aquatic species for food and nutritional

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reliability. It is the fastest increase in the food production industry to reduce wild stock overexploitation and keep the ecosystem from declining. (Fao, 2014; Hassan et al., 2021c; Hussain et al., 2021). Inland aquaculture production has been increased from 29.9 to 41.9 million tons from 2012 to 2017. Rainbow trout, *Oncorhynchus mykiss* is one of the most suitable aquaculture species with high profit and nutritional value. This cold-water species has a high customer preference due to its taste and flesh quality (Adel and Khara, 2016). Rainbow trout is an exotic carnivore species that can be kept at 0 to 25 °C, while 13 to 18 °C is most favorable for their growth and feeding. Rainbow trout flesh contains high proteins and low carbohydrates, which is good for human health (Polakof et al., 2012).

During the early life stages, larvae and juveniles are particularly susceptible to certain diseases or infections due to various environmental stresses, producing significant economic losses for the fish hatchery. Using synthetic chemicals in fish farms or hatcheries to control some parasitic diseases and predators might cause ecological damage and threaten aquatic animal health. The inclusion of different herbal products as a dietary supplement plays a crucial role in maintaining the health status and survival of fish species. Herbs are currently being used as growth-promoting substances, antimicrobial agents, immunestimulants and nutrient sources in commercially formulated feeds for fish and shellfish species (Citarasu, 2010; AftabUddin et al., 2017). Therefore, researchers are searching for cheap and environment-friendly bioactive compounds from herbs, which have very low or non-toxic impacts on most aquaculture species. Recently, many plants, including *Aloe vera, Andrographis pariculata, Annona squamosa,* Neem tree *Azadirachta indica, Citrus aurantifolia, Coriandrum sativum, Ocimum sanctum, Ollium cepa and Psidium guajava,* and different seaweeds have been used as a natural immunostimulant for preventing several fish and shellfish diseases (Nya and Austin, 2009; Pedge and Ahirrao, 2012; AftabUddin et al., 2017; Kaur et al., 2020; AftabUddin et al., 2021; Syed et al., 2021). Among these various herbs, *A. indica* is the most promising medicinal and commercially exploitable plant widely available in most tropical and sub-tropical regions of the world.

A. indica is a large evergreen tree with aromatic leaves and edible fruits and has widely been used for various purposes because of its immunological, anti-inflammation, and anti-ulcer properties (Shah et al., 2009; Talpur and Ikhwanuddin, 2013; Kaur et al., 2020). Moreover, every part of *A. indica* tree contain a wide range of pharmacological properties against certain fungal, bacterial, and viral infections and boost antioxidant properties (Talpur and Ikhwanuddin, 2013; Adamu et al., 2018). It can be used as a natural insecticide, pesticides, and molluscicide to kill or control these aquatic parasites (El-Badawi et al., 2015). Similarly, several neem-based products have been widely used in fish farms as an alternative to some toxic pesticides or antibiotics for controlling various fish parasites and fish fry predators during their culture in lakes, rivers, or streams. Hence, using *A. indica* plant extracts in the fish industry has been encouraging due to their operative role in enhancing immunity against certain fish diseases and suppressing specific pathogens (FAO, 2007; Binh, 2016). However, the potential effects of *A. indica* leaf extract on the growth and survival of cold-water species, e.g., rainbow trout, have been less studied. Therefore, the objective of the present study was to investigate the effects of *A. indica* leaf extracts on the growth performance and survival of rainbow trout fry.

2. Materials and methods

2.1. Experimental trials and diet preparation

This trial was conducted in the Trout Fish Hatchery of Madyan valley of Swat District of Khyber Pakhtunkhwa province (35°14′0732″N, 72°54′9529″E) of Pakistan (Fig. 1) from August to October 2020. The current study was conducted in eight tanks. There were four treatments designated as T1 (with 5% *A. indica* leaf extract), T2 (with 7% *A. indica* leaf extract), T3 (with 10% *A. indica* leaf extract) and T4 (Control group without *A. indica* leaf extract) were fed with experimental diets for 90 days. Each treatment had two replications. Rainbow trout fry had an average initial body weight of 0.4 ± 0.14 g and an average body length of 2.2 ± 0.03 cm. These fish were acclimatized for two weeks in the rearing tank before the experiment. Two hundred individuals were randomly distributed at a stocking density of 25 fish/tank with 1000 L of water capacity and feeding rate adjusted according to fish biomass on a weekly basis. The dietary protein level was 35%, and feeding frequency was four times per day. The ingredients used for these formulated feeds include, i.e., (a) Butylated hydroxytoluene (BHT), (b) wheat flour, (c): Vitamin minerals, (d) Vitamin premix, (e) bone meal, (f) Soya bean meal, (g) dried milk, (h) Brewer yeast, (i) rice bran, (j) soya bean oil, (k) fish meal, (l) Choline chloride, (m) meat meal, (n) Vitamin C (see Table 1 and Table 2). The daily water exchange rate was approximately 30%. Continuous aeration was provided using an air blower and submerged air diffusers.

2.2. Physicochemical parameters

To maintain the water pH of these tanks, sodium hydroxide (NaOH) was added to raise the pH, and Hydrochloric acid (HCl) was added to reduce pH if required. The total water hardness was maintained by adding the Calcium sulfate ($CaSO_4$) salts to increase its hardness and magnesium sulfate (MgSO4) to decrease the hardness. The scheduled photoperiod during the experimental period was 12L: 12D. The water quality parameters were determined at twelve hours intervals during the experiment. Total dissolved oxygen (mg/L^{-1}) was measured using a portable digital DO-meter (Model: HI 9146, Hanna, UK), the water temperature was determined with a Celsius glass thermometer, pH with a digital pen pH meter (Orion, model 201, USA), and salinity with a handheld refractometer (Hanna, UK). Total hardness ($mg\ L^{-1}$), electric conductivity ($mScm^1$) and total ammonia ($mg\ L^{-1}$) were measured with a ProPlus multiparameter (YSI, USA). After acclimation, 25 healthy *O. mykiss* fry of the same size was randomly stocked in each experimental tank.

2.3. Calculation of growth parameters, survival rate and health indices

The growth parameters, survival rate and health indices were calculated by the following equations (Hassan et al., 2021c).

$$\text{Weight gain (WG, g)} = \text{Final weight - Initial weight}$$

$$\text{Specific growth rate (SGR, \%/day)} = [(\ln \text{FBW} - \ln \text{IBW})/t] \times 100$$

where ln = natural logarithm, FBW = final body weight and IBW = initial body weight, t = time in days.

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake/Weight gain}$$

$$\text{Survival rate (SR, \%)} = (\text{Number of fish at the end/Initial number of fish}) \times 100$$

$$\text{Viscerosomatic index (VSI, \%)} = (\text{Weight of viscera/Gutted weight}) \times 100$$

$$\text{Hepatosomatic index (HSI, \%)} = (\text{Liver weight/Gutted weight}) \times 100$$

$$\text{Fulton's condition factor (K)} = (BW/L^3) \times 100$$

where BW = total body weight and L = total body length.

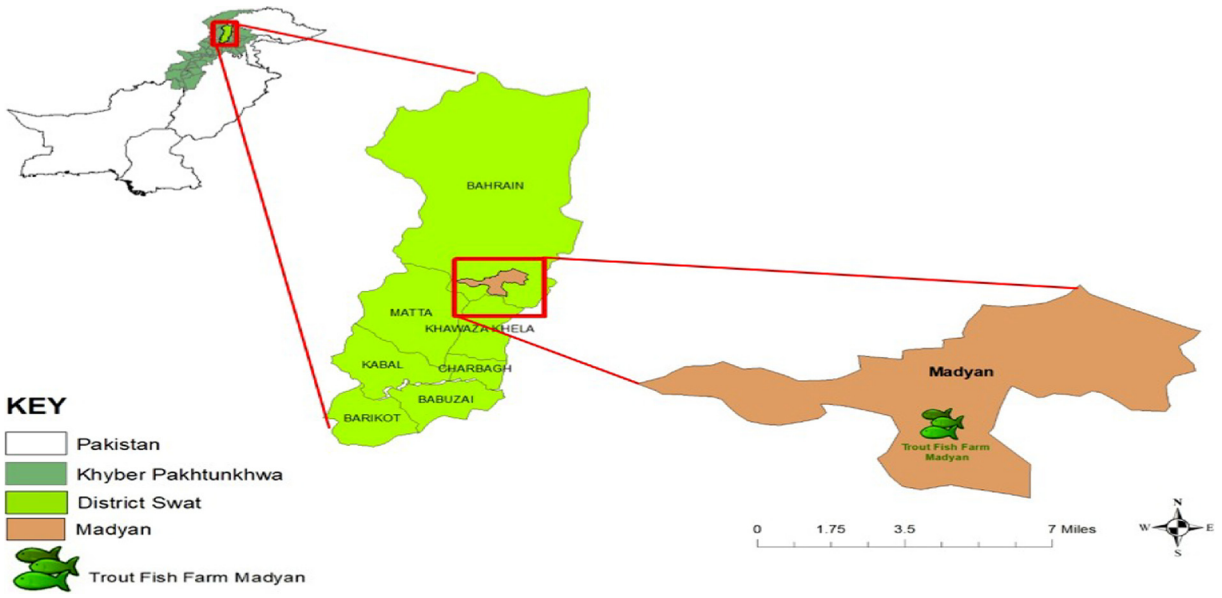

Fig. 1. Study area of Madyan valley of Swat in Khyber Pakhtunkhwa province of Pakistan.

Table 1
Ingredients used to prepare experimental diets for rainbow trout, *O. mykiss*.

Ingredients	T1 (g/100 g)	T2 (g/100 g)	T3 (g/100 g)	Control T4 (g/100 g)
Butylated hydroxytoluene (BHT)	0.1	0.1	0.1	0.1
Wheat flour	20.0	20.0	20.0	20.0
Vitamin Mineral	0.075	0.075	0.075	0.075
Vitamin premix	0.55	0.55	0.55	0.55
Bone meal	1.0	1.0	1.0	1.0
Soya bean meal	12.0	10.0	7.0	12.0
Dry milk	5.0	5.0	5.0	5.0
Brewer yeast	2.0	2.0	2.0	2.0
Rice bran	3.0	3.0	3.0	8.0
Oil (soya bean)	4.0	4.0	4.0	4.0
Fish meal	35.0	35.0	35.0	35.0
Choline chloride	0.2	0.2	0.2	0.2
Meat meal	12.0	12.0	12.0	12.0
Vitamin C	0.075	0.075	0.075	0.075
Azadirachta indica , leaf extract	5	7	10	0
Total	100 g	100 g	100 g	100 g

Table 2
Proximate composition of experimental feed for different treatments.

Treatments	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Moisture (%)	Ash (%)
T1	34.90 ± 0.22	06.22 ± 0.32	08.95 ± 0.22	08.11 ± 0.11	09.60 ± 0.33
T2	35.01 ± 0.01	07.66 ± 0.38	09.39 ± 0.04	08.32 ± 0.22	10.11 ± 0.12
T3	35.03 ± 0.02	07.88 ± 0.08	09.48 ± 0.08	09.21 ± 0.02	10.25 ± 0.33
T4	34.95 ± 0.05	06.01 ± 0.06	07.88 ± 0.89	09.38 ± 0.03	10.33 ± 0.24

2.4. Proximate composition of carcass

After the experimental feeding trials, about five fish samples were collected from each treatment group and then subjected for their proximate composition analysis. Total ash contents, carbohydrate, crude fats, total fibres, crude proteins, dry matters, and moisture contents were analyzed following the methods of AOAC (2000), Siddique et al. (2012) and Khan et al. (2013).

2.5. Statistical analysis

In the present study, data were tested for normality and homogenous variance using a Shapiro-Wilk and Levene's test, respectively. A one-way (ANOVA) using (SAS) version 9.1 were performed to determine the effects of Neem leaf extracts on the growth parameters of rainbow trout fry treated with different formulated feeds. A one-way ANOVA was also performed to compare the water quality parameters in fry-rearing tanks that were treated with different formulated feeds. Data were presented as mean ± SD. Alpha was set at $p < 0.05$.

3. Results

3.1. Growth performance and morphological indices

After 90 days of the experimental trial, One-way ANOVA showed significant differences in final body weight, weight gain, specific growth rate, feed conversion ratio, and survival rate among

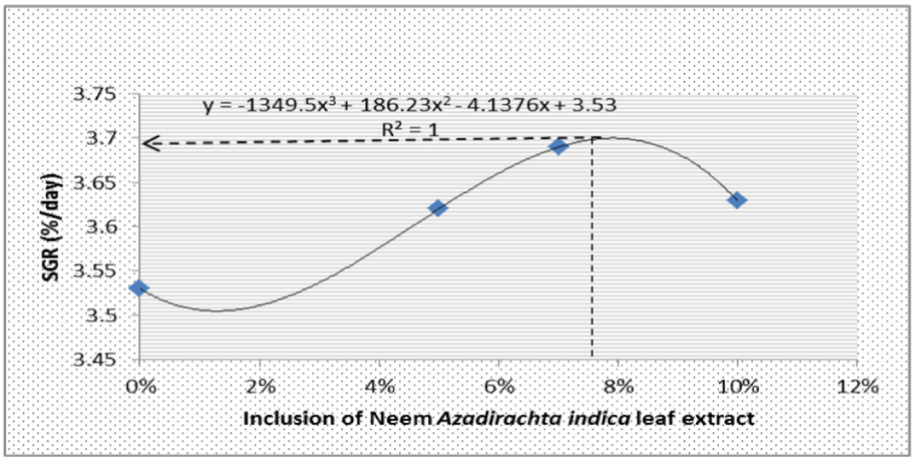

Fig. 2. The optimum supplementation of Neem extract in the dietary protein level of rainbow trout determined by the phenomenal regression.

the treatment groups ($p < 0.05$) (Table 3). The highest final body weight (48.10 g) and weight gain (47.70 g) was observed in T2 with 7% *A. indica* leaf extract, which was significantly different from the other treatments ($p < 0.05$). The lowest FCR was recorded in T2 (1.90), which was also significantly different compared to other treatment groups ($p < 0.05$). In the present study, 5 to 10% inclusion of *A. indica* leaf extract in formulated feed showed significantly higher SGR in T1, T2 and T3, respectively ($p < 0.05$) compared to the control group (with no *A. indica* leaf extract). Inclusion of *A. indica* leaf extract in formulated feed for rainbow trout had significant effects in the hepatosomatic index, viscerosomatic index and Fulton's condition factor ($p < 0.05$, see Table 3), but there was no significant difference in the survival rate of rainbow trout fry treated with different experimental diets ($p > 0.05$). On the other hand, the maximum point of SGR carve was 7.8%, while the determination coefficient (r^2) = 1. The phenomenal regression indicated that the inclusion of 7.5% *A. indica* extract is optimum for best growth performance and survival of rainbow trout under a controlled environment (see Fig. 2).

3.2. Proximate composition of carcass

The proximate composition of fish samples was performed after 12 weeks of the experimental trial. Four fish specimens (whole carcass) were collected from each treatment group to determine the crude proteins, crude lipids, carbohydrates, moisture, dry matter, crude fibre and ash contents (see Table 4). The highest percentage of crude proteins was 70.18 in fishes treated with 5% *A. indica* leaf extract diets, which was significantly different from the other treatments ($p < 0.05$). Neem *A. indica* leaf extract containing diets had significant effects on carcass total carbohydrates, crude lipids, ash, and fibre, among other treatments ($p < 0.05$, see Table 4).

3.3. Physicochemical parameters of experimental trials

During the experimental period, physicochemical parameters of water samples in all tanks were recorded daily. The mean values of every fortnight were calculated for water temperature, pH, total hardness, ammonia, DO, electrical conductivity (Table 5). The overall physicochemical parameters, i.e., water temperature, pH, total hardness, ammonia, DO, and electric conductivity for different treatments groups (T1, T2, T3 and T4) were ranged from 14.5–15.0 °C, 6.9–7.1, 110.7–122.6, 0.4–0.8 mg L^{-1}, 6.2–6.7 mg L^{-1}, and 2.5–32.9 $mScm^{-1}$, respectively. Among the four treatment groups, a significant difference ($p < 0.05$) was observed in total hardness and total ammonia concentration.

4. Discussion

The present study showed that *A. indica* leaf extract is beneficial for the growth of rainbow trout. The maximum weight gain was observed with a mean value of 48.1 g in T2, while the minimum weight gain was recorded at 43.80 g in control (T4). The maximum and minimum ingestion of food was observed in the T1 and T4 (control group) with average values of 90.3 ± 1.0 g and 33.99 ± 0.65 g, respectively. The feed conversion ratio (FCR) was recorded as poor in T1 (2.9), while better for T2 (1.9), respectively. Significant differences were recorded in Fulton's condition factor (CF) throughout the experiment, which was analogous to the findings of Zeng and Naylor (1996). Zeng and Naylor (1996) reported that adding 50 mg/kg formed allicin (a component of garlic) for tilapia

Table 3
Effects of *Azadirachta indica* on growth, feed utilization and survival indices of *Oncorhynchus mykiss*. Values with different superscript in the same raw indicate significant differences ($p < 0.05$).

Attributes	Inclusion of neem *A. indica* leaf extract			
	T1 (5%)	T2 (7%)	T3 (10%)	T4 (0%)
Initial body weight (g)	0.40 ± 0.01	0.40 ± 0.08	0.42 ± 0.11	0.41 ± 0.11
Final body weight (g)	46.10 ± 2.50^{b}	48.10 ± 0.50^{a}	47.28 ± 0.35^{a}	44.20 ± 3.10^{c}
Weight gain (g)	45.10 ± 0.80^{b}	47.70 ± 0.70^{a}	$46.60 \pm .80^{b}$	43.80 ± 0.50^{c}
Feed conversion ratio	2.90 ± 0.0^{a}	1.90 ± 0.0^{d}	2.60 ± 0.0^{b}	2.10 ± 0.0^{c}
Specific growth rate (%/day)	3.62 ± 0.03^{a}	3.68 ± 0.02^{a}	3.65 ± 0.01^{a}	3.50 ± 0.02^{b}
Hepatosomatic index (%)	1.20 ± 0.15^{c}	1.30 ± 0.14^{b}	1.50 ± 0.14^{a}	1.10 ± 0.13^{c}
Viscerosomatic index (%)	3.40 ± 0.31^{b}	3.80 ± 0.21^{a}	3.70 ± 0.41^{a}	3.10 ± 0.21^{c}
Fulton's condition factor	2.80 ± 0.0^{a}	2.90 ± 0.1^{a}	2.80 ± 0.0^{a}	2.20 ± 0.0^{b}
Survival rate (%)	100.00 ± 0.0	100.00 ± 0.0	98.00 ± 0.0	100.00 ± 0.0

Table 4
Proximate composition of rainbow trout *O. mykiss* (whole carcass) treated with different formulated feeds. Data are represented as mean ± standard values for all treatments. Values with different superscript in the same column indicate significant differences ($p < 0.05$).

Treatments	Crude proteins (%)	Carbohydrates (%)	Total lipids (%)	Ash (%)	Total fibre (%)	Moisture (%)	Dry matter (%)
T1	70.18 ± 2.70^{a}	8.83 ± 0.10^{c}	9.17 ± 1.00^{c}	11.45 ± 0.90^{c}	0.37 ± 0.05^{a}	0.35 ± 0.04^{c}	99.65 ± 0.08^{a}
T 2	68.00 ± 1.70^{b}	9.05 ± 0.90^{c}	8.53 ± 1.30^{c}	14.21 ± 0.80^{b}	0.21 ± 0.01^{b}	0.64 ± 0.06^{b}	99.36 ± 0.06^{a}
T 3	65.84 ± 1.60^{b}	13.63 ± 0.30^{a}	10.59 ± 0.90^{b}	9.53 ± 0.60^{d}	0.41 ± 0.02^{a}	0.17 ± 0.04^{d}	99.82 ± 0.04^{a}
T4 (Control)	61.9 ± 1.20^{c}	10.55 ± 0.08^{b}	11.20 ± 0.50^{a}	16.11 ± 0.40^{a}	0.24 ± 0.03^{b}	1.50 ± 0.34^{a}	99.98 ± 0.03^{a}

Table 5
Physicochemical parameters of water samples collected from different treatment groups of rainbow trout, *O. mykiss*. Data are presented as Mean ± Standard deviation. Values with different superscript in the same raw indicate significant differences ($p < 0.05$).

Parameters	T1 (5%)	T2 (7%)	T3 (10%)	Control (T4)
Temperature (°C)	14.9 ± 0.3^{a}	15.0 ± 0.3^{a}	15.0 ± 0.2^{a}	14.5 ± 0.2^{a}
pH	7.0 ± 0.1^{a}	7.1 ± 0.1^{a}	7.1 ± 0.1^{a}	6.9 ± 0.1^{a}
Total hardness	110.7 ± 1.3^{b}	110.9 ± 1.8^{b}	116.0 ± 1.3^{ab}	122.6 ± 1.0^{a}
Total ammonia (mg L^{-1})	0.4 ± 0.3^{b}	0.5 ± 0.2^{b}	0.8 ± 0.3^{a}	0.5 ± 0.1^{b}
Dissolved oxygen (mgL^{-1})	6.5 ± 0.3^{a}	6.5 ± 0.2^{a}	6.7 ± 0.4^{a}	6.2 ± 0.1^{a}
Electrical conductivity (mScm^{1})	2.9 ± 0.2^{a}	2.7 ± 0.1^{a}	2.6 ± 0.1^{a}	2.5 ± 0.1^{a}

fish helped increase 2–3% of weight gain and survival rates after 45 days of trial. The present study revealed that the inclusion of *A. indica* leaf extract in formulated diets of rainbow trout positively influenced the growth of the rainbow trout, which was opposite to the statement given by Omorgerie and Okpanachi (1997), who stated that a low quantity of *A. indica* extracts delayed the growth of cichlid fish. The proximate composition analysis showed significantly higher percentages of proteins, crude fat, fibre, and carbohydrates due to *A. indica* leaf extract supplementation. The mean percentages of total crude proteins, crude lipids, carbohydrates, and ash contents were very close to Craft et al. (2016) and Naeem et al. (2016). Şahin et al. (2011) observed the proximate composition of cultured brook trout (*Salvelinus fontinalis*) and black sea trout (*Salmo trutta labrax*) in comparison with their hybrid, which was also supported our present results. Kaur et al. (2020) observed the effects of dietary supplementations of *A. indica* extracts on the feeding efficiency, growth, survival, immunological, and reproduction properties of certain carp species.

The inclusion of *A. indica* extracts showed a non-toxicity impact on most non-target aquatic species (Oniovosa et al., 2017); therefore, its extract is mostly with fish feed as dietary supplements are considered safe for the culture of most fish species. Oniovosa et al. (2017) investigated that most fish diseases, mainly dragon-fly larval stages that act as fish fry predators, could be easily controlled by using the aqueous extract of *A. indica* leaves in the farming of African catfish, *Clarias gariepinus*. In addition, the haematological and biochemical analysis of Clarias gariepinus was found within normal range, and the level of total proteins, globulin, and lymphocytes increases, which indicates immune stimulation against specific pathogens. Hence, using *A. indica* extracts in the fish industry has been encouraging due to their operative role in enhancing immunity against certain fish diseases and suppressing particular pathogen growth. Binh (2016) has also reported the beneficial or eco-friendly impact of *A. indica* leaf extract on the phytoplankton community and water quality of catfish *Pangasiushy pophthalmus* ponds located in Binh Duong province of Vietnam. A comprehensive study has shown that all water quality parameters were found unchanged except the reduction of dissolved carbon dioxides and no change in the species composition and frequency of occurrence in whole phytoplankton communities located in these fishponds. Dinda et al. (2020) and Kaur et al. (2020) observed that 1.0 g/kg of a dietary leaf extract from *A. indica* when incorporated in the basal diet like rice bran and mustard meal in 1:1 ratio) of *Cyprinus carpio* revealed the high reproductive capacity by increasing fecundity rates or ova diameter and increasing its feeding efficiency, growth performance, and immunological response against certain diseases. Thus, it increases the growth and survival rates of *C. carpio*.

Several studies revealed that most extracts from various parts of *A. indica* plants might have significant antioxidant properties that can considerably protect the animals from oxidative stress. Nowadays, *A. indica* has successfully used aquaculture systems to control certain fish parasites or predators. Though the Neem-based extracts are mostly considered target-specific and non-toxic to untargeted aquatic life, the aqueous extracts of the bark from *A. indica* might sometimes produce respiratory issues in tilapia upon its long exposure (El-Badawi et al., 2015). Even low concentrations of crude extracts from the *A. indica* can delay the growth performance of cichlid fishes (El-Badawi et al., 2015). It has been observed that various Neem-based pesticides, particularly 'Achook' were toxic for the zebrafish (Martinez, 2002; Kumar et al., 2010).

More recently, *A. indica* has been used for increasing aquaculture production because of its potential benefits. The successful usage of *A. indica* indicating aquaculture practices mostly depends upon various factors, including, i.e., parts of plant selected for treatment, methods of the preparation of plant extract as well as the procedure of its different application for the treatments of certain diseases, its dosages according to fish age or species. From all recent studies, it was proved that *A. indica* leaf and its extracted products are effective in varying purposes and helpful in controlling predators and parasites during fish culture systems. It has been reported that the extracts of *A. indica* flower can increase the total WBCs count, which can provide protection against myelosuppression or have a stimulatory impact on the activity of bone marrow (Shah et al., 2009). The leaf extract of *A. indica* has been reported to increase red and white blood cells and lymphocyte counts, thus enhancing the immune response and antibodies production against most pathogenic diseases. Martinez (2002) examined the impact of leaf extracts on the water quality and protozoa community during the culture of freshwater catfish. Kaur et al. (2020) observed the effects of dietary supplementations of *A. indica* extracts on the feeding efficiency, growth, survival, immunological, and reproduction properties of certain carp species.

A. indica could also play a vital role in controlling or preventing various fish diseases (FAO, 2014; Oniovosa et al., 2017). Obaroh and Achionye-Nzeh (2011) observed the impact of different percentages of dietary supplements of crude leaf extracts of *A. indica* as an antifertility agent for controlling the overproduction of tilapia *Oreochromis niloticus*, which leads to shut the growth during culture. It has been proved that 2.0 to 8.0 g/kg diet of leaf extracts of *A. indica* could be useful in controlling the overpopulation and sustainable development in tilapia fish farming (Obaroh and Achionye-Nzeh, 2011). Talpur and Ikhwanuddin (2013) also observed the impact of dietary leaf extract of *A. indica* on enhancing the immune response in fingerlings of Asian seabass *(Lates calcarifer)* against viral infections. They proved that *A. indica* leaf extract in diet could significantly increase the phagocytic activity of WBCs and affect the haematological and immunological parameters and survival rate of fingerlings of that species against viral infection.

5. Conclusions

Present study would be helpful to develop the artificial feed with variable composition to increase the healthy production of *O. Mykiss* in the aquaculture system of Pakistan. During the present research the rainbow trout were fed with four treatments groups, it was concluded that the best diet was Neem leaf plant extract 7% which was the high growth of fish. The optimal supplementation of Neem enhances growth and lowers operating costs. Therefore, further studies are necessary for effective use of *Aloe vera* extract with optimal dose and suitable duration

Data availability statement

All data analyzed during this study are included in this published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

ZUA, HUH and ZM designed the study and executed the experimental work. NR and MIA performed chemical analysis and formulated feed. KB and AG helped in data analysis. AU and TZ helped in literature search. HUH, BAP and MAMS conceive the concept of the review. HUH wrote the article.

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