

ORIGINAL RESEARCH ARTICLE

Evaluating the Growth-Promoting potential of *Nigella sativa* (Habbatussauda) Seeds in *Clarias gariepinus* (Kullume)

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ABSTRACT

High demand, growing feed prices, and worries about synthetic medicine use are all posing problems for the aquaculture sector. In order to improve fish growth during times of scarcity, it is necessary to investigate particular natural products as substitutes. This study determined the effects of *Nigella sativa* seed extract on *Clarias gariepinus* growth. Accordingly, the phytochemicals in the extract of *Nigella sativa* seeds were screened. Experimental fish were acquired and randomly allocated into four (4) treatment groups in triplicate. Crude extracts of *Nigella sativa* were added to a basal diet that contained 35% crude protein at inclusion levels of 0.0g/kg, 1g/kg, 1.5g/kg, and 2g/kg. Growth performance and survival rate were assessed accordingly. Data were evaluated using one-way ANOVA to identify significant differences between treatment groups ($p < 0.05$). This study found that tannins and saponins were present in considerable quantities (++), while alkaloids, anthraquinones, flavonoids, steroids, and terpenoids were present sparsely (+). The 2 g/kg *N. sativa* group had the highest final growth weight (16.25 g), weight increase (12.45 g), and SGR (1.54%). The 2 g/kg group had a little higher FCR (7.75). In addition, the findings indicated a significant difference ($P < 0.05$) between the 2 g/kg group, other treatment groups, and the control group in all growth parameters, with the exception of FCR. Furthermore, the study further revealed that *Nigella sativa* has a significant relationship with weight gained and FCR of catfish. The findings could have practical implications for using *N. sativa* as a natural supplement in aquaculture to increase resilience and health in farmed fish. Thus, more research into the toxicological and histopathological consequences of *N. sativa* seed extract on *Clarias gariepinus* is required.

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INTRODUCTION

Clarias gariepinus, commonly known as "Kullume" in Hausa, is an important catfish species with a high growth rate, adaptability to varying aquatic conditions, and tough performance under intensive farming conditions (Gabriel et al., 2021). However, sustainable output depends on fish health, feed efficiency, and survival, all of which have often posed a challenge with the expansion of aquaculture operations. Amongst other potential solutions, nutritional supplements from natural sources such as herbal extracts may be used to augment the development of fish, immunity, and general health.

Nigella sativa, popularly known as black cumin black seed or *habbatussauda*, has recently attracted interest in aquaculture as a supplementary agent for the reason that it possesses a rich bioactive profile comprising of antimicrobials, antioxidants, and essential fatty acids which enhance production and health in fish (Abdel-Tawwab and Ahmad, 2020; Rehman et al., 2019). *Nigella*

sativa is a medicinal plant belonging to the family Ranunculaceae. It has been used traditionally for the treatment for more than 2000 years (Ziaee et al., 2012). *N. sativa* seeds have been used to treat a variety of disorders affecting the respiratory system, digestive tract, kidney and liver function, cardiovascular system, immune system support, and overall well-being (Ahmad et al., 2013). Research on adding *N. sativa* to aquaculture diets has yielded promising outcomes in terms of fish body composition, growth, feed utilization, and survival. Thymoquinone is an active molecule in *N. sativa* that has been demonstrated to improve stress resilience, reduce fat deposition, and increase protein metabolism (Reverter et al., 2021; Osman et al., 2021). More so, *N. sativa* was found to significantly ameliorate water quality parameters-an important aspect in aquaculture-through the maintenance of pH, temperature, and dissolved oxygen within operational ranges, hence creating an

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optimal culture environment (Rahman et al., 2020). In relation to such benefits, the incorporation of *N. sativa* into aquafeeds could be one of the strategic options for enhancing the production and welfare of reared species like *C. gariepinus*.

Plant extracts are potential alternatives to synthetic drugs, hormones, and chemotherapeutics in aquaculture, as they provide useful active metabolites with various benefits such as immune system modulation (Zanuzzo et al., 2015), growth promotion, digestion enhancement, appetite-stimulating effects, among others when properly administered (Zahran et al., 2014).

These positive effects of plant products on fish feed utilization, growth enhancement, disease resistance, sex reversal, and other biological activity, as previously reported by Sani et al. (2018), Saad and Ahmed (2019); Tope-Jegede et al. (2019); Nyadjeu et al. (2019); Omeje et al. (2020); Ivan et al. (2022).

Specifically, *N. sativa* has a wide range of applications in aquaculture, such as enhancement of immunity, growth, sex reversal and shelf life of fish as shown by many studies (Elkamel and Mosaad, 2012; Alishahi et al., 2012; Awad et al., 2013; Khatun et al., 2015; Khondoker et al., 2016; Ozpolat and Duman, 2016; ÖZ et al., 2016; Sani et al., 2018; Zübeyde et al., 2018; Sani et al., 2019; Hussein et al., 2020; Latif et al., 2021). Furthermore, the positive effects of *N. sativa* seeds have been shown on the growth performance, biochemical and immuno-haematological parameters of *Carrasius auratus*, *Oreochromis niloticus*, *Lates calcarifer*, *Oncorhynchus mykiss*, *Sparus aurata*, *Anabas testudineus*, *Cyprinus carpio*, *Labeo rohita*, and *Tilapia zillii* as reported by Fadeifard et al., (2018); Asad et al., (2020); Latif et al. (2021) and Yousefi et al., (2021).

The present study was conducted to determine the effect of different levels of *Nigella sativa* seed extract on the survival rate and growth performance of *Clarias gariepinus*. Based on these parameters, this work aims to shed light on *N. sativa*'s potential as a natural dietary supplementation to enhance aquaculture performance. Results obtained in this study may contribute to the elaboration of inexpensive and eco-friendly feeding strategies to ensure the long-term development of this industry. The specific objectives of this study are to screen the active phytochemicals found in *Nigella sativa* seed extract and determine the effects of survival rates growth of catfish

MATERIALS AND METHOD

Ethical Approval

Ethical clearance was obtained from the Biological Sciences Research and Ethical Committee, Federal University Birnin Kebbi, with Reference No. BIOSCI-AREC-FUBK-2024/026.

Study Area

The study was carried out at the Department of Biological Sciences, Federal University Birnin Kebbi, Kebbi State, Nigeria. Kebbi State lies in the northwestern part of Nigeria and covers an area of approximately 36,229 square kilometers with a population of about 3,630,931. With an annual population growth rate of 2.38%, the estimated population for 2019 was around 4,988,067. The State lies between latitudes 10° 05' and 13° 27' N and longitudes 3° 35' and 6° 03' E.

Collection and Identification of Experimental Fish

Clarias gariepinus (Kullume) fingerlings were collected from the National Institute for Fresh Water Research in New Bussa, Niger State. Identification was based on ISSG (2014) by using morphological characteristics of the experimental fish. The fish were transported in oxygenated bags and then released into a plastic tank filled with water, allowing them to swim out and acclimate for two weeks.

Collection, Identification, and Preparation of Plant Samples

Dried *Nigella sativa* (Habbatussauda) seeds were purchased from the central market in Birnin Kebbi. A taxonomist from the Department of Biological Sciences, Federal University Birnin Kebbi, identified the plant with voucher number FUBK-BIOSCI-156, and authenticated samples were stored in the department's herbarium. The seeds were washed, shade-dried for 48 hours, and ground into powder. One hundred grams of this powder was soaked in 500 mL of aqueous solvent for 72 hours, with constant shaking, as described by AOAC (2019). The solution was filtered, and the filtrate was concentrated by drying at 40°C for 8 hours. The extracted sample was stored in a refrigerator prior to analysis.

Phytochemical Analysis of Plant Extract

Qualitative phytochemical screening of the *N. sativa* extract was done following the AOAC (2019) procedure.

Test for Alkaloids

The plant extract (2g) was acidified with acetic acid, and a drop of Mayer's reagent was added. A white precipitate indicated the presence of an alkaloid.

Test for Anthraquinones

Exactly 5g of extract was hydrolysed with dilute Sulphuric acid and extracted with benzene. Then, 1 ml of dilute ammonia was added to the above mixture. Pink colorization suggested a positive response for anthraquinones.

Test for Tannins

The extract (1g) was added to 5mL of 1% ferric chloride, and the colour was observed. The bluish-black colour appeared, which disappeared with the addition of dilute H₂SO₄; a yellow-brown precipitate showed the presence of tannins.

Test for Saponins

The 2g of the extract was diluted with water to 20 ml and this was shaken for 15min. The formation of a 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

A drop of concentrated HCl acid was added to the 2g of extract, and a red colour appeared, which indicated the presence of flavonoids.

Test for Steroids

The extract (5g) was mixed with 3 ml of Chloroform, and 2 ml concentration of Sulfuric acid was poured from the side of the test tube, and the colour of the ring at the junction of two layers was noted. A red colour showed the presence of steroids.

Test for Terpenoids

Exactly 5g of the extract was mixed with 2ml chloroform and concentrated sulphuric acid to form a layer. A reddish-brown colour is formed, indicating the presence of Terpenoids.

Experimental Diet Preparation and Analysis

Feed ingredients used for the formulation of the diet included maize, groundnut cake, fish meal, lysine, methionine, blood meal, bone meal, and vitamin premix, which were obtained from Birnin Kebbi metropolis. A basal diet containing 35% crude protein was formulated and added with the crude extracts of *Nigella sativa* at inclusion levels of 0.0g/kg, 1g/kg, 1.5g/kg, and 2g/kg (Sani *et al.*, 2018). Then all the ingredients were mixed with an electric mixer and pelleted into 4 mm diameter pellets, after which it was shade-dried for one week. Four experimental diets were prepared and kept until the feeding trial.

Experimental Design

The experiment was carried out with aerated rectangular plastic experimental tanks of 100L. The experiment used a completely randomized design with four treatment groups, each with three replicates as follows:

Control group: Fish received a regular diet without *N. sativa* extract.

1 g/kg *N. sativa* extract group: Fish fed a feed with 1 g/kg of *N. sativa* extract.

1.5 g/kg *N. sativa* extract group: Fish were fed a meal containing 1.5 g/kg of *N. sativa* extract.

2 g/kg *N. sativa* extract group: Fish fed a feed containing 2 g/kg *N. sativa* extract.

Each group had 20 fingerlings; the trial lasted for 12 weeks, as adopted by Aly *et al.* (2023), and the water was borehole water. The fish were fed 5% of their body weight daily, but this was divided into two times a day: at 9:00 a.m. and 5:00 p.m., Modifications were made every week based on the increase in weight. The water levels and quality were maintained appropriately.

Weekly water quality parameters were monitored to provide optimal culture conditions and to assess the effect of *N. sativa* on these parameters. A digital thermometer was used to measure the temperature. A pH meter was used to measure the pH. A conductivity meter was used to measure electrical conductivity (EC). A TDS meter was used to test the total dissolved solids (TDS). A DO meter was used to measure the amount of dissolved oxygen (DO).

Evaluation of Growth, Feed Utilization Parameters, and Survival rate

Growth and feed utilization parameters were evaluated following Sadek *et al.* (2022). Growth indices included were final weight (FW), feed utilization indices included feed intake (FI), weight gain (WG), specific growth rate (SGR), absolute growth rate (AGR), feed efficiency ratio (FER), and feed conversion ratio (FCR), survival rate.

Data Analysis

One-way ANOVA was applied to test for significant differences between treatment groups, and regression analysis was used in determining the relationship between *Nigella sativa* concentrations, weight gained, and FCR at a significance probability level of $p < 0.05$. Duncan's Multiple Range Test was used as a follow-up test whenever differences were significant. All analyses were performed using SPSS Version 20.

RESULTS

Phytochemicals screening of *Nigella sativa* used

The *Nigella sativa* seed extract included a variety of bioactive compounds, including alkaloids, anthraquinones, tannins, saponins, flavonoids, steroids, and terpenoids, as determined by phytochemical screening. While alkaloids, anthraquinones, flavonoids, steroids, and terpenoids were present in traces (+), tannins and saponins were present in moderate amounts (++) (Table 1). From these results, it can be adduced that a diverse range of phytochemicals from *N. sativa* seed extract has biological merit in aquaculture for the possible roles in fish immunity and growth or general health.

Growth performance of *Clarias gariepinus* (Kullume) fed varying concentrations of *Nigella sativa* seed extract

Results in Table 2 showed the effects of various dosage of *Nigella sativa* extract on the growth performance and feed consumption of *Clarias gariepinus* (Kullume). The final weight and weight gain were significantly higher in the 2 g/kg *N. sativa* group compared to the control group at P < 0.05 level of significance, while the latter had the highest final growth of 16.25 g and weight gain of 12.45 g. This indicates that an increase in *N. sativa* concentrations of *N. sativa* extract can enhance growth performance, presumably from the growth-stimulating bioactive ingredients of the extract. Additionally, with the highest dosage of *N. sativa* extract, the 2 g/kg group expressed the highest SGR at 1.54%, reflecting better growth with time.

The SGR increased as the extract concentration did. Similar to SGR, the 2 g/kg group had the greatest AGR (0.13), indicating a positive relationship between *N. sativa*

concentration and AGR. The group fed with 2 g/kg had the highest feed intake of 88.56, indicating that the *N. sativa* extract probably enhances feed palatability or digestibility, hence leading to a higher intake. Low FCR values refer to better feed conversion efficiency. However, the FCR value was insignificantly higher in the group fed with a 2 g/kg diet (7.75) compared to all groups, including the control. The fish in the 2 g/kg group also had the highest values of Feed Efficiency Ratio -FER of 0.13, thereby indicating that the latter had been successful in converting a higher amount of feed into body weight.

The results show that increasing doses of *N. sativa* extract, notably 2 g/kg, improve *Clarias gariepinus* ' growth performance and feed efficiency. In comparison to lower concentrations and the control, fish fed with the highest concentration (2 g/kg) of *N. sativa* extract showed significantly increased growth parameters (final growth, weight gain, SGR, and AGR) and feed utilization indices (FI and FER). As a result, 2 g/kg of *N. sativa* extract could be an effective supplement for improving *Clarias gariepinus* (Kullume) development and feed efficiency.

Table 1: Qualitative Phytochemicals Result of *Nigella sativa* seed extract

S/N	Phytochemicals	Status
1	Alkaloids	+
2	Antraquinone	+
3	Tannins	++
4	Saponins	++
5	Flavonoids	+
6	Steroids	+
7	Terpenoids	+

Key: += sparingly present; ++= moderately present

Table 2: Effects of *N. sativa* on Growth performance of *Clarias gariepinus*

TG	IW (g)	FW (g)	WG (g)	SGR	AGR	FI	FCR (g/g)	FER
A	3.80±0.00	8.00±0.50 ^a	4.20±0.50 ^a	0.83±0.07 ^a	0.05±0.01 ^a	45.85±3.30 ^a	10.96±0.64 ^b	0.09±0.01 ^a
B	4.01±0.00	13.83±0.76 ^b	9.82±0.76 ^b	1.38±0.06 ^b	0.11±0.01 ^b	81.53±6.72 ^b	8.30±0.17 ^a	0.12±0.00 ^b
C	3.80±0.00	16.25±1.65 ^c	12.45±1.65 ^c	1.54±0.11 ^c	0.13±0.02 ^b	88.56±8.70 ^b	7.75±0.23 ^a	0.13±0.00 ^c
CT	3.90±0.00	14.70±0.36 ^b	11.45±1.46 ^b	1.47±0.03 ^{bc}	0.12±0.00 ^b	88.29±4.75 ^b	8.17±0.18 ^a	0.12±0.01 ^{bc}

KEY: TG = Treatment Groups; **A** = 1g/kg *N. sativa* Extract; **B** = 1.5g/kg *N. sativa* Extract; **C** = 2g/kg *N. sativa* Extract; **CT** = control **IW** = Initial weight; **FW** = Final weight; **WG** = Weight Gained; **SGR** = Specific growth rate; **AGR** = Absolute growth rate; **FI** = Feed utilization indices will be feed intake; **FCR** = Feed conversion ratio; **FER** = Feed efficiency ratio. The values are presented as mean ± SEM (N=3) values having the same superscript are not significantly different at (p>0.05) analyzed using one-way ANOVA and vice versa, followed by the Duncan Multiple Comparison test with SPSS Version 20

The survival rate of *Clarias gariepinus* fed varying concentrations of *Nigella sativa* seed extract experimental diet

The survival rates of *Clarias gariepinus* in response to varying concentrations of *Nigella sativa* seed extract are displayed in Figure 1. According to the result, no significant difference was found between the control and the other treatments, as shown by P > 0.05. The highest survival rate was found in the group subjected to the 2g/kg concentration of *N. sativa* seed extract, with a survival rate of 100%, while the lowest was in the control, with a survival rate of 97%.

Physicochemical properties of water used in culturing *Clarias gariepinus* fed diets with different concentrations of *Nigella sativa* extract.

Table 3 presents the physicochemical properties of water used for culturing *Clarias gariepinus* that received test meals containing different concentrations of *Nigella sativa* extract. These parameters include temperature, pH, electrical conductivity, TDS, and dissolved oxygen, which were measured throughout the study to monitor the stability and suitability of the culture medium for fish growth. The result showed no significant difference between the control and the other treatments in all

physicochemical parameters, as shown by $P > 0.05$. All treatment groups and the control group had relatively stable temperatures, ranging from 27.0°C to 27.7°C. The optimal temperature for the development and metabolism of *Clarias gariepinus* lies between 25-30°C (Gabriel et al., 2021). While the control group showed a slightly lower pH of 6.53, all of the groups that received *N. sativa* extract maintained a pH of approximately 6.93. The pH values remained within a narrow range throughout the treatments. Generally, the ideal Ph for *C. gariepinus* is within the range of 6.5–8.5, which minimizes stress and is conducive to fish health (Tayem et al., 2022). This narrow range of pH indicates that the treatment of fish with *N. sativa* does not influence the acidity or alkalinity of the water. Electrical conductivity (EC), measuring the total concentration of ions within the water, acts to point out the quality of the water along with the levels of dissolved nutrients. Between 26.67 $\mu\text{S}/\text{cm}$ and 30.67 $\mu\text{S}/\text{cm}$, there were no appreciable differences between

treatments. These ranges are within the permissible limits for aquaculture, and higher conductivity may indicate increased pollution or nutrients that can affect fish health (Rahman et al., 2020).

The TDS values varied between 11.67 and 14.00 mg/L, with no significant difference among groups. TDS reflects the concentration of dissolved chemicals, which impacts water quality. Since TDS in *Clarias gariepinus* should not be above 50 mg/L to prevent stress, these results are obviously within permissive ranges (Miyittah et al., 2018). The DO values for each group were relatively steady, ranging from 1.10 to 1.20 mg/L. Due to the fact that *Clarias gariepinus* is able to breathe atmospheric air, it can, therefore, tolerate low oxygen levels, though this is somewhat low for intensive aquaculture, as obtained by Adeyemi et al. (2019). Low levels of DO suggest aeration should be considered and possibly improved to enhance fish growth and well-being.

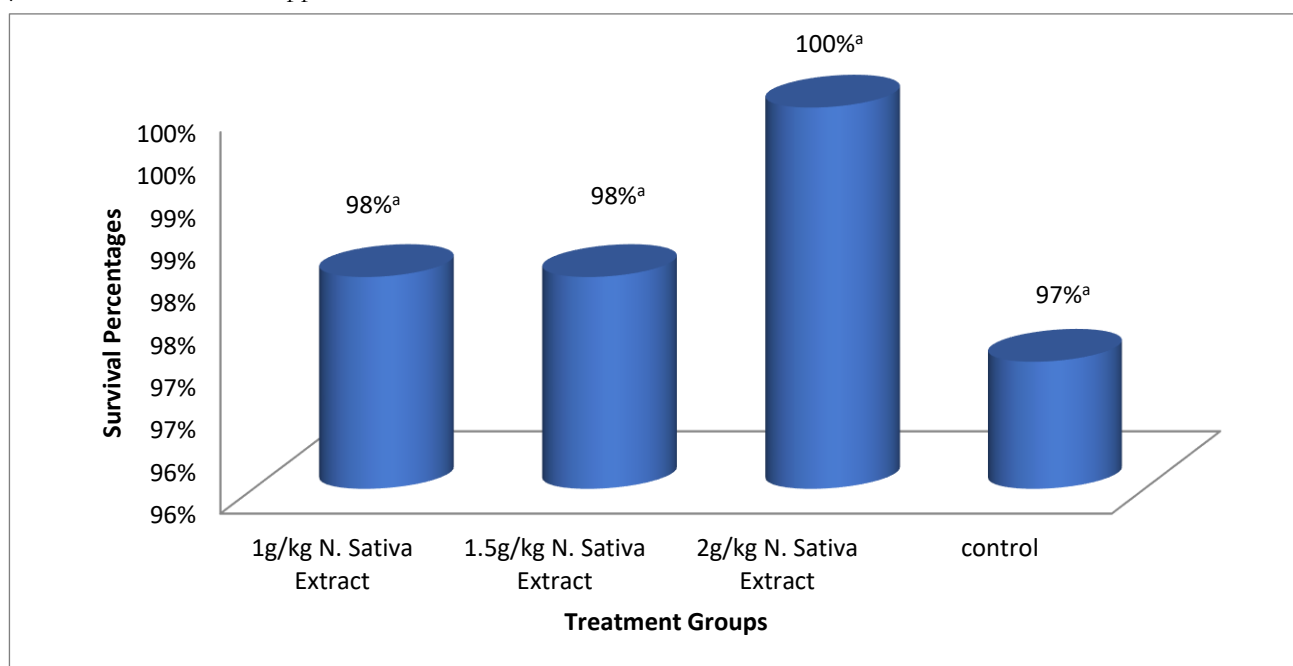


Figure 1: Effects of *N. sativa* on Survival rate of *Clarias gariepinus*

The values having the same superscript are not significantly different at ($p > 0.05$) analyzed using one way ANOVA, followed by Duncan Multiple Comparison test with SPSS Version 27.1.

Table 3: Physicochemical Properties of water used for culture

Treatment Groups	Temperature	pH	EC	TDS	DO
1g/kg <i>N. sativa</i> Extract	27.50±0.50 ^a	6.93±0.65 ^a	28.00±6.00 ^a	11.67±2.08 ^a	1.10±0.10 ^a
1.5g/kg <i>N. sativa</i> Extract	27.70±0.30 ^a	6.93±0.47 ^a	26.67±4.16 ^a	14.00±3.61 ^a	1.17±0.12 ^a
2g/kg <i>N. sativa</i> Extract	27.60±0.20 ^a	6.93±0.45 ^a	28.67±6.11 ^a	14.00±3.00 ^a	1.17±0.15 ^a
control	27.00±1.00 ^a	6.53±0.40 ^a	30.67±3.06 ^a	12.00±2.65 ^a	1.20±0.10 ^a

KEY: EC = Electric conductivity; TDS: Total Dissolved Solid; DO = Dissolved Oxygen. The values having the same superscript are not significantly different at ($p > 0.05$) analyzed using one way ANOVA, followed by Duncan Multiple Comparison test with SPSS Version 27.1.

Relationship between *N. sativa* concentrations, Weight Gained, and Feed conversion ratio metrics using linear regression model

A regression study was conducted to determine the relationship between *N. sativa* concentrations, weight gain,

and feed conversion ratio. In terms of weight gain, the study found an R-value of 0.810, indicating a substantial positive association between *Nigella sativa* concentration and catfish weight gain. This suggests that as the concentration of *Nigella sativa* grows, so does weight gain, indicating a close relationship between the two variables.

The R² value of 0.656 indicates that the concentration of *Nigella sativa* accounts for 65.6% of the variability in catfish weight gain. This is a pretty high percentage, showing that *Nigella sativa* concentration has a significant impact on growth; however other factors not included in the model may account for the remaining variance. The Adjusted R² score of 0.622 accurately measures the model's fit, taking into consideration the number of predictors utilized. The score indicates that the model explains a large percentage of the variability in weight increase, even after controlling for the number of variables in the model. The intercept (Constant) value was determined to be 3.712, implying that in the absence of *Nigella sativa* (zero concentration), the catfish's average weight gain would be around 3.712 grams.

The slope (Concentrations) coefficient of 2.143 implies that every unit increase in *Nigella sativa* concentration results in a 2.143-gram weight gain for the catfish. This demonstrates a positive and linear association between *Nigella sativa* concentration and weight gain, with greater

concentrations resulting in more substantial growth. The analysis of variance yielded an F-statistic of 19.081 with a p-value of 0.001, indicating that the model is statistically significant. This suggests that the concentration of *Nigella sativa* considerably explains the difference in weight gain, and the impact is highly unlikely to be caused by coincidence.

The regression analysis revealed a significant correlation (R = 0.767) between *Nigella sativa* concentration levels and FCR, accounting for approximately 58.8% of the variability (R² = 0.588). The regression coefficient for the concentration variable was -0.892, indicating that larger concentrations of *Nigella sativa* were related to a lower FCR. As the concentration of *Nigella sativa* increased, feed conversion efficiency improved, resulting in superior catfish development performance. The analysis of variance (ANOVA) revealed that the model was statistically significant, with a p-value of 0.004, implying that *Nigella sativa* concentrations significantly influenced FCR.

Table 4: Relationship between *N. sativa* concentrations, Weight Gained, and Feed conversion ratio metrics using linear regression model

Analysis	Parameter	Weight gained	Feed conversion ratio (FCR)
Regression Model	R	0.810	0.767
	R Square	0.656	0.588
	Adjusted R Square	0.622	0.547
	Intercept (Constant)	3.712	11.027
	Slope (Concentrations) coefficient	2.143	-0.892
	P-value	0.001	0.004
ANOVA	F	19.081	14.264
	P-value	0.001	0.004

DISCUSSION

Many substances present in *Nigella sativa* seed extract justify the traditional history of this plant for medicinal purposes and also prove it as a useful ingredient in aquaculture. Since alkaloids are antibacterial and antiparasitic, they could contribute to better health status and higher survival rates in fish due to the low infection rate (Osman and Abouelmagd, 2021). Alkaloids have been proven to improve feed efficiency and nutrient utilization in aquaculture by being used as natural growth promoters (Rahman et al., 2020). According to Rehman et al. (2019), anthraquinones possess laxative, anti-inflammatory, and antioxidant features that can boost fish immune systems and healthy digestion.

The *Nigella sativa* extract has moderate levels of tannins, which can support the growth of favorable microbes (Kalhoru et al., 2018). The extract's moderate saponin content may help to increase protein consumption and reduce feed conversion ratios. Furthermore, saponins are known to have anti-inflammatory and immune-boosting effects that can benefit fish health, especially in intense aquaculture settings (Abdel-Tawwab and Ahmad, 2020). Saponins have been shown to affect lipid metabolism and reduce fat storage, promoting development (Reverter et al.,

2021). Despite their low abundance, flavonoids are powerful antioxidants with anti-inflammatory effects that aid in fish stress reduction and immune response (Zahran et al., 2022). Apart from enhancing blood circulation, flavonoids could contribute to protein synthesis and the development of muscles. The small dosages of steroids are also reported to influence metabolic activity and can be beneficial towards body composition and muscle growth. According to Gabriel et al. (2021), natural steroids present in plants have a potential effect on fish development by influencing protein synthesis and assimilation of food. Terpenoids' antioxidant and antibacterial properties, found in modest concentrations, may help boost the immune system and reduce fish's susceptibility to illness (Rehman et al., 2019).

The study's findings are consistent with previous research on the favorable effects of *Nigella sativa* (Habbatussauda) on aquatic species' growth performance and feed efficiency. Higher concentrations of *N. sativa* extract were associated with increased final growth, weight gain, specific growth rate (SGR), absolute growth rate (AGR), and feed efficiency, implying that *N. sativa*'s phytochemical properties are crucial for growth promotion. The bioactive components in *N. sativa* account for the significant increase in weight gain and final growth in

groups treated with higher concentrations of *N. sativa* extract, particularly at 2 g/kg. Studies have demonstrated that *N. sativa*'s essential fatty acids, vitamins, and phenolic compounds boost fish growth by increasing food absorption and accelerating metabolic processes (Al-Ghazzewi and Tester, 2014; Rehman *et al.*, 2019). Similar studies by Abdel-Tawwab (2019) found that supplementation with *N. sativa* caused significantly higher growth rates in the carp compared to the control groups.

In aquaculture, the feed conversion ratio (FCR) and feed efficiency ratio (FER) are two important indicators of feed utilization. The 2 g/kg group in this study manifested increasing feed efficiency with higher *N. sativa* concentrations reflected by a moderately higher FCR and highest FER across all groups. This finding agrees with the studies conducted on tilapia and other fish species where supplementation with *N. sativa* improved feed efficiency through enhanced nutrient absorption and higher protein synthesis, as evidenced by Osman *et al.* (2021) and Hassaan *et al.* (2020). This increase might be due to the increased palatability and digestibility brought in by *N. sativa*, which may contain bioactive compounds that enhance gut integrity and enzymatic activity, thereby promoting the utilization of feed more effectively, as was shown by Kalhoro *et al.* (2018). The SGR and AGR were highest in the 2g/kg *N. sativa* group. Similar responses were also noted for Nile tilapia, where the supplemented fish with *Nigella sativa* grew better than the unsupplemented ones. Such a performance is presumed to be due to its high concentration of antioxidants and its immunomodulatory property, which reduces disease incidence and enhances health status (El-Khaldi *et al.*, 2018; Zahran *et al.*, 2022). From these growth parameters, *N. sativa* might exert its growth-enhancing effects on *Clarias gariepinus* by improving feed intake and reducing oxidative stress.

The percentage survival of *C. gariepinus* fed the *Nigella sativa* seed extract agrees with the reported studies indicating that herbal dietary supplementation could improve the survival and health status of fish. The highest survival rate (100%) in the group given 2 g/kg of *N. sativa* extract suggests a potential protective effect, most likely due to the bioactive compounds found in *N. sativa*, even if no statistically significant differences were found between the groups. Thymoquinone was identified as a bioactive component of black cumin seeds and proved to possess immunomodulating, anti-inflammatory, and antioxidant properties (Abdel-Tawwab and Ahmad, 2020). Maybe these compounds make the fish healthier and more robust, hence reducing mortality rates.

The ability of herbal supplements, such as *N. sativa*, to boost immune response and provide fish with additional protection against diseases and environmental stress is increasingly being researched in aquaculture (Reverter *et al.*, 2021). According to research, adding herbal extracts

to the meal can improve fish survival rates by boosting their immune systems and improving their antioxidant capacity (Hassaan *et al.*, 2020). Similar findings were observed in Nile tilapia, where *N. sativa* supplementation improved fish survival and health (Abdel-Tawwab *et al.*, 2020).

The slight decrease in survival in the control group (97%) as compared to the 2 g/kg treatment group may highlight *N. sativa*'s potential beneficial impact on fish diets. The water of the present study showed physicochemical parameters within acceptable limits for *Clarias gariepinus* culture. This might mean that different *Nigella sativa* extract concentrations have no deleterious effects on water quality. Aquaculture needs to be operated under consistent water quality due to the effect of its fluctuations on fish health, metabolism, and overall development performance. In studies about the use of herbal supplements in aquaculture, there has been no harmful effect of herbal extracts, like *Nigella sativa*, on the water quality measurements of pH and TDS, including conductivity (Hassaan *et al.*, 2020). The constant condition of pH and temperature during the study supports the hypothesis that *N.*

The extracts of *sativa* do not add destabilizing elements to the culture water. This finding is in agreement with those of Rahman *et al.* (2020), who established that because herbal additives are biodegradable and natural, they rarely affect any significant water quality measurement. *Nigella sativa* extract is, therefore, a suitable candidate for sustainable aquaculture since it seems to be a nontoxic dietary supplement that does not have adverse effects on water quality.

Nigella sativa concentrations significantly increase catfish weight gain, according to the results of regression analysis. This outcome is in line with other research that looked into *Nigella sativa*'s application in aquaculture. Similar to what we found in this work, Abd El-Rahman *et al.* (2020) discovered that feeding *Nigella sativa* to African catfish greatly increased their growth rates. They ascribed this growth enhancement to *Nigella sativa*'s antioxidant qualities, which may lessen oxidative stress and enhance fish metabolism. These results are further supported by a study conducted by Ali *et al.* (2021), which showed that supplementing tilapia with *Nigella sativa* enhanced their immunological response and growth performance.

They pointed out that thymoquinone, one of *Nigella sativa*'s active components, may improve weight gain by improving nutrient absorption and general wellness. It is crucial to remember that although *Nigella sativa* has demonstrated beneficial effects, different species, and environmental circumstances may require different concentrations for optimal growth. According to Mollah *et al.* (2019), fish species, temperature, and water quality can all affect how *Nigella sativa* affects aquaculture. Therefore, when implementing these findings in other aquaculture contexts, it is imperative to take these factors into account.

Nigella sativa may improve feed utilization efficiency, which is crucial for aquaculture methods, as evidenced by the reported increase in FCR. *Nigella sativa* concentration and FCR have a negative connection, indicating that larger concentrations promote feed conversion and, in turn, growth performance. The results of this study are corroborated by a study by Mollah *et al.* (2019), which found that feeding tilapia (*Oreochromis niloticus*) *Nigella sativa* oil enhanced their growth performance and feed consumption. In particular, their study found that adding *Nigella sativa* oil at different quantities significantly reduced FCR. The authors ascribed this impact to *Nigella sativa*'s anti-inflammatory and antioxidant qualities, which may improve growth rates by lowering metabolic stress and enhancing nutrient absorption.

In a similar vein, Abd El-Rahman *et al.* (2020) examined how *Nigella sativa* seeds affected the growth and feed conversion of African catfish (*Clarias gariepinus*) and discovered notable enhancements in feed usage and growth performance. According to their research, supplementing with *Nigella sativa* decreased FCR. This was ascribed to the plant's bioactive components, such as thymoquinone, which are thought to boost immunological and metabolic functions. This is in line with the current study's findings, which showed that higher *Nigella sativa* concentrations increased FCR and maybe growth. Though identical results have been reported in a number of investigations, the processes underlying these effects may differ.

According to El-Banna *et al.* (2019), the active compounds in *Nigella sativa*, like thymoquinone, have antioxidant qualities that lower oxidative stress in fish, which in turn improves metabolic efficiency and lowers the energy expenditure needed for immune responses. These findings are consistent with the current study's findings, which indicate that improved FCR may be due to the lower stress and improved metabolic efficiency brought about by *Nigella sativa* supplementation. However, it is crucial to remember that not all studies have demonstrated such consistent effects. In a study on *Nigella sativa* supplementation in freshwater fish, for example, Ali *et al.* (2021) found no discernible increase in FCR at specific concentrations. This suggests that the effects of *Nigella sativa* may differ based on the species of fish, the concentration used, and other dietary or environmental factors. This variation emphasizes the need for more investigation to identify ideal concentrations and get a deeper comprehension of the processes by which *Nigella sativa* influences aquaculture development and feed conversion.

CONCLUSION

Given the low amount of additional bioactive components and the relatively low amounts of tannins and saponins present, *N. sativa* may be a balanced and nutritious additive for health in *Clarias gariepinus*. Based on the results, supplementation with *N. sativa*, especially at an inclusion rate of 2 g/kg, considerably enhances

growth performance as well as feed utilization in *Clarias gariepinus*. Based on these results, this study supports previous research work on *N. sativa* on the development of fish and feed intake; thus, this may serve as a potential feed additive for aquaculture. Results indicated that supplementation with *N. sativa* seed extract, especially at 2 g/kg, enhanced survival in *Clarias gariepinus*, though without statistical significance. The study further revealed that *Nigella sativa* has a significant relationship with weight gained and FCR of catfish. This may have practical implications for the application of *N. sativa* as a natural nutritional supplement in aquaculture to help keep farmed fish healthy and resilient. More research is needed to determine the toxicological and histopathological effects of *N. sativa* seed extract on *Clarias gariepinus*.

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