



The Effectiveness of Black Seed Extract (*Nigella Sativa*) on the Movement of *Ascaridia Galli* Worms in Vitro

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Article Info

Article history:

Received 27 March 2026

Received in revised form 16 April 2026

Accepted 1 May 2026

Keywords:

Nigella Sativa
Anthelmintic Activity
Ascaridia Galli
Worm Motility
Herbal Medicine

Abstract

*This study concludes that Black Seed (*Nigella sativa*) extract demonstrates significant anthelmintic activity against *Ascaridia galli* in vitro, as evidenced by decreased worm motility and reduced time to death across treatment groups. The findings highlight a clear dose-response relationship, where higher extract concentrations produce stronger and faster anthelmintic effects. Statistical analyses, including the Friedman test and One-Way ANOVA, confirm that most treatment groups show significant differences in worm motility and mortality compared to the negative control, while the highest concentration (100%) exhibits effectiveness comparable to the positive control (pyrantel pamoate). The presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and triterpenoids further supports the biological mechanism underlying this effect, particularly through pathways involving neuromuscular disruption, protein denaturation, and inhibition of energy metabolism in worms. Overall, these results indicate that Black Seed extract has strong potential as a natural alternative anthelmintic agent, although further in vivo studies and clinical validation are required to confirm its safety, efficacy, and practical application in both veterinary and human health contexts.*

Introduction

Plants play a role in maintaining environmental balance, producing oxygen, serving as a food source, and as an important ingredient in the development of modern medicine (Manisha et al., 2025; Goyal & Chauhan, 2024; Ashraf et al., 2024). Habbatussauda, or *Nigella sativa* L., from the Ranunculaceae family, is an annual herb characterized by linear leaves, bluish flowers, and capsule-shaped fruit, and is commonly known as black cumin. Black cumin, known as Habba al-Barakah or the miracle herb, has long been considered an "herb from paradise." In the hadith of the Prophet, it is mentioned as a cure for all diseases except death, thus earning it the nickname "prophetic medicine" among Muslims. *N. sativa* plants contain secondary compounds such as alkaloids, saponins, flavonoids, and tannins that play a role in drug development, including fighting microorganisms and malaria parasites by inhibiting protein synthesis and protecting against oxidative stress (Pawłowska et al., 2023; Gomes et al., 2022; Netongo et al., 2025).

Worm infections remain a public health problem both globally and in Indonesia (Lubis et al., 2025; Astuti et al., 2024; Riadi et al., 2026; Kusumasari et al., 2025). Intestinal parasitic infections are conditions in which parasites attack the human intestine. One of the main causes of this infection is soil-transmitted intestinal worms.

The prevalence of worm infections in Indonesia remains relatively high, especially among school-age children and communities with poor sanitation, with the national figure being around 28.12%. Ascariasis is the most common intestinal worm infection in humans, particularly children in developing countries (Mallo et al., 2026; Bonja et al., 2026; Azizi et al., 2026). The WHO reports that more than 760 million children worldwide are at risk of *Ascaris lumbricoides* infection each year, while in Indonesia the prevalence among children remains very high, at around 60–90%.

Livestock disease is a common problem faced by farmers. According to previous research by Levine (1990), ascariasis infection in poultry is caused by *Ascaridia galli*, a nematode belonging to the same family as *Ascaris lumbricoides* in humans. *Ascaridia galli* is a type of roundworm from the large nematode group that does not require an intermediate host in its life cycle (Azam et al., 2025; Miao et al., 2026). When infective *A. galli* eggs are ingested by poultry, the worms will grow, develop, and reproduce in the digestive tract of the bird, which serves as its definitive host. This worm generally infects the digestive tract of chickens, especially the small intestine (Walter et al., 2026; Haryatmi et al., 2026; Abbas et al., 2024). This worm infection can cause weight loss and growth disorders due to disruption of the nutrient absorption process. According to research by Febriani et al. (2018), the disease caused by *A. galli* infection is known as ascariasis. Meanwhile, research by Zakiah et al. (2020) explains that ascariasis is a parasitic disease that occurs due to the ingestion of worm eggs or larvae through contaminated food, generally related to poor hygiene habits. Free-range systems are usually used to raise free-range chickens, which forage directly from the environment, including soil, insects, and organic waste. This dietary pattern increases the risk of exposure to various infectious agents, particularly intestinal parasites (Sadek & Mahmoud, 2026; Patouillat et al., 2026; Boueroy et al., 2026).

Inadequate environmental sanitation and the presence of toilets that do not meet health standards can increase the risk of intestinal parasitic infections (Tolera et al., 2026; Abdulaziz et al., 2026; Buyanjargal et al., 2026). Dirty environments and the lack of isolation of waste from toilets can potentially contaminate the soil and water around residential areas.

Ascaris lumbricoides is difficult to obtain alive because humans are its definitive host, and worm removal usually involves administering anthelmintic drugs that kill the worms (Nath et al., 2026; Inyang et al., 2022; Davie et al., 2024). Therefore, this study used *Ascaridia galli*, a roundworm that infects chickens, as the test animal (Berihun et al., 2026; Raza et al., 2016; Anisha et al., 2025). *A. galli* was selected based on its family similarity and morphological similarity to *A. lumbricoides*, making it suitable for use as a nematode model in *in vitro* studies.

Based on this description, the active compounds in *Nigella sativa*, such as thymoquinone, flavonoids, and saponins, are suspected to have potential as natural antiparasitic agents. Therefore, this study aims to test the effectiveness of Black Seed extract on the movement of *Ascaridia galli* worms *in vitro* as a biological model of intestinal nematodes.”

Methods

This research is a pure experiment (true experimental design) with a Posttest-Only Control Group Design, in which *Ascaridia galli* that has been isolated from chicken intestines will be divided into several treatment groups based on the concentration of black seed extract. This research was conducted at the UP3M Research Laboratory, Faculty of Medicine, Muslim University of Indonesia, and the Faculty of Pharmacy, Hasanuddin University. The study will be conducted from November 2025 to March 2026. The independent variable in this study is

the concentration of Black Seed (*Nigella*) extract. The dependent variable in this study is the movement of *Ascaridia galli* worms. Data were obtained through direct observation of the motility of *Ascaridia galli* worms during treatment. Each concentration group was tested three times (triplicate), and results recorded included the time to onset of loss of motility and the number of surviving worms at each time interval. Quantitative data were calculated as a mortality percentage or motor activity score. Data analysis in this study included univariate and bivariate analyses. Univariate analysis was conducted to describe the distribution of *Ascaridia galli* motility data based on motility scores at each observation time, as well as to describe the time to death of the worms in each treatment group. The results of the univariate analysis are presented in the form of a distribution table and mean values. Bivariate analysis was conducted to assess changes in worm motility in each treatment group based on observation time using the Friedman test, as worm motility data are ordinal and subject to repeated observations. Furthermore, bivariate analysis was also conducted to compare the time to death of the worms between treatment groups. Prior to the comparative test, the time to death data were first tested for normality and homogeneity. Data that met the assumptions of normality and homogeneity were analyzed using a one-way ANOVA test, followed by a post hoc test to more specifically identify differences between treatment groups. A p-value <0.05 was considered statistically significant.

Result and Discussion

Qualitative Phytochemical Test Results

Table 1. Phytochemical Test Results of Black Seed Extract

Test Type	Reagent	Test Result	Result Interpretation
Flavonoids	Sitroborate	Positive (+)	Color change to greenish yellow
Tannins	FeCl ₃ (Ferric Chloride)	Positive (+)	Color change to greenish black
Alkaloids	Dragendorff's Reagent	Positive (+)	Color change to reddish orange
Saponins	Distilled Water	Positive (+)	Formation of stable foam in the test tube
Triterpenoids	Liebermann Burchard	Positive (+)	Color change to greenish

Based on Table 1, the phytochemical test on black cumin seed extract conducted in January at the Phytochemistry Laboratory, Faculty of Pharmacy, Hasanuddin University, used a qualitative test method. This test included the identification of flavonoids, tannins, alkaloids, saponins, and triperpenoids using specific reagents for each test. Based on the results of the phytochemical test, the ethanol extract of black cumin seeds showed positive results for all tested compounds.

Morphology of *Ascaridia galli* Worms

Morphological observations of *Ascaridia galli* worms were conducted to confirm the identity of the samples used in the study. Macroscopic and microscopic observations were conducted to describe the worms' morphological characteristics and ensure that the observed characteristics matched descriptions of *Ascaridia galli* in the literature. The results of these observations were used as supporting data to ensure the biological validity of the samples in the in vitro study. The results are presented in Figures 1 and 2.

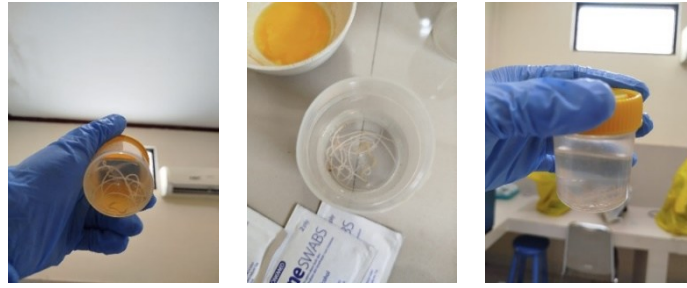


Figure 1. Macroscopic Image of *Ascaridia galli* Worms

Based on macroscopic observations in Figure 1, *Ascaridia galli* worms appear elongated, cylindrical, yellowish-white to cream-colored, and have a smooth body surface. The anterior and posterior portions appear tapered, with female worms generally being longer and larger than males.

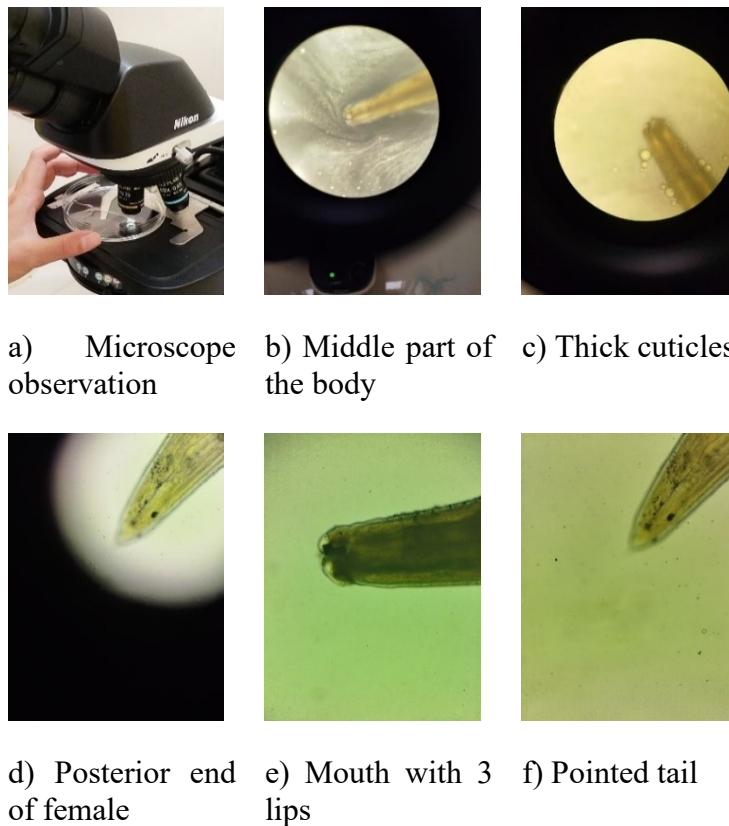


Figure 2. Microscopic image of *Ascaridia galli* worm

Based on Figure 2, microscopic observations show several morphological structures typical of *Ascaridia galli* worms, including the anterior portion, mouthparts, cuticle, and posterior portion, which demonstrate morphological differences between male and female worms. These findings support the identification of the worms used in the study as *Ascaridia galli*.

Results of Black Seed Extract Test on *Ascaridia galli* Worm Movement

The in vitro study of the anthelmintic effect of black cumin (*Nigella sativa*) seed extract on *Ascaridia galli* was conducted by observing worm motility and time to death after administration of the black cumin extract. Worm motility was observed using a scoring system to reflect changes in the worms' biological response to the extract. The worms were divided into six treatment groups consisting of black cumin seed extract solutions at concentrations of

25%, 50%, 75%, and 100%, a negative control containing distilled water, and a positive control containing pyrantel pamoate solution.

In this study, observations and recordings were made every 10 minutes until all worms in each treatment group died. This observation interval was used to provide a clearer description of the gradual decline in worm activity, starting from active movement, reduced activity, near immobility, and complete immobility. Through this approach, the study was able to compare the effectiveness of each extract concentration in influencing the paralysis and mortality of *Ascaridia galli*. The complete results of the black cumin seed extract test on *Ascaridia galli* worms are presented in Figure 3 and Tables 2 to 6.

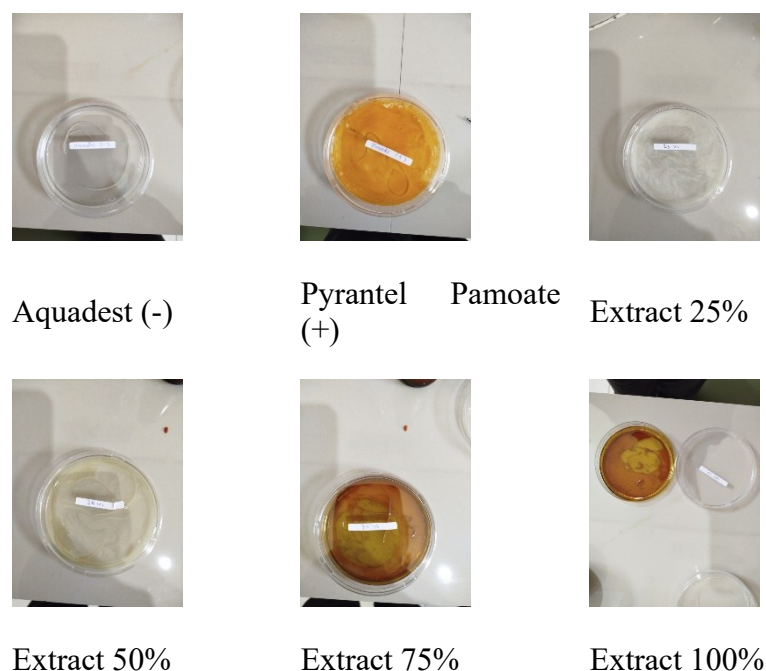


Figure 3. Black Seed Extract Effectiveness Test

Figure 3 shows visual documentation of the control and treatment groups' effects of Black Seed extract at various concentrations on *Ascaridia galli* worms in vitro. This documentation was used to support observations of the extract's effectiveness in each treatment group.

Table 2. Motility Scoring Criteria for *Ascaridia galli* Worms

Score	Category	Observation Criteria
3	Active	Worm is alive with normal motor activity
2	Weakening	Decreased motor activity is observed
1	Nearly Immobile	Worm is alive with very minimal motor activity
0	Immobile	Worm is declared dead

Based on Table 2, the results of the study were graded based on the observed worm movement activity. The highest score indicates that the worms are still actively moving, while the lowest score indicates that the worms no longer respond to stimulation and are declared dead. This criterion was used as a reference for assessing changes in worm movement in each treatment group and each observation period.

Table 3. Motility Score Results in the 25% Black Seed Extract Group for *Ascarida galli* Worms

Movement Criteria	Number of Worms by Observation Time (minutes)						P-Value
	10	20	30	40	50	60	
Active	3	3	0	0	0	0	0.0108
Reduced Activity	0	0	3	3	0	0	
Nearly Immobile	0	0	0	0	2	0	
Immobile	0	0	0	0	1	3	

Based on Table 3, in the 25% Black Seed extract group, all worms still showed active movement until the 20th minute. At the 30th and 40th minutes, all worms were in the weakened movement category. Furthermore, by the 50th minute, most worms had entered the nearly immobile category, and by the 60th minute, all worms were immobile. The Friedman test results showed a p-value of 0.0108 ($p < 0.05$), indicating a statistically significant change in worm movement in the 25% Black Seed extract group during the observation period.

Table 4. Motility Score Results in the 50% Black Seed Extract Group for *Ascarida galli* Worms

Movement Criteria	Number of Worms by Observation Time (minutes)						P-Value
	10	20	30	40	50	60	
Active	3	3	0	0	0	0	0.0108
Reduced Activity	0	0	3	3	0	0	
Nearly Immobile	0	0	0	0	2	0	
Immobile	0	0	0	0	1	3	

Based on Table 4, in the 50% Black Seed extract group, all worms remained active until the 20th minute. At the 30th and 40th minutes, all worms showed weakened movement. By the 50th minute, most worms were nearly immobile, while others were immobile. Furthermore, by the 60th minute, all worms were immobile. The Friedman test showed a p-value of 0.0108 ($p < 0.05$), indicating a statistically significant change in worm movement in the 50% Black Seed extract group during the observation period.

Table 5. Motility Score Results in the 75% Black Seed Extract Group for *Ascarida galli* Worms

Movement Criteria	Number of Worms by Observation Time (minutes)						P-Value
		20	30	40	50	60	
Active	0	0	0	0	0	0	0.0297
Reduced Activity	2	1	0	0	0	0	
Nearly Immobile	1	1	2	0	0	0	
Immobile	0	1	1	3	3	3	

Based on Table 5, in the 75% Black Seed extract group, no active worms were found after 10 minutes. At 10 minutes, most worms were in the weakened category, and some were nearly immobile. At 20 minutes, there was a further decrease in movement, with worms in the weakened, nearly immobile, and immobile categories. By 30 minutes, most worms were nearly immobile, and some were immobile. Furthermore, by 40 minutes, all worms were immobile, and this condition persisted until the end of the observation. The Friedman test showed a p-value of 0.0297 ($p < 0.05$), indicating a statistically significant change in worm movement in the 75% Black Seed extract group during the observation period.

Table 6. Motility Score Results in the 100% Black Seed Extract Group for *Ascarida galli* Worms

Movement Criteria	Number of Worms by Observation Time (minutes)						P-Value
		20	30	40	50	60	
	10	20	30	40	50	60	
Active	0	0	0	0	0	0	0.0297
Reduced Activity	2	1	0	0	0	0	
Nearly Immobile	1	1	2	0	0	0	
Immobile	0	1	1	3	3	3	

Based on Table 6, the 100% Black Seed extract group saw the most rapid decrease in worm motility. By the 10th minute, most worms were nearly immobile, while others were immobile. Furthermore, by the 20th minute, all worms were immobile, and this condition persisted until the end of the observation period. The Friedman test results showed a p-value of 0.0752 ($p > 0.05$), indicating that the change in worm motility in the 100% Black Seed extract group was not statistically significant. However, descriptively, the 100% concentration still showed the most rapid decrease in motility compared to the other concentrations.

Statistical Tests

Friedman Test

The Friedman test is a non-parametric statistical test used to analyze differences in ordinal data within the same group. This test is used when data does not meet parametric assumptions and aims to determine whether there are significant changes over time or between related observation conditions. These results can be seen in Table 7.

Table 7. Friedman Test

Variable	Chi-Square	df	P-Value	Interpretation
K1 (25%)	11.667	5	0.1080	Significant
K2 (50%)	11.667	5	0.1080	Significant
K3 (75%)	10.417	5	0.0297	Significant
K4 (100%)	8.333	5	0.0752	Not Significant

The Friedman test results showed a difference in *Ascaridia galli* motility scores during the observation period in the 25%, 50%, and 75% Black Seed extract groups, with p-values of 0.0108, 0.0108, and 0.0297, respectively ($p < 0.05$). This indicates a statistically significant change in worm motility in all three groups. Conversely, the 100% concentration group obtained a p-value of 0.0752 ($p > 0.05$), indicating that the change in worm motility scores in that group was not statistically significant.

Normality Test

The normality test is a statistical procedure performed to determine whether a set of data follows a normal distribution. These results can be seen in Table 9.

Table 8. Normality Test

Variables	P Value	Description
K1 (25%)	0,702	Normal
K2 (50%)	0,298	Normal
K3 (75%)	1,000	Normal

K4 (100%)	0,537	Normal
K+	0,637	Normal
K-	0,780	Normal

Based on the results of the normality test presented in the table, it is known that all research variables have a normal data distribution. This is evidenced by the significance value (P-Value) for each variable group, which is greater than 0.05. Specifically, variable K1 (25%) has a P-value of 0.702, K2 (50%) has a P-value of 0.298, K3 (75%) has a P-value of 1.000, K4 (100%) has a P-value of 0.537, and the control groups K+ and K- have P-values of 0.637 and 0.780, respectively. Because all groups meet the criterion of $P > 0.05$, it can be concluded that the assumption of normality has been met and the data is suitable for further analysis using parametric statistics.

Homogeneity Test

The homogeneity test is conducted to determine whether several data groups have the same variance (diversity). These results can be seen in Table 9.

Table 9. Homogeneity Test

Variable	P-Value	Description
Treatment	0,759	Homogeneous

The homogeneity test for the treatment variable also showed positive results with a P-value of 0.759. Because this significance value is greater than 0.05, it can be concluded that the variances between the data groups are equal or homogeneous. Fulfilling the assumptions of normality and homogeneity provides a strong basis for continuing data analysis using parametric statistics, such as the One-Way ANOVA test.

One-Way ANOVA Test

One-Way ANOVA (Analysis of Variance) is conducted to determine whether there are statistically significant differences in the averages between three or more independent (unrelated) groups. These results can be seen in Table 10.

Table 10. One-Way ANOVA Test

A. Variable	B. P-Value	C. Description
D. Treatment	E. 0,000	F. Significant

After the assumptions of normality and homogeneity were met, the hypothesis was tested using a One-Way ANOVA test. Based on the calculation results in the table, a significance value (P-Value) of 0.000 was obtained. Considering that this significance value is much smaller than the 0.05 level of significance ($P < 0.05$), it can be concluded that there is a statistically significant difference in the average values between the treatment groups. This indicates that the administration of various treatment dosage levels has a significant effect on the observed variables.

Table 11. Post Hoc Test

Variable	Mean Difference	P Value	Description
K+ Vs K1	-53,000	0,000	Significant
K+ VS K2	-46,333	0,000	Significant
K+ Vs K3	-31,667	0,000	Significant
K+ Vs K4	-2,667	1,000	Not Significant
K+ Vs K-	-66,000	0,000	Significant
K- Vs K1	12,333	0,027	Significant
K- Vs K2	19,667	0,001	Significant

K- Vs K3	34,333	0,000	Significant
K- Vs K4	63,333	0,000	Significant
K1 Vs K2	7,333	0,529	Not Significant
K1 Vs K3	22,000	0,000	Significant
K1 Vs K4	51,000	0,000	Significant
K2 Vs K3	14,667	0,007	Significant
K2 VS K4	43,667	0,000	Significant
K3 Vs K4	29,000	0,000	Significant

Based on the results of the prerequisite assumption test, this research data met the criteria for analysis using parametric statistics. The normality test results in the table indicate that all variable groups had a normal distribution with a significance value (P-Value) > 0.05. Furthermore, the homogeneity test for treatment yielded a significance value of 0.759, indicating that the data variance between groups was homogeneous. After the assumptions were met, a one-way ANOVA test was conducted to determine the overall effect of the treatment. The results in Table 4.6 show a significance value of 0.000 ($P < 0.05$), thus concluding that there was a statistically significant difference in the average values between the treatment groups.

To identify specific differences between groups, further analysis was conducted using a post-hoc test, as presented in the table. The test results show that almost all pairs of groups had significant differences ($P < 0.05$). However, two comparisons were found to be insignificant: the K+ vs. K4 group ($P = 1.000$) and the K1 vs. K2 group ($P = 0.529$). This indicates that treatment at a dose of K4 (100%) has an effectiveness equivalent to the positive control (K+), while doses of K1 (25%) and K2 (50%) do not provide a significant difference in effect from each other. Overall, the gradual increase in treatment doses (K1 to K4) shows a tendency for a significant increase in effect when compared to the negative control (K-).

Based on the results of the research, black cumin seed extract (*Nigella sativa*) was shown to have anthelmintic activity against *Ascaridia galli* in vitro. This activity was demonstrated by differences in the death time of the worms in each group.

The negative control group (distilled water) showed the longest death time with an average of 77.33 ± 2.52 minutes, indicating no anthelmintic effect. Conversely, the positive control group (pyrantel pamoate) showed the fastest death time with an average of 11.33 ± 3.06 minutes, thus being considered highly effective.

In the black seed extract treatment group, there was a tendency for a decrease in the death time of *Ascaridia galli* as the extract concentration increased. The 25% concentration showed a moderate effect, while the 50% and 75% concentrations showed strong effects. The 100% concentration had a very strong effect with an average death time of 14.00 ± 3.61 minutes, which was not statistically significantly different from the positive control. This pattern indicates a dose-response relationship, where the higher the extract concentration, the greater the anthelmintic effect.

Statistical test results support the findings of this study. Normality and homogeneity tests indicate that the data meet the assumptions for parametric analysis. One-way ANOVA showed a significant difference between treatment groups ($P < 0.05$), while post-hoc tests confirmed that increasing extract concentration had a significant effect compared to the negative control. The absence of a significant difference between the 100% concentration and the positive control indicates that black cumin extract at high concentrations is comparable in effectiveness to standard anthelmintic drugs.

Black cumin seeds (*Nigella sativa*) are known to contain non-volatile and volatile compounds that play a role in various biological activities. Furthermore, phytochemical screening of the

extract revealed the presence of secondary metabolites in the form of alkaloids, flavonoids, saponins, tannins, and triterpenoids, which have potential antihelminthic properties.

Saponins and alkaloids work by inhibiting the enzyme acetylcholinesterase or cholinesterase, causing nerve damage and muscle paralysis in worms, leading to death. Flavonoids and phenolic compounds can damage the protein structure of worm tissue through the process of protein denaturation. In research (Noviana et al., 2017), tannins act as anthelmintic agents through the mechanism of inhibiting the oxidative phosphorylation process, which results in disrupted energy production and ultimately causes death in nematode worms. Meanwhile, triterpenoids are thought to play a role in disrupting the polar balance and excessive nerve stimulation, which ultimately causes paralysis and death of worms. The mechanism of action of these compounds supports the potential of black cumin seed extract as an antihelminthic agent in vitro.11,37 (Islam et al., 2025)

This is in line with research conducted by (Vanda et al. 2023) which showed that *Nigella sativa* seed extract significantly reduced the motility and death time of *Ascaridia galli* worms, with varying mortality depending on the extract concentration.42

Previous research by (Tita Rifatul Mahmudah, 2010) showed that black cumin seed extract against *Ascaris suum goeze* in vitro had an effective concentration of 10% with an average death time of 24.67 hours. These results align with this study's pattern of relationship between extract concentration and worm death time, where increasing the concentration of black seed extract accelerated worm death.33

Meanwhile, in a study (Anjani et al., 2023) on herbal medicine X, which reported that a concentration of 70% v/v had the fastest average death time and was close to the positive control, and concentrations of 35% and 17% v/v resulted in longer death times, the similarity in the patterns indicates that the anthelmintic effect is influenced by the concentration of the active compound contained in the test material. Therefore, despite using different materials, this study and previous studies show a similar anthelmintic mechanism.

Research by Kusuma et al. showed that turmeric extract infusion had an average death time for *Ascaridia galli* of 31.66 minutes, faster than the negative control (47.77 minutes) and slower than the positive control (24.44 minutes). This pattern indicates that distilled water, as the negative control, lacks anthelmintic activity, as it produced the longest death time. This finding aligns with the results of this study, where distilled water, used as a solvent and negative control, also showed the longest death time for worms compared to the treatment group.43

Previous research by (Rahayu & Sundari, 2007) showed that pyrantel pamoate works by affecting the worm's neuromuscular system. This drug disrupts muscle depolarization, leading to spastic paralysis. Furthermore, it can inhibit cholinesterase activity, resulting in continuous increased muscle contractions, ultimately leading to worm death.44 This finding, in line with this study, explains why the positive control treatment group showed a faster death time than the other groups.

According to previous research (Desai, 2009), it was stated that the saponin content in kebar grass has an anthelmintic effect which is also found in black cumin seed extract in this study.

Conclusion

Black Seed (*Nigella sativa*) extract has been shown to reduce the motility of *Ascaridia galli* worms in vitro. Administering Black Seed extract at various concentrations showed differences in the motility response of *Ascaridia galli* worms. Higher extract concentrations showed a more rapid decrease in worm motility than lower concentrations.

Suggestion

Further research is recommended to use in vivo methods on test animals (chickens) so that the effectiveness of the extract or drug can be observed under more realistic physiological conditions. Further research is recommended to use other standard anthelmintic drugs such as ivermectin or albendazole as positive controls, as well as to conduct quantitative tests on the main active compounds of black cumin, such as thymoquinone, to determine the relationship between active compound levels and the resulting antiparasitic effectiveness. For healthcare professionals, the results of this research are expected to serve as considerations and initial scientific references in the development of natural ingredients as alternative or supportive antiparasitic therapies, particularly those based on herbal plants. For the public, this research is expected to increase knowledge regarding the potential of black cumin as a natural ingredient with health benefits, while still maintaining rational use based on scientific evidence.

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