

Evaluation of the anthelmintic activity of an acetone extract of *Coriandrum sativum* L. seeds against *Haemonchus contortus* in sheep

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Article info

Received: 06 May 2026

Received in revised form: 09 August 2026

Accepted: 11 August 2026

Published online: 04 September 2026

Keywords

Haemonchus contortus

Acetone extract

Albendazole

Coriandrum sativum

HPLC

Phenolic metabolites

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Consent to publish the names of other two reviewers could not be obtained

Abstract

The gastrointestinal nematode *Haemonchus contortus* is a major economic and pathogenic challenge to livestock productivity worldwide. Extensive use of synthetic drugs like albendazole and ivermectin, the mainstays for controlling these infections, has resulted in the emergence and spread of anthelmintic resistance in gut nematodes. Hence, there is growing interest in plant-based alternatives that utilize secondary metabolites to disrupt parasite physiology. The present study was conducted to evaluate the anthelmintic activity of an acetone extract of *Coriandrum sativum* L. seeds against different developmental stages of *H. contortus*. The extract was characterized for chemical composition using high-performance liquid chromatography (HPLC). *In vitro* assays were conducted to evaluate the effects of the extract on adult worms, larvae, and eggs at concentrations of 0.5%, 1%, 2%, and 4%. Adult worm motility and mortality, larval migration and mortality, and egg hatchability were determined and compared with albendazole as positive control and distilled water as negative control. HPLC analysis revealed the presence of phenolic and related metabolites in the extract with quercetin identified at the highest concentration of 38.89 µg/g. The extract showed a significant dose- and time-dependent anthelmintic activity in all the parameters studied. The motility of adult worms at highest concentration (4%) was reduced to 6.33% after 12 hours and mortality was 100% within 48 hours. Similarly, larval migration was inhibited by 93.33% and larval mortality was 96.33% after 48 hours. Furthermore, egg hatchability was completely inhibited (0%) after 72 h of exposure to the 4% concentration. The effect of the extract at 4% was almost comparable to the commercial drug albendazole. The acetone seed extract of *C. sativum* displayed a potent natural anthelmintic activity against different developmental stages of *H. contortus*. These inhibitory effects may be related to the phenolic compounds identified in the extract. These findings suggest that further investigations should be conducted on the economic feasibility, sustainability, and scalability of *C. sativum* seed extract as a sustainable and cost-effective alternative for parasite management in livestock.

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1. Introduction

Small ruminants, especially sheep and goats, contribute significantly to rural livelihoods and food security. Gastrointestinal nematode infections are a major problem for health and production of small ruminants worldwide (Khan et al. 2023; Hassan et al. 2024). *Haemonchus contortus* is one of the major economically important and pathogenic helminths affecting sheep and goats (Arsenopoulos et al. 2021; Komáromyová et al. 2025). This parasite is present in the abomasum of the host and causes hypoproteinemia, anemia, and decreased animal weight and milk production (Bhat et al. 2024; Abuelo 2026). *H. contortus* can greatly contaminate pastures with infective larvae, leading to persistent infection cycles in grazing animals (Flay et al. 2022; Wei et al.

2025). Haemonchosis is an important challenge to livestock productivity, especially in tropical regions where the environmental conditions favor parasite development (Shrivastava et al. 2022; Sacarrão-Birrento et al. 2024). Control of *H. contortus* infection has conventionally relied on the administration of synthetic drugs, including ivermectin, albendazole, thiabendazole, oxfendazole, and levamisole (Ali et al. 2021; Adduci et al. 2022; Jabbar et al. 2022). Though these drugs are highly efficient, their extensive use has led to the development and dissemination of anthelmintic resistance in gut nematodes (Zajčková et al. 2020; Charlier et al. 2024; Arshad et al. 2025). The *H. contortus* resistance has now been described in livestock-producing areas, rigorously compromising the efficacy of treatments

(Bassetto et al. 2024; Rodrigues et al. 2025). Moreover, concerns regarding drug residues in meat products have increased interest in alternative parasite-control methods, including the use of botanicals, nanoparticles, and probiotics (Sander et al. 2020; Mohsin et al. 2024; Chen et al. 2025; Okoye et al. 2025; Suthar 2025).

Recently, plant-based drugs have gained more attention as alternatives to traditional methods (Nasim et al. 2022; Ullah et al. 2025). Medicinal plants have different classes of compounds such as flavonoids, phenolics, alkaloids, terpenoids, and tannins. Many of these metabolites show antiparasitic potential (Al-Quraghuli and Ali 2025; Faisal et al. 2025). These metabolites may interfere with various physiological processes in parasites and exert effects such as breakdown of the cuticle, metabolic disturbance, and inhibition of larval development (Mukherjee et al. 2016; Karabacak et al. 2025). The use of plant-based metabolites represents an environmentally friendly and promising method for controlling intestinal parasites in livestock (Cissokho et al. 2024; Zeeshan et al. 2025). Among the medicinal plants, *Coriandrum sativum* (Apiaceae family) has gained considerable attention for its medicinal properties (Al-Khayri et al. 2023). *C. sativum* seeds are contain a variety of secondary metabolites such as phenolics, terpenoids, flavonoids, and essential oils (Elshafie et al. 2023). Most of these phytochemicals are responsible for various types of biological activities (Antonia 2025; Xu et al. 2025). Previous studies have reported that plant extracts have antibacterial (Maskur et al. 2025), antifungal (Abbas et al. 2024), antioxidant (Phupaboon et al. 2025), insecticidal (Satybaev et al. 2025), and antiparasitic potential (Paul and Chohan 2024), suggesting their potential application in the production of natural bioactive agents (Morsy et al. 2018; Ullah 2024). Recent studies have also demonstrated that extracts and essential oils obtained from the Apiaceae family have the ability to reduce parasite survival and adult and larval motility against gastrointestinal nematodes both *in vitro* and *in vivo* (Helal et al. 2024).

The organic solvent plays a vital role in the extraction of secondary metabolites. Their polar nature influences the solubility and recovery of different classes of bioactive compounds (Barthwal and Mahar 2024). Solvents such as acetone are efficient at extracting various types of bioactive metabolites such as flavonoids, phenolics, and terpenoids that may greatly contribute to bioactivities (Lezoul et al. 2020; Fatima 2025). Acetone extracts have shown more stronger antiparasitic potential as compared to water extracts (Ademola and Eloff 2011). However, limited information regarding the detailed anthelmintic activity of the acetone extract of *C. sativum* against *H. contortus* is available. Furthermore, the anthelmintic potential of this extract against different developmental stages of *H. contortus*, including adult worm mortality, larval mortality, and egg hatchability, was insufficiently investigated. Moreover, the phytochemicals responsible for its anthelmintic potential have not been comprehensively characterized. Thus, the present research aims to measure the anthelmintic activity of the acetone extract of *C. sativum* seeds against *H. contortus*. Specifically, the study evaluates the effects of the prepared extract on parameters of this parasite, such as adult worm motility, mortality, larval migration, and egg hatchability, in order to evaluate its potential as a sustainable and natural approach for controlling nematodes in livestock.

2. Materials and Methods

2.1 Collection of *C. sativum* L. seeds

Seeds of *C. sativum* were collected from the local market of Faisalabad,

Pakistan in March 2025. Seeds were selected based on uniformity in color and size to ensure healthy seeds were collected. Then the seeds were washed with tap water to remove dust particles and dried at room temperature in a shaded area. The dried seeds were stored in an airtight glass container under dry and dark conditions at room temperature.

2.2 Acetone extract preparation

The seeds were crushed using an electric crusher and subjected to solvent phase extraction using acetone (analytical grade). One hundred grams of crushed seeds were weighed and added to 1 L of acetone. The mixture of crushed seeds and acetone was maintained for 7 days at room temperature with intermittent shaking on an orbital shaker at 120 rpm. After that, the solution was filtered through Whatman No. 1 filter paper, and the collected filtrate was concentrated using a rotary evaporator at 50 °C to evaporate the acetone solvent (Mahlo et al. 2013). The prepared extract was transferred to a vial and stored at – 20 °C for further analysis. The yield was not determined gravimetrically in the present study, so percentage recovery was not calculated.

2.3 High-performance liquid chromatography (HPLC) analysis of phenolic metabolites

The HPLC analysis was conducted by adding 100 mg of seed extract to 1 mL of acetone. The mixture was then filtered through a 0.22 µm nylon membrane filter. The extract was analyzed by an HPLC system (LC-10A Shimadzu, Kyoto, Japan) equipped with a reverse-phase C18 column (250 × 4.6 mm, 5 µm particle size). The injection volume was 20 µL, the flow rate was 1 mL/min, and the detection was conducted at 281 nm. The isocratic elution was carried out using mobile phase A (acetic acid: water :: 6:94 v/v) and mobile phase B (acetonitrile) at a ratio of 70:30 (v/v) throughout the 45-min run. Identification of phenolic compounds was performed by comparing retention times and UV spectra with authentic standards of quercetin, gallic acid, caffeic acid, benzoic acid, chlorogenic acid, syringic acid, p-coumaric acid, ferulic acid, cinnamic acid, and sinapic acid (Hefny Gad et al. 2018). Quantification was performed using external calibration curves prepared from the corresponding standards. The concentrations of the identified compounds were expressed as µg/g of the dry extract.

2.4 Collection of Adult *H. contortus*

Adult *H. contortus* worms were obtained from the abomasum of slaughtered sheep at the local slaughterhouse in Faisalabad, Pakistan. The worms were then placed in normal saline solution to maintain viability and transported to the Chemotherapy Lab, Agriculture University Faisalabad, Pakistan. The worms were subsequently washed with phosphate buffer saline and healthy motile worms were transferred to Petri plates. The worms were maintained at room temperature and monitored during the assessment period.

2.5 Collection of parasitic eggs and harvesting of larvae

Fecal samples were collected from sheep infected with *H. contortus*. The samples were transported to the Chemotherapy Laboratory, Agriculture University Faisalabad, Pakistan, homogenized in normal saline. The mixture was then filtered through sieves to remove debris. The filtrate was centrifuged, and the sediment was resuspended in a saturated sodium chloride solution to recover the eggs, which were washed with distilled water several times and used for egg hatchability assay. For the larval mortality assay, a portion of the fecal samples was cultured for 7-10 days under moist conditions at 27-28 °C to facilitate larvae (L3) development. Using the Baermann technique, the larvae were

recovered, washed with distilled water, and temporarily stored at 4 °C before assay. The larvae were examined under a microscope for confirmation of *H. contortus* infection.

2.6 Tested solution preparation, experimental groups, and exposure conditions

The *C. sativum* seed extract was diluted to prepare solutions at concentrations of 0.5%, 1%, 2%, and 4% (v/v), and a commercial medicine (albendazole, 10% EC) was used as a positive control. Distilled water was used as a negative control. The experiment was performed under controlled conditions at 28 °C and relative humidity of 75%. Ten worms were placed in each Petri plate having the respective extract concentrations of *C. sativum* seed extract or controls [T0 = negative control (deionized water), T1 = 0.5%, T2 = 1%, T3 = 2%, T4 = 4%, and T5 = albendazole (10% EC)]. Each treatment was tested in triplicate as technical replicates, maintaining the extract composition same. For each treatment, three Petri plates were used and treatment groups were randomly assigned to minimize experimental bias. Adult worm motility was assessed by noting movement after 2, 4, 8, and 12 h. Adult mortality was assessed based on the absence of movement after 12, 24, and 48 h. Worms were designated as dead when no movement was observed after gentle mechanical stimulation. Larval migration and mortality were evaluated after 24 and 48 h, the egg hatchability was noted after 24, 48, and 72 h. The larval migration inhibition assay was performed using infective third-stage L3 larvae. One hundred infective L3 larvae were exposed to different concentrations of acetone extract of *C. sativum* seed and incubated at 28 °C. After incubation, larvae were transferred to migration chambers, and migrated larvae were counted microscopically. The percentage inhibition of larval migration was calculated relative to the untreated control. One hundred infective L3 larvae were exposed to different concentrations of acetone extract of *C. sativum* seeds. Incubation was done at 28 °C and larval mortality was assessed. Larvae showing no movement after gentle stimulation were considered dead and the percentage mortality was calculated. Approximately 100 eggs were incubated at 28 °C with different concentrations of acetone extract of *C. sativum* seeds. Egg hatching was evaluated microscopically by counting unhatched eggs and developed larvae. Egg hatch inhibition was calculated as the % reduction in larval development as compared with the negative control.

2.7 Statistical analysis

Data were analyzed using analysis of variance (ANOVA) to determine significant differences between treatments, and Tukey’s test was applied to compare the means. All the tests were performed in triplicate. A statistically significant difference was considered at $p < 0.05$. All statistical analyses were done using Minitab statistical software.

3. Results

3.1 HPLC-based phytochemical analysis

HPLC analysis showed the presence of various phenolic compounds, among which quercetin was detected at the highest concentration. However, other phenolic compounds were also present in varying concentrations (Table 1 and Fig. 1). The presence of these metabolites indicates that the seed extract of *C. sativum* is a rich source of bioactive metabolites.

3.2 Adult worm motility

Statistical analysis revealed that the extract of *C. sativum* showed a marked reduction in worm motility in a time- and dose-dependent manner. The lowest concentration (0.5%) decreased motility from 96.33% at 2 h to 51.66% at 12 h ($P < 0.05$). However, the highest concentration (4%) reduced motility from 81.66% at 2 h to 6.33% at 12 h ($P < 0.05$). The positive control also exhibited a decline in motility to 0% at 12 h. However, the negative control showed 100% motility throughout the experiment (Table 2). Hence, significant differences were observed among various concentrations of acetone extract of *C. sativum*, with higher concentrations and longer exposure times producing greater inhibition of worm motility ($P < 0.05$).

3.3 Worm mortality

Mortality of worms increased in a time- and dose-dependent manner. After 12 h, mortality was 8.66% at 0.5% and 51% at 4%. Mortality reached 100% after 48 h of exposure to the 4% concentration. However,

Table 1. High-performance liquid chromatography (HPLC) study of acetone extract of <i>C. sativum</i> seeds for identification of polyphenolic compounds			
S. No.	Compound	Retention time (RT)	Value (µg/g)
1	Quercetin	2.713	38.89
2	Gallic acid	4.313	4.38
3	Caffeic acid	12.220	17.5
4	Benzoic acid	14.327	31.91
5	Chlorogenic acid	15.587	6.23
6	Syringic acid	16.380	4.42
7	p-coumaric acid	18.087	24.27
8	H-coumaric acid	20.680	9.36
9	Ferulic acid	22.213	14.67
10	Cinnamic acid	24.973	23.52
11	Sinapic acid	26.260	14.37

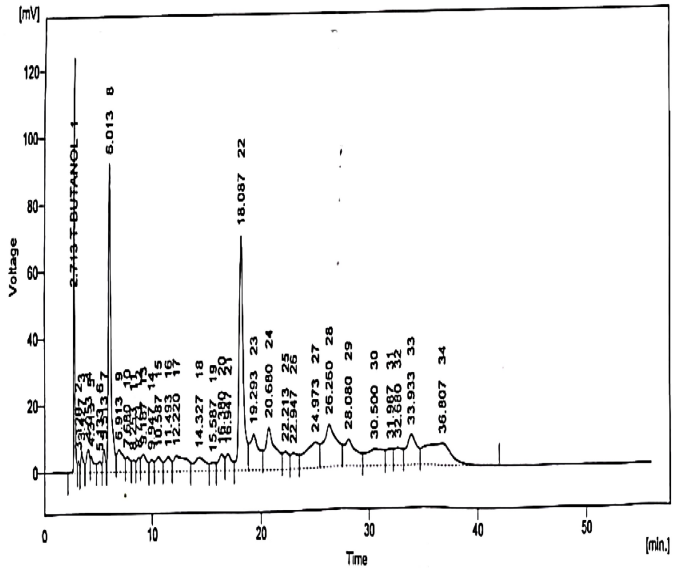


Fig. 1. High performance liquid chromatogram of acetone extract of *C. sativum* seeds

Table 2. Adult motility of *H. contortus* treated with various concentrations of acetone extract of *C. sativum* seeds

Time of exposure (h)	Concentration (%)					
	0.5%	1.0%	2.0%	4.0%	Positive control	Negative control
2	96.33 ± 0.66 ^A	91.66 ± 0.87 ^B	84.50 ± 0.57 ^C	81.66 ± 1.20 ^D	70.34 ± 0.88 ^E	100 ± 0.00 ^A
4	86.66 ± 0.86 ^B	76.33 ± 0.66 ^C	62.50 ± 1.21 ^D	51.34 ± 0.88 ^E	42.00 ± 1.15 ^F	100 ± 0.00 ^A
8	72.00 ± 1.14 ^B	61.00 ± 1.00 ^C	38.75 ± 0.85 ^D	22.66 ± 1.45 ^E	15.00 ± 0.57 ^F	100 ± 0.00 ^A
12	51.66 ± 1.20 ^B	33.00 ± 1.15 ^C	16.25 ± 1.16 ^D	6.33 ± 0.88 ^E	0.00 ± 0.00 ^F	100 ± 0.00 ^A

Different superscripts within a row indicate significant differences according to Tukey's test

no death was observed in the negative control (Table 3). Statistical analysis revealed significant differences in worm mortality among the different concentrations and exposure times ($P < 0.05$).

3.4 Inhibition of larval migration

Migration of larvae was considerably inhibited in a concentration and time-dependent way. At 24 h, the inhibition ranged from 22% to 75% (0.5 to 4% concentration). However, the inhibition was further increased at 48 h, reaching 41.00%, 62.33%, 85.50%, and 93.33% for 0.5, 1, 2, and 4%, respectively. The positive control showed 100% larvae migration at 48 h, whereas no inhibition was observed in negative control (Table 4).

The current study investigated the anthelmintic potential of *C. sativum* extract against *H. contortus* by assessing its effects on adult motility and mortality, larvae migration and larvae mortality, and egg hatchability. The results showed that the *C. sativum* seed extract revealed a concentration- and time-dependent activity. These results validated the potential of this extract as a natural anthelmintic agent and also support the earlier reports on this activity of phenolic-rich plant extracts (Spiegler et al. 2017; Abubakar et al. 2024). The mortality of adult worms was considerably decreased within 12 h of exposure, especially at the high dose of extract. These observations suggested that the

Table 3. Adult mortality of *H. contortus* treated with various concentrations of acetone extract of *C. sativum* seeds

Time of exposure (h)	Concentration (%)					
	0.5%	1.0%	2.0%	4.0%	Positive control	Negative control
12	8.66 ± 0.87 ^E	21.67 ± 1.20 ^D	36.75 ± 1.45 ^C	51.00 ± 0.57 ^A	41.66 ± 1.20 ^B	0.00 ± 0.00 ^F
24	25.00 ± 0.58 ^D	52.00 ± 1.15 ^C	75.00 ± 1.21 ^B	82.66 ± 1.45 ^A	83.33 ± 1.76 ^A	0.00 ± 0.00 ^E
48	43.00 ± 1.52 ^D	71.33 ± 0.88 ^C	94.50 ± 1.45 ^B	100.00 ± 0.00 ^A	100.00 ± 0.00 ^A	0.00 ± 0.00 ^E

Different superscripts within a row indicate significant differences according to Tukey's test

3.5 Larval mortality

Larvae mortality also showed a similar pattern, increasing with time and concentration. After 24 h, the larval mortality increased from 15.33% (at 0.5%) to 62.33% (at 4%). After 48 h, the mortality increased to 32.66%, 61.66%, 87.5%, and 96.33% at concentrations of 0.5%, 1%, 2%, and 4%, respectively. The positive control showed 100% mortality after 48 h of exposure. No mortality was observed in the negative control (Table 5).

3.6 Egg hatchability

Egg hatchability decreased considerably with increasing extract concentration and time of exposure. After 24 h the hatchability was 41.33% at 4% concentration, but eventually decreased to 0% after 72 h. The positive control also completely inhibited egg hatching after 72 h of exposure (Table 6).

4. Discussion

extract has potential anthelmintic effects, likely mediated by phenolic compounds identified by HPLC (Chiavaroli et al. 2021; Kandil et al. 2025). A decline in motility is an essential indicator of lethal stress in worms and may indicate impairment of muscular contraction (Wu et al. 2023). Similar findings have also been reported in *Allium sativum* and *Azadirachta indica* extracts, where plant-based compounds disrupted neuromuscular functions (Adetuyi et al. 2023). Moreover, at the high concentration (4%), the extract showed a high mortality rate (100%) within 48 h, comparable to the commercial drug. These observations suggested that *C. sativum* extract has nematocidal potential. Further, the larvae migration was affected in a time- and dose-dependent way. The inhibition of larval migration indicates that the seed extract interferes with larvae motility, impairing the ability of the parasite to establish an infection in the host (Schytz Andersen-Civil et al. 2024). The mortality of larvae also followed the same pattern, and these outcomes are consistent with earlier studies showing that phenolic plant extracts containing tannins and flavonoids can alter both adult and larval

Table 4. Larval migration inhibition of *H. contortus* treated with various concentrations of the acetone extract of *C. sativum* seeds

Time of exposure (h)	Concentration (%)					
	0.5%	1.0%	2.0%	4.0%	Positive control	Negative control
24	22.00 ± 1.15 ^D	35.33 ± 0.88 ^C	60.25 ± 1.45 ^B	75.00 ± 1.15 ^A	71.00 ± 1.00 ^A	0.00 ± 0.00 ^E
48	41.00 ± 1.00 ^E	62.33 ± 1.20 ^D	85.50 ± 1.21 ^C	93.33 ± 1.21 ^B	100 ± 0.00 ^A	0.00 ± 0.00 ^F

Different superscripts within a row indicate significant differences according to Tukey's test

Table 5. Larval mortality of *H. contortus* treated with various concentrations of the acetone extract of *C. sativum* seeds

Time of exposure (h)	Concentration (%)					
	0.5%	1.0%	2.0%	4.0%	Positive control	Negative control
24	15.33 ± 0.87 ^E	32.00 ± 1.15 ^D	55.00 ± 1.15 ^C	70.33 ± 1.45 ^A	62.33 ± 1.44 ^B	0.00 ± 0.00 ^F
48	32.66 ± 1.43 ^D	61.66 ± 1.20 ^C	87.50 ± 0.57 ^B	96.33 ± 1.21 ^A	100.00 ± 0.00 ^A	0.00 ± 0.00 ^E

Different superscripts within a row indicate significant differences according to Tukey's test

Table 6. Egg hatchability of *H. contortus* treated with various concentrations of acetone extract of *C. sativum* seeds

Time of exposure (h)	Concentration (%)					
	0.5%	1.0%	2.0%	4.0%	Positive control	Negative control
24	92.00 ± 1.15 ^B	82.33 ± 1.20 ^C	55.50 ± 1.21 ^D	41.33 ± 0.88 ^F	50.33 ± 1.45 ^E	100.00 ± 0.00 ^A
48	75.33 ± 0.87 ^B	53.00 ± 1.52 ^C	29.25 ± 1.20 ^D	11.00 ± 1.52 ^F	20.66 ± 1.76 ^E	100.00 ± 0.00 ^A
72	60.66 ± 1.20 ^B	32.66 ± 1.45 ^C	11.25 ± 1.15 ^D	0.00 ± 0.00 ^E	0.00 ± 0.00 ^E	100.00 ± 0.00 ^A

Different superscripts within a row indicate significant differences according to Tukey's test

worms by disrupting metabolic processes and inducing oxidative stress (Punia et al. 2023; Al-Hoshani et al. 2024; Bashir et al. 2025).

Egg hatchability was also considerably inhibited in a concentration-dependent way. The extract's ability to block egg hatching confirms that its biologically active metabolites can penetrate the eggshell (Oliveira et al. 2022). The phenolic metabolites have been reported to exert such functions through enzyme inhibition, oxidative injury, and disruption of the membrane, eventually preventing the larvae from emerging (Punia et al. 2023). These results suggest that the extract acts potentially not only against the motile stage but also against the developing stages of the parasite. Similar results have been described with other plant extracts, where egg hatchability provided evidence of the extract's anthelmintic potential (Alemu et al. 2014). The anthelmintic potential is attributable to the chemical composition of the extract. HPLC analysis revealed the presence of various phenolic metabolites. These metabolites may act synergistically to impair nematode motility and cause death. The phenolic metabolites have been shown to interfere with cellular integrity (Tan et al. 2019; Bagheri et al. 2024).

Further, when comparing the seed extract to a commercial drug, it was shown that 4% of seed extract produced the same outcomes in all parameters. However, albendazole acts by altering microtubule functions in nematodes, whereas the extract acts on multiple sites of nematodes such as metabolic pathways and neuromuscular functions (Martin and Robertson 2010). This targeted action not only suggests its broad-spectrum efficiency but also shows that natural plant-based products could play a vital role in parasite management (Liu et al. 2020). The observations of this study may have potential practical applications because *C. sativum* is easily available and has been widely used in different medicines. Its activity against *H. contortus* suggests it may be a potential cost-effective, plant-based anthelmintic. However, these *in vitro* outcomes may not replicate in the *in vivo* environment, where factors like metabolism and absorption can influence the efficacy. Thus, further studies are necessary to evaluate the pharmacokinetics of this extract in an appropriate animal model.

5. Conclusions

The present study showed the potential anthelmintic effect of the

acetone extract of *C. sativum* against *H. contortus* affecting multiple developmental stages, including adult worms, larvae, and eggs. The extract significantly reduced adult motility and mortality, inhibited larval migration, induced larval death, and prevented egg hatching in a dose- and time-dependent manner, with higher concentrations being as effective as the commercial albendazole. These effects are probably due to the synergistic action of flavonoids and phenolic compounds present in the extract, which interfere with nematode neuromuscular function, metabolic activity, and embryonic development of nematodes. The multi-stage activity and natural origin of *C. sativum* seeds indicate its potential as a cost-effective and sustainable alternative for controlling gastrointestinal nematodes in livestock. Further *in vivo* studies are required to validate its efficacy, determine the optimal dosage, and assess its safety aspects in the field conditions towards the development of plant-based anthelmintic formulations.

Declarations

Funding: None

Conflict of interest: The author declare no conflicts of interest

Acknowledgements: None

Ethics approval: The study was reviewed and approved by Institutional Animal Ethics Committee of the University

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Citation

Abeer K, Iftikhar R, Hasan MS, Tahir UB, Jahangir A, Fatima K, Khan AMA, Ruzieva M, Umirkulova E, Fernando A. (2026). Evaluation of the anthelmintic activity of an acetone extract of *Coriandrum sativum* L. seeds against *Haemonchus contortus* in sheep. Letters in Animal Biology 06(1): 146 – 154. <https://doi.org/10.62310/liab.v6i1.369>