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In Vitro Efficacy of the Monoterpene Linalool Isolated or Combined with the Nematophagous Fungus *Duddingtonia flagrans* in the Control of Sheep Gastrointestinal Nematodes

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Abstract: New alternatives for controlling resistant populations of gastrointestinal nematodes are being studied, including the use of plant compounds and biological control with nematophagous fungi. The objective of this study was to evaluate the in vitro anthelmintic effect of linalool and its association with the fungus *Duddingtonia flagrans* (isolated AC001) in controlling gastrointestinal nematodes in sheep. The ovicidal activity of linalool was assessed via the Egg Hatch Test (EHT), and the larvicidal activity of linalool, alone and in combination with *D. flagrans* conidia, was evaluated via the Larval Motility Inhibition Test (LMIT) on infective larvae (L3). In the EHT, 100% inhibition occurred (at 1.25 and 2.5 mg/mL), with an LC50 of 0.49 mg/mL. In the LMIT, linalool alone inhibited 100% of larval motility (at 4% and 8%), with an LC50 of 0.42% or 4.2 mg/mL. In the combination of linalool with *D. flagrans*, there was a significant reduction in larvae, starting at 24 h, with 100% reduction after 14 days, thus being more effective in reducing L3 compared to the use of the fungus alone. It is concluded that linalool exhibits ovicidal and larvicidal activity, and its association with *D. flagrans* enhances the fungal predation capacity and potentiates anthelmintic efficacy.

Keywords: helminths; anthelmintic resistance; phytotherapy; monoterpene; biological control; nematophagous fungi; small ruminants

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1. Introduction

Gastrointestinal nematodes represent one of the main threats to the health and productivity of small ruminants, causing significant economic losses, which are reflected in reduced weight gain, decreased productivity, and increased susceptibility to diseases [1,2].

The conventional treatment for controlling gastrointestinal nematodes is based on the administration of anthelmintic drugs. However, the indiscriminate use of these drugs has led to the development of resistance in nematode populations, resulting in treatment ineffectiveness [3].

In light of this situation, different strategies are being explored in the search for alternative methods to control helminth infections in small ruminants. Integrated parasite control consists of implementing combined strategic measures to reduce contamination in animals and pastures, while preserving the efficacy of anthelmintics, and is mainly used in cases of anthelmintic resistance [4]. Thus, the combination of control strategies has been proposed as a viable alternative, aiming for effective management, minimizing the use of drugs and maintaining nematode populations in refugia [5–7].

Biological control with nematophagous fungi has been widely used as a safe and effective alternative to conventional chemical methods, promoting a significant reduction in the availability of free-living parasite stages in pastures [8,9], and can be implemented in an integrated control strategy to improve animal health as well as the sustainability of production [10]. *Duddingtonia flagrans* stands out as one of the most promising species, with proven efficacy in controlling gastrointestinal parasites in livestock through its use alone, via co-administration, and, more recently, with a commercial formulation made from the fungus's chlamydospores (Bioverm®) [11–16].

The use of plant-derived compounds, such as monoterpenes, has also been the subject of multiple studies, showing efficacy in controlling gastrointestinal nematode populations [17–19]. Monoterpenes are the most abundant natural secondary metabolites in plants, renowned for their diverse biological properties [20]. Among them, linalool, an alcoholic monoterpene, is a prominent compound found as a primary constituent in the essential oils of over 200 plant species. This compound is notable for its wide range of biological activities and has been extensively studied as a potential alternative for controlling helminthiases due to its bioactive properties [20–22]. The mode of action of monoterpenes, such as linalool, is suggested to be related to the disruption of membrane function and the inhibition of acetylcholinesterase (AChE) receptors, leading to neurotoxicity [23].

Studies have already been conducted to evaluate the association of natural plant-derived products in the control of nematode infections in small ruminants [18,21,24,25], and promising results have been obtained in studies investigating the combination of different fungal species, as well as their association with chemical compounds [26–28]. However, there are no reports on the evaluation of the combination of nematophagous fungi with plant-derived compounds.

Therefore, the objective of the present study was to evaluate the in vitro anthelmintic effect of linalool and its association with the fungus *D. flagrans* in controlling gastrointestinal nematodes in sheep.

2. Materials and Methods

2.1. Collection of Eggs and Larvae of Gastrointestinal Nematodes

For the collection of eggs and larvae of gastrointestinal nematodes for the experimental assays, feces from naturally infected Santa Inês sheep from the Sheep Farming Sector of the Federal Institute of Paraíba (IFPB), Sousa campus, were used. The flock consists of approximately 60 animals, maintained for educational purposes and regularly monitored due to a selective parasitic control program [29]. Fecal samples for egg and larval isolation were collected between January and May 2023. Samples were obtained directly from the rectal ampulla of the animals, stored in sterile plastic bags, and immediately sent for processing. The collection followed a randomized protocol, including all sheep in the flock that were naturally infected during the experimental period. To standardize conditions and minimize environmental variations, all samples were collected in the morning, during the coolest part of the day. Coprocultures were performed according to Roberts and O'Sullivan [30], and subsequently, for the recovery of infective larvae (L3), the recommendations of the Baermann method were followed [31]. The composition of the

parasitic population was determined by identifying infective larvae (L3): *Haemonchus* sp., 86%; *Trichostrongylus* spp., 10%; and *Oesophagostomum*, sp. 4%.

2.2. Fungal Isolate and Conidia Solution Preparation

The *D. flagrans* nematophagous fungus isolate AC001 was used, obtained from a commercial product (Bioverm®, GhenVet Animal Health, Paulínia, São Paulo, Brazil). Fungal conidia were inoculated onto 9 cm diameter Petri dishes containing 2% potato dextrose agar for 10 days, in the dark. After this period, to obtain the conidia solution, 10 mL of distilled water was added to the cultivated Petri dishes [14]. The release of conidia fragments was facilitated by gently brushing the culture with a brush, and the resulting suspension was collected using a Pasteur pipette and transferred to 15 mL Falcon tubes. The quantification of conidia was carried out using a Neubauer chamber.

2.3. Linalool and Synthetic Anthelmintics

The monoterpene linalool (≥97% purity) (Sigma Chemical Co., St. Louis, MO, USA) was used. Also, technical-grade thiabendazole (Sigma Chemical Co., St. Louis, MO, USA) and ivermectin (Sigma Chemical Co., St. Louis, MO, USA) were used as positive controls in the assays.

2.4. Experimental Assays

2.4.1. Assay A—Egg Hatching Test (EHT)

The assay was conducted to evaluate the ovicidal effect of linalool on gastrointestinal nematode eggs from sheep, following the methodology described by Coles et al. [32]. Initially, a pre-experiment was carried out to assess the anthelmintic resistance of the gastrointestinal nematode population in the sheep flock at IFPB. The discriminatory dose established for the experiment was 0.1 µg/mL for thiabendazole, the lethal dose required to inhibit 50% of a susceptible population (LD50), according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) [33]. The nematode population was therefore classified as resistant, as it showed an LD50 of 6.40 µg/mL, with this dose being used as the positive control.

Fecal samples were homogenized and filtered using a set of sieves with serial sections from 1 mm to 25 µm, where gastrointestinal nematode eggs are retained on the 25 µm sieve. The sieve was flipped, and the eggs were recovered using distilled water. The suspension containing the eggs was centrifuged at 1000× *g* for 10 min to obtain a concentrate containing 20 eggs/50 µL.

For the assays, 250 µL of the concentrate containing 100 eggs was incubated with an equal volume of linalool at six different concentrations: 0.007% (0.07 mg/mL); 0.015% (0.15 mg/mL); 0.031% (0.31 mg/mL); 0.062% (0.62 mg/mL); 0.125% (1.25 mg/mL); and 0.25% (2.5 mg/mL). Thiabendazole, used as the positive control, was diluted in a 3% DMSO solution (Sigma-Aldrich®, St. Louis, MO, USA). The negative control consisted only of the diluent (3% DMSO).

For each experimental group, 8 replicates (100 eggs each) were performed, and the tests were incubated for 48 h in a Biochemical Oxygen Demand (BOD) incubator, with temperatures ranging from 27 to 28 °C, with relative humidity between 85 and 90%. A 2% iodine solution was added to interrupt larval hatching. The efficacy percentage of linalool in inhibiting egg hatching was determined using the following formula:

$$\%\ \text{Egg Hatching Inhibition} = \frac{\text{Number of eggs}}{\text{Number of eggs} + \text{Number of larvae (L1)}} \times 100$$

2.4.2. Assay B—Larval Motility Inhibition Test (LMIT)

The test was conducted to evaluate the effect of linalool on the inhibition of L3 motility and was developed as described by Ferreira et al. [34]. A pre-experiment determined the lethal dose to inhibit 50% of the population (LD50) with ivermectin, at concentrations of 0.15; 0.31; 0.62; 1.25; 2.5; 5 and 10 mg/mL, resulting in a LD50 of 0.53 mg/mL (IC95% = 0.38–0.67), which was used in the positive control group.

Infective larvae (L3) were obtained from co-procultures [30] and recovered using the Baermann method [31], resulting in a concentration of 20 L3/50 µL. For the assay, 250 µL of the concentrate containing 100 L3 were incubated with an equal volume of linalool at the following concentrations: 0.12% (1.25 mg/mL); 0.25% (2.5 mg/mL); 0.5% (5 mg/mL); 1% (10 mg/mL); 2% (20 mg/mL); 4% (40 mg/mL); and 8% (80 mg/mL). Distilled water was used as the negative control, and ivermectin was used as the positive control. Eight replicates (100 egss each) were made for each group, and the tests were incubated for 72 h in BOD. After this period, the motility of the L3 in each well was counted using a light microscope at 100× magnification. For motility criteria, L3 were considered alive if they were mobile and dead if they were immobile. The motility inhibition efficacy of L3 was determined by the following formula:

$$\% \text{ Larval Motility Inhibition} = \frac{\text{L3 mobile in NCG} - \text{L3 mobile in TG}}{\text{L3 mobile no NCG}} \times 100$$

NCG: negative control group; GT: treated group.

2.4.3. Assay C—Larval Motility Inhibition Test with Linalool and *D. flagrans*

To evaluate the effect of *D. flagrans* associated with linalool, the L3 motility inhibition test was performed. Ten (10) experimental groups were set up in 1.5 mL microtubes, with a final volume of 500 µL, in eight replicates containing 100 eggs each (Table 1). In the groups containing linalool, the LD25 and LD50 were obtained from the results of Assay B. The groups were maintained in a BOD incubator, and after 24 h, 7 days, and 14 days of exposure to the treatments, the motility of the larvae was assessed using optical microscopy (100× magnification), with quantification of live and dead larvae. The motility inhibition efficacy of L3 was determined by the following formula:

$$\% \text{ Larval reduction} = \frac{\text{Mean number of L3 in NCG} - \text{Mean number of L3 in TG}}{\text{Mean of L3 in NCG}} \times 100$$

NCG: negative control group; GT: treated group.

Table 1. Experimental groups designed to evaluate the isolated or combined use of linalool and *D. flagrans* (AC001) on the infective larvae of sheep gastrointestinal nematodes.

Experimental Groups	Composition
Group 1 (Negative control)	250 µL (100 L3) + 250 µL (distilled water)
Group 2 (Positive control)	250 µL (100 L3) + 250 µL (LD50 ivermectin)
Group 3	250 µL (100 L3) + 250 µL (10,000 conidia)
Group 4	250 µL (100 L3) + 250 µL (100,000 conidia)
Group 5	250 µL (100 L3) + 250 µL (LD25 linalool)
Group 6	250 µL (100 L3) + 250 µL (LD50 linalool)
Group 7	250 µL (100 L3) + 250 µL (10,000 conidia + LD25 linalool)
Group 8	250 µL (100 L3) + 250 µL (10,000 conidia + LD50 linalool)
Group 9	250 µL (100 L3) + 250 µL (100,000 conidia + LD25 linalool)
Group 10	250 µL (100 L3) + 250 µL (100,000 conidia + LD50 linalool)

2.5. Statistical Analysis

The Probit analysis was performed on the results of Assays A and B, using the software Polo-Plus (LeOra Software, 2003) to determine the LD25 and LD50 with a 95% confidence interval (CI 95%). The results obtained from the assays were interpreted using one-way analysis of variance (ANOVA), followed by Tukey's Test at a 5% probability level, using GraphPad Prism 8.0. Tukey's test was chosen due to the nature of the comparisons, with balanced groups and homogeneous variances, characteristics that make this test suitable for performing multiple comparisons between experimental groups while controlling for Type I errors and ensuring the robustness of the results.

3. Results

3.1. Ovicidal Activity of Isolated Linalool (Assay A—EHT)

He results obtained in Assay A (EHT) are presented in Figure 1. Linalool showed inhibitory activity on egg hatching, with 100% efficacy at concentrations of 1.25 and 2.5 mg/mL and a LD50 of 0.49 mg/mL (CI 95% = 0.47–0.51), which differed statistically from both the negative and positive controls ($p \leq 0.05$). At a lower concentration of 0.62 mg/mL, 78.8% inhibition was observed, with results similar to the positive control with thiabendazole (57%), showing no statistically significant difference ($p > 0.05$).

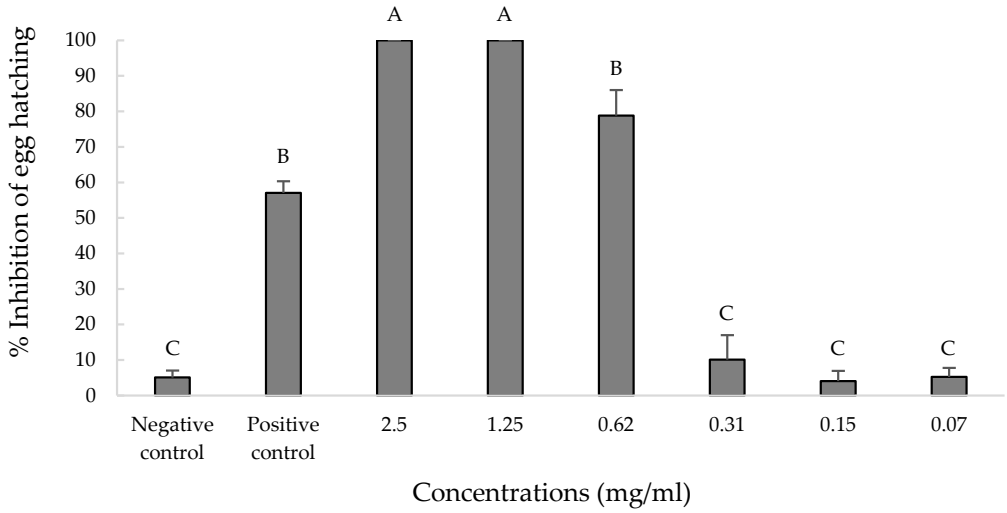

Figure 1. Percentage inhibition of egg hatching of gastrointestinal nematodes in sheep, with standard deviation. Negative control: 3% DMSO; positive control: 6.40 µg/mL thiabendazole. Different letters above the bars indicate statistical differences according to Tukey's test at a 5% significance level.

3.2. Larvicidal Activity of Isolated Linalool (Assay B—LMIT)

In Assay B (LMIT), linalool inhibited 100% of the motility of L3 at the highest concentrations tested (4% and 8%) after 72 h of incubation, differing statistically from the other experimental groups ($p \leq 0.05$; Table 2). Inhibition greater than 80% was observed at the 2% concentration, significantly differing ($p \leq 0.05$) from the positive control (ivermectin). The LD50 value for linalool was 0.42% or 4.2 mg/mL (CI 95% = 0.29–0.57) and the LD25 was 0.15% or 1.5 mg/mL (CI 95% = 0.08–0.23).

Table 2. Mean number of live larvae ± standard deviation and percentage of inhibition of motility of infective larvae of gastrointestinal nematodes in sheep after 72 h of incubation.

Experimental Groups	Mean ± SD	% Inhibition
Negative control (NC)	96.9 ± 2.7^{A}	-
Positive control (PC)	70.7 ± 6.8^{B}	20.9
0.12% (1.,2 mg/mL)	56.2 ± 18.3^{C}	41.9
0.25% (2.5 mg/mL)	50 ± 25.9^{C}	48.4
0.5% (5 mg/mL)	64.5 ± 20.2^{C}	34.4
1% (10 mg/mL)	38.6 ± 20.6^{D}	60.1
2% (20 mg/mL)	17.9 ± 7.9^{D}	81.5
4% (40 mg/mL)	0 ± 0^{E}	100
8% (80 mg/mL)	0 ± 0^{E}	100

Negative control: distilled water; positive control: 0.53 mg/mL ivermectin. Means followed by different letters in the columns denote statistical differences ($p \leq 0.05$) according to Tukey's Test at 5% probability.

3.3. Larvicidal Activity of Linalool Combined with D. flagrans Conidia (Assay C—LMIT)

In Assay C, a significant reduction ($p \leq 0.05$) in the number of infective larvae was observed in the treated groups compared to the control group, starting at 24 h of treatment (Table 3). Groups 5 (LD25 linalool) and 6 (LD50 linalool) showed an average reduction in larvae of 41.5% and 72.8%, respectively, after 24 h, reaching 100% reduction after 14 days (D14). It was also observed that groups 7 to 10, treated with *D. flagrans* conidia associated with linalool, also inhibited larval motility starting at 24 h, with a significant increase in L3 reduction throughout the experiment, showing high percentages of reduction after 14 days (100%). Throughout the experimental period, groups G3 (10,000 conidia) and G4 (100,000 conidia), treated only with conidia, were significantly less effective ($p \leq 0.05$) compared to groups treated with both the fungus and linalool (G7 to G10). During the evaluation, in groups consisting of conidia, either alone or combined with linalool, the formation of traps and the predation of infective larvae were observed, confirming the predatory action of *D. flagrans*. There was no statistical difference ($p > 0.05$) between the larvicidal activity of the groups treated only with linalool (G5 and G6) and the groups treated with conidia associated with linalool (G7 to G10).

Table 3. Mean number of live larvae ± standard deviation and percentage of reduction of infective larvae of sheep gastrointestinal nematodes.

Experimental Groups	Hours/Days After Treatments					
	24 h		Day 7		Day 14	
	Mean ± SD	R (%)	Mean ± SD	R (%)	Mean ± SD	R (%)
Group 1	$96.3^{A} \pm 2.2$	-	$94.2^{A} \pm 1.8$	-	$92.1^{A} \pm 1.6$	-
Group 2	$86.2^{A} \pm 3.9$	10.5	$0^{C} \pm 0$	100	$0^{C} \pm 0$	100
Group 3	$76.7^{B} \pm 5$	21	$68.3^{B} \pm 6.3$	27.5	$30.8^{B} \pm 4.4$	66.5
Group 4	$85^{A} \pm 4.7$	11.7	$71^{B} \pm 7.1$	24.6	$27^{B} \pm 4$	70.7
Group 5	$56.3^{B} \pm 9.4$	41.5	$15.6^{C} \pm 4.1$	83.4	$0^{C} \pm 0$	100
Group 6	$26.2^{B} \pm 6.4$	72.8	$5^{C} \pm 0$	94.7	$0^{C} \pm 0$	100
Group 7	$45.3^{B} \pm 5.6$	53	$5^{C} \pm 1$	94.7	$0^{C} \pm 0$	100
Group 8	$16.2^{B} \pm 6.5$	83.2	$0^{C} \pm 0$	100	$0^{C} \pm 0$	100
Group 9	$37.3^{B} \pm 5.2$	61.3	$12.1^{C} \pm 3.6$	87.1	$0^{C} \pm 0$	100
Group 10	$29.4^{B} \pm 3.6$	69.5	$0^{C} \pm 0$	100	$0^{C} \pm 0$	100

SD: standard deviation; R (%): percentage of reduction. Means followed by different letters in the columns denote statistically significant differences ($p \leq 0.05$) according to Tukey's Test at 5% probability.

4. Discussion

Numerous studies have demonstrated the anthelmintic activity of bioactive compounds derived from plants, as well as the action of nematophagous fungi on gastrointestinal nematodes [15,22,35–38]. In an effort to enhance these effects, combinations of control strategies have been proposed. This study represents the first investigation into the efficacy of a combination of a nematophagous fungus and a monoterpene for controlling gastrointestinal nematodes in sheep.

The procedures established for evaluating the efficacy of new molecules, drugs, and natural compounds are carried out using in vitro techniques [21]. In this research, linalool was evaluated in isolation for its inhibitory activity on egg hatching, as well as its effectiveness in inhibiting the motility of infective larvae.

In Assay A (EHT), linalool caused a complete inhibition of egg hatching (100%) at a concentration of 1.25 mg/mL. Similar results were obtained by Ureña et al. [39], who reported a 94.7% inhibition of eggs (2 mg/mL) when evaluating the essential oil of *Lippia dominguensis*, rich in linalool (33.85%). In the present study, an inhibition greater than 70% (0.62 mg/mL) and an LD50 of 0.49 mg/mL (CI 95% = 0.47–0.51) were observed, which is similar to what was reported by Katiki et al. [24], who found an LD50 of 0.29 mg/mL (CI 95% = 0.27–0.31) in drug-resistant strains using linalool with 99.63% purity, demonstrating the promising anthelmintic potential of linalool at lower doses. However, Zhu et al. [40] reported a lower ovicidal activity of linalool at a higher concentration (10 mg/mL), with a maximum inhibition of 65.8% and a LD50 of 5.63 mg/mL (CI 95% = 4.66–7.07) against *Haemonchus contortus* eggs. Sousa et al. [18] obtained an LD50 of 1.75 mg/mL (CI 95% = 1.65–1.86) against a *H. contortus* strain resistant to benzimidazoles, which was higher than that observed in the present study, which also involved a population classified as resistant. This suggests that the efficacy of linalool in inhibiting egg hatching varies depending on the population studied and its resistance to anthelmintics.

The EHT was developed for the phenotypic diagnosis of anthelmintic resistance of nematodes to benzimidazoles [33]. However, with the aim of exploring new strategies for controlling gastrointestinal nematodes, it has also been applied to assess the efficacy of natural products with anthelmintic properties and biological control agents, considering eggs that do not hatch as non-viable [41].

In the present study, the EHT was performed to evaluate the efficacy of linalool as an ovicidal agent, as studies with other monoterpenes have already demonstrated this effect [17,19,42,43]. It is worth noting that in the present study, linalool was not evaluated in combination with *D. flagrans* in inhibiting egg hatching, as previous investigations have shown that this fungus does not possess ovicidal activity, with only the adherence and colonization of conidia on the surface of the eggs being observed, without any morphological damage. Its action is, however, confirmed to be predatory [14,44–47]. Considering the efficacy of linalool in inhibiting egg hatching, further studies could be conducted to investigate its association with fungi that have ovicidal activity.

For the analysis of larval predation, the LMIT serves as an indicator of the viability of parasites after exposure to different anthelmintic substances and is recognized as the gold standard [48,49]. Linalool demonstrated 100% efficacy in inhibiting the motility of L3 gastrointestinal nematode larvae at concentrations of 4% and 8% after 72 h of exposure in Assay B. Additionally, at a concentration of 2%, an inhibition greater than 80% was observed. These findings are consistent with those of Helal et al. [21] and Helal et al. [22], who reported 87% and 97% inhibition when testing 1% and 10 µg/mL (0.01 mg/mL) of linalool, respectively. These results show that even at lower concentrations, this biocompound promotes a high rate of inhibition of larval motility.

The LD50 value for linalool in Assay B was 0.42% or 4.2 mg/mL (95% CI = 0.29–0.57) after 72 h of exposure. However, for Helal et al. [21], the values obtained ranged from 0.51% to 1.76% after 24 h of exposure. These differences can be attributed to variations in incubation conditions and exposure time to linalool. Prolonged exposure may have led to greater toxicity, resulting in a lower LD50, while shorter exposure might not have been sufficient for linalool to exert a more toxic effect.

This study was the first to evaluate the predatory activity of a nematophagous fungus associated with a monoterpene in the control of sheep gastrointestinal nematodes. In Assay C, the combined use of *D. flagrans* and linalool, comprising Group 7 (10,000 conidia + LD25 linalool), Group 8 (10,000 conidia + LD50 linalool), Group 9 (100,000 conidia + LD25 linalool), and Group 10 (100,000 conidia + LD50 linalool), significantly outperformed the use of the fungus alone ($p \leq 0.05$) starting from seven days of interaction, resulting in a 100% reduction of L3 larvae after 14 days, indicating that the combined treatment was more effective. There are reports on the antifungal action of linalool [50–52]. However, when using the LD25 and LD50 doses in combination with *D. flagrans*, no antagonistic effect was observed, as linalool did not interfere with trap formation or the larval predation capacity of the fungus.

Studies have already been conducted evaluating the association between *D. flagrans* and synthetic anthelmintics on nematodes [16,27,53,54]. The results obtained in the present study corroborate those reported by Hao et al. [28], who also observed a greater reduction of L3 nematodes in sheep with the combination of *D. flagrans* and ivermectin, without compromising the predatory activity of the fungus. These findings emphasize that the combination of biological agents, such as nematophagous fungi, with drugs or natural compounds can enhance the anthelmintic efficacy.

In the present study, the larvicidal activity observed in the groups treated exclusively with linalool (G5—LD25 and G6—LD50) and the groups treated with conidia associated with the monoterpene (G7 to G10) did not show a statistically significant difference ($p > 0.05$). However, the results obtained in the groups with the combination revealed promising effects and advantages over the isolated treatments. While linalool may exert a direct effect on the parasitic stages present in the host organism, *D. flagrans* can act on the infective larvae in the environment. Its combined use is capable of disrupting the nematode life cycle, providing a more effective and sustainable control strategy.

The mechanism of action of linalool is not yet fully elucidated; however, it is suggested that it causes neurotoxic lesions similar to those of organophosphates, inhibiting acetylcholinesterase (AChE) receptors and causing damage to the nervous system of nematodes through intense lipolytic activity [23,55]. On the other hand, the use of *D. flagrans* represents a biological control strategy that stands out for its action targeted at combating the free-living larvae of nematodes, its ability to germinate in fecal material, and its resistance to adverse conditions, such as passage through the digestive tract [11,56]. It works by producing adhesive, three-dimensional hyphae that ensure the immobilization, penetration, and destruction of infective larvae, promoting a reduction of larvae in the pasture and reinfection of animals [9,15,57].

In the groups treated only with *D. flagrans* conidia (G3 and G4), the maximum larval reduction was 66.5% and 70.7%, respectively, after 14 days (D14). These results reinforce that the combination of *D. flagrans* with other control agents can minimize the shortcomings of isolated administration and increase fungal predation capacity [12].

Thus, the combination of *D. flagrans* with linalool represents an innovative and promising approach in the management of parasitic infections. However, in vivo experiments are needed to clarify the synergistic interaction between these agents, ensuring their effectiveness in field conditions for the control of helminth infections.

5. Conclusions

It is concluded that linalool exhibits ovicidal and larvicidal activity and that its combination with *D. flagrans* enhances the predation capacity of the fungus and potentiates the anthelmintic efficacy, making it an alternative method for the control of gastrointestinal nematodes in small ruminants.

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Institutional Review Board Statement: The experiments conducted in this study were approved and performed according to the recommendations of the Ethics Committee on the Use of Animals of the Federal Institute of Paraíba, Campus Sousa (CEUA-IFPB), under protocol number 23000.002370.2023-87.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Karshima, S.N.; Maikai, B.V.; Kwaga, J.K.P. Helminths of veterinary and zoonotic importance in Nigerian ruminants: A 46-year meta-analysis (1970–2016) of their prevalence and distribution. *Infect. Dis. Poverty* **2018**, *7*, 52. [CrossRef] [PubMed]
2. Smith, D.; Price, D.R.G.; Burrells, A.; Faber, M.N.; Hildersley, K.A.; Chintoan-Uta, C.; Chapuis, A.F.; Stevens, M.; Stevenson, K.; Burgess, S.T.G.; et al. The Development of Ovine Gastric and Intestinal Organoids for Studying Ruminant Host-Pathogen Interactions. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 733811. [CrossRef]
3. Fissiha, W.; Kinde, M.Z. Anthelmintic Resistance and Its Mechanism: A Review. *Infect. Drug Resist.* **2021**, *14*, 5403–5410. [CrossRef]
4. Costa, V.M.M.; Simões, S.V.D.; Riet-Correa, F. Gastro-intestinal nematodes control in goats and sheep in the semiarid region northeastern Brazil. *Pesq. Vet. Bras.* **2011**, *31*, 65–71. [CrossRef]
5. Soares, F.B.; Monteiro, A.C. Compatibilidade de *Metarhizium anisopliae* com carrapaticidas químicos. *Arq. Inst. Biol.* **2011**, *78*, 385–391. [CrossRef]
6. Ribeiro, W.L.C.; Camurça-Vasconcelos, A.L.F.; Santos, J.M.L.; Macedo, I.T.F.; Ribeiro, J.C.; Oliveira, E.F.; Paula, H.C.B.; Bevilaqua, C.M.L. The use of *Eucalyptus staigeriana* nanoemulsion for control of sheep haemonchosis. *Pesq. Vet. Bras.* **2017**, *37*, 221–226. [CrossRef]
7. Vieira, J.N.; Filho, F.S.M.; Ferreira, G.F.; Mendes, J.F.; Gonçalves, C.L.; Villela, M.M.; Pereira, D.I.B.; Nascente, P.S. In vitro susceptibility of nematophagous fungi to antiparasitic drugs: Interactions and implications for biological control. *Braz. J. Biol.* **2017**, *77*, 476–479. [CrossRef]
8. Vieira, I.S.; Oliveira, I.C.; Campos, A.K.; Araújo, J.V. Association and predatory capacity of fungi *Pochonia chlamydosporia* and *Arthrobotrys cladodes* in the biological control of parasitic helminths of bovines. *Parasitology* **2019**, *146*, 1347–1351. [CrossRef]
9. Araújo, J.V.; Braga, F.R.; Mendoza-de-Gives, P.; Paz-Silva, A.; Vilela, V.L.R. Recent Advances in the Control of Helminths of Domestic Animals by Helminthophagous Fungi. *Parasitologia* **2021**, *1*, 168–176. [CrossRef]
10. Zegbi, S.; Sagües, F.; Saumell, C.; Ceballos, L.; Domínguez, P.; Guerrero, I.; Junco, M.; Iglesias, L.; Fernández, S. Effect of Different Anthelmintic Drugs on the Development and Efficacy of *Duddingtonia flagrans*. *Ruminants* **2024**, *4*, 10–21. [CrossRef]
11. Wang, B.; Wang, F.; Xu, Q.; Wang, K.; Xue, Y.; Ren, R.; Zeng, J.; Liu, Y.; Zhang, H.; Wang, H.; et al. In vitro and in vivo studies of the native isolates of nematophagous fungi from China against the larvae of trichostrongylides. *J. Basic Microbiol.* **2016**, *57*, 265–275. [CrossRef]
12. Luns, F.D.; Assis, R.C.L.; Silva, L.P.C.; Ferraz, C.M.; Braga, F.R.; Araújo, J.V. Coadministration of Nematophagous Fungi for Biological Control over Nematodes in Bovine in the South-Eastern Brazil. *Biomed. Res. Int.* **2018**, *2018*, 2934674. [CrossRef]

13. Fausto, G.C.; Fausto, M.C.; Vieira, I.S.; Freitas, S.G.; Carvalho, L.M.; Oliveira, I.S.; Silva, E.N.; Campos, A.K.; Araújo, J.V. Formulation of the nematophagous fungus *Duddingtonia fagrans* in the control of equine gastrointestinal parasitic nematodes. *Vet. Parasitol.* **2021**, *295*, 109458. [CrossRef] [PubMed]
14. Rodrigues, J.A.; Roque, F.L.; Álvares, F.B.V.; Silva, A.L.P.; Lima, E.F.; Filho, G.M.S.; Feitosa, T.F.; Araújo, J.V.; Braga, F.R.; Vilela, V.L.R. Efcacy of a commercial fungal formulation containing *Duddingtonia fagrans* (Bioverm®) for controlling bovine gastrointestinal nematodes. *Rev. Bras. Parasitol. Vet.* **2021**, *30*, e026620. [CrossRef] [PubMed]
15. Rodrigues, J.A.; Roque, F.L.; Lima, B.A.; Filho, G.M.S.; Oliveira, C.S.M.; Sousa, L.C.; Silva, A.L.P.; Lima, E.F.; Feitosa, T.F.; Braga, F.R.; et al. Control of sheep gastrointestinal nematodes on pasture in the tropical semiarid region of Brazil, using Bioverm® (*Duddingtonia flagrans*). *Trop. Anim. Health Prod.* **2022**, *54*, 179. [CrossRef] [PubMed]
16. Mendes, L.Q.; Ferraz, C.M.; Motta, C.P.; Araújo, J.C.; Ferrari, E.S.; Rodrigues, J.A.; Luz, J.R.; Rocha, R.O.; Vilela, V.L.R.; Moreira, T.F.; et al. Asociación in vitro de *Duddingtonia flagrans* con ivermectina en el control de nematodos gastrointestinales de búfalos. *Rev. MVZ Cordoba* **2022**, *27*, 3. [CrossRef]
17. André, W.P.P.; Cavalcante, G.S.; Ribeiro, W.L.C.; Santos, J.M.L.D.; Macedo, I.T.F.; Paula, H.C.B.; Morais, S.M.; Melo, J.V.; Bevilaqua, C.M.L. Anthelmintic effect of thymol and thymol acetate on sheep gastrointestinal nematodes and their toxicity in mice. *Rev. Bras. Parasitol. Vet.* **2017**, *26*, 323–330. [CrossRef] [PubMed]
18. Sousa, A.I.P.; Silva, C.R.; Costa-Júnior, H.N.; Silva, N.C.S.; Pinto, J.A.O.; Blank, A.F.; Soares, A.M.S.; Costa-Júnior, L.M. Essential oils from *Ocimum basilicum* cultivars: Analysis of their composition and determination of the effect of the major compounds on *Haemonchus contortus* eggs. *J. Helminthol.* **2021**, *95*, e17. [CrossRef]
19. Aguiar, A.A.R.M.; Filho, J.V.A.; Pinheiro, H.N.; Campelo, M.D.S.; Ribeiro, W.L.C.; Melo, A.C.F.L.; Rocha, L.O.D.; Ribeiro, M.E.N.P.; Ricardo, N.M.P.S.; Abreu, F.O.M.D.S.; et al. In vitro anthelmintic activity of an R-carvone nanoemulsions towards multiresistant *Haemonchus contortus*. *Parasitology* **2022**, *149*, 1631–1641. [CrossRef] [PubMed]
20. Forezi, L.S.M.; Ferreira, P.G.; Hüther, C.M.; Silva, F.C.; Ferreira, V.F. There is Chemistry Here: Part IV. Terpenes in perfumery. *Rev. Virtual Quim.* **2022**, *14*, 1005–1024. [CrossRef]
21. Helal, M.A.; Abdel-Gawad, A.M.; Kandil, O.M.; Khalifa, M.M.E.; Cave, G.W.V.; Morrison, A.A.; Bartley, D.J.; Elsheikha, H.M. Nematocidal Effects of a Coriander Essential Oil and Five Pure Principles on the Infective Larvae of Major Ovine Gastrointestinal Nematodes In Vitro. *Pathogens* **2020**, *9*, 740. [CrossRef]
22. Helal, M.A.; Abdel-Gawad, A.M.; Kandil, O.M.; Khalifa, M.M.E.; Morrison, A.A.; Bartley, D.J.; Cave, G.W.V.; Elsheikha, H.M. Microfluidic-Based Formulation of Essential Oils-Loaded Chitosan Coated PLGA Particles Enhances Their Bioavailability and Nematocidal Activity. *Pharmaceutics* **2022**, *14*, 2030. [CrossRef] [PubMed]
23. López, M.D.; Pascual-Villalobos, M.J. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Ind. Crops Prod.* **2010**, *31*, 284–288. [CrossRef]
24. Katiki, L.M.; Barbieri, A.M.E.; Araujo, R.C.; Veríssimo, C.J.; Louvandini, H.; Ferreira, J.F.S. Synergistic interaction of ten essential oils against *Haemonchus contortus* in vitro. *Vet. Parasitol.* **2017**, *243*, 47–51. [CrossRef]
25. Katiki, L.M.; Araujo, R.C.; Ziegelmeyer, L.; Gomes, A.C.P.; Gutmanis, G.; Rodrigues, L.; Bueno, M.S.; Veríssimo, C.J.; Louvandini, H.; Ferreira, J.F.S.; et al. Evaluation of encapsulated anethole and carvone in lambs artificially- and naturally-infected with *Haemonchus contortus*. *Exp. Parasitol.* **2019**, *197*, 36–42. [CrossRef] [PubMed]
26. Vilela, V.L.R.; Feitosa, T.F.; Braga, F.R.; Araújo, J.V.; Santos, A.; Morais, D.F.; Souto, D.V.; Athayde, A.C. Coadministration of nematophagous fungi for biological control over gastrointestinal helminths in sheep in the semiarid region of northeastern. Brazil. *Vet. Parasitol.* **2016**, *221*, 139–143. [CrossRef] [PubMed]
27. Vilela, V.L.R.; Feitosa, T.F.; Braga, F.R.; Vieira, V.D.; Lucena, S.C.; Araújo, J.V. Control of sheep gastrointestinal nematodes using the combination of *Duddingtonia fagrans* and Levamisole Hydrochloride 5%. *Rev. Bras. Parasitol. Vet.* **2018**, *27*, 26–31. [CrossRef]
28. Hao, L.; Guo, Y.; Wang, X.; Gao, M.; Liu, T.; Ma, Y.; Zhang, Y.; Li, Q.; Wang, R.; You, X. Preparation and application of biocontrol formulation of nematode-trapping fungus—*Duddingtonia flagrans*. *Vet. Parasitol.* **2024**, *327*, 110119. [CrossRef] [PubMed]
29. Vilela, V.L.R.; Bezerra, H.M.F.F.; Bezerra, R.A.; Dantas, M.O.; Alcântara, E.T.; Oliveira, L.V.S.; Nóbrega, K.S.; Calazans, F.B.; Feitosa, T.F.; Braga, F.R.; et al. Sustainable agriculture: The use of FAMACHA method in Santa Ines sheep in the Semi-arid region of Brazil. *Semin. Ciênc. Agrár.* **2021**, *42*, 1647–1662. [CrossRef]
30. Roberts, F.H.S.; O'Sullivan, J.P. Methods of egg counts and larval cultures for Strongyles infesting the gastrointestinal tract of cattle. *Aust. J. Agric. Res.* **1950**, *1*, 99–102. [CrossRef]
31. Willcox, H.P.; Coura, J.R. Nova concepção para o método de BaermannMoraes-Coutinho na pesquisa de larvas de nematódeos. *Mem. Inst. Oswaldo Cruz.* **1989**, *84*, 563–565. [CrossRef]
32. Coles, G.C.; Jackson, F.; Pomroy, W.E.; Prichard, R.K.; Von Samson-Himmelstjerna, G.; Silvestre, A.; Taylor, M.A.; Vercruysse, J. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* **2006**, *136*, 67–185. [CrossRef] [PubMed]

33. Coles, G.C.; Bauer, C.; Borgsteede, F.H.; Geerts, S.; Klei, T.R.; Taylor, M.A.; Waller, P.J. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* **1992**, *44*, 35–44. [CrossRef]
34. Ferreira, L.E.; Castro, P.M.; Chagas, A.C.; França, S.C.; Beleboni, R.O. In vitro anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. *Exp. Parasitol.* **2013**, *134*, 327–332. [CrossRef] [PubMed]
35. Kļaviņa, A.; Keidāne, D.; Ganola, K.; Lūsis, I.; Šukele, R.; Bandere, D.; Kovalcuka, L. Anthelmintic Activity of *Tanacetum vulgare* L. (Leaf and Flower) Extracts against Trichostrongylidae Nematodes in Sheep In Vitro. *Animals* **2023**, *13*, 2176. [CrossRef] [PubMed]
36. Štrbac, F.; Krnjajić, S.; Stojanović, D.; Ratajac, R.; Simin, N.; Orčić, D.; Rinaldi, L.; Ciccone, E.; Maurelli, M.P.; Cringoli, G.; et al. In vitro and in vivo anthelmintic efficacy of peppermint (*Mentha x piperita* L.) essential oil against gastrointestinal nematodes of sheep. *Front. Vet. Sci.* **2023**, *10*, 1232570. [CrossRef] [PubMed]
37. Liu, X.Y.; Chang, F.F.; Zhao, T.Y.; Huang, H.Y.; Li, F.D.; Wang, F.; Wang, B.B.; Wang, F.H.; Liu, Q.; Luo, Q.H.; et al. Biological control of sheep gastrointestinal nematode in three feeding systems in Northern China by using powder drug with nematophagous fungi. *Biocontrol Sci. Technol.* **2020**, *30*, 701–715. [CrossRef]
38. Cubides-Cárdenas, J.A.; Duarte, J.J.V.; Lombana, H.G.; Céspedes-Gutiérrez, E.; Gómez-Álvarez, M.I.; Cortés-Rojas, D.F. Evaluation of new formulations of nematophagous fungi *Duddingtonia flagrans* to control gastrointestinal nematodes in post-weaning lambs in Colombia Andean region. *Small Rumin. Res.* **2023**, *223*, 106980. [CrossRef]
39. Ureña, E.M.J.; Katchborian-Neto, A.; Trinidad, A.B.; Ramírez, M.; Vásquez, M.T.; Araújo-Filho, J.V.; Ribeiro, W.L.C.; Castelo-Branco, D.S.C.M.; Oliveira, L.M.B.; Bevilaqua, C.M.L.; et al. Chemical Composition, Anthelmintic Activity, and Mechanism of Action of *Lippia dominguensis* Mold. Essential Oil on *Haemonchus contortus*. *Chem. Biodivers.* **2023**, *20*, e202300135. [CrossRef]
40. Zhu, L.; Dai, J.; Yang, L.; Qiu, J. Anthelmintic activity of Arisaema franchetianum and Arisaema lobatum essential oils against *Haemonchus contortus*. *J. Ethnopharmacol.* **2013**, *148*, 311–316. [CrossRef] [PubMed]
41. Lacey, E.; Brady, R.L.; Prichard, R.K.; Watson, T.R. Comparison of inhibition of polymerisation of mammalian tubulin and helminth ovicidal activity by benzimidazole carbamates. *Vet. Parasitol.* **1987**, *23*, 105–119. [CrossRef]
42. Barbosa, M.L.F.; Ribeiro, W.L.C.; Araújo Filho, J.V.; Pereira, R.C.A.; André, W.P.P.; Melo, A.C.F.L.; Castelo-Branco, D.S.C.M.; Oliveira, L.M.B.; Bevilaqua, C.M.L. In vitro anthelmintic activity of *Lippia alba* essential oil chemotypes against *Haemonchus contortus*. *Exp. Parasitol.* **2023**, *244*, 108439. [CrossRef]
43. Amel, A.; Sebai, E.; Mhadhbi, M.; Akkari, H. In vitro and in vivo anthelmintic effect of essential oil obtained from *Thymus capitatus* flowers against *Haemonchus contortus* and *Heligmosomoides polygyrus*. *Exp. Parasitol.* **2024**, *262*, 108778. [CrossRef] [PubMed]
44. Braga, F.R.; Araújo, J.V.; Campos, A.K.; Araújo, J.M.; Carvalho, R.O.; Silva, A.R.; Tavela, A.O. In vitro evaluation of the action of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Fasciola hepatica eggs*. *World J. Microbiol. Biotechnol.* **2008**, *24*, 1559–1564. [CrossRef]
45. Motta, C.P.; Ferraz, C.M.; Soares, F.E.F.; Schmidt, T.F.; Stabenow, R.S.; Araújo, J.V.; Tobias, F.L.; Rossi, G.A.M.; Feitosa, T.F.; Vieira, G.S.; et al. Ovicidal Activity of Chemical Disinfectants and the Nematophagous Fungus *Duddingtonia flagrans* and *Toxocara canis*. *Adv. Biotechnol. Microbiol.* **2022**, *17*, 555952. [CrossRef]
46. Roque, F.L.; Filho, G.M.S.; Oliveira, C.S.M.; Rodrigues, J.A.; Feitosa, T.F.; Braga, F.R.; Araújo, J.V.; Vilela, V.L.R. Evaluation of the fungus *Duddingtonia flagrans* (Bioverm®) on *Ascaris suum* eggs and infective larvae of *Oesophagostomum* spp. and *Hyostrongylus rubidus* from swine. *Semin. Ciênc. Agrár.* **2023**, *44*, 1587–1596. [CrossRef]
47. Ferreira, T.S.; Ferraz, C.M.; Santos, P.H.D.; Soares, F.E.F.; Segantine, V.B.S.; Vilela, V.L.R.; Araújo, J.V.; Braga, F.R. Isolated and Associated Use of the Nematophagous Fungi *Pochonia chlamydosporia* and *Duddingtonia flagrans* to Control *Taenia saginata* Eggs. *Parasitologia* **2024**, *4*, 238–245. [CrossRef]
48. Ritler, D.; Rufener, R.; Sager, H.; Bouvier, J.; Hemphill, A.; Lundström-Stadelmann, B. Development of a movement-based in vitro screening assay for the identification of new anti-cestodal compounds. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005618. [CrossRef] [PubMed]
49. Partridge, F.A.; Brown, A.E.; Buckingham, S.D.; Willis, N.J.; Wynne, G.M.; Forman, R.; Else, K.J.; Morrison, A.A.; Matthews, J.B.; Russell, A.J.; et al. An automated high-throughput system for phenotypic screening of chemical libraries on *C. elegans* and parasitic nematodes. *Int. J. Parasitol. Drugs Drug Resist.* **2018**, *8*, 8–21. [CrossRef]
50. Singulani, J.L.; Pedroso, R.S.; Ribeiro, A.B.; Nicolella, H.D.; Freitas, K.S.; Damasceno, J.L.; Vieira, T.M.; Crotti, A.E.M.; Tavares, D.C.; Martins, C.H.G.; et al. Geraniol and Linalool Anticandidal Activity, Genotoxic Potential and Embryotoxic Effect on Zebrafish. *Future Microbiol.* **2018**, *13*, 1637–1646. [CrossRef]
51. Barros Gomes, P.R.; Oliveira Cunha, M.J.; de Sousa, D.A.; Reis, J.B.; Melo, A.V.; Freitas, A.C.; Hunaldo, V.K.L.; Paula, M.L.; Louzeiro, H.C.; Rodrigues, J.R.P.; et al. Chemical Study and Antifungal Activity of the Essential Oil of the Branches of *Aniba duckei* Kostermans. *JEOBP* **2019**, *22*, 1554–1561. [CrossRef]

52. Li, X.; Wang, Q.; Li, H.; Wang, X.; Zhang, R.; Yang, X.; Jiang, Q.; Shi, Q. Revealing the Mechanisms for Linalool Antifungal Activity against *Fusarium oxysporum* and Its Efficient Control of Fusarium Wilt in Tomato Plants. *Int. J. Mol. Sci.* **2023**, *24*, 458. [CrossRef] [PubMed]
53. Ferraz, C.M.; Sobral, S.A.; Senna, C.C.; Junior, O.F.; Moreira, T.F.; Tobias, F.L.; Soares, F.E.F.; Geniêr, H.L.A.; Vilela, V.L.R.; Lima, J.A.C.; et al. Combined use of ivermectin, dimethyl sulfoxide, mineral oil and nematophagous fungi to control *Rhabditis* spp. *Vet. Parasit.* **2019**, *275*, 108924. [CrossRef]
54. Sobral, S.A.; Ferraz, C.M.; Souza, R.I.L.; Queiroz, L.M.; Reinó, N.; Junior, O.L.F.; Tobias, F.L.; Correia, J.A.L.; Araújo, J.V.; Vilela, V.R.L.; et al. Association between *Duddingtonia flagrans*, dimethylsulfoxide and ivermectin for the control of *Rhabditis* spp. in cattle. *Trop. Anim. Health Prod.* **2022**, *54*, 198. [CrossRef] [PubMed]
55. Nadeem, M.; Anjum, F.M.; Khan, M.I.; Tehseen, S.; El-Ghorab, A.; Sultan, J.I. Nutritional and medicinal aspects of coriander (*Coriandrum sativum* L.). *Br. Food J.* **2013**, *115*, 743–755. [CrossRef]
56. Larsen, M.; Wolstrup, J.; Henriksen, S.A.; Gronvold, J.; Nansen, P. In vivo passage through calves of nematophagous fungi selected for biocontrol of parasitic nematodes. *J. Helminthol.* **1992**, *66*, 137–141. [CrossRef] [PubMed]
57. Baiak, B.H.B.; Gasparina, J.M.; Ianke, L.; Sousa, K.T.; Deniz, M.; Pereira, L.M.; Araújo, J.V.; Rocha, R.A.; Dittrich, J.R. Predatory activity of nematophagus fungus *Duddingtonia fagrans* in infective larvae after gastrointestinal transit: Biological control in pasture areas and in vitro. *J. Helminthol.* **2021**, *95*, e31. [CrossRef]