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Species-Specific Chemotactic Responses of Entomopathogenic and Slug-Parasitic Nematodes to Cannabinoids from *Cannabis sativa* L.

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Abstract: The increasing environmental and health concerns associated with synthetic pesticides underscore the need for sustainable alternatives in pest management. This study investigates the chemotactic responses of five nematode species—*Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*, *Phasmarhabditis papillosa*, and *Oscheius myriophilus*—to three major cannabinoids from *Cannabis sativa*: Δ^9-tetrahydrocannabinol (THC), cannabigerol (CBG), and cannabidiol (CBD). Using a standardized chemotaxis assay, we quantified infective juvenile movement and calculated Chemotaxis Index (CI) values across varying cannabinoid concentrations. Our results revealed strong species-specific and dose-dependent responses. THC and CBG elicited significant attractant effects in *P. papillosa*, *S. feltiae*, and *H. bacteriophora*, with CI values ≥ 0.2, indicating their potential as behavioral modulators. In contrast, CBD had weaker or repellent effects, particularly at higher concentrations. *O. myriophilus* exhibited no consistent response, underscoring species-specific variation in chemosensory sensitivity. These findings demonstrate the potential utility of cannabinoids, especially THC and CBG, as biocompatible cues to enhance the efficacy of nematode-based biological control agents in integrated pest management (IPM). Further field-based studies are recommended to validate these results under realistic agricultural conditions.

Keywords: biological control; chemosensory sensitivity; cannabinoids; cannabidiol; cannabigerol; chemotaxis; entomopathogenic nematodes; integrated pest management; slug-parasitic nematodes; tetrahydrocannabinol

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1. Introduction

The widespread use of synthetic chemical pesticides in agriculture has raised serious concerns due to their detrimental effects on environmental health and biodiversity [1]. These compounds not only contaminate ecosystems but also pose significant risks to human health [2]. Despite these well-documented hazards, global pesticide use has continued to rise at an average annual rate of 1% over the past decade [3]. This trend highlights the urgent need for sustainable alternatives, particularly plant-derived or biological pest control agents—commonly referred to as botanical pesticides or biopesticides [4].

According to FAOSTAT [3], biological pesticides accounted for a mere 0.01% of the 4.1 million kilograms of pesticides used in global agriculture in 2022. This stark imbalance underscores the critical importance of identifying and promoting new "green" pesticide formulations that minimize ecological harm while maintaining effective pest control.

Among the most promising candidates for botanical pesticides is *Cannabis sativa* L. (Rosales: Cannabaceae), a species renowned for its rich array of biologically active secondary metabolites. Numerous studies have investigated various plant parts and preparations of *C. sativa* for their insecticidal, acaricidal, and nematicidal properties [5–7]. Cannabis-based formulations have demonstrated efficacy against a range of phytophagous arthropods, including the European mole cricket (*Gryllotalpa gryllotalpa* L.; Orthoptera: Gryllotalpidae), western flower thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae), cotton bollworm (*Helicoverpa armigera* Hübner; Lepidoptera: Noctuidae), Japanese beetle (*Popillia japonica* Newman; Coleoptera: Scarabaeidae), fall armyworm (*Spodoptera frugiperda* [J.E. Smith]; Lepidoptera: Noctuidae), and two-spotted spider mite (*Tetranychus urticae* Koch; Trombidiformes: Tetranychidae). These findings suggest that cannabinoid-based biopesticides could serve as a sustainable and selective alternative to conventional synthetic pesticides [7,8].

The pesticidal properties of *C. sativa* are attributed to its diverse secondary metabolites, such as alkaloids, flavonoids, lignans, steroids, and, most notably, cannabinoids. Over 100 cannabinoids have been identified, with Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) being the most abundant and pharmacologically significant [9,10]. Based on the concentration of THC in female inflorescences, *C. sativa* is classified into two categories: industrial hemp (fiber-type), which contains less than 0.3% THC as per Regulation (EU) No 2021/2115 [11], and medical cannabis, which exceeds this threshold and is cultivated for therapeutic use [12].

Nematodes, ubiquitous in terrestrial and aquatic ecosystems, may be either free-living or parasitic [13]. Among these, entomopathogenic nematodes (EPNs) play a vital role in integrated pest management due to their ability to seek and infect insect hosts using a range of chemical cues [14,15]. Members of the families Heterorhabditidae such as *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterirhabditidae) and Steinernematidae such as *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) have been widely applied as biological insecticides. Additionally, species from the genus *Phasmarhabditis*, known for their efficacy against pest gastropods, and some from *Oscheius*, which exhibit opportunistic entomopathogenic behavior, have gained increasing interest in biological control research [16,17].

Beyond insect control, recent studies have demonstrated that *Phasmarhabditis papillosa* (Schneider) (Rhabditida: Rhabditidae) Andrássy and *Oscheius myriophilus* (Poinar) (Rhabditida: Rhabditidae) are also effective against mollusk pests, particularly slugs, which are significant agricultural pests in many cropping systems [17]. These nematodes parasitize slugs by entering through natural openings or penetrating the body wall, subsequently invading internal organs such as the mantle cavity, digestive tract, and reproductive system. This internal colonization disrupts critical physiological processes, leading infected slugs to stop feeding within days—a key benefit for reducing crop damage before death occurs. Mortality typically follows within 4 to 21 days, influenced by environmental conditions and infection intensity. Inside the host cadaver, nematodes reproduce, and new infective juveniles eventually emerge to seek out additional hosts in the surrounding soil. This life cycle not only enables sustained suppression of slug populations but also promotes nematode persistence in agroecosystems, making them promising candidates for sustainable pest management strategies [18].

A critical component of nematode foraging behavior is the ability to detect and respond to chemical cues such as carbon dioxide (CO_2) and volatile organic compounds (VOCs) emitted by plants and soil organisms [14,15,19,20]. VOCs serve as essential belowground signals, shaping multitrophic interactions between plants, herbivores, and their natural enemies—including nematodes [14,15]. While it is well established that VOCs can attract

entomopathogenic nematodes as part of indirect plant defenses [14,15], the response of slug-parasitic nematodes such as *P. papillosa* and *O. myriophilus* to plant-emitted volatiles remains largely uncharacterized [21,22].

A prior study by Laznik et al. [23] investigated the impact of ethanol extracts from various *C. sativa* genotypes and plant organs on the chemotactic behavior of three EPN species. Results revealed genotype- and organ-specific effects, with extracts from the inflorescences of medical cannabis 'MX-CBD-707' and hemp varieties 'Futura 75' and 'Tiborszallasi' producing varied nematode responses. These findings suggest that specific cannabis-derived metabolites, particularly cannabinoids, may influence nematode behavior and could be leveraged as novel attractants or repellents in pest management.

Building on this research, the present study aims to (1) evaluate the influence of selected cannabinoids on the chemotactic behavior of five nematode species—*Heterorhabditis bacteriophora* Poinar, *O. myriophilus*, *P. papillosa*, *Steinernema carpocapsae* (Weiser), and *Steinernema feltiae* (Filipjev); (2) determine whether chemotactic responses are species-specific; and (3) assess how cannabinoid concentration affects nematode behavior. A better understanding of these interactions may contribute to the development of targeted, ecologically sound pest control methods based on nematode sensory biology.

2. Materials and Methods

2.1. Source and Maintenance of Parasitic Nematodes

Three species of commercially available entomopathogenic nematodes (EPNs)—*H. bacteriophora*, *S. carpocapsae*, and *S. feltiae*—were procured from Koppert B.V. (Berkel en Rodenrijs, The Netherlands) for use in chemotaxis experiments. These nematodes were maintained via in vivo propagation, using last-instar larvae of the wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) as hosts, following established procedures [13]. Infective juveniles (IJs) were recovered and held in M9 buffer at 4 °C at a density of 4000 IJs per mL. Only specimens younger than two weeks were used for bioassays. Nematode counts were performed using the enumeration protocol detailed by Laznik et al. [23]. Before each assay, nematode viability was verified under a microscope; only batches with viability greater than 95% were selected for testing.

In addition to the EPNs, this study also involved two mollusk-parasitic nematode species: *P. papillosa* (GenBank accession no. MT800511.1) and *O. myriophilus* (GenBank accession no. OP684306.1). These strains were recently isolated from *Arion vulgaris* Moquin-Tandon (Stylommatophora: Arionidae), following the approach described by Laznik et al. [17]. Cultures were maintained in vivo using freeze-killed slugs as a substrate. Ten days after exposure, infective juveniles were isolated using the same protocol employed for the EPN species. Resulting suspensions were stored in M9 buffer at 4 °C. Only those less than 14 days old and showing more than 95% viability were included in experimental trials.

2.2. Tested Cannabinoids

Cannabidiol (CBD) and cannabigerol (CBG) were sourced in powder form from Candropharm B.V. (Weeze, Germany), while tetrahydrocannabinol (THC) was acquired as Dronabinol from Spectrum Therapeutics (Kelowna, BC, Canada). Each cannabinoid was initially dissolved by adding 1 g of the compound to 1 mL of 96% ethanol, creating a stock solution defined as 100% concentration. This stock was subsequently diluted with ethanol to achieve working concentrations of 50%, 25%, and 12%. Accurate volumetric measurements were used during dilution to ensure precise concentrations. Before use in the bioassays, all solutions were vortexed to achieve uniform dispersion. This method ensured consistent cannabinoid exposure across all experimental replicates and supported the reliability and reproducibility of the chemotaxis assays.

2.3. Chemotaxis Assay

The chemotaxis bioassay (Figure 1) followed a protocol originally established by O'Halloran and Burnell [24], with subsequent modifications introduced by Laznik et al. [20,21]. The tests were conducted in 9 cm Petri dishes containing 25 mL of 1.6% technical agar (Biolife, Milan, Italy). This agar was prepared using a buffered solution consisting of 5 mM potassium phosphate (pH 6.0), 1 mM calcium chloride ($CaCl_2$), and 1 mM magnesium sulfate ($MgSO_4$), which facilitated nematode movement.

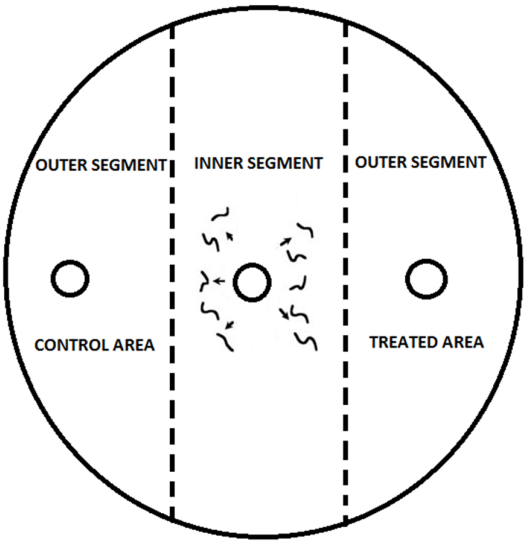

Figure 1. Schematic representation of the chemotaxis assay. Each Petri dish (ø = 9 cm) was marked with three 1 cm diameter reference points on the bottom: one at the center and two 1.5 cm from the edge on opposing sides. A 10 µL droplet of the test cannabinoid solution, prepared at a predefined concentration, was applied to the right side of the agar surface (treated area), while the left side received 10 µL of 96% ethanol (control area). Immediately thereafter, 100 infective juveniles (IJs) were placed at the center point in a 50 µL droplet. For negative controls, both sides received 10 µL of 96% ethanol, with the IJs applied in the same manner. Cannabinoids were administered just prior to nematode inoculation to ensure activity during the assay period.

Each treatment condition was replicated ten times, and the entire experiment was independently repeated on three separate occasions to ensure reproducibility. To prevent any risk of cross-contamination from volatile organic compounds (VOCs), only one compound was tested per experimental set. Petri dishes were sealed with Parafilm™ (Bemis Company, Inc., Neenah, WI, USA) to confine the treatment within the designated exposure area and avoid diffusion to other plates.

Plates were incubated in a controlled environment chamber (RK-900 CH, Kambič Laboratory Equipment, Semič, Slovenia) maintained at 22 °C and 75% relative humidity in complete darkness. These conditions were selected to simulate the nematodes' natural soil habitat and eliminate interference from light or temperature fluctuations. After a 24 h exposure period, nematode movement was halted by briefly freezing the plates at −20 °C for 3 min, preserving their final positions for accurate analysis.

The distribution of nematodes was assessed under a Nikon SMZ800N stereo microscope (Nikon Corporation, Tokyo, Japan) equipped with a 4K UHD Multi-output HDMI camera (Model XCAM4K8MPB; Tucsen Photonics Co., Ltd., Fuzhou, China), using 25× magnification. The nematodes' behavioral responses were quantified using the Chemo-

taxis Index (CI), calculated with a modified formula based on Bargmann and Horvitz [25] and Laznik et al. [20,21], as follows:

$$CI = (\% \text{ of IJs in the treated area} - \% \text{ of IJs in the control area})/100\% \quad (1)$$

This index provided a standardized measure of nematode preference or avoidance in response to the tested volatiles.

CI values ranged from 1.0 (indicating complete attraction) to −1.0 (indicating complete repulsion). Based on the calculated CI, compounds were classified as follows: attractants ($CI \geq 0.2$), weak attractants ($0.2 > CI \geq 0.1$), neutral ($-0.1 \leq CI < 0.1$), weak repellents ($-0.2 \leq CI < -0.1$), and repellents ($CI \leq -0.2$) [20,21].

2.4. Statistical Analysis

Directional movement of nematodes within the chemotaxis assay—specifically, migration from the inner segment to either the treated or control area—was evaluated using a paired Student's *t*-test. A significance threshold of $p < 0.05$ was used to determine statistically meaningful preference or avoidance behaviors. Prior to all parametric tests, data were assessed for normality using the Shapiro–Wilk test.

To assess interspecies variation in behavioral responses, the mean percentage of infective juveniles (IJs) that migrated to the outer segments or remained in the inner segment was calculated for each replicate. These data were analyzed using a three-way analysis of variance (ANOVA), with fixed factors including cannabinoid identity, cannabinoid concentration, and nematode species. Interaction effects among these variables were also tested. Among all interactions, only the three-way interaction (cannabinoid identity × nematode species × cannabinoid concentration) was statistically significant and considered biologically relevant.

Additionally, a separate two-way analysis of variance (ANOVA) was applied to the Chemotaxis Index (CI) data in order to assess the general responsiveness of the nematode species to various cannabinoid treatments. Where significant differences were identified, Duncan's multiple range test was employed for post hoc comparisons, using a significance threshold of $p < 0.05$. Data are reported as mean values accompanied by their corresponding standard errors (SE).

All statistical evaluations were carried out using Statgraphics Plus for Windows, version 4.0 (Statistical Graphics Corp., Manugistics, Inc., Rockville, MD, USA), while graphical representations of the results were prepared in Microsoft Excel 2010.

3. Results

3.1. Nematode Motility

In this study, motility was defined as the movement of infective juveniles (IJs) from the inner segment to the outer segments of the chemotaxis assay plate (Figure 1). The proportion of IJs reaching the outer segments was used as a quantitative measure to assess the influence of species identity, cannabinoid type, concentration, and their interactions (Table 1).

The analysis revealed that nematode species identity had the most significant impact on IJ motility ($F = 137.6$, $p < 0.0001$), reflecting marked behavioral differences among the five tested taxa. Cannabinoid type also exerted a strong effect ($F = 11.9$, $p < 0.0001$), highlighting the relevance of chemical identity in modulating nematode movement. Additionally, cannabinoid concentration significantly influenced the chemotactic response ($F = 18.0$, $p < 0.0001$), suggesting a dose-dependent effect.

Table 1. ANOVA results for the directional movement of infective juveniles (IJs) from the inner to the outer segments of the Petri dish.

Factor	Sum of Squares	Df	F	*p*
Nematode species (S)	39,465.0	4	137.6	0.0001
Cannabinoids (C)	1702.1	2	11.9	0.0001
Cannabinoid concentration (Cc)	5157.1	4	18.0	0.0001
Temporal replication	933.1	9	1.4	0.1627
Spatial replication	210.0	2	1.5	0.2330
S × C	6057.9	8	10.6	0.0001
S × Cc	1166.1	16	1.0	0.4374
C × Cc	1262.7	8	2.2	0.0280
S × C × Cc	6014.1	32	2.6	0.0001
Residual	21,220.5	296		
Total (Corrected)	83,188.6	381		

Neither temporal replication (F = 1.4, p = 0.1627) nor spatial replication (F = 1.5, p = 0.2330) had a significant effect, confirming the robustness and consistency of the experimental setup.

Significant interaction effects further revealed the complexity of nematode behavior. The interaction between species and cannabinoid type (S × C) was highly significant (F = 10.6, $p < 0.0001$), indicating that nematode responses varied with the type of cannabinoid. Although the species × concentration (S × Cc) interaction was not significant (F = 1.0, p = 0.4374), the cannabinoid × concentration (C × Cc) interaction reached statistical significance (F = 2.2, p = 0.0280), suggesting that the influence of cannabinoids may vary across different concentrations, though not consistently across all species.

Importantly, the three-way interaction species × cannabinoid × concentration (S × C × Cc) was highly significant (F = 2.6, $p < 0.0001$), demonstrating that nematode chemotactic behavior is both species-specific and context-dependent, influenced by the particular combination of chemical identity and dose.

These findings collectively underscore that species identity, cannabinoid type, and concentration are the primary drivers of nematode motility. The interaction effects emphasize the nuanced and dynamic nature of nematode chemosensation and suggest potential for tailoring plant-derived compounds to enhance biological control strategies. The lack of significant variability due to replication also supports the experimental reliability.

Figure 2 shows the mean percentage (±SE) of IJs migrating to the outer segments of the Petri dishes across the five nematode species. *P. papillosa* exhibited the highest motility (~45%), followed by *S. carpocapsae* and *S. feltiae* (~35–37%). On the other hand, *H. bacteriophora* showed intermediate movement (~30%), while *O. myriophilus* displayed the lowest response (~13%).

Figure 3 presents nematode responses to Δ^9-tetrahydrocannabinol (THC) at 100%, 50%, 25%, and 12%, compared to a 96% ethanol control. *P. papillosa* displayed the highest responsiveness across all THC levels, peaking at 50% (~70%). *S. feltiae* responded strongly at 100%, while *O. myriophilus* remained minimally responsive at all concentrations. *H. bacteriophora* and *S. carpocapsae* showed moderate, dose-dependent responses.

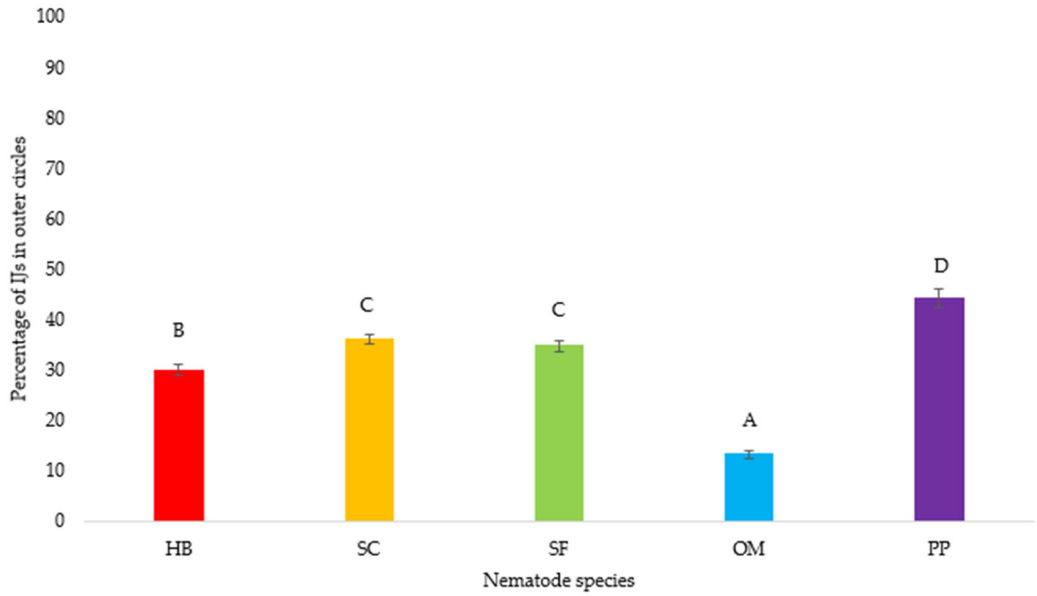

Figure 2. Mean percentage (±standard error) of infective juveniles (IJs) of five nematode species—*Heterorhabditis bacteriophora* (HB), *Steinernema carpocapsae* (SC), *Steinernema feltiae* (SF), *Oscheius myriophilus* (OM), and *Phasmarhabditis papillosa* (PP)—that migrated to the outer segments of the chemotaxis assay dish. Different capital letters above the bars indicate statistically significant differences according to Duncan's multiple range test ($p < 0.05$).

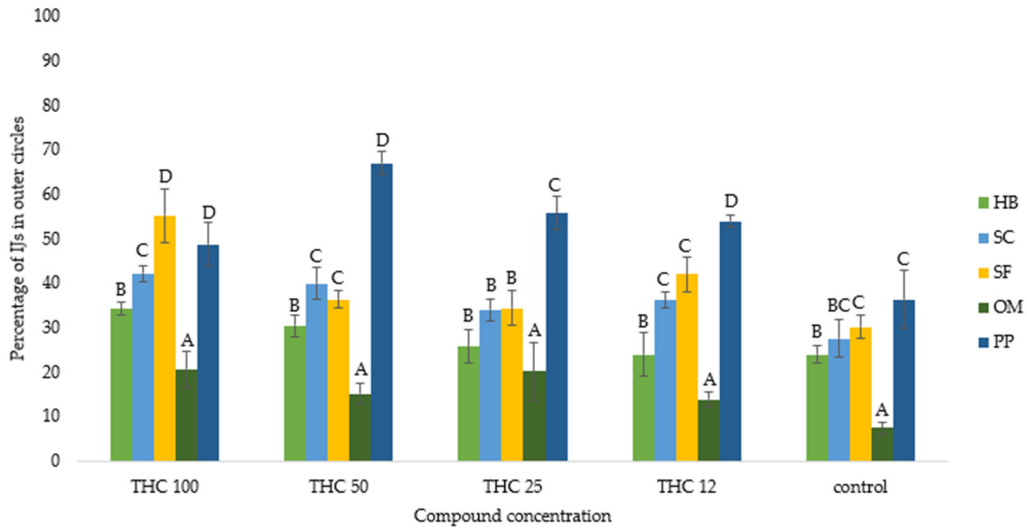

Figure 3. Mean percentage (±standard error) of infective juveniles (IJs) of five nematode species to varying concentrations of Δ^9-tetrahydrocannabinol (THC; 100%, 50%, 25%, 12%) and a 96% ethanol control. Different capital letters above the bars indicate statistically significant differences among nematode species within the same THC concentration, based on Duncan's multiple range test ($p < 0.05$). Legend: *Heterorhabditis bacteriophora* (HB), *Steinernema carpocapsae* (SC), *Steinernema feltiae* (SF), *Oscheius myriophilus* (OM), and *Phasmarhabditis papillosa* (PP).

Figure 4 illustrates the effect of cannabigerol (CBG) on nematode motility. *P. papillosa* again showed the highest responsiveness, particularly at 25% CBG. *S. carpocapsae* and *H. bacteriophora* were moderately attracted, especially at 100% and 50% CBG. *O. myriophilus* exhibited limited movement, while *S. feltiae* displayed variable responses.

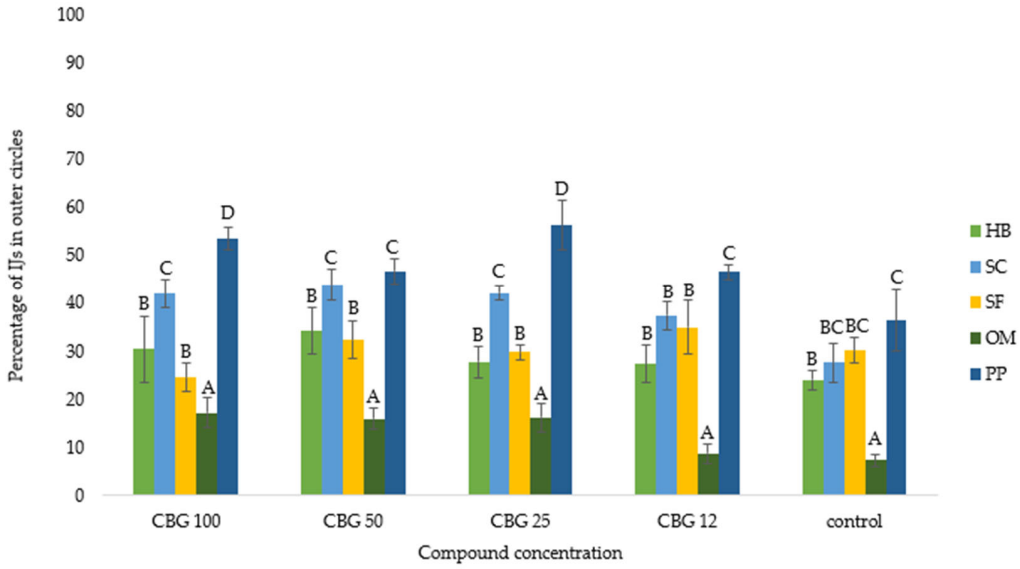

Figure 4. Mean percentage (±standard error) of infective juveniles (IJs) of five nematode species to varying concentrations of cannabigerol (CBG; 100%, 50%, 25%, 12%) and a 96% ethanol control. Different capital letters above the bars indicate statistically significant differences among nematode species within the same CBG concentration, based on Duncan's multiple range test ($p < 0.05$). Legend: *Heterorhabditis bacteriophora* (HB), *Steinernema carpocapsae* (SC), *Steinernema feltiae* (SF), *Oscheius myriophilus* (OM), and *Phasmarhabditis papillosa* (PP).

Figure 5 summarizes responses to cannabidiol (CBD). Motility was generally lower than for THC or CBG. *P. papillosa* responded well to 100% CBD but less so at lower concentrations. *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* showed consistent, moderate responses, while *O. myriophilus* had the weakest responses, even at peak concentration.

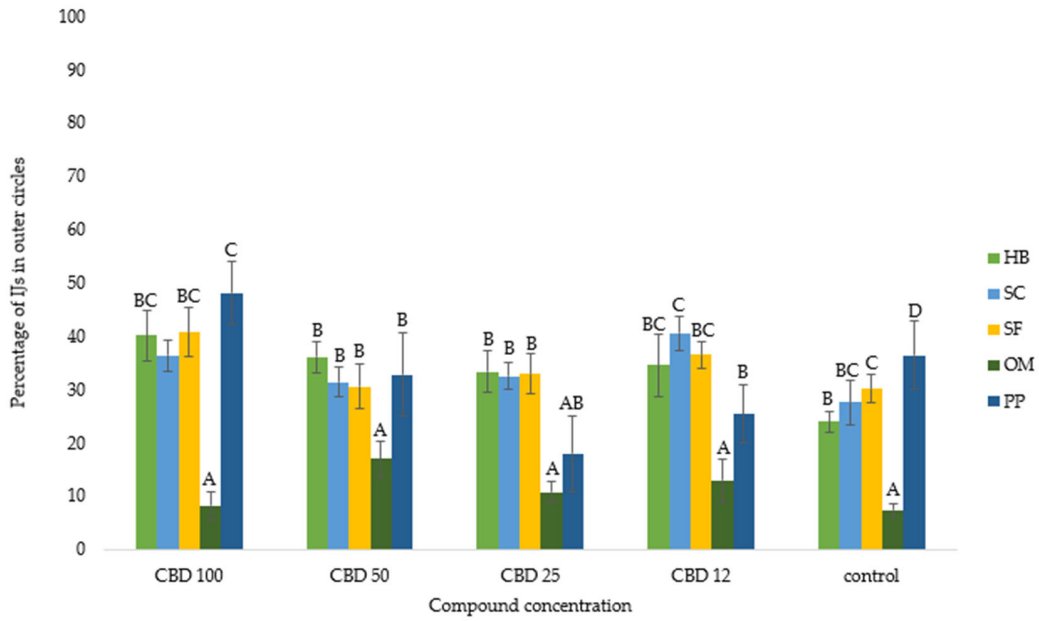

Figure 5. Mean percentage (±standard error) of infective juveniles (IJs) of five nematode species to varying concentrations of cannabidiol (CBD; 100%, 50%, 25%, 12%) and a 96% ethanol control. Different capital letters above the bars indicate statistically significant differences among nematode species within the same CBD concentration, based on Duncan's multiple range test ($p < 0.05$). Legend: *Heterorhabditis bacteriophora* (HB), *Steinernema carpocapsae* (SC), *Steinernema feltiae* (SF), *Oscheius myriophilus* (OM), and *Phasmarhabditis papillosa* (PP).

In summary, nematode motility was strongly influenced by species identity, cannabinoid type, and concentration. *P. papillosa* consistently demonstrated the highest responsiveness across all treatments, particularly to THC and CBG, while *O. myriophilus* showed minimal movement regardless of compound or concentration. Statistically significant interactions between species, cannabinoids, and their concentrations underscore the species-specific and dose-dependent nature of the chemotactic response. Conversely, CBD induced weaker and more variable effects, suggesting a more limited role in driving nematode attraction. The absence of significant effects from temporal or spatial replication supports the reliability and reproducibility of the experimental design. These findings collectively highlight the potential of selected cannabinoids—especially THC and CBG—to modulate nematode behavior in a species-specific manner, with implications for their application in targeted biological control strategies.

3.2. Chemotaxis Index

The directional movement of infective juveniles (IJs) in response to cannabinoids was quantified using a chemotaxis assay, and the results were analyzed via ANOVA (Table 2). The analysis revealed that cannabinoid type was the most influential factor affecting nematode chemotaxis (F = 214.29, p = 0.0001), followed by nematode species (F = 38.78, p = 0.0001) and cannabinoid concentration (F = 13.78, p = 0.0001). In contrast, temporal replication (F = 0.67, p = 0.7350) and spatial replication (F = 2.04, p = 0.1310) were not significant, confirming the stability and consistency of the experimental setup. Several interaction terms were also highly significant, including the species × cannabinoid interaction (F = 31.88, p = 0.0001), species × concentration (F = 4.71, p = 0.0001), cannabinoid × concentration (F = 16.33, p = 0.0001), and the three-way interaction between species, cannabinoid, and concentration (F = 4.53, p = 0.0001). These results highlight the complex and species-specific nature of nematode responses to plant-derived compounds, with significant modulation depending on both the identity and concentration of the cannabinoids tested. The relatively low residual variance indicates that the experimental model captured most of the biologically relevant variation in IJ motility.

Table 2. ANOVA results for the Chemotaxis Index values.

Factor	Sum of Squares	Df	F	p
Nematode species (S)	0.76	4	38.78	0.0001
Cannabinoids (C)	2.10	2	214.29	0.0001
Cannabinoid concentration (Cc)	0.27	4	13.78	0.0001
Temporal replication	0.03	9	0.67	0.7350
Spatial replication	0.02	2	2.04	0.1310
S × C	1.25	8	31.88	0.0001
S × Cc	0.37	16	4.71	0.0001
C × Cc	0.64	8	16.33	0.0001
S × C × Cc	0.71	32	4.53	0.0001
Residual	1.45	296		
Total (Corrected)	7.58	381		

The Chemotaxis Index (CI) values for five nematode species in response to varying concentrations of Δ^9-tetrahydrocannabinol (THC), cannabigerol (CBG), and cannabidiol (CBD) are summarized in Table 3.

Table 3. Chemotaxis Index (CI ± standard error) of five nematode species—*Heterorhabditis bacteriophora* (HB), *Steinernema carpocapsae* (SC), *Steinernema feltiae* (SF), *Oscheius myriophilus* (OM), and *Phasmarhabditis papillosa* (PP)—in response to varying concentrations of Δ^9-tetrahydrocannabinol (THC), cannabigerol (CBG), and cannabidiol (CBD), including a 96% ethanol control. CI values are interpreted as follows: values ≥ 0.2 indicate an attractant effect; values between 0.1 and 0.2 indicate a weak attractant; values between −0.1 and 0.1 indicate no effect; values between −0.1 and −0.2 indicate a weak repellent; and values ≤ −0.2 are considered a repellent. Different capital letters within columns indicate statistically significant differences ($p < 0.05$) among nematode species for the same cannabinoid type and concentration, while different lowercase letters within rows indicate significant differences ($p < 0.05$) among concentrations for the same nematode species, based on Duncan's multiple range test.

	THC 100%	**THC 50%**	**THC 25%**	**THC 12%**	**Control**
HB	0.13 ± 0.04 Bb	0.16 ± 0.05 Bb	0.12 ± 0.04 Bb	0.10 ± 0.03 BCb	−0.01 ± 0.03 ABa
OM	0.01 ± 0.01 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
PP	0.09 ± 0.01 Bb	0.38 ± 0.04 Cd	0.15 ± 0.02 Bc	0.13 ± 0.01 Cc	0.00 ± 0.03 ABa
SC	0.24 ± 0.05 Cd	0.15 ± 0.02 Bc	0.12 ± 0.02 Bbc	0.07 ± 0.03 Bb	0.01 ± 0.02 ABa
SF	0.26 ± 0.05 Cc	0.15 ± 0.02 Bb	0.13 ± 0.02 Bb	0.13 ± 0.04 Cab	0.05 ± 0.04 Ba
	CBG 100%	CBG 50%	CBG 25%	CBG 12%	Control
HB	0.20 ± 0.05 Db	0.17 ± 0.03 Cb	0.04 ± 0.05 ABa	−0.03 ± 0.05 Aa	−0.01 ± 0.03 ABa
OM	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	−0.01 ± 0.03 Aa	0.00 ± 0.00 Aa
PP	0.11 ± 0.03 Cb	0.09 ± 0.02 Bb	0.18 ± 0.03 Cc	0.19 ± 0.03 Cc	0.00 ± 0.03 ABa
SC	0.05 ± 0.04 Bb	0.04 ± 0.06 ABab	0.13 ± 0.04 BCb	−0.06 ± 0.06 Aa	0.01 ± 0.02 ABab
SF	0.12 ± 0.03 CDab	0.15 ± 0.04 BCb	0.14 ± 0.03 Cb	0.13 ± 0.01 Bb	0.05 ± 0.04 Ba
	CBD 100%	CBD 50%	CBD 25%	CBD 12%	Control
HB	−0.14 ± 0.04 Ba	−0.12 ± 0.03 Aa	−0.17 ± 0.04 Ba	−0.16 ± 0.02 Ba	−0.01 ± 0.03 ABb
OM	0.01 ± 0.01 Ca	0.00 ± 0.00 Ba	0.00 ± 0.00 Da	−0.02 ± 0.02 Da	0.00 ± 0.00 Aa
PP	0.16 ± 0.05 Dc	0.16 ± 0.02 Cc	0.06 ± 0.01 Cb	0.06 ± 0.03 Cb	0.00 ± 0.03 ABa
SC	−0.11 ± 0.01 Bc	−0.15 ± 0.02 Ab	−0.22 ± 0.03 ABa	−0.27 ± 0.02 Aa	0.01 ± 0.02 ABd
SF	−0.24 ± 0.04 Aa	−0.16 ± 0.03 Ab	−0.28 ± 0.05 Aa	−0.30 ± 0.02 Aa	0.05 ± 0.04 Bc

P. papillosa exhibited the strongest attractant responses, particularly to THC at 50% concentration (CI = 0.38 ± 0.04), clearly classifying it as a strong attractant. This species also responded positively to CBG at 25% and 12% concentrations, with CI values around 0.18–0.19, indicating a weak attractant effect. *S. feltiae* and *S. carpocapsae* responded positively to THC and CBG at higher concentrations, with several responses—such as *S. feltiae* at 100% THC (CI = 0.26 ± 0.05) and *S. carpocapsae* at 100% THC (CI = 0.24 ± 0.05)—falling within the strong attractant range. *H. bacteriophora* showed a similar response to CBG 100% (CI = 0.20 ± 0.05), also meeting the strong attractant threshold.

In contrast, *O. myriophilus* consistently showed CI values near zero or negative across all treatments and concentrations, indicating no chemotactic effect or weak repellency. Responses to CBD were generally less favorable across all species. For example, *S. carpocapsae* and *S. feltiae* displayed repellent or weak repellent behavior at higher CBD concentrations, with CI values as low as −0.28 and −0.30, respectively. *P. papillosa* remained the only species with a consistently positive response to CBD, although the values (0.06–0.16) fell into the weak attractant range.

The ethanol control treatments yielded CI values between −0.01 and 0.05, all falling within the "no effect" category, confirming that the solvent itself did not influence nematode movement. These results underscore the species-specific and dose-dependent nature of nematode responses to different cannabinoids, with THC and CBG eliciting stronger behavioral responses than CBD—particularly in *P. papillosa*—supporting their potential application in targeted biocontrol strategies.

4. Discussion

This study reinforces the growing evidence that plant-derived secondary metabolites, among them cannabinoids from *C. sativa* in particular, can significantly influence the chemotactic behavior of entomopathogenic and mollusk-parasitic nematodes. In the face of escalating environmental and health concerns linked to synthetic pesticides, the development of sustainable and biologically compatible alternatives has become a pressing need [1,2]. Our findings demonstrate that specific cannabinoids, notably Δ^9-tetrahydrocannabinol (THC) and cannabigerol (CBG), function as potent behavioral cues for several nematode species. In contrast, cannabidiol (CBD) generally exhibited limited or even repellent effects, especially at higher concentrations.

Chemotactic responses were highly species-specific and concentration-dependent. *P. papillosa* consistently showed the strongest attraction, particularly to THC at 50% (CI = 0.38), which classifies it as a strong attractant. *S. feltiae* and *S. carpocapsae* also responded favorably to THC and CBG, with several CI values exceeding the threshold of 0.2. Conversely, *O. myriophilus* demonstrated negligible or neutral responses across all cannabinoid treatments, suggesting either a limited receptor profile or different ecological foraging strategies [19,20]. These interspecific differences underscore the need to tailor biocontrol interventions to the behavioral ecology of the targeted nematode species.

The observed patterns support earlier research on the organ-specific chemosensory effects of *C. sativa* extracts, where inflorescences yielded more pronounced responses compared to leaves or roots [23]. This aligns with the higher concentration of terpenoids and cannabinoids in floral tissues, particularly sesquiterpenes like trans-caryophyllene, which have been previously identified as potent attractants for *Heterorhabditis megidis* Jackson & Klein. [14]. It is plausible that similar mechanisms underlie the responses observed in this study, particularly those of *S. carpocapsae* and *P. papillosa*.

Our results also corroborate the hypothesis that nematode chemotaxis is influenced by foraging strategy. *H. bacteriophora*, a cruiser forager, exhibited the highest overall motility, consistent with its need to traverse larger areas to locate hosts. In contrast, the ambusher *S. carpocapsae* was less mobile, reflecting its adaptation to await passing hosts [19,20].

The strong and reproducible chemotactic responses to THC and CBG indicate that these compounds could be strategically employed to direct nematodes toward pest targets. Nonetheless, field application remains a critical bottleneck. While synthetic and microbial VOCs have shown promise in lab settings, field trials have yielded inconsistent outcomes, likely due to environmental volatility and compound degradation [26]. Developing delivery systems that maintain compound stability and bioactivity in situ—such as encapsulated formulations—may enhance the practical viability of this approach. Encapsulation technologies, including polymer-based and lipid-based carriers, have been explored in other systems to protect volatile compounds and extend their efficacy. For instance, encapsulated attractants have shown improved stability and field performance in insect pest management. Although studies specific to nematode-guiding compounds are limited, these successes suggest that similar strategies could be adapted for nematode biocontrol. Further research is needed to optimize encapsulation materials and release kinetics tailored to soil environments [27].

From a practical standpoint, the identified nematode responses to THC and CBG could be operationalized by co-applying encapsulated cannabinoids with infective juveniles in baited formulations or soil drenches near pest hotspots [26]. This would direct nematodes toward pest-infested zones, enhancing their efficacy. Alternatively, cannabinoids could be used to precondition soil zones with attractant gradients that increase nematode encounter rates with pests.

Cost considerations remain speculative at this stage, but initial estimates suggest that pure cannabinoid isolation is significantly more expensive than traditional nematicides or microbial biocontrol agents [4,5]. However, advances in low-cost cannabinoid production through microbial biosynthesis or hemp waste valorization may reduce these costs substantially in the near future. For context, commercial nematode formulations typically cost $100–300/ha depending on the species and application method, whereas cannabinoid-based formulations could initially exceed this, unless produced as byproducts from existing hemp processing streams [4,5]. Comparative cost-effectiveness will ultimately depend on improved field persistence, enhanced pest control efficiency, and reduced application frequency.

Importantly, this research provides the first comprehensive examination of how pure cannabinoids affect the chemotactic behavior of a diverse group of beneficial nematodes. The consistent attraction observed in *P. papillosa*, *S. feltiae*, and *H. bacteriophora* to THC and CBG positions these cannabinoids as valuable behavioral modulators in integrated pest management (IPM). Moreover, the absence of phytotoxicity and the eco-friendly profile of *C. sativa* products make them particularly attractive as botanical alternatives to synthetic pesticides [4–7]. Future work should focus on isolating and testing individual compounds—both cannabinoids and terpenes—and evaluating their synergistic effects on nematode behavior. Expanding such research under field conditions will be crucial for determining the ecological and agronomic feasibility of cannabinoid-based biocontrol strategies.

5. Conclusions

This study provides compelling evidence that selected cannabinoids from *C. sativa* L., particularly Δ^9-tetrahydrocannabinol and cannabigerol, can significantly influence the chemotactic behavior of entomopathogenic and mollusk-parasitic nematodes in a species-specific and dose-dependent manner. Among the five tested nematode species, *P. papillosa*, *S. feltiae*, and *H.bacteriophora* exhibited strong attractant responses, suggesting that cannabinoids may function as effective behavioral modulators in biological pest control strategies. In contrast, cannabidiol showed weaker or even repellent effects, underscoring the importance of compound identity in shaping nematode responses.

Our findings highlight the potential of integrating cannabinoid-based formulations into environmentally friendly pest management systems. The species-specific nature of nematode responses emphasizes the need to tailor attractant-based strategies to target pests effectively. Further research under field conditions, including the role of individual cannabinoids and their interaction with environmental variables, will be essential for optimizing their practical application. Overall, this study contributes to the growing recognition of *C. sativa* as a multifunctional crop with potential benefits beyond its traditional uses, offering a novel direction for sustainable agriculture.

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