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Nematicidal *trans*-Anethole Blends Paralyzing *Meloidogyne incognita*

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Abstract: Nematodes have a negative impact on crop production and yield. The use of synthetic formulations to control plant parasitic nematodes carries both environmental and human health risks. As these agrochemicals are gradually being phased out, recent research has been focused on finding more environmentally friendly, plant-based alternatives. This study aims to investigate the effectiveness of botanicals, used alone or in artificial blends, in paralyzing *Meloidogyne incognita* second-stage juveniles (J2s) immersed in test solutions or exposed to vapors. We tested thymol, *trans*-anethole, and two lavender essential oils, referred to as LEOA and LEOB, which vary in their flower and stem compositions. We also employed in our study *Melia azedarach* aqueous extract (MWE), already proven to have considerable nematicidal activity. According to our findings, all treatments used individually exhibited considerable efficacy, even LEOA and LEOB first reported herein. In addition, all blends exhibited significant synergism, and the best-performing were *trans*-anethole/thymol, being synergic to paralyzing J2s for up to two days, and *trans*-anethole/LEOB as well as *trans*-anethole/MWE, provoking irreversible paralysis since the first day of J2 immersion in test solutions. Most importantly, the blend of *trans*-anethole with LEOA displayed the best effective synergism against *M. incognita* both for immersion and fumigation methods. Lastly, the chemical composition analysis displayed linalyl acetate and β-linalool as the major components of LEOA and β-linalool and eucalyptol as the major components of LEOB.

Keywords: root-knot nematodes; *trans*-anethole; lavender essential oil; *Melia azedarach*; nematicidal activity; synergistic action; additive effects

1. Introduction

The organisms classified under the phylum Nematoda, which are commonly known as roundworms, have been recognized to have existed for around one billion years, making them one of the most ancient animal groups on the planet [1]. The species belonging to the genus *Meloidogyne* spp., more commonly referred to as root-knot nematodes (RKNs), pose a considerable and pervasive risk to agricultural production, resulting in annual losses surpassing USD 80 billion in value [2]. To date, there have been documented descriptions of over 100 *Meloidogyne* species worldwide. The four most predominant species are *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*, and *Meloidogyne hapla*. Moreover, research has identified over 3000 plant species, encompassing nearly all cultivated crops, as potential hosts for these nematodes [3].

Traditionally, nematode management has been heavily relied on the use of synthetic nematicides [4]; however, it has been revealed that a significant number of these nematicidal products present substantial risks to both human health and the environment. As a result, several of these nematicides have been banned [5]. The objective of Directive 91/414/EEC was to establish a unified European policy regarding the market for plant protection products, with a specific focus on safety criteria. In this frame, the approval of pesticides required a demonstration of safety, as outlined in Article 5. This directive was published on 19 August 1991 and was later replaced by Regulation 1107/2009, which came into effect on 14 June 2014 [6–8]. The approval process for plant protection products has since involved a time-consuming procedure that unfolds through a sophisticated system of assessment and decision-making. By the year 2000, the European Union had more than 900 licensed active substances, but this number decreased to 425 by 2008 and was further lessened to 352 by 2018 [9]. Currently, the total number of active substances is 440, out of which 73 are considered low-risk pesticides, meeting the low-risk criteria as specified in Annex II, point 5 of Regulation (EC) 1107/2009. In recent years, the European Green Deal aims to achieve a climate-neutral Europe by 2030, with a target of reducing greenhouse gas emissions by 50 to 55% compared to the 1990s. The Farm to Fork strategy advocates for a more environmentally friendly approach to agriculture, proposing a minimum of 25% organic farming in Europe and a 50% reduction in the use of plant protection products. The contribution of member states to reaching the Green Deal targets on the reduced and sustainable use of pesticides can be contextualized with indicators providing information on the significance of the challenge for the member states [10]. Because of their favorable properties, low-risk products are preferred to manage pests [11]. Therefore, the importance of finding alternative management methods, such as natural substances, has been increasing [12].

Plants synthesize a wide range of compounds with distinctive chemical compositions that are crucial for their growth and development. Primary metabolites are essential for fundamental biological processes such as photosynthesis, nutrient transport, and respiration. In contrast, secondary metabolism involves the production of metabolites in specific tissues and organs at distinct stages of plant development. These secondary metabolites play a crucial role in protecting plants against changes in both biotic and abiotic environmental factors [13,14]. Secondary metabolites have been shown to possess a wide array of biological activities, such as fungicidal, antibiotic, antiviral, antibacterial, nematicidal, and other properties [15].

Despite the acknowledged potential of essential oils and secondary metabolites in controlling *Meloidogyne* spp. [16–19], there remains a significant gap in the knowledge concerning synergic interactions and nematicidal effects. Previously, we have shown the strong synergism of *trans*-anethole and thymol with other terpenes [20] in managing root-knot nematodes, but we had yet to study the synergism of the two terpenes when blended with nematicidal extracts.

In this frame, herein, we evaluate the effectiveness of *trans*-anethole or thymol blends with the aqueous extract of *M. azedarach* (MWE) or lavender essential oil (LEO) against the root-knot nematode *M. incognita*. As an efficacy parameter, we assess the caused paralysis on second-stage juveniles (J2s) both after immersion in treatment wells and after exposure to the treatment vapors.

We have already published research on the substantial nematicidal activity of MWE [18,19,21–23], and on this basis, we have involved it in this synergic study. On the other hand, the nematicidal potential of lavender essential oil has only been partially demonstrated [24], although some cultivars in the lavandin group have even been found to be resistant to *M. incognita* [25]. To further correlate the differences in the chemical composition of the essential oil with the induced J2 paralysis levels, we tested two lavender samples that originated from different proportions of flowers and shoots. Specifically, LEOA comprised 60% stems, whereas LEOB comprised 60% flowers. Initially, a comprehensive

chemical analysis of the two lavender samples was conducted. Subsequently, the concentrations of LEOB and LEOA necessary to immobilize 50% of J2s were determined. Moreover, an investigation was carried out to examine whether LEOA, LEOB, and MWE had an additive, synergistic, or antagonistic impact on inducing paralysis in J2s if blended with *trans*-anethole or thymol.

2. Materials and Methods

2.1. Preparation of Lavender Essential Oils (LEOA and LEOB)

Lavender essential oils, namely LEOA and LEOB, were produced with steam distillation from plant material that originated from organic cultures provided by AiTHeRIA Essential Oils & More, Velvento Kozanis, Greece. The two different botanical materials were different mixtures of stems and flowers. In particular, LEOA was produced by the hydrodistillation of plant material consisting of 60% stems and 40% flowers, while LEOB used 60% lavender flowers and 40% stems.

2.2. Gas Chromatography Mass Spectrometry Analysis for Essential Oil Composition

The separation and identification of the main components of essential oils were conducted using a Trace GC Ultra gas chromatograph (Thermo Finnigan, San Jose, CA, USA), coupled with a Trace ISQ MS detector and equipped with a split–splitless injector and a TriPlus RSH autosampler (Switzerland). Data analysis was performed using the Xcalibur MS platform. After extraction, the essential oil was diluted to a 1:1000 (*v/v*) ratio in hexane, and subsequently, one microliter of the diluted samples was injected onto a 5% phenyl methylsiloxane fused silica capillary column (TR-5MS, dimensions: 30 m length × 0.250 mm inner diameter, with a film thickness of 0.25 μm) with a split ratio of 50:1. The injector and transfer line were at 220 °C and 220 °C, respectively, the interface at 250 °C, and the electron energy in electron impact was 70 eV. The GC oven temperature followed a specific temperature program, starting at 70 °C for 5 min and gradually increasing to 240 °C at a rate of 8 °C/min, then being held at that final temperature for 15 min. Helium was employed as the carrier gas, at a constant flow rate of 1 mL/min. Following a solvent delay of 5 min, a mass range spanning from *m/z* 50 to 600 was recorded. Mass spectrometry acquisition was conducted in the continuous electron impact ionization (EI) mode. The Xcalibur processing program was utilized for peak area integration and chromatogram visualization. Peak identification and evaluation of mass spectra ticks were accomplished using the NIST11 database (NIST Mass Spectral Library 2011), along with comparing retention indices (RIs) for alkanes C9–C24 against those reported by Adams [26]. A match quality exceeding 90% was required for valid identification to ensure accurate substance identification. Additionally, for certain compounds, authentic standards used to confirm identity.

2.3. Development and Maintenance of the M. incognita Population

The population of *M. incognita* was reared in tomato plants (*Solanum lycopersicum* L.), specifically the variety Belladonna. These plants were artificially inoculated with *M. incognita*, obtained from naturally infested tomato roots, and were grown in plastic containers with a diameter of 18 cm, filled with peat. They were kept at 27 ± 5 °C, at a humidity level of 60% and a photophase of 16 h. In this controlled environment, the biological life cycle lasted for approximately 40 days.

2.4. Method for Collecting J2s

The artificial inoculation of the tomato plants with the nematodes was performed with 2000 J2s per tomato plant at the fifth fully developed leaf stage, when plants had well-established root systems that facilitated the growth and reproduction of the nematodes. After a period of thirty to forty days, and the completion of a biological cycle, the roots of the tomato plants were washed to remove soil residues and cut into segments measuring 2 cm. These segments were then placed in a solution consisting of 20 mL of 1%

NaOCl and 80 mL of H_2O, and the suspension was stirred for 5 min. Subsequently, the segments were rinsed with running water at a low flow rate through sieves with diameters of 250 and 38 μm. This process was followed by the collection of nematode eggs and their transfer to modified Baermann funnels, which were kept at room temperature, approximately 25 °C [27]. The J2s were collected every 48 h for biological testing.

2.5. Aqueous Extract of M. azedarach

To obtain MWE, we followed the protocol of Ntalli et al., 2018 [21]. Briefly, mature fruits weighing 1 g were finely pulverized and placed in a falcon tube. Subsequently, 10 mL of distilled water was added to the tube. The falcon tube was then transferred to an ultrasonic bath (Sonicator) and subjected to sonication for a duration of 10 min. After this step, the mixture was filtered using cotton, and the resulting filtrate was used for the biological tests.

2.6. Evaluation of trans-Anethole, Thymol, and Essential Oils for Inducing Paralysis in M. incognita J2s

This study investigated the nematicidal effects of *trans*-anethole, thymol, and LEOA and LEOB on *M. incognita* J2 paralysis. There were five different concentration levels: 800, 400, 200, 100, and 50 μL/L for *trans*-anethole and thymol, and 2000, 1000, 500, 250, and 125 μL/L for the essential oils. Concentrated mother solutions (5000 μL/L) were prepared using ethanol and Tween-20 in water (0.3% *v/v*). Subsequent dilutions in water led to solutions with twice the intended testing concentration, to be added to 96-well polystyrene plates, mixed in a 1:1 ratio with a J2-containing water suspension. Each well received 30 J2s for paralysis assessment, ensuring that the ethanol concentration in the control solutions remained below 1% (*v/v*), thus had no effect on J2s.

To minimize evaporation and concentration variations, the plates were covered with lids. Separate polystyrene plates were used per treatment to prevent cross-contamination. Furthermore, wells adjacent to the treatment wells contained J2s in water to evaluate the fumigant effect. The plates were kept in a 27 °C chamber, and J2 paralysis was assessed at 24, 48, and 96 h post-treatment using an inverted microscope at 40× magnification. Juveniles were categorized as either mobile or immobile. Irreversible paralysis till the termination of the experiment and dilution of test solutions using water was considered death.

The MWE was tested at concentrations of 1000, 500, 250, 125, and 62.5 mg/L per dry extract after exhaustive evaporation, and test solutions were prepared in water. This procedure aimed to determine the EC_{50} value of MWE, which had been previously established at 500 mg/L [23].

2.7. Evaluation of Paralysis Induction of Terpenes and Essential oils on M. incognita J2s (Synergistic Action)

Binary mixtures of *trans*-anethol, thymol, LEOA, LEOB, and MWE were tested at final concentrations of 100 μL/L, 250 μL/L, 125 μL/L, 125 μL/L, and 125 mg/L, respectively. The concentration levels for each component, employed in each binary mixture, were chosen according to Ntalli et al., 2011 [20], so that the expected exhibited efficacy was less than 50%, thus below the respective EC_{50} value. The expected and observed nematicidal activities were compared according to the effect addition model [28,29]. Mother solutions were prepared at a quadruple concentration for each component, and the blend of botanicals was the combination of two components at a 1:1 ratio (*v/v*). In the 96-well polystyrene plates, a further mixture of the blend with the J2 suspension at a 1:1 ratio (*v/v*) was prepared so that the final number of J2s in each well was 30.

To prevent evaporation and ensure consistent test concentrations, lids were placed on the plates. Adjacent wells to the treatment wells contained J2s in water to observe fumigant effects. The plates were incubated at 28 °C, and the mobility of J2s was assessed under a microscope at 40x magnification at 24, 48, and 96 h after immersion in test solutions. The J2s were classified as either mobile or paralyzed. The efficacy of each mixture

was compared to the sum of efficacies of the individual components tested separately to determine whether the interaction was synergistic, antagonistic, or additive.

2.8. Statistical Analysis

The paralysis treatments for individual substances or their combinations were replicated six times using a completely randomized experimental design. Each experiment was conducted twice. The data analysis was combined for both time points (two experiments) since there was no significant interaction between the time of the experiments and the interventions. The mean values of the two temporal experimental repetitions are presented because the combined analysis of variability showed no significant time–treatment interaction. Paralysis data were expressed as a percentage increase over the water control using Schneider-Orelli's equation:

Increase in paralysis % = {(paralysis % in treatment − paralysis % in control)/(100 − paralysis % in control)} × 100.

For the calculation of EC_{50} values, the control-adjusted data were subjected to analysis of variance (ANOVA) and applied to the logistic–logarithmic equation of Seefeldt et al., 1995 [30]:

$$y = C + \frac{D - C}{1 + \left(\frac{x}{EC_{50}}\right)^b} \tag{1}$$

where D = upper limit, C = lower limit, b = slope of the line at EC_{50}, and EC_{50} = concentration of substances required for a 50% increase in paralyzed J2s compared to the control. In this covariance equation, the concentration of substances (μL/L or mg/L) was the independent factor (x), and the paralyzed J2s (percentage increase over control) was the dependent variable (y).

Regarding paralysis data after immersion in binary mixtures for synergism study, the control-adjusted data were subjected to ANOVA with a significance level of $p < 0.05$ for each immersion time (24, 48, and 96 h).

3. Results

3.1. Analysis of Lavender Essential Oil Composition (LEOA and LEOB)

The chemical compositions of LEOA and LEOB exhibited significant differences. LEOA contained a total of 45 compounds, which accounted for 96.59% of the oil content. The main constituents of LEOA were linalyl acetate (26.27%) and *β*-linalool (24.63%), along with lower levels of *trans-β*-ocimene, *β*-ocimene, (±)-lavandulyl acetate, 4-terpineol, camphol, caryophyllene, and eucalyptol (*p*-cineole). In contrast, LEOB comprised 50 distinct compounds, making up 96.94% of its total composition. The predominant component in LEOB was *β*-linalool (43.28%), with eucalyptol (*p*-cineole) following closely at 22.12%. Additionally, other components like *α*-terpineol and germacrene D were also present. For a comprehensive breakdown of these compositions, including the percentage content of lavender essential oil in both samples, please refer to the subsequent table (Table 1).

Table 1. Chemical composition of lavender essential oils (LEOA and LEOB).

Compound Name in Order of Elution [b]	Retention Time (Rt)	[a] RIest	[b] RIexp	Percentage Content (%) LEOA	Percentage Content (%) LEOB
1. 2-thujene	6.85	930	924	0.13	0.0
2. [c] *d-α*-pinene	7.08	937	932	0.40	0.92
3. camphene	7.57	952	946	0.23	0.13
4. sabinene (4-thujene)	8.26	975	969	0.23	0.69
5. *β*-pinene	8.38	978	974	0.39	1.88
6. 1-octen-3-ol	8.45	980	974	0.13	-
7. *n*-octanone-3	8.59	985	979	0.44	-

8. ᶜ β-myrcene	8.71	990	988	0.57	1.05
9. butanoic acid, butyl ester	8.88	993	990 *	0.08	-
10. ᶜ 3-octanol	8.93	994	988	0.08	-
11. α-phellandrene	9.19	1004	1002	0.09	-
12. 3-carene	9.26	1011	1008	0.75	0.02
13. *n*-hexyl acetate	9.34	1011	1007	0.18	-
14. *o*-cymene	9.70	1027	1022	0.79	0.03
15. ᶜ *d*-limonene	9.83	1030	1024	0.58	-
16. ᶜ eucalyptol (*p*-cineole)	9.91	1032	1026	2.74	22.12
17. *trans*-β-ocimene	10.01	1038	1032	6.15	-
18. β-ocimene	10.30	1049	1050 *	4.92	0.14
19. γ-terpinene	10.61	1060	1054	0.24	0.20
20. α-terpinolene	11.32	1063	1067 *	0.31	0.11
21. *cis*-linalool oxide (furanoid)	11.36	1064	1067	-	0.23
22. ᶜ β-linalool	11.70	1099	1095	24.63	43.28
23. 1-octen-3-yl-acetate	11.85	1119	1110	0.80	0.02
24. *cis*-β-terpineol	12.31	1144	1140	0.19	0.35
25. camphor, (*1R,4R*)-(+)-	12.85	1147	1141	1.34	0.39
26. (±)-lavandulol	13.24	1168	1165	0.88	-
27. camphol	13.45	1166	1165	3.43	1.24
28. (-)-4-terpineol	13.64	1168	1178 *	4.08	0.44
29. ethyl linalool (ethoxy)	13.84	1170	1174 *	0.67	-
30. α-terpineol	13.96	1172	1186	0.99	-
31. *L*-isopulegol	14.72	1174	1167	0.23	0.06
32. linalyl acetate	15.14	1254	1272	26.27	-
33. (±)-lavandulyl acetate	15.82	1284	1288	5.67	-
34. borneol acetate,(*1S,2R,4S*)-(-)-	15.90	1289	1284	-	1.28
35. ᶜ *p*-cymen-3-ol (thymol)	16.09	1290	1289	0.64	-
36. exo-2-hydroxicineole acetate	16.99	1323	1341 *	-	0.10
37. ᶜ eugenol	17.29	1360	1356	-	0.96
38. nerol acetate	17.37	1364	1359	0.80	-
39. α-copaene	17.78	1376	1374	-	0.30
40. β-elemene, (-)-	18.02	1391	1389	-	0.95
41. α-cubebene	18.12	1396	1387	-	0.07
42. α-santalene	18.58	1420	1416	1.37	-
43. ᶜ caryophyllene	18.64	1424	1417	3.44	0.14
44. cedrene	18.74	1431	1419	-	0.05
45. α-bergamotene	18.83	1436	1432	0.55	1.71
46. α-guaiene	18.89	1440	1437	-	0.70
47. isocaryophyllene	19.04	1442		0.07	-
48. (E)-β-famesene	19.14	1456	1454	0.77	0.13
49. humulene,(α-caryophyllene)	19.29	1459	1452	-	0.56
50. β-caryophyllen -epi(E)	19.30	1454	1464	0.22	-
51. *cis*-muurolene-4(14),5diene	19.41	1461	1465	-	0.40
52. germacrene *D*	19.76	1480	1484	0.97	2.24
53. γ-elemene	20.02	1484	1482 *	-	0.57
54. azulene, (α-bulnesene)	20.11	1505	1509	-	1.40
55. β-bisabolene	20.18	1508	1505	0.06	-
56. γ-cadinene	20.32	1511	1513	0.35	2.52

57. δ-cadinene	20.38	1524	1522	-	0.18
58. *L*-calamenene	20.44	1529	1528	-	0.26
59. (-)-spathulenol	21.45	1572	1577	-	1.14
60. caryophylene oxide	21.58	1581	1582	0.35	0.17
61. humulene epoxide 2	22.02	1606	1608	-	0.07
62. (-)-cubenol	22.09	1638	1645	-	0.86
63. iso-spathulenol	22.38	1640	1644 *	-	0.10
64. cadinol *T*	22.56	1644	1652	0.12	5.77
65. *β*-eudesmol	22.76	1652	1649	-	0.43
66. alloaromadendrene oxide-(2)	22.83	1659	1678 *	-	0.10
67. *trans*-longi pinocarveol	23.36	1640	1634 *	-	0.20
68. Ledene oxide-(II)	23.62	1672	1682 *	-	0.15
69. 6-isopropenyl-4,8a-dimethyl 1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	23.92	1680	1690 *	-	0.10
Total identified compounds (%)				96.59	96.94
Number of identified compounds				45	50

[a] Retention indices determined experimentally on a TR-5MS column, 30 m length × 0.250 mm i.d., film thickness 0.25 μm and calculated according to Kovats, 1978, for alkanes C9 to C24. [b] Reported Kovats indices (RI) by Adams [26]; * refers to the RI reported on https://webbook.nist.gov/chemistry/ (accessed on 18 May 2024). [c] Authentic standards used to confirm identity. Identification of compounds was based on the comparison of mass spectra and their calculated Kovats retention indices (RIs) with the respective data of NIST Mass Spectral Library (2011) in continuous electron impact ionization (EI) mode and those data reported in the literature.

3.2. Paralyzing Effect of LEOA on J2s

The impact of LEOA on the paralysis of second-stage larvae was assessed at 24, 48, and 72 h following the commencement of the experiment (Table 2). At 24 h, the EC_{50} value was determined to be 1073.8 μL/L with a standard error of 75.7. At 48 h, the EC_{50} value decreased to 677.5 μL/L with a standard error of 47.6, and at 72 h, it further decreased to 381.1 μL/L with a standard error of 27.2. Similarly, the fumigant action was tested in adjacent wells on the same plate containing a nematode suspension and water at a 1:1 ratio. The EC_{50} value for the fumigant action was found to be 1284.8 μL/L with a standard error of 93.6 at 24 h, 850.4 μL/L with a standard error of 74.3 at 48 h, and 483.6 μL/L with a standard error of 32.0 at 72 h.

Table 2. Effect of LEOA on J2 *M. incognita*.

Lavender Essential Oil A (LEOA) Paralysis Effect on *Meloidogyne incognita* Second-Stage Juveniles (J2s) after 24, 48, and 72 h of Immersion in Test Solutions.						
	Immersion Well			**Fumigant Activity**		
LEOA	**EC_{50} (μL/L)**	**Std. Error**	**95% Conf. Int.**	**EC_{50} (μL/L)**	**Std. Error**	**95% Conf. Int.**
24 h	1073.8	75.7	917.2–1230.4	1284.8	93.6	1091.1–1478.5
48 h	677.5	47.6	579.1–775.9	850.4	74.3	696.8–1004.1
72 h	381.1	27.2	324.8–437.5	483.6	32.0	417.4–549.8

3.3. Paralyzing Effect of LEOB on J2s

The impact of LEOB on the paralysis of J2s of *M. incognita* was assessed at 24, 48, and 72 h post-experiment-initiation (Table 3). At 24 h post-experiment-initiation, the EC_{50} value was determined to be 1917.3 μL/L with a standard error of 177.9. At 48 h, the EC_{50} value was 350.2 μL/L with a standard error of 79.9. The EC_{50} value at 72 h was not determined since the lowest tested concentration (125 μL/L) resulted in over 50% paralysis. In terms

of fumigant activity, it is anticipated that the EC$_{50}$ value at 24 and 48 h would surpass 2000 µL/L. At 72 h, the EC$_{50}$ value was established as 762.5 µL/L with a standard error of 126.7.

Table 3. Effect of LEOB on J2 *M. incognita*.

Lavender Essential Oil B (LEOB) Paralysis Effect on *Meloidogyne incognita* Second-Stage Juveniles (J2s) after 24, 48, and 72 h of Immersion in Test Solutions.						
	Immersion Well			**Fumigant Activity**		
LEOB	**EC$_{50}$ (µL/L)**	**Std. Error**	**95% Conf. Int.**	**EC$_{50}$ (µL/L)**	**Std. Error**	**95% Conf. Int.**
24 h	1917.3	177.9	1549.1–2285.4	>2000	n.a.	n.a.
48 h	350.2	79.9	184.8–515.6	>2000	n.a.	n.a.
72 h	<125	n.a. [a]	n.a.	762.5	126.7	500.4–1024.6

[a] not applicable.

3.4. Paralytic Effect of MWE on J2s

The MWE was examined at concentrations of 2000, 1000, 500, 250, and 125 mg/L in order to assess its impact on the paralysis of J2s of *M. incognita*. A previous investigation conducted by N. Ntalli et al. (2010) [18] determined the EC$_{50}$ value of the aqueous extract of ground mature fruits of *M. azedarach* to be 500 mg/L after 24 h. Based on the findings of the present study, the test concentration of 125 mg/L of the aqueous extract exhibited a mortality rate of 5% after 24 h, 19% after 48 h, and 25% after 72 h. Consequently, this concentration was employed in binary mixtures to study synergism effects.

3.5. Impact of the Binary Mixture of Thymol and trans-Anethole on Paralysis Induction in M. incognita

Initially, the individual substances were tested at test concentrations resulting in less than 50% mortality. The selected doses for the synergistic blend test were 100 µL/L for *trans*-anethole and 250 µL/L for thymol, and assessments were made at three different immersion times (24, 48, 72 h). The findings regarding the paralysis of J2s are presented in Table 4, which also includes the results obtained for the fumigant effects of these substances, that is the paralysis activity exhibited on J2s immersed in water, adjacent to the treatment wells.

Table 4. Synergistic and antagonistic interaction observed between *trans*-anethole and thymol against *M. incognita*.

		J2 Paralysis over Control, % (± SD) (n = 4)			
	Concentration (µL/L)	**Observed [a]**	**Expected [b]**	**Significance of Difference [c]**	**Interaction**
		Combination *trans*-anethole/thymol in immersion well			
24 h		17 ± 5.0	6.6 ± 2.2	**	Syn
48 h	100/250	33 ± 7.8	14.3 ± 5.3	**	Syn
72 h		51 ± 3.2	38.6 ± 16.0	*	Additive effect
		Combination *trans*-anethole/thymol fumigant activity			
24 h		2 ± 2.4	2.7 ± 1.9	*	Additive effect
48 h	100/250	40 ± 14.0	26.7 ± 12.1	*	Additive effect
72 h		37 ± 8.5	50.0 ± 13.9	*	Additive effect

[a] Observed % paralysis, corrected according to the control, after immersion of J2s in paired terpene solutions. [b] Expected % paralysis, corrected according to the control, calculated as the sum of paralysis observed after immersion of J2s in pure terpene solutions. [c] Significance of the difference between observed and expected paralysis as presented by each row in the table ($p < 0.05$). * No significant difference; ** significant difference.

Based on our findings, it was observed that an increase in immersion time led to a greater paralysis of J2s. The paralysis observed in the binary mixtures, particularly *trans*-anethole/thymol, was significantly higher for both immersion periods (24 and 48 h) compared to the cumulative effects of their individual components, implying synergism. However, this synergistic effect disappeared after 72 h. Regarding the fumigant effect, an additive effect was observed for all assessment dates. Therefore, this particular combination of substances demonstrated a synergistic action against the nematodes when immersed in the control solutions for two days, which is to be further considered for efficacy expression *in vivo*.

3.6. Impact of the Binary Mixture of LEOA with trans-Anethole on M. incognita J2 Paralysis

Individual tests were conducted on *trans*-anethole using concentrations that resulted in less than 50% mortality. Doses of 125 μL/L were administered for a synergism test for both *trans*-anethole and LEOA. Subsequently, the paralysis activity of the mixture and the individual treatments were tested over three different immersion periods (24, 48, 72 h). The following table (Table 5) presents the paralysis effect for J2 immersion in treatment wells, as well as fumigant activity.

Table 5. Synergistic and antagonistic interaction observed between *trans*-anethole and LEOA against *M. incognita*.

	Concentration (μL/L)	J2 Paralysis over Control, % (48 h) (±SD) (n = 4)
	Concentration (μL/L)	Observed [a]	Expected [b]	Significance of Difference [c]	Interaction
Combination *trans*-anethole/LEOA in immersion well
24 h		57 ± 15	4 ± 3.1	**	Syn
48 h	125/125	60 ± 15.4	15 ± 4.6	**	Syn
72 h		68 ± 11.1	31 ± 7.1	**	Syn
Combination *trans*-anethole/LEOA fumigant activity
24 h		45 ± 9.9	18 ± 5.5	**	Syn
48 h	125/125	48 ± 11.4	32 ± 7.6	**	Syn
72 h		56 ± 13.1	66 ± 24.2	*	Additive effect

[a] Observed % paralysis, corrected according to the control, after immersion of J2s in paired treatment solutions (*trans*-anethole and extract). [b] Expected % paralysis, corrected according to the control, calculated as the sum of paralysis observed after immersion of J2s in single treatment solutions (*trans*-anethol or extract). [c] Significance of the difference between observed and expected paralysis as presented by each row in the table ($p < 0.05$). * No significant difference; ** significant difference.

Based on the provided table, it is evident that the paralysis of J2s increased progressively over time. *trans*-anethole and LEOA consistently exhibited synergistic effects throughout the entire duration of observation. Regarding their fumigant action, synergy was observed at 24 and 48 h, but not at 72 h (Table 5).

3.7. The Influence of LEOB with trans-Anethole on the Paralysis of M. incognita J2s

Table 6 demonstrates the impact on the motility of J2s when subjected to a blend of *trans*-anethole and LEOB for 24, 48, and 72 h. The consistent occurrence of a synergistic effect between these compounds was observed throughout all observation periods. Nevertheless, it is crucial to acknowledge that an additive effect was noticed in the wells utilized to assess the fumigant activity.

Table 6. Synergistic and antagonistic interaction observed between *trans*-anethole and LEOB against *M. incognita*.

	J2 paralysis over Control, % (48 h) (± SD) (n = 4)				
	Concentration (µL/L)	Observed [a]	Expected [b]	Significance of Difference [c]	Interaction
Combination *trans*-anethole/LEOB in immersion well					
24 h		35 ± 3.8	13 ± 1.9	**	Syn
48 h	125/125	46 ± 5.6	28 ± 5.1	**	Syn
72 h		58 ± 7.9	43 ± 10.2	**	Syn
Combination *trans*-anethole/LEOB fumigant activity					
24 h		30 ± 10.7	28 ± 6.3	*	Additive effect
48 h	125/125	36 ± 9.2	36 ± 3.6	*	Additive effect
72 h		47 ± 11.4	41 ± 6.2	*	Additive effect

[a] Observed % paralysis, corrected according to the control, after immersion of J2s in paired treatment solutions (*trans*-anethole and extract). [b] Expected % paralysis, corrected according to the control, calculated as the sum of paralysis observed after immersion of J2s in single treatment solutions (*trans*-anethole or extract). [c] Significance of the difference between observed and expected paralysis as presented by each row in the table (*p* < 0.05). * No significant difference. ** significant difference.

3.8. The Influence of the Aqueous Extract of M. azedarach Blended with trans-Anethole on the Paralysis of M. incognita

The investigation concluded by examining the joint impact of *trans*-anethole and the MWE at standardized concentrations of 125 µL/L and 125 mg/L, respectively. These combinations of treatments were assessed for J2 paralysis at three different immersion periods (24, 48, 72 h). The outcomes of J2 paralysis and the evaluation of the substances' fumigant properties are presented in Table 7.

Table 7. Synergistic and antagonistic interaction observed between *trans*-anethole and MWE *M. incognita.*

Synergistic and Antagonistic Interactions Observed between *trans*-Anethole and Nematicidal Chinaberry Extracts against *Meloidogyne incognita.*					
	J2 Paralysis over Control, % (48 h) (±SD) (*n* = 4)				
	Concentration (µL/L and mg/L)	Observed [a]	Expected [b]	Significance of Difference [c]	Interaction
Combination *trans*-anethole/ MWE in immersion well					
24 h		53 ± 7.8	5 ± 3.8	**	Syn
48 h	125/125	60 ± 7.1	19 ± 3.7	**	Syn
72 h		68 ± 3.9	29 ± 8.4	**	Syn
Combination *trans*-anethole/ MWE fumigant activity					
24 h		42 ± 13.9	27 ± 8.3	*	Additive effect
48 h	125/125	40 ± 6.8	36 ± 8.6	*	Additive effect
72 h		45 ± 8.6	43 ± 5.2	*	Additive effect

[a] Observed % paralysis, corrected according to the control, after immersion of J2s in paired treatment solutions (*trans*-anethole and extract). [b] Expected % paralysis, corrected according to the control, calculated as the sum of paralysis observed after immersion of J2s in single treatment solutions (*trans*-anethole or extract). [c] Significance of the difference between observed and expected paralysis as presented by each row in the table (*p* < 0.05). * No significant difference; ** significant difference.

As the duration of exposure to escalating concentrations of nematodes prolongs, the degree of paralysis becomes increasingly pronounced. It is important to note that paralysis becomes evident rapidly within the first 24 h. The combined effect of *trans*-anethole

and the MWE is evident throughout the entire duration. Furthermore, an additional effect was observed in the neighboring wells containing the control concentration (Table 7).

4. Discussion

The European Union's European Green Deal aims to revolutionize agriculture through a "Farm to Fork" approach, placing utmost importance on environmental sustainability and reducing the utilization of chemical products. One of the crucial objectives is to achieve a 50% decrease in the utilization of plant protection products by the year 2030. Fulfilling this target necessitates the adoption of innovative and sustainable agricultural practices [31,32]. A significant challenge in this transformation is the efficient management of nematodes, particularly the *Meloidogyne* spp., which have detrimental effects on numerous plant species and result in substantial crop losses [33]. Conventional synthetic agrochemicals, now subject to regulation under European standards, present risks to both the environment and human health. As a result, there is a shift towards eco-friendly alternatives, such as plant-derived extracts possessing nematicidal properties. Essential oils obtained from aromatic plants, which are abundant in compounds like terpenes and phenolics, have demonstrated effectiveness against various pests, including nematodes. These oils offer a promising and sustainable solution that aligns with the objectives of the European Green Deal [31,33,34].

In our study, we focused on the evaluation of the nematicidal properties of individual substances and plant extracts and their binary mixtures. We conducted an examination of the synergistic, antagonistic, or additive effects of thymol, *trans*-anethole, and lavender essential oils obtained from two distinct samples (LEOA and LEOB). These samples were characterized based on their distinctive flower and stem compositions. Additionally, we included in the synergism study a water extract from *M. azedarach* of significant nematicidal potential as reported in our previous studies.

Our findings revealed that all blends exhibited significant synergism effects after J2 immersion in test solutions. Specifically, *trans*-anethole/thymol was synergic to paralyzing J2s for up to two days, solutions of *trans*-anethole/LEOB blend provoked irreversible paralysis on the first day of J2 immersion in test solutions, and the same stands for the binary mixture of *trans*-anethole/MWE. Most importantly, the combination of *trans*-anethole with LEOA exhibited the most potent synergistic effects against *M. incognita* both for immersion and fumigation methods. Thus, this blend is a promising candidate for effective nematode management.

In another study, thymol has demonstrated significant nematicidal activity against *M. javanica*. Specifically, it effectively immobilized second-stage juveniles (J2) after a 96 h exposure at concentrations of 500 and 1000 μL/L. Additionally, thymol hindered egg maturation in *M. javanica*, resulting in a reduction of approximately 35% after a 21-day incubation period at 1000 μL/L. These findings highlight the potential of thymol as a nematicidal agent and justify further investigation into its mechanisms of action and potential synergistic interactions, particularly for managing *Meloidogyne* spp. infestations. Variations in responses between *M. incognita* and *M. javanica* underscore the need for species-specific studies to fully comprehend the nematicidal capabilities of thymol and optimize its application in sustainable agricultural practices [35].

Also, in the conducted experiment involving lavender essential oil, a notable anti-nematode activity against the root-knot nematode *M. incognita* was observed. Specifically, the aqueous by-product derived from the steam-distillation extraction of lavender essential oil exhibited significant *in vitro* activity against *M. javanica*, affecting both the mortality rate of J2s and their hatching process. Additionally, application of this by-product to the soil led to a reduction in nematode reproduction [16]. Another experiment was performed to examine the impact of *trans*-anethole on egg hatching and the occurrence of galls in tomato roots. The findings demonstrated that the administration of *trans*-anethole hindered egg hatching and reduced the prevalence of galls in potted tomato plants [36]. Fi-

nally, a research study was undertaken to examine the inhibitory impact of thymol at different concentrations on the process of egg differentiation following a 21-day exposure to *M. javanica* [35]. Our research contributes to the development of potential nematicides based on plant secondary metabolites by demonstrating the strong synergism of *trans*-anethole with LEOA, LEOB, and MWE against *M. incognita*. *Trans*-anethole has already demonstrated synergism effects when tested with a vast number of different terpenes. In particular, the combination of *trans*-anethole and geraniol has emerged as the most effective, followed by other successful combinations such as *trans*-anethole and eugenol, carvacrol and eugenol, and geraniol and carvacrol [20]. Herein, we demonstrate for the first time *trans*-anethole synergism with essential oils and a plant extract. This discovery is of significant importance as extracts can be utilized to effectively combat resistance. Extracts possess the ability to target multiple mechanisms due to their intricate chemical composition [37]. This highlights the potential of utilizing plant-based substances in sustainable agriculture, aligning with global research trends and emphasizing the diversity and promise of natural substances in pest control. It offers valuable insights for future agricultural practices.

In the same frame, in a separate study conducted on potato crops, a synergistic approach utilizing a water extract from the outer shell of *Punica granatum* fruit and the bacterial strains *B. subtilis* and *B. pumilus* (Bp) was implemented for root-knot nematode control. This combined approach yielded a significant reduction in J2s, achieving an 84% decrease with the *B. subtilis* and water extract mixture and an 82.3% decrease with the *B. pumilus* and water extract mixture. Consequently, these findings demonstrate a notable synergistic effect in nematode management [38].

In the discussion of our findings, it is essential to provide contextual information regarding the efficacy of the synergistic effects of thymol, *trans*-anethole, and lavender essential oils (LEOA and LEOB) in comparison to existing strategies used for nematode control. Previous studies have primarily focused on evaluating the individual effectiveness of various biological and chemical agents against *M. incognita*. For example, Avid, while effective in stone wool substrate when applied during nematode inoculation, has limited efficacy in soil due to issues related to adsorption, as highlighted in related research [39]. Similarly, the utilization of *B. subtilis*, particularly isolate B10, has shown significant suppression of nematode reproduction and infestation under laboratory and greenhouse conditions. However, the primary emphasis of the study was on the broad-spectrum application rather than the interactions among different agents [40].

Our research builds upon these findings by demonstrating that the combination of specific natural compounds can lead to improved outcomes in nematode control through synergistic effects.

We chose to study two distinct levanter essential oils because differences in chemical composition may be translated as differences in efficacy. In this context, Malhotra, A., et al. reported different nematicidal activity for essential oils of *Artemisia annua* L. (Asteraceae) extracted during the rainy or winter seasons. The rainy season yielded essential oil containing camphor, germacrene-D, *β*-caryophyllen, and eucalyptol, while winter oil consisted mainly of camphor, eucalyptol, and artemisia alcohol. The rainy season oil exhibited a higher percentage of nematode immobility, with noticeable efficacy observed at a concentration of 5.0 µL/mL after 72 h. On the other hand, the winter oil demonstrated a stronger ability to inhibit egg hatching, showing significant effects at various concentrations [41].

In addition, limited research has been conducted on the nematicidal action of lavender essential oil. One study reported an LC$_{50}$ of 20.24 mg mL^{-1} at 48 h [42]. In 1982, it was found that linalool, the main component of *Ocimum basilicum* (Lamiaceae), exhibited nematicidal activity against *M. incognita*. However, a study in 2000 showed no nematicidal activity of lavender essential oil extracted from the foliage. According to the literature, the concentration of linalool in *L. angustifolia* inflorescences is 10 times higher compared to other parts of the plant [43].

In the context of our research on the nematicidal properties of lavender essential oils against root-knot nematodes, an analysis was conducted on two samples. LEOA was derived from a combination of 60% lavender plant stems and 40% flowers, while LEOB had the inverse ratio of 60% flowers to 40% stems. The chemical analysis revealed that LEOA consisted of 54 distinct compounds, which accounted for 98.92% of the oil. The main constituents were linalyl acetate (26.27%) and β-linalool (24.63%). On the other hand, LEOB contained 57 compounds, making up 99.47% of the oil. The dominant concentration in LEOB was β-linalool (43.28%), followed by eucalyptol (22.12%). The disparity in chemical composition, specifically the variation in β-linalool content, between LEOA and LEOB might be correlated to differences in efficacy. It is important to note that the methodological approach, including the selection of plant parts for distillation, significantly influenced the profiles of the essential oils. This finding is consistent with the existing literature, which suggests that the chemical composition of essential oils can vary greatly based on factors such as the specific plant part employed, geographical origin, and distillation technique [44,45].

The results of this research underscore the need for further investigation into the effectiveness of specific combinations. Specifically, it is crucial to explore further the efficacy of *trans*-anethole with lavender essential oil samples A and B, as well as *trans*-anethole with *M. azedarach* water extract *in vivo*. Additionally, it would be valuable to assess their effectiveness against different species of nematodes in future studies as well as on soil beneficial organisms. Further research is needed to validate the consistency of these findings across botanical species from different geographical origins and to examine any temporal variations. Such research has the potential to yield significant insights.

The emerging formulations for nematicides show promise for achieving success in the market. These formulations offer a combination of high efficacy against nematodes and minimal toxicity to non-target organisms. However, the market for nematicides based on natural substances is still relatively small. Thymol, geraniol, and eugenol are the primary substances featured in this market. The widespread adoption and utilization of these products face several challenges. These challenges include a lack of comprehensive understanding regarding the biochemical mechanisms by which they act on nematodes, as well as issues related to their volatility and distribution through irrigation systems. To overcome these challenges, the development of effective stabilization techniques is necessary. These techniques will ensure the gradual release of active ingredients and enhance water solubility [31,46].

5. Conclusions

In conclusion, this research underscores the noteworthy potential of employing combinations of natural substances for efficacious nematode management. Notably, the amalgamation of *trans*-anethole with lavender essential oil and *M. azedarach* water extract exhibits synergistic effects. These findings establish the foundation for subsequent investigations aimed at augmenting the scope of nematode control. Nevertheless, the successful integration of these formulations into the market necessitates grappling with challenges pertaining to their biochemical comprehension and practical stabilization. We are now in the process of performing pot bioassays to assess phytotoxicity and efficacy at a larger scale. This research constitutes a substantial contribution to the realm of sustainable nematode management and introduces novel prospects for environmentally conscious agricultural practices.

References

1. Lambert, K.; Bekal, S. Introduction to Plant-Parasitic Nematodes. *Plant Health Instr.* **2002**. https://doi.org/10.1094/PHI-I-2002-1218-01.
2. Pu, W.; Xiao, K.; Luo, S.; Zhu, H.; Yuan, Z.; Gao, C.; Hu, J. Characterization of Five *Meloidogyne Incognita* Effectors Associated with PsoRPM3. *Int. J. Mol. Sci.* **2022**, *23*, 1498. https://doi.org/10.3390/ijms23031498.
3. Gulzar, R.M.A.; Rehman, A.U.; Umar, U.U.D.; Shahid, M.; Khan, M.F. Evaluation of Genetic and Induced Resistance Phenomena in Cucumbers against the Root-Knot Nematode (*Meloidogyne incognita*). *Plant Prot. Sci.* **2022**, *58*, 338–350. https://doi.org/10.17221/130/2021-PPS.
4. Ahmad, S.; Khan, M.; Ullah, I. Perspective Chapter: Integrated Root-Knot Nematodes (*Meloidogyne*) Management Approaches. In *Parasitic Helminths and Zoonoses—From Basic to Applied Research*; Morales-Montor, J., Hugo Del Rio-Araiza, V., Hernadez-Bello, R., Eds.; Intechopen: Rijeka, Croatia, 2022.
5. Castro-Toro, A.M.; Rivillas-Osorio, C.A. Bio Regulation of *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood Complex in Coffee Roots. *Rev. Cienc. Agrícolas* **2023**, *39*, 128–142. https://doi.org/10.22267/rcia.223902.189.
6. European Parliament. Directive 2009/128/EC European Union. Available online: https://eur-lex.europa.eu/legal-content/EL/TXT/PDF/?uri=CELEX:32009L0128&qid=1684328867663 (accessed on 25 March 2024).
7. European Parliament. Regulation 1107/2009. *Off. J. Eur. Union* **2009**. Available online: http://data.europa.eu/eli/reg/2009/1107/oj (accessed on 24 April 2024).
8. Harada, H.; Ota, M.; Tatsu, M. Changes in Procedures and Approval Criteria for Active Substances in Plant Protection and Biocidal Products in the European Union. *Sumitomo Kagaku* **2019**. Available online: https://www.sumitomo-chem.co.jp/english/rd/report/files/docs/2019E_4.pdf (accessed on 24 April 2024).
9. Punja, N. *The Impact of Regulation (EC) No/2009 on Innovation and Development of Alternatives and New Protection Products*; European Commission: Brussels, Belgium, 2018.
10. Metis Ecorys; Agrosynergy. *Mapping and Analysis of CAP Strategic Plans Assessment of Joint Efforts for 2023–2027*; European Commission: Brussels, Belgium, 2023.
11. Boix-Fayos, C.; de Vente, J. Challenges and Potential Pathways towards Sustainable Agriculture within the European Green Deal. *Agric. Syst.* **2023**, *207*, 103634. https://doi.org/10.1016/j.agsy.2023.103634.
12. Silva Faria, J.M. Essential Oils as Anti-Nematode Agents and Their Influence on In Vitro Nematode/Plant Co-Cultures. Ph.D. Thesis, Universidade de Lisboa, Faculdade de Ciências, Lisboa, Portugal, 2015.
13. Isah, T. Stress and Defense Responses in Plant Secondary Metabolites Production. *Biol. Res.* **2019**, *52*, 39. https://doi.org/10.1186/s40659-019-0246-3.
14. Twaij, B.M.; Hasan, M.N. Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses. *Int. J. Plant Biol.* **2022**, *13*, 4–14. https://doi.org/10.3390/ijpb13010003.
15. Hussein, R.A.; El-Anssary, A.A. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. In *Herbal Medicine*; Builders, P.F., Ed.; IntechOpen: Rijeka, Croatia, 2019. https://doi.org/10.5772/intechopen.76139.
16. D'Addabbo, T.; Avato, P. Chemical Composition and Nematicidal Properties of Sixteen Essential Oils—A Review. *Plants* **2021**, *10*, 1368. https://doi.org/10.3390/plants10071368.
17. Andrés, M.F.; González-Coloma, A.; Sanz, J.; Burillo, J.; Sainz, P. Nematicidal Activity of Essential Oils: A Review. *Phytochem. Rev.* **2012**, *11*, 371–390. https://doi.org/10.1007/s11101-012-9263-3.
18. Ntalli, N.G.; Menkissoglu-Spiroudi, U.; Giannakou, I. Nematicidal Activity of Powder and Extracts of *Melia azedarach* Fruits against *Meloidogyne incognita*. *Ann. Appl. Biol.* **2010**, *156*, 309–317. https://doi.org/10.1111/j.1744-7348.2009.00388.x.
19. Ntalli, N.G.; Ferrari, F.; Giannakou, I.; Menkissoglu-Spiroudi, U. Phytochemistry and Nematicidal Activity of the Essential Oils from 8 Greek Lamiaceae Aromatic Plants and 13 Terpene Components. *J. Agric. Food Chem.* **2010**, *58*, 7856–7863. https://doi.org/10.1021/jf100797m.
20. Ntalli, N.G.; Ferrari, F.; Giannakou, I.; Menkissoglu-Spiroudi, U. Synergistic and Antagonistic Interactions of Terpenes against *Meloidogyne incognita* and the Nematicidal Activity of Essential Oils from Seven Plants Indigenous to Greece. *Pest Manag. Sci.* **2011**, *67*, 341–351. https://doi.org/10.1002/ps.2070.
21. Ntalli, N.; Monokrousos, N.; Rumbos, C.; Kontea, D.; Zioga, D.; Argyropoulou, M.D.; Menkissoglu-Spiroudi, U.; Tsiropoulos, N.G. Greenhouse Biofumigation with *Melia azedarach* Controls *Meloidogyne* spp. and Enhances Soil Biological Activity. *J. Pest Sci.* **2018**, *91*, 29–40. https://doi.org/10.1007/s10340-017-0909-1.

22. Aoudia, H.; Ntalli, N.; Aissani, N.; Yahiaoui-Zaidi, R.; Caboni, P. Nematotoxic Phenolic Compounds from *Melia Azedarach* against *Meloidogyne incognita*. *J. Agric. Food Chem.* **2012**, *60*, 11675–11680. https://doi.org/10.1021/jf3038874.

23. Cavoski, I.; Chami, Z.A.; Bouzebboudja, F.; Sasanelli, N.; Simeone, V.; Mondelli, D.; Miano, T.; Sarais, G.; Ntalli, N.G.; Caboni, P. *Melia azedarach* Controls *Meloidogyne incognita* and Triggers Plant Defense Mechanisms on Cucumber. *Crop Prot.* **2012**, *35*, 85–90. https://doi.org/10.1016/j.cropro.2012.01.011.

24. D'Addabbo, T.; Laquale, S.; Argentieri, M.P.; Bellardi, M.G.; Avato, P. Nematicidal Activity of Essential Oil from Lavandin (Lavandula × Intermedia Emeric Ex Loisel.) as Related to Chemical Profile. *Molecules* **2021**, *26*, 6448. https://doi.org/10.3390/molecules26216448.

25. Bayındır, D.; Uysal, G.; Erbaş, S.; Devran, Z. The Response of Lavender and Lavandin Cultivars to *Meloidogyne incognita* and *Meloidogyne arenaria*. *J. Plant Dis. Prot.* **2023**, *130*, 1049–1055. https://doi.org/10.1007/s41348-023-00771-3.

26. Adams, P. Robert. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publ: Carol Stream, IL, USA, 2007.

27. Hussey, R.S.; Barker, K.R. A Comparison of Methods of Collecting Inocula of *Meloidogyne* spp. Including a New Technique. *Plant Disease Report.* **1973**, *57*, 1025–1028.

28. Berenbaum, M.C. What Is Synergy? *Pharmacol. Rev.* **1989**, *41*, 93.

29. Lee, J.J.; Kong, M.; Ayers, G.D.; Lotan, R. Interaction Index and Different Methods for Determining Drug Interaction in Combination Therapy. *J. Biopharm. Stat.* **2007**, *17*, 461–480. https://doi.org/10.1080/10543400701199593.

30. Seefeldt, S.S.; Jensen, J.E.; Feurst, E.P. Log-logistic analysis of herbicide dose-response relationships. *Weed Technol.* **1995**, *9*, 218-227.

31. Catani, L.; Manachini, B.; Grassi, E.; Guidi, L.; Semprucci, F. Essential Oils as Nematicides in Plant Protection—A Review. *Plants* **2023**, *12*, 1418. https://doi.org/10.3390/plants12061418.

32. Silva, V.; Yang, X.; Fleskens, L.; Ritsema, C.J.; Geissen, V. Environmental and Human Health at Risk—Scenarios to Achieve the Farm to Fork 50% Pesticide Reduction Goals. *Environ. Int.* **2022**, *165*, 107296. https://doi.org/10.1016/J.ENVINT.2022.107296.

33. Ntalli, N.G.; Ozalexandridou, E.X.; Kasiotis, K.M.; Samara, M.; Golfinopoulos, S.K. Nematicidal Activity and Phytochemistry of Greek Lamiaceae Species. *Agronomy* **2020**, *10*, 1119. https://doi.org/10.3390/agronomy10081119.

34. Ajith, M.; Pankaj; Shakil, N.A.; Kaushik, P.; Rana, V.S. Chemical Composition and Nematicidal Activity of Essential Oils and Their Major Compounds against *Meloidogyne graminicola* (Rice Root-Knot Nematode). *J. Essent. Oil Res.* **2020**, *32*, 526–535. https://doi.org/10.1080/10412905.2020.1804469.

35. Nasiou, E.; Giannakou, I.O. Nematicidal Potential of Thymol against *Meloidogyne javanica* (Treub) Chitwood. *Plants* **2023**, *12*, 1851.

36. Oka, Y.; Nacar, S.; Putievsky, E.; Ravid, U.; Yaniv, Z.; Spiegel, Y. Nematicidal Activity of Essential Oils and Their Components against the Root-Knot Nematode. *Phytopathology* **2000**, *90*, 710–715.

37. Khameneh, B.; Iranshahy, M.; Soheili, V.; Fazly Bazzaz, B.S. Review on Plant Antimicrobials: A Mechanistic Viewpoint. *Antimicrob. Resist. Infect. Control* **2019**, *8*, 118. https://doi.org/10.1186/s13756-019-0559-6.

38. El-Nagdi, W.M.A.; Youssef, M.M.A.; Abd-El-khair, H.; Elkelany, U.; Abd-Elgawad, M.M.M.; Dawood, M.G. Effect of Integration of Two Bacterial Bioagents and a Plant Residue Extract for Biocontrolling Root-Knot Nematode, *Meloidogyne incognita* Infesting Potatoes. *Egypt. Pharm. J.* **2023**, *22*, 67–77. https://doi.org/10.4103/epj.epj_119_22.

39. López-Pérez, A.; Edwards, S.; Ploeg, A. Control of Root-Knot Nematodes on Tomato in Stone Wool Substrate with Biological Nematicides. *J. Nematol.* **2011**, *43*, 110–117.

40. Basyony, A.G.; Abo-Zaid, G.A. Biocontrol of the Root-Knot Nematode, *Meloidogyne incognita*, Using an Eco-Friendly Formulation from *Bacillus subtilis*, Lab. and Greenhouse Studies. *Egypt. J. Biol. Pest Control* **2018**, *28*, 87. https://doi.org/10.1186/s41938-018-0094-4.

41. Malhotra, A.; Rawat, A.; Prakash, O.; Kumar, R.; Srivastava, R.M.; Kumar, S. Chemical Composition and Pesticide Activity of Essential Oils from *Artemisia annua* L. Harvested in the Rainy and Winter Seasons. *Biochem. Syst. Ecol.* **2023**, *107*, 104601. https://doi.org/10.1016/j.bse.2023.104601.

42. Asadi Sardari, A.; Hojat Jalali, A.A.; Bahraminejad, S.; Safaee, D. Effect of Plant Extracts on the Mortality of Root-Knot Nematodes' J2, *Meloidogyne javanica*. *Arch. Phytopathol. Plant Prot.* **2015**, *48*, 365–375. https://doi.org/10.1080/03235408.2014.889342.

43. Filipa Antónia Mota Ferreira da Costa, M. Biological Activities of *Lavandula angustifolia* Mill. Essential Oil. Master's Thesis, Universidade De Coimbra, Coimbra, Portugal, 2012.

44. Hassiotis, C.N.; Ntana, F.; Lazari, D.M.; Poulios, S.; Vlachonasios, K.E. Environmental and Developmental Factors Affect Essential Oil Production and Quality of *Lavandula angustifolia* during Flowering Period. *Ind. Crops Prod.* **2014**, *62*, 359–366. https://doi.org/10.1016/j.indcrop.2014.08.048.

45. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological Effects of Essential Oils—A Review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. https://doi.org/10.1016/j.fct.2007.09.106.

46. Laquale, S.; Candido, V.; Avato, P.; Argentieri, M.P.; D'Addabbo, T. Essential Oils as Soil Biofumigants for the Control of the Root-Knot Nematode *Meloidogyne incognita* on Tomato. *Ann. Appl. Biol.* **2015**, *167*, 217–224. https://doi.org/10.1111/AAB.12221.