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Nematicidal and Toxicity Effects of *Eupatorium adenophorum* Spreng against the Root-Knot Nematode *Meloidogyne incognita* in Soil Producing Cucumber

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Abstract: The root-knot nematode (*Meloidogyne incognita*) is a plant pathogen that causes significant economic damage to important food crops. The nematicidal and insecticidal effects of the essential oil and extract of the root and stem of different species of *Eupatorium* have been studied in several countries. We investigated the impact of root stems and leaves of the *E. adenophorum* on the second-stage juveniles (J2s) of *M. incognita*. Nematode mortality decreased by root-stem treatment and increasing temperature and time. Nematodes (J2) were more sensitive to root-stem treatment than leaf treatment at all tested conditions. For example, the half maximal effective concentration (EC_{50}) root-stems at 35 °C was estimated as 10.3 mg/g and in the 8th week as 7.8 mg/g, while the maximal effective concentration (EC_{max}) in leaf treatment is 20.3 and 10.1 mg/g, respectively. The *E. adenophorum* 40 mg/g concentration of root stems also produced the highest height of the cucumber stem and the highest cucumber fresh weight in the greenhouse. (E)-beta-farnesene, α-pinene and D-limonene, as the main identified components in fumigant plants, increased the mortality rate of J2s in root-stems treatment. We conclude that *E. adenophorum* dried root stems added to the soil in greenhouses have the potential as a bio-fumigant for *M. incognita* management.

Keywords: bio-fumigant; *Eupatorium adenophorum*; *Meloidogyne incognita*; root-knot nematode; RKN; (E)-beta-farnesene; α-pinene; D-limonene

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1. Introduction

Protective structures such as greenhouses create favorable environmental conditions not only for plant growth but also for increasing pathogen populations [1]. The root-knot nematode *Meloidogyne incognita* causes severe damage to economically important food crops produced in greenhouses [2]. The *M. incognita* are endoparasites that can limit agricultural productivity when their economic threshold is exceeded [3]. Cucumber has grown in China for over 3000 years [4]. The *M. incognita* causes root galling that reduces root growth and causes yellowing of leaves. When the infestation is severe, the whole plants and crops can be destroyed by nematodes [5,6]. Most chemical fumigants applied to control soil-borne pathogens can pollute groundwater, damage human health and reduce beneficial organism presence in the soil [7–9]. Nematologists have explored various cheaper and more environmentally-benign methods to control plant-parasitic nematodes, including using plants as bio-fumigants [10]. Bio-fumigation may be a more sustainable method to manage pathogens, nematodes, insects, and weeds in developing countries

than synthetic fumigants [11]. Field research has shown that nematicidal secondary plant metabolites are usually also environmentally-benign [12]. Asteraceae family belongs to the class of eudicotyledons, one of the big families. Various types of its aromatics are known for their therapeutic and cosmetic properties and are essential from environmental and economic points of view. Their anti-inflammatory, antimicrobial, anticancer and insecticidal properties have been specifically studied [13].

Eupatorium is a flowering plant from the Asteraceae family. The *Eupatorium* genus in the Asteraceae family has approximately 1200 species widely distributed on five continents. The largest number of species has been observed in tropical, subtropical and temperate America [14]. *Eupatorium adenophorum* species [Syn. *Agratina adenophora* (Spreng.) R.M. King & H. Rob.] is native to Mexico and is now exclusively distributed worldwide [15]. It is used as an antimicrobial in traditional medicine [16]. A single plant group containing *Eupatorium* (about 42 white-flowered plants) exists in North America, Europe and Asia [17]. The *Eupatorium adenophorum* Spreng, from composite plant species, was first seen in China in the 20th century [18].

Several *Eupatorium* spp. are used in traditional medicine to relieve various diseases [19,20]. However, many species of the *Eupatorium* genus have no use in traditional medicine. These species should be studied phytochemically because they may have good potential to produce essential products for humans. Therefore, ethnobotanical knowledge has special importance for the rational use of natural resources. Furthermore, the mentioned cases are essential arguments regarding the recognition and preservation of plant biodiversity [21]. *Eupatorium* phytochemical studies identified sesquiterpene lactones, flavonoids, triterpenes, benzofuran compounds, pyrrolizidine alkaloids, chromene and steroids [22–25]. Furthermore, the *Eupatorium* spp. has various biotechnological applications, such as the preparation of essential oils for insect control and insecticidal effects in the larval stage of the vector mosquito *Aedes aegypt* [26]. There are many reports of the *Eupatorium's* insecticidal properties [27,28] but relatively few of its nematicidal properties [29].

Our research aimed to (1) study the mortality of the second-stage (J2) juveniles of *M. incognita* exposed to various concentrations of leaves and root stems of *E. adenophorum* after eight weeks and at two incubation temperatures by non-linear regression. (2) Investigate the impact of the leaves and root stems of the *E. adenophorum* on cucumber stem length and yield. (3) Identify key chemical compounds in the gases extracted from the leaves and root stems of *E. adenophorum*. We hoped that our research would show the potential for selected plant materials from *E. adenophorum* to replace synthetic fumigants for *M. incognita* control in greenhouses.

2. Materials and Methods

2.1. Biofumigant Plant

Root and stems of the *E. adenophorum* were collected from uncultivated areas of Xichang City, Sichuan Province, China, in 2019. The plant was identified by Prof. A. C. Cao (Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China). The root stems and leaves are placed in a dark and dry place at room temperature of about 28 °C for a week to dry.

The dried root stems and leaves were powdered by a grinder (Qufu Shunyang Machinery Co. Ltd.; Jining, China). Then, the dried root stems and leaves were stored in vacuum-packed Ziplock plastic bags at 28 °C.

2.2. Identification of Chemical Compounds

Gas chromatography–mass spectroscopy (GC-MS) analysis of the dried roots-stems was carried out using a SHIMADZU GC-MS QP 2010 (SHIMADZU Co. Ltd.; Kyoto, Japan) set in the Select Ion Monitoring mode. The operating conditions that followed a previously described method [29] were: RTX-5MS capillary column (30 m long, 0.25 mm ID, 0.25 µm thick); helium carrier gas at a flow rate of 1.5 mL min^{-1}; oven temperature initially at 65 °C for 1 min., then raised at 3 °C min^{-1} until 250 °C; 230 °C interface temperature and 350 °C

ion source temperature. Mass acquisition parameters were ion source 180 °C, transfer line temperature 70 °C. The relevant chemicals in the GC column were identified by referencing the spectra stored in the NIST spectral library.

2.3. In Vitro Studies

Soil and root samples were collected from the rhizosphere of tomato plants in a nematode-infested tomato greenhouse in Shunyi, Beijing, China. First, for soil sampling, 3 to 5 cm of the top layer of the soil was removed, and about 250 m^2 of soil and 10 g of feed roots were collected up to a depth of 30 cm [30]. Ten soil samples were randomly selected from different soil locations in the greenhouse. Single egg mass was used to establish a population of nematode on tomato (*Solanum lycopersicum* L. cv Aojinfutian) (Tomato plants were purchased from China Vegetable Seed Technology Co., Ltd., Beijing, China) seedlings (3 or 4-true-leaf stage) in plastic pots. After 60 days, the aboveground parts of the plants were separated, and the soil was gently washed from the roots with tap water. After adding 1.5% NaOCl to the 2–3 cm pieces of the roots, they were vigorously shaken for 2 min [31,32]. After shaking, the nematode suspension was poured onto a 25 µm mesh sieve and washed with tap water to eliminate NaOCl residues. The egg suspension was aerated in the dark for 7 to 10 days for the second-stage juveniles hatching. Freshly hatched J2s were separated from unhatched eggs by Baermann 1917 [33] method. The species was identified as *M. incognita* based on characteristics, morphometric attributes of the juveniles and the perineal pattern of the females [34].

Soil samples were collected from the top 20 cm of soil in another cucumber (cv "Jinyou 35") greenhouse in the same location. After wetting, the soil was sterilized (at a temperature of 85 °C for 30 min in an autoclave) and physiochemically analyzed. The soil comprised 12.3% sand, 64.4% silt and 23.3% clay, organic matter content of 33.5 g kg^{-1} soil and pH 6.5. The "cucumber soil" was sieved through 2 mm mesh and mixed thoroughly. The soil moisture of 17.8% (w/w) was measured by heating the soil in a drying oven at 105 ± 5 °C until mass constancy was achieved [35].

To determine the *M. incognita* J2s mortality after exposure to the *E. adenophorum*, dried and powdered root stems and leaves were added to 100 g of sterilized greenhouse soil to provide final concentrations of 20, 40, 60, 80, and 100 mg/g. Each was infected with 200 *M. incognita* J2s (2 juveniles/gr soil) [36] before each was transferred to 250 g capacity plastic test tubes. The control comprised wet soil with nematodes but without any plant material added. All the tubes were incubated at 25 and 35 °C for 1, 2, 3, 4, and 8 weeks. Each concentration comprised three replicates. The mortality of the *M. incognita* J2s was assessed with the aid of a binocular microscope.

2.4. In Vivo Experiments

Two demonstration experiments were carried out in 2019 and 2020 in greenhouses important for cucumber cultivar (Jinyou 35) susceptible to *M. incognita* production on a commercial property in Shunyi, Beijing (40°03′10.48″ N; 116°56′2.12″ E).

Biofumigant concentrations tested 20 mg/g and 40 mg/g dried root stems and leaves from July 2019 to February 2020 at 35 °C. The treatments were designed as wholly randomized, with three replicates and four treatments. The control contained no bio-fumigant material—the five plots comprised four treatment plots and one control. There were 30 pots per plot laid out in 3 rows of 10.

The pots were filled with 5 kg of sterilized soil, as described above. Soil comprised 12.3% sand, 64.4% silt, and 23.3% clay, with organic matter content of 33.5 g kg^{-1} 122 soil and pH 6.5. The soil moisture of 17.8% (w/w) was measured by heating the soil in a drying oven at 105 ± 5 °C until mass constancy was achieved. Dried and powdered root stems or leaves were added to each pot except the control. Fifteen-day-old cucumber "cv Jinyou 35" susceptible to *M. incognita* seedlings were planted in the pots. Then, 10,000 J2 *M. incognita* (2 juveniles/gr soil) [36] were inoculated into each of the pots immediately. The inoculum

was injected into the soil of the pot through 3 holes 2 cm deep around the roots of the seedlings. The plants in the treated pots were watered with 2 g per liter of water [37].

2.5. Parameter Measurement

Nematode mortality in vitro studies and vivo experiments were calculated according to the following equation [35]:

$$X = \frac{N1}{N1 + N2} \times 100 \quad (1)$$

where X is the percentage of nematode mortality, N1 is the number of dead J2 juveniles, and N2 is the number of living J2 juveniles. Nematode mortality was assessed using a binocular microscope. Nematodes were immobile when touched lightly with a dissecting needle and were scored as dead. Otherwise, they were scored as alive [35].

Cucumber plant height and fresh weight were measured to determine the impact of the biofumigants on RKN.

2.6. Data Analysis

All the assays were carried out in a completely randomized factorial design. Each treatment comprised three replicates. Statistical significance was assessed at the level $p < 0.01$. In vivo data analysis, when the analysis was statistically significant, Duncan's Multiple Range Test (SSR Test) was applied to test mean separations among the mean values of each treatment. For determination of regression trend of mortality response of *M. incognita* used of non-linear—regression function of Logistic (2) and Weibull (3) by R 2.4.1 software:

$$Y = \frac{Y_{max}}{1 + \left(-\left(\frac{x}{EC_{50}}\right)\right)^{slope}} \quad (2)$$

$$Y = Y_{max}\left[1 - e^{[X - ECmax + slope\ln 2^{\frac{1}{2}}]^c}\right] \quad (3)$$

where Y_{max} is maximum y, EC_{50}: is half maximal effective concentration, EC_{max}: maximal effective concentration,

R^2 and RMSE were applied to determine the best estimates of the parameters. R^2 was calculated using the following formula: R^2 = SSR/SST, Where SSR denotes the sum of squares (SS) for regression ($\sum n\ i = 1\ L - \bar{L}$) and SST the total SS ($\sum n\ i = 1\ Li - \bar{L}$). Li is the observed value, and $\bar{L}$ is the corresponding estimated value. In addition, the root mean square error (RMSE) is calculated using the following formulae: RMSE = $\sqrt{(1/n)} \sum$ (Yobs−Ypred) 2, Where Yobs denotes the observed value, Ypred predicted value, and n is the number of samples [38]. Principal component analysis (PCA) was done by R 2.4.1 software.

3. Results

3.1. In Vitro Mortality of J2 Juveniles

The mortality response of *M. incognita* J2 exposed to soil containing dried root stems or leaves of the *E. adenophorum* was significantly different ($p \leq 0.05$) when the larvae were exposed for different lengths of time, and different plant concentrations and incubation temperatures (Table 1).

Fitted the Logistic model for mortality response of *M. incognita* J2 influenced by the interaction of temperature and concentrations, time and concentrations of root-stems shown in (Figure 1A,B). Based on the output parameters of the model, this model was suitable for changing the mortality response (R^2 = 0.991–0.999 and RMSE = 1.18–3.76). Increasing concentrations of root-stems *E. adenophorum* resulted in increased mortality in *M. incognita* J2. Also, temperature and time cause increased toxicity of root-stems *E. adenophorum* and increased mortality. For example, maximum mortality (Y_{max}) and (EC_{50}) under 25 °C were estimated at 77.4% and 11.3 mg/g, respectively, which reached 96.8% and 10.2 mg/g under

35 °C. The (Y_{max}) and (EC_{50}) in 1 week were estimated at 76.8% and 10.3 mg/g and reached 96.2% and 7.8 mg/g in 8 weeks (Table 2).

Table 1. In vitro analysis of variance of the mortality response of *Meloidogyne incognita* J2 exposed to soil containing dried root-stems and leaves of the *Eupatorium adenophorum*.

Source of Variation	DF	Means Square	
		Root-Stems	Leaves
Temperature	1	11,394.75 **	6328.48 **
Time	4	1804.91 **	4425.35 **
Concentrations	5	27,635.65 **	2436.66 **
Temperature × Time	4	931.90 **	80.07 **
Temperature × Concentrations	5	620.92 **	761.76 **
Time × Concentrations	20	166.30 *	285.97 **
Temperature × Time × Concentrations	20	48.24 ^{ns}	54.77 ^{ns}
Error	120	51.55	38.48
Coefficient of Variation (%)	-	9.73	32.10

ns, * and ** are non-significant and significant at 5% and 1%.

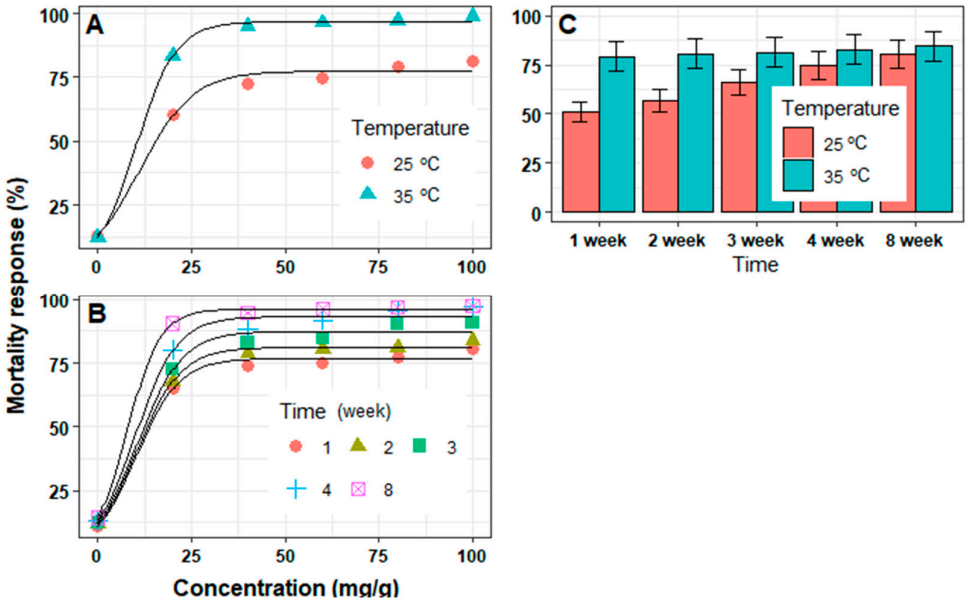

Figure 1. Regression relationship between in vitro percentage mortality of *Meloidogyne incognita* J2 and different concentrations of root-stems *Eupatorium adenophorum* at different temperatures (**A**), time (**B**) and the relationship between temperatures and time (**C**) by Logistic model. Points were observation values, and lines predicted values.

Table 2. Estimate parameters models of regression relationship between in vitro percentage mortality of *Meloidogyne incognita* J2 and different concentrations of root-stems *Eupatorium adenophorum* at different temperatures and times.

Treatments	Levels	Parameters Model			R^2	RMSE
		Y_{max} (%)	Slope	EC_{50} (mg/g)		
Temperature (°C)	25	77.4 ± 1.8	7.1 ± 1.1	11.3 ± 1.5	0.989	3.54
	35	96.8 ± 0.72	5.3 ± 0.3	10.3 ± 0.5	0.999	1.43
Time (week)	1	76.8 ± 1.5	5.8 ± 0.7	10.3 ± 1.3	0.993	2.95
	2	81.1 ± 0.9	5.9 ± 0.4	10.4 ± 0.7	0.997	1.80
	3	87.5 ± 1.9	6.0 ± 0.8	10.7 ± 1.4	0.991	3.72
	4	93.2 ± 1.9	5.6 ± 0.7	10.0 ± 1.4	0.992	3.76
	8	96.2 ± 0.6	4.4 ± 0.3	7.8 ± 0.5	0.999	1.18

Y_{max}: maximum mortality, EC_{50}: half maximal effective concentration, R^2; coefficient of determination, RMSE: Root Mean Square Error.

Fitted the Weibull model for mortality response of *M. incognita* J2 influenced by the interaction of temperature and concentration, time and concentration of leaves shown

in (Figure 2A,B). Based on the output parameters of the model, this model was suitable for changing the mortality response (R^2 = 0.986–0.999 and RMSE = 0.12–3.13). Increasing concentrations of leaves *E. adenophorum*, about 25 mg/g, increased mortality in *M. incognita* J2, but higher values caused decreased mortality and increased survival in *M. incognita* J2. Temperature and time in this experiment resulted in reduced effect of leaves. For example, the (Y_{max}) and (EC_{max}) under 25 °C were estimated at 45.0% and 17.6 mg/g, respectively, which reached 19.3% and 20.3 mg/g under 35 °C. The (Y_{max}) and (EC_{max}) in 1 week were estimated at 48.8% and 25.9 mg/g and reached 20.3% and 10.1 mg/g in 8 weeks (Table 3).

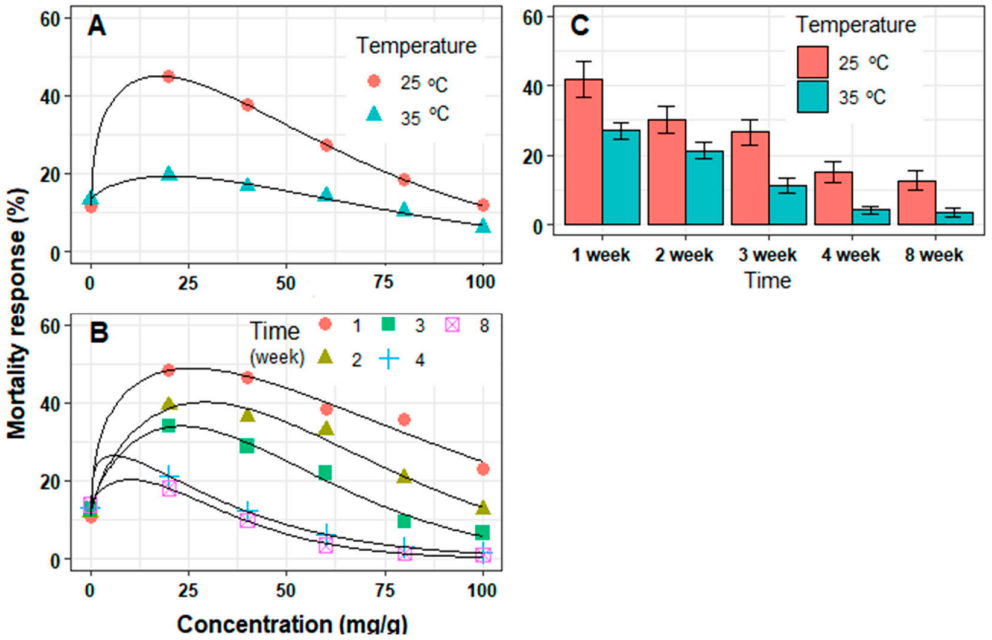

Figure 2. Regression relationship between in vitro percentage mortality of *Meloidogyne incognita* J2 and different concentrations of leaves *Eupatorium adenophorum* at different temperatures (**A**), time (**B**), and the relationship between temperatures and time (**C**) by the Weibull model. Points were observation values, and lines predicted values.

Table 3. Estimate parameters models of regression relationship between in vitro percentage mortality of *Meloidogyne incognita* J2 and different concentrations of leaves *Eupatorium adenophorum* at different temperatures and times.

Treatments	Levels	Parameters Model				R^2	RMSE
		Y_{max} (%)	Slope	c	EC_{max} (mg/g)		
Temperature (°C)	25	45.0 ± 0.2	57.1 ± 0.3	1.3 ± 0.1	17.6 ± 0.5	0.999	0.19
	35	19.3 ± 0.8	67.2 ± 3.7	1.4 ± 0.2	20.3 ± 4.8	0.986	0.88
Time (week)	1	48.8 ± 2.3	82.1 ± 8.3	1.3 ± 0.1	25.9 ± 7.9	0.981	3.13
	2	40.1 ± 1.8	62.8 ± 3.6	1.5 ± 0.2	29.2 ± 4.4	0.984	2.46
	3	34.0 ± 1.8	51.3 ± 2.9	1.5 ± 0.2	23.3 ± 4.4	0.986	2.09
	4	26.4 ± 0.9	33.9 ± 0.3	1.2 ± 0.1	6.1 ± 0.9	0.999	0.12
	8	20.3 ± 1.9	32.0 ± 3.2	1.4 ± 0.2	10.1 ± 2.9	0.997	0.57

Y_{max}: maximum mortality, EC_{max}: maximal effective concentration, C; Model coefficient, R^2; coefficient of determination, RMSE: Root Mean Square Error.

The results of the effect of temperature and time on mortality of J2 juveniles in two experiments showed, through time, mortality of J2 decreased under root-stems condition, and increased temperature caused enhanced this effect. However, in leaves, conditions through time reduced mortality, and this change decreased by increased temperature (Figures 1 and 2C).

3.2. In Vivo Mortality of J2 Juveniles

In the greenhouse, three items, the percentage mortality of J2 juveniles, plant height and fresh fruit weight, were studied in two concentrations of 20 and 40 mg/g of *E. adenophorum* and compared with the control. *E. adenophorum* concentrations of 20 and 40 mg/g of dried root stems and leaves significantly affected ($p < 0.01$) the percentage mortality of J2 juveniles in the greenhouse at 35 °C. Mortality percentage was studied from July to November. In the dried root-stems treatment, the results obtained from both concentrations were higher than the control in all three items. *E. adenophorum* 40 mg/g root-stems concentration caused significantly higher percentage mortality of J2 juveniles (50.9%) than a 20 mg/g concentration (46.1%). Cucumbers were significantly taller in the 40 mg/g (185 cm) than 20 mg/g (177.3 cm) root-stems treatment. The fresh fruit weight in two concentrations of 20 and 40 mg/g were observed as (>50,000 kg/h) and (>58,000 kg/h), respectively. In the study of dried leaves treatment of *E. adenophorum* and all three items, the percentage mortality of J2s, plant height and fruit fresh weight in the greenhouse were observed. In all three items, 20 mg/g concentration had the highest result compared to 40 mg/g concentration and control (Table 4).

Table 4. In vivo, analysis of variance and comparison of means the mortality response of *Meloidogyne incognita* J2s nematode and plants height and fruit fresh weight of cucumber influenced by two concentrations of dried root-stems and leaves of *Eupatorium adenophorum*.

Analysis	Source of Variation	DF	Mortality (%)		Plants Height (cm)		Fruit Fresh Weight (kg/h)	
			Root-Stems	Leaves	Root-Stems	Leaves	Root-Stems	Leaves
Means square	Concentration	2	3703.9 **	242.76 **	2084.1 **	1276.77 **	309,238,018 **	108,297,840 **
	Error	6	3.23	3.57	2.44	2	268,788.2	241,007.2
	CV (%)	-	4.9	9.6	0.94	0.9	9.8	10.1
Means	Control		13.6 ± 1.1 ^{c}	13.9 ± 1.2 ^{c}	136.0 ± 2.1^{c}	134.7 $_{c}$ ± 5.4	39.4 ± 3.3 ^{c}	26.7 ± 1.6 ^{b}
	20 mg/g		46.1 ± 1.6 ^{b}	23.9 ± 0.7 ^{a}	177.3 ± 2.5 ^{b}	174.3 $_{a}$ ± 2.3	54.3 ± 1.5 ^{b}	37.9 ± 1. 3 ^{a}
	40 mg/g		50.9 ± 0.6 ^{a}	21.3 ± 0.7 ^{b}	185.0 ± 3.5 ^{a}	164.3 $_{b}$ ± 1.2	58.8 ± 2.6 ^{a}	28.9 ± 0.9 ^{b}

** are significant at 1%. CV; Coefficient of Variation. The letters indicate comparison of means for e each separate column.

At the same concentration, root stems, and leaf treatments in the greenhouse resulted, indicating that the active substance was more potent in the root stems.

3.3. Chemical Compounds Extracted

Results of gas chromatography–mass spectroscopy of root-stems and leaves of *E. adenophorum* were shown in Tables 1 and 2 supplemental. In the root stems of this plant, chemical compounds were detected, such as butanol, α-pinene, camphene, butanoic acid, propanoic acid, carene, α-phellandrene, o-cymene, D-limonene, α-bergamotene and (E)-beta- farnesene, etc. and in leaves also, methylene chloride, butanal, α-pinene, camphene, cyclohexene, carene, α-phellandrene, o-cymene, linalool, and nonanal, etc detected.

3.4. Principal Component Analysis (PCA)

The result of Principal Component Analysis (PCA) chemical compounds extracted from root stems indicated two PCA (PCA1 = 48.4% and PCA2 = 16.6%) could explain 65% of the resulting change (Figure 3). Based on the result of this section, (E)-beta-farnesene, D-limonene, Bicyclo-methylene, Furan, 2,5-dimethyl, Bicyclo-methylene, and α-Pinene had more similarity by mortality response of *M. incognita* J2s of nematode and were placed on II and IV of coordinate diagram of PCA. According to the fact that the extracted gas of this plant caused the mortality of nematodes, the existence of these compounds can cause the mortality of nematodes in this part of the plant.

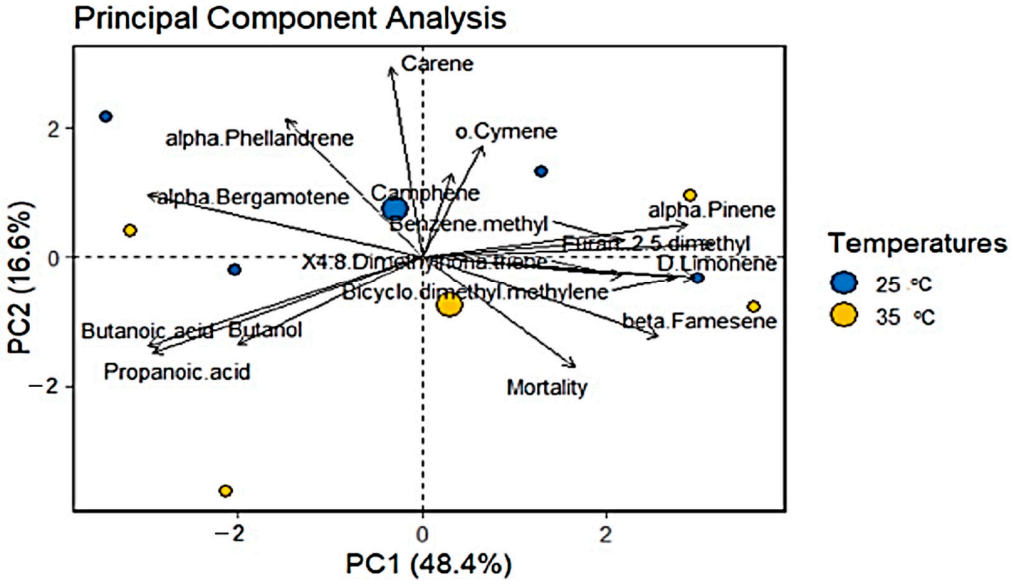

Figure 3. Principal component analysis chemical compounds extracted from root-stems of *Eupatorium adenophorum* over four weeks at 25 or 35 °C using gas chromatography–mass and mortality of *Meloidogyne incognita* J2 juveniles.

The result in PCA (71%) compounds extracted from leaves and mortality response is shown in (Figure 4). Also, according to use of leaves resulted in increased survived nematodes. The result of PCA chemical compounds extracted from leaves and mortality response showed onanal, Camphene, Methylene chloride, α-Bergamotene, Carene, and Linalool had the loss similarity by mortality response of *M. incognita* J2s nematode a. It replaced I and III of the coordinate diagram of PCA.

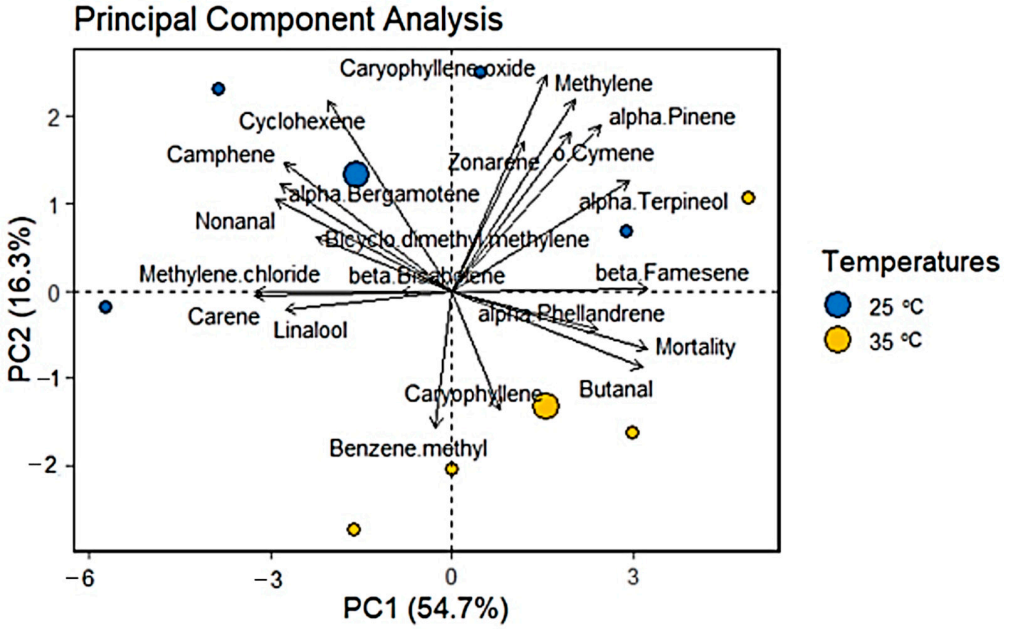

Figure 4. Principal component analysis chemical compounds extracted from leaves of *Eupatorium adenophorum* over four weeks at 25 or 35 °C using gas chromatography–mass and mortality of *Meloidogyne incognita* J2 juveniles.

4. Discussion

The dried root stems of *E. adenophorum* have a nematicidal effect on *M. incognita*. Most researchers consider using plant materials as nematicidal agents eco-friendly, and to

date, nematode resistance to them has not been detected or reported [39,40]. In laboratory studies, we obtained the nematicidal activity of *E. adenophorum* root-stems influenced by temperature and time cycle against *M. incognita*. The EC_{50} or half maximal effective concentration as the degree of toxicity of this plant decreased (increased toxicity) by temperature and time. For example, EC_{50} at 25 and 35 °C were 11.3 and 10.3 mg/g; in 1 and 8 weeks, they were 10.3 and 7.8 mg/g (Tables 2 and 3). The nematicidal effects of essential oils of several species of *Eupatorium* plants, such as *E. buniifolium*, *E. inulaefolium*, *E. arnotii*, and *E. viscidum* on the RKN *M. javanica* were studied, in which *E. viscidum* showed a high nematicidal efficacy [41]. Kundu et al. [29] reported that *Eupatorium* spp. can control *M. javanica* J2ss. The nematicidal effects of *Tagetes erecta*, *Tithonia diversifolia*, *Chromolaena odorata*, and *Occimum gratissimun* against *M. incognita* in a greenhouse were reviewed [42]. The *T. erecta*, *C. odorata*, and *O. gratissimun* treatments reduced the gall index by 63, 65, and 76%, respectively. The *M. incognita* population in the *T. erecta* treatment was reduced by 85%; *C. odorata* decreased the population by 88%; and carbofuran decreased the population by 93% [42]. In an experiment, soil nematode community structure under temperature and humidity was studied in a semiarid shrubland. Based on the conventional variable analysis, both drying and warming parameters greatly affected species with low density (Wilk's lambda: 0.02) and high density (Wilk's lambda: 0.002). The life cycles of nematodes became shorter with increased temperature [43]. However, as the temperature approached the upper limits, nematode sensitivity to plant compounds of *E. adenophorum*. The increase in metabolic temperature, on the one hand, and the increase in the release rate of compounds to the soil at higher temperatures can reason for the increase in mortality in the nematode [44].

The results indicate that the dried root stems from *E. adenophorum* have a high potential to reduce the population and control the pathogenic activity of the RKN J2 juveniles both at ambient temperature and in the greenhouse. Although the result of gas-mass analysis in root stems indicated many compounds were extracted (Supplementary Table S1). But based on the information available from reliable sources, some compounds such as (E)-beta-farnesene (sesquiterpenes), α-pinene (terpene) and D-limonene (monoterpene) those known to increase the mortality of *M. incognita* J2s.

Our chemical analysis of gases extracted from the dried root stems of *E. adenophorum* led to three the identification of (E)-beta-farnesene, α-pinene and D-limonene. Results of PCA analysis also showed a significant relationship between (E)-beta- farnesene, D-limonene, and α-pinene and the mortality of *M. incognita* (Figure 3). Nematicidal effects of some aromatic plants enriched with terpenes and their main compounds were also reported in recent studies. The root parasitic nematode *Xiphinema index* damage by increasing biogenic volatile organic compounds (α-farnesene and α-bergamotene) was proved on *Vitis vinifera* L. [45]. The nematicidal activity of the essential oils of the aerial parts of *Achillea wilhelmsii*, *Tanacetum polycephalum* and *Teucrium polium* against *M. incognita* J2s with β-pinene and α-pinene respectively, at concentrations of 100 and 200 ppm were reported [46]. Li et al. [47] reported that α-pinene at a low concentration of 56.33 mg/mL had inhibited effect on *Bursaphelenchus xylophilus*, as fatal to pine trees. The other study reported the nematicidal effect of essential oil and two synthesized compounds, limonene tetrabromide and carvone, at 1500 $\mu g/mL^{-1}$ on the *M. incognita* [48].

Leaves of *E. adenophorum* did not have power nematicidal activity. For example, the EC_{max} of leave was about 20–25 mg/g and decreased temperature and passing of time resulted in a reduced this parameter to 17.3 and 6.1 mg per g in 25 °C and four weeks but use more of this part of the plant cause resulted in increased reproduction in nematodes (Table 2, Figure 2). Our chemical analysis of gases extracted from the dried leaves of *E. adenophorum* led to identifying linalool, nonanal and methylene chloride (Supplementary Table S2). The PCA analysis showed nonanal, camphene, methylene chloride, carene, and linalool had the loss similarity by mortality response of *M. incognita* (Figure 4). Unlike the dried root stems that increased nematode mortality, our results showed that chemicals from the leaves were not as pathogenic. Previous research also extracted volatile oil and secondary metabolites, including cadinene sesquiterpenes from the leaves of *E. adenophorum* that

resulted in *M. incognita* EC_{50} values of 189.2, 154.0 and 133.7 μg ml^{-1} after 24, 48 and 72 h, respectively [29]. In another study, the chemical composition of linalool secreted from two host plants of potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, revealed that J2 juveniles of both species were attracted to linalool that was non-toxic to them [49]. Studies on *Hylastes opacus* Erichson, a species of bark beetle found in North America, reported that the species detects volatile substances such as nonanal to locate hosts suitable for feeding and egg-laying [50]. Another study examined reported female beetles (*Callosobruchus maculatus*) as an essential pest of legumes that were attracted to mixtures of 1-pentanol, 1-octen-3-ol, (E)-2-octenal, nonanal and 3-carene compounds, but male beetles were attracted only to two binary mixtures that contained only aldehydes [51].

We understood from the above-mentioned and our results that dried plants of *E. adenophorum* can be relatively effective for nematode management. The root stems of *E. adenophorum* were much stronger and more effective than leaves. In the pots where root stems were used, plant height and fresh fruit weight were higher than those treated with dried leaves. However, this is the first time that the dried plant of *E. adenophorum* has been reported to be used in a cucumber greenhouse as a biofumigant plant to control the pathogenic activity of second-stage juvenile larvae of root-knot nematode (*M. incognita*). So far, no similar study has been recorded. We observed that elevated incubation temperature and increased exposure time to increased concentrations of *E. adenophorum* dried root stems increased the in vitro mortality of *M. incognita* J2 juveniles. The percentage mortality of J2 juveniles exposed to dried root stems was significantly higher than the mortality of J2 juveniles exposed to dried leaves under the same conditions. We surmise that key chemical compounds in the dried root stems have greater nematicidal activity at 35 °C than at 25 °C, and *E. adenophorum* could replace synthetic chemicals for managing *M. incognita*.

5. Conclusions

The nematicidal properties of dried root stems of the *E. adenophorum* provide sufficient control of *M. incognita* in greenhouses. Our studies showed that relatively low concentrations of the plant in the soil would reduce the ability of *M. incognita* to survive in a greenhouse, especially during the warm months of the year in August. Considering that the main compounds of dried leaves of *E. adenophorum* increase the root-knot nematode population, more research is needed to identify its mechanism.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13061109/s1, Supplementary Table S1: Chemical composition of dried roots-stems obtained from *Eupatorium adenophorum* with headspace at two temperature and four week; Supplementary Table S2: Chemical composition of dried leaves obtained from *Eupatorium adenophorum* with headspace at two temperature and four week.

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