

Chemical composition and nematicidal activity of wasabi(*Eutrema japonicum*) rhizome extracts against *Meloidogyne enterolobii*

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Abstract

Aims

Eutrema japonicum a perennial herb belonging to the *Eutrema* genus in the Crucifer family. In recent years, numerous substances with notable pharmacological activities have been successfully isolated from *E.japonicum*. Despite significant advancements in related research, the efficacy of *E.japonicum* extracts against rhizome-knot nematodes remained unknown.

Methods

This study utilized extracts obtained from the rhizome of *E.japonicum* to evaluate their activity against J2 *Meloidogyne enterolobii* and single-egg hatching through a soaking method, demonstrating inhibition and killing activity against *M.enterolobii*.

Results

The results showed that the LC_{50} of *E.japonicum* extract on J2 were 44.633 mg/mL and 22.840 mg/mL at 24 h and 48 h post-treatment, respectively. The mortality rate of J2 reached 88.93% at 48h post-treatment when the concentration was 200 mg/mL, and the inhibition rate of single egg hatching reached 88.14%. This study conducted an analysis of the chemical composition of the ethanol extract of *E.japonicum*. we preliminarily screened out 10 organosulfur compounds and lipid compounds with insecticidal and antibacterial effects. Including Sec-butyl isothiocyanate and geraniol. Sec-butyl isothiocyanate and geraniol were further investigated for their nematocidal activity, demonstrating high efficacy against *M.enterolobii*.The results indicate that the extract of *E.japonicum* shows promise in inhibiting *M.enterolobii*.

Conclusions

These findings offer a scientific foundation and theoretical framework for utilizing *E.japonicum* as a potential raw material for the development of novel natural plant nematicides.

Introduction

Meloidogyne enterolobii is recognized as one of the most destructive root-knot nematodes(Lian et al.2015).The nematode has a wide host range of most crops including monocotyledonous, dicotyledonous herbaceous and woody crops(Liu et al.2024). It can also parasitize tomato (*Mi-1* gene), pepper (*N* gene), and cowpea (*Rk* gene) crops carrying disease resistance genes, resulting in yield losses of more than 65% of the crop(Kicwnick et al.2024) *M. enterolobii* is widely distributed in tropical and subtropical regions, especially in Hainan Province, China, which has a tropical climate conducive to the

rapid spread of nematode populations without overwintering, facilitating the occurrence and dissemination of *M. enterolobii*. Research by Huang et al(2024). focused on characterizing root-knot nematode populations in cucurbit vegetables on Hainan Island, revealing a high incidence of *M.enterolobii*, reaching up to 80%. Li et al(2020).reported a detection rate of over 60% for *M.enterolobii* on vegetables in Hainan Province. This finding suggests that *M.enterolobii* has replaced *M.incognita* as the primary pathogenic nematode species impacting vegetables in the region. Consequently, the prevention and control of *M.enterolobii* are crucial for agricultural production in Hainan Province, China(Long et al.2015 ; Li et al.2020).

E.japonicum, commonly known as *wasabi*, is a perennial herbaceous plant belonging to the Cruciferae family. It thrives in cold and humid environments and is native to Japan. In China, mainly planted in Yunnan, western and southwestern Sichuan and other places in some of the central mountainous areas(Xiao et al.2017) . The skin of the *E.japonicum* plant is green, known as “green gold”, mainly consumed for its distinctive spicy flavor when crushed, rhizomes are widely utilized for flavoring various dishes(Miles et al.2019 and Yang 2015). *E.japonicum* is recognized as a valuable medicinal plant with a range of pharmacological benefits, attributed to its high concentration of isothiocyanate compounds(Chen et al.2022 and Hu et al.2004). Studies have shown that *E.japonicum* has a strong inhibitory effect on the gray mold and penicillium that are prone to infecting fruits, as well as common pathogen such as *Staphylococcus aureus*, *Bacillus cereus*, and *Helicobacter pylori*(Du et al.2011 ; Li 1999; Lu et al.2016; Jiang et al.2005;Shin et al.2004 and Szewczyk et al.2023), and early research have indicated that the pungent flavor compound, isothiocyanate, found in *E.japonicum* can kill the *Anisakis*(Hu et al.2015). Furthermore, *E.japonicum* can serve as an effective antimicrobial agent, insecticide, and herbicide(Liang et al.2018). *E.japonicum* stands out for its distinctive pharmacological properties, positioning it as a noteworthy herbal medicine deserving of further exploration and development.

Currently, chemical methods are predominantly utilized for the management of root-knot nematodes, with the issue of residues from conventional chemical control agents posing a significant challenge. Since the 1980s, there has been a growing interest in the use of plant extracts for controlling root-knot nematodes(Liang 2019).Nematicidal compounds derived from natural plants offer advantages such as biodegradability in soil, beneficial effects on biological health, and environmental safety, making them a focal point in biopesticide research(Ping 2019; San et al.2011 and Wang et al.2013).In this study, we examined the inhibitory effects of the ethanol extract derived from the rhizome of *E.japonicum* on *M. enterolobii*. Additionally, we conducted a preliminary screening of potential active compounds and identified promising nematicidal substances for further indoor activity testing. These findings aim to establish a scientific foundation and theoretical framework for utilizing *E.japonicum* as a novel natural botanical nematicide.

Materials and methods

Plant Material

E.japonicum was purchased online in July 2023. The species identification was confirmed by Dr. Chen. from the Hainan Branch of the Institute of Medicinal Plant Development, Chinese Academy of Medicinal Sciences & Peking Union Medical College. The specific variety *Wasabia japonica* cv. *Mazuma* (Fig.1-1).

Nematodes

M.enterolobii were collected from Lingkou Village, Lingkou Town, Ding'an County, Hainan Province, China (N:19.345925 E:110.310199), purified using a single egg mass and preserved in the laboratory of the Institute of Plant Protection, Hainan Academy of Agricultural Sciences.

Preparation of *E.japonicum* extracts

The *E.japonicum* rhizomes were thoroughly cleaned with distilled water to remove soil and surface impurities. The cleaned rhizomes were then sliced using a knife and dried in an oven at a constant temperature(DHG-9023A oven (Yiheng Scientific Instrument Co., Ltd., Shanghai, China) of 60°C until a constant mass was achieved. The dried rhizomes were crushed using a wall-breaking machine(JXFSTPRP-24 grinder Jingxin Technology Co., Ltd., Shanghai, China) and sieved through a 50-mesh sieve. A 10-g sample of the dried *E.japonicum* powder was placed in a 500-mL Erlenmeyer flask, and 100 mL of 85% ethanol solution((Pure ethanol of analytical grade was obtained from Hengshun Chemical Co., Ltd. in Wenzhou, China.) was added to achieve a 1:10 ratio. The mixture was extracted by ultrasonication(YM-080S Fangao Microelectronics Co., Ltd., Shenzhen, China) at 35°C and 80W for 2 hours. After filtration, the filtrate was in the form of extractum paste after evaporated by rotary evaporator(Heidelberg, Schwarzbach, Germany) at 40°C for 35min, and then stored in the refrigerator at 4°C for spare use.

Comparison test of Cosolvents for plant extracts

Because the ethanolic extract of *E.japonicum* rhizome was in the form of an infusion after rotary evaporation, acetone and dimethyl sulfoxide(DMSO,Shanghai McLean Biochemical Technology Co., Ltd. is located in China.) were used to solubilize the infusion, respectively. In a 35 mm Petri dish, 1 mg of extract infusion was weighed and added sequentially to 1 mL of acetone, DMSO. Each treatment was repeated three times, and the occurrence of precipitation in the extract was observed after 24 h and 48 h of treatment. In a 12-well cell culture plate, nematode suspension (Around 100 nematodes) was added to each well. Subsequently, 500 µL of acetone and DMSO were added respectively, and sterile water was used to make up the volume to 1 mL. Sterile water was utilized as a blank control. Nematode deaths were observed and recorded after incubation in a constant temperature chamber at 25°C for 24 h and 48 h. The mortality rate was calculated following the method described by Wang, et al ^[23].

Determination of Nematicidal Activity

Using the impregnation method, a nematode suspension (Around 100 nematodes) was added to each well in a 24-well cell culture plate with 500 μL of *E.japonicum* extract solution. The volume was then replenished to 1 ml with sterile water. The *E.japonicum* extract solution concentrations were finalized at 200, 100, 50, 25, and 12.5 mg/mL after several trials. Sterile water was used as the negative control, while 90% Abamectin original(Aino Pharmaceutical Co., Ltd. North China) powder served as the pharmaceutical control. Each concentration was repeated three times. The treated cell culture plates were incubated at 25 °C for 24 h and 48 h. Subsequently, the treated nematodes were transferred to centrifuge tubes, centrifuged, and washed twice with sterile water. The nematodes were then transferred to a 3-cm Petri dish, resuscitated by adding sterile water, and left for 48 h. After 48 h of resuscitation, the nematodes were observed under a microscope. Live nematodes exhibited an arched curved body with coiling and peristaltic movement, while dead nematodes had stiff bodies. The needle-prick test was used to confirm death, as the nematodes remained immobile upon stimulation(Wang et al.2013). The nematode mortality rate and corrected mortality rate were then calculated.using equations(1.1) and (1.2):

Mortality rate = Number of dead nematodes/Number of nematodes in the treatment $\times$ 100%

1.1

Corrected mortality rate = (treatment nematode mortality rate - control nematode mortality rate) / (1-control nematode mortality rate) $\times$ 100%

1.2

Effect of *E.japonicum* extracts on hatching of single-egg of *M. enterolobii*

Fresh, plump, and yellow-brown egg sacs were carefully selected for incubation experiments. The egg sacs underwent a series of steps, starting with immersion in a 1.0% sodium hypochlorite solution and agitation for 1 minute, followed by centrifugation. The supernatant was discarded and the bottom solution was washed three times with sterilized water. Subsequently, water was added and the mixture was shaken to collect the supernatant to obtain a single-egg suspension(Lu et al.2006 and Qiu et al.2011). Each well of a 12-well cell culture plate received a single-egg suspension (Around 100 particles/well), followed by the addition of 500 μL of *E.japonicum* extracts solution to each well. Sterile water was then used to adjust the volume in each well to 1 mL, achieving final *E.japonicum* extracts solution concentrations of 200, 100, 50, 25, or 12.5 mg/mL. Sterile water was utilized as a control, and each treatment was replicated three times. Following treatment at 25 °C for durations of 4, 8, 16, 24, and 48 hours, the extraction solution adhering to the egg surface was centrifuged and rinsed with sterile water. Subsequently, the eggs were transferred to sterile water for further incubation at 25 °C in an incubator. After four days, egg hatching was observed under a stereomicroscope, and the hatching inhibition rate was calculated following the method described by Qiu et al(2011).using equations (1.3) and (1.4):

Hatching rate = Total number of incubations/ Total number of eggs × 100% (1.3)

Incubation inhibition rate = Control hatching rate –treatment hatching rate/ Control hatching rate× 100% (1.4)

Analysis of the chemical composition of *E.japonicum* extract

To elucidate the active compounds present in the ethanolic extract of wasabi rhizome, the active substances were identified through the application of Gas Chromatography-Mass Spectrometry (GC-MS) - Volatile Organic Compounds (VOCs) technique.

GC-MS - VOCs analysis

The solid-phase microextraction (SPME) cycle of the PAL rail system comprised the following steps: incubation temperature set at 60 °C; preheat time of 15 minutes; incubation time lasting 30 minutes, and desorption time of 4 minutes. The GC-MS analysis was conducted utilizing an Agilent 7890 gas chromatograph system connected to a 5977B(Agilent, Santa Clara, California, USA) mass spectrometer(Lico, San Jose, USA) . The system employed DB-Wax as the medium, with injection performed in splitless mode. Helium served as the carrier gas, with a front inlet purge flow of 3 mL min⁻¹, and a gas flow rate through the column of 1 mL min⁻¹. The initial temperature was maintained at 40 °C for 4 minutes, then ramped up to 245 °C at a rate of 5 °C min⁻¹, and held at that temperature for 5 minutes. The temperatures of the injection port, transfer line, ion source, and quadrupole were set at 250, 250, 230, and 150 °C, respectively. The electron impact mode operated at an energy of -70 eV. Mass spectrometry data were collected in scan mode within an m/z range of 20-400, with a solvent delay of 2.13 minutes.

Data Processing and Analysis

Data Processing for Root-Knot Nematode Bioactivity Assay

The data were analyzed utilizing Excel, SPSS 27, and GraphPad Prism 9.5 statistical analysis software to compute the mortality rate for each treatment and ascertain the LC₅₀ value of the rhizome of *E. japonicum* extract.

Qualitative analysis of was conducted on chemical composition of *E.japonicum*

The mass spectral data analysis of the ethanolic extract of the rhizome *E. japonicum* involved peak extraction, baseline correction, deconvolution, peak integration, peak alignment, and mass spectral matching using ChromaTOF software (Version 4.3x, LECO)(Dunn et al.2011 and Kind et al.2009). Matching of mass spectral and retention time indices with the compounds in the LECO-Fiehn Rtx5 database was conducted, followed by comparison with databases such as KEGG and HMDB, as well as a self-constructed database by Shanghai Biotree Biomedical Technology Co.

Determination of activity of *E.japonicum* extract Sec-butyl isothiocyanate against J2 *M.enterolobii* and Single-egg hatching

Determination of activity of Sec-butyl isothiocyanate against J2 *M. enterolobii*

Using the impregnation method, a nematode suspension (Around 100 nematodes) was added to each well in a 24-well cell culture plate with 500µL of sec-butyl isothiocyanate solution(purchased from Aladdin Reagent Shanghai Co). The volume was then replenished to 1 ml with sterile water. The sec-butyl isothiocyanate solution concentrations were finalized at 10, 5, 2.5, 1.25, and 0.625 µg/mL after several trials. Sterile water was used as the negative control, while 90% Abamectin original powder served as the pharmaceutical control. Each concentration was repeated three times.The methodology aligns with the description provided in section 1.3, where J2 mortality and corrected mortality are computed based on equations (1.1 and 1.2).

Effect of Sec-butyl isothiocyanate on the hatching of Single-egg of *M. enterolobii*

Each well of a 12-well cell culture plate received a single-egg suspension (Around 100 particles/well), followed by the addition of 500µL of sec-butyl isothiocyanate solution to each well. Sterile water was then used to adjust the volume in each well to 1 mL, achieving final sample concentrations of 10, 5, 2.5, 1.25, or 0.625µg/mL. Sterile water was utilized as a control, and each treatment was replicated three times.The methodology aligns with the procedure outlined in section 1.4. Single-egg hatchability and Single-egg inhibition were determined using equations (1.3 and 1.4), as specified.

Determination of activity of *E.japonicum* extract Geraniol against J2 *M. enterolobii* and Single-egg hatching

Determination of activity of Geraniol against J2 *M. enterolobii*

Using the impregnation method, a nematode suspension (Around 100 nematodes) was added to each well in a 24-well cell culture plate with 500 µL of geraniol solution(purchased from Aladdin Reagent ShanghaiCo). The volume was then replenished to 1 ml with sterile water. The geraniol solution concentrations were finalized at 5, 2.5, 1.25, 0.625 or 0.3125µg/mL, after several trials. Sterile water was used as the negative control, while 90% Abamectin original powder served as the pharmaceutical control. Each concentration was repeated three times.The methodology aligns with the description provided in section 1.3, where J2 mortality and corrected mortality are computed based on equations (1.1 and 1.2).

Effect of Geraniol on the hatching of Single-egg of *M.enterolobii*.

Each well of a 12-well cell culture plate received a single-egg suspension (Around 100 particles/well), followed by the addition of 500 µL of geraniol solution to each well. Sterile water was then used to adjust the volume in each well to 1 mL, achieving final geraniol solution concentrations of 5, 2.5, 1.25, 0.625 or 0.3125µg/mL. Sterile water was utilized as a control, and each treatment was replicated three times.The methodology aligns with the procedure outlined in section 1.4. Single-egg hatchability and Single-egg inhibition were determined using equations (1.3 and 1.4), as specified.

Results

Effects of different Cosolvents on *E.japonicum* extract

In order to select an appropriate co-solvent for *E.japonicum* extract, three reagents were tested. The results indicated that DMSO provided a superior solubilizing effect for the *E.japonicum* extract, as the extract remained clear without precipitation, and the nematodes remained alive at 24 and 48 h post-treatment with DMSO; When acetone was used for extract dissolution, the solution precipitated at 24 h post-treatment, and the nematodes died; Using sterile water for extract dissolution resulted in partial insolubility and precipitation at 24 h post-treatment; hence, DMSO was selected for solubilizing the extract.

Table 2-1 Toxic effects of different Cosolvent against J2 *M.enterolobii*.

Treatment	Precipitation (24h)	Average mortality rate (% at 24 h)	Average mortality rate (%at 48 h)
DMSO	No Precipitate	0.00	0.00
Acetone	Precipitate	5.333±0.578	10.333±1.528
Sterile water	Precipitate	0.00	0.00

Nematicidal effect rhizome of *E.japonicum* extracts

The results of the bioactivity assay indicated that the ethanolic extract obtained from the rhizome of *E.japonicum* displayed significant bioactivity against J2 *M.enterolobii*. This bioactivity was notably higher in comparison to the blank control (sterile water). Additionally, the bioactivity exhibited a tendency to increase with time. The LC₅₀ values were determined to be 44.633mg/mL and 22.840mg/mL after 24 and 48 hours, respectively. Moreover, at a concentration of 200 mg/mL of the *E.japonicum* rhizome extract, the nematode mortality rate reached 88.93% after 48 hours (please refer to Table 2-2 and 2-3 for detailed information on the *E.japonicum* extract).

Table 2-2 Toxicity of different treatments against J2 *M. enterolobii*.

Treatment	Concentration mg/mL	Average mortality rate % at 24 h	Average mortality rate %at 48 h
Rhizome of <i>E.japonicum</i>	200	71.81±2.082a	88.93±2.082a
	100	60.06±1.453b	72.49±1.453b
	50	47.65±1.528c	59.06±2.333c
	25	42.28±1.453d	50.67±2.082d
	12.5	33.89±2.33e	42.28±1.453e
90% Abamectin original powder	0.1	63.33±1.527 b	68.33±1.527b
Sterile water	—	0.00	0.00

Note: Within the same column (a, b, ab), significant differences are denoted ($p<0.05$). The maximum value of the corrected mortality rate is designated as "a". For all other corrected mortality rates, if no

significant difference is observed, they are labeled with the letter "a". Until a significantly different corrected mortality rate is identified, it is marked with the letter "b", and so on.

Table 2-3 Toxicity of rhizome of *E.japonicum* extract on *M.enterolobii*.

Extract	Period of treatment h	Linear equation $y=ax+b$	LC ₅₀ mg/mL	LC ₉₀ mg/mL	Correlation coefficient (R)
Rhizome of <i>E.japonicum</i>	24	$y=0.801x-1.321$	44.633	1776.132	0.937
	48	$y=1.081x-1.468$	22.840	350.561	0.955

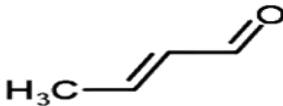
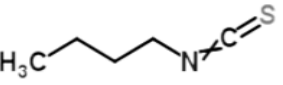
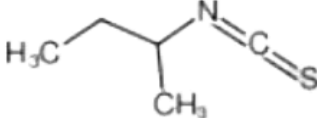
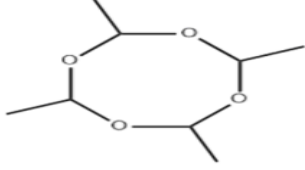
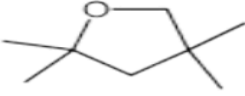
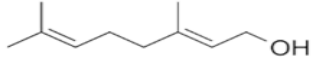
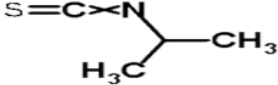
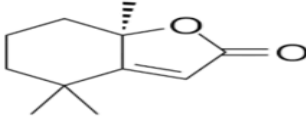
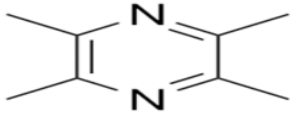
Effects of Rhizome of *E.japonicum* Extract on the Hatching of Nematode Single-egg

The ethanol extract of the rhizome of *E.japonicum* at various concentrations exhibits inhibitory effects on the hatching of nematode single-egg in *M.enterolobii*. As the treatment duration extends and the concentration increases, the inhibition rate of egg hatching also rises. The concentration of 200 mg/mL shows the most significant effect on nematode egg hatching, with the inhibition rate reaching 88.14% after 24 hours of treatment(Fig. 2-1).

Chemical composition analysis of *E.japonicum* extracts

GC-MS -VOCs analysis yielded 1210 peaks. By reviewing the literature and other studies and analysis, 10 organosulfur compounds, lipids, organic heterocyclic compounds and other substances with insecticidal and bacteriostatic effects were initially screened from the extract.

Table 2-4 GC-MS-VOCs analysis of some chemical constituents of the ethanolic extract from the rhizome of *E.japonicum*

No.	Compound Name	Molecular Formula	Biotree Class	Relative content %	Chemical Structure
1	2-Butenal, (E)-	C ₄ H ₆ O	Organooxygen compounds	0.086	
2	Butane, 1-isothiocyanato-	C ₅ H ₉ NS	Isothiocyanates	0.0423	
3	sec-butyl isothiocyanate	C ₅ H ₉ NS	Isothiocyanates	5.629	
4	Acetaldehyde, tetramer	C ₈ H ₁₆ O ₄	Organooxygen compounds	3.184	
5	Furan, tetrahydro-2,2,4,4-tetramethyl-	C ₈ H ₁₆ O	Tetrahydrofurans	0.6638	
6	Geraniol	C ₁₀ H ₁₈ O	Prenol lipids	0.387	
7	Isopropyl isothiocyanate	C ₄ H ₇ NS	Isothiocyanates	1.041	
8	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	C ₁₁ H ₁₆ O ₂	Benzofurans	0.185	
9	2(5H)-Furanone	C ₄ H ₄ O ₂	Dihydrofurans	0.1781	
10	Pyrazine, tetramethyl-	C ₈ H ₁₂ N ₂	Diazines	0.8013	

Results of activity assay of *E.japonicum* extract sec-butyl isothiocyanate against J2 *M. enterolobii* and Single-egg hatching

Activity of sec-butyl isothiocyanate against J2 *M.enterolobii*

The outcomes of the activity assay indicated that the individual component, sec-butyl isothiocyanate, exhibited substantial biological activity against *J2 M. enterolobii*, surpassing that of the negative control sterile water. Additionally, the inhibitory effect showed a tendency to escalate over time. The LC₅₀ was 0.902 and 0.780µg/mL after 24 and 48 h of treatment, respectively. Moreover, the mortality rate of the nematodes reached 100% after 48 hours when exposed to 10µg/mL of sec-butyl isothiocyanate (Table 2-5 and Table 2-6).

Table 2-5 Toxicity of different treatments against J2 *M. enterolobii*.

Treatment	Concentration µg/mL	Average mortality rate (% at 24 h)	Average mortality rate (%at 48 h)
Sec-butyl isothiocyanate	10	92.62±2.5166a	100±0.00a
	5	72.49±2.5166b	82.55±2.5166b
	2.5	65.10±2.5166c	70.13±1.5275c
	1.25	57.38±2.5166d	62.67±1.5275d
	0.625	44.96±2.5166e	51±1.1547e
90% Abamectin original powder	0.1mg/mL	63.33±1.527 c	67.9±1.53c
Sterile water	—	0.00	0.00

Note: Within the same column (a, b, ab), significant differences are denoted (p<0.05). The maximum value of the corrected mortality rate is designated as "a". For all other corrected mortality rates, if no significant difference is observed, they are labeled with the letter "a". Until a significantly different corrected mortality rate is identified, it is marked with the letter "b", and so on.

Table 2-6 Analysis of Toxicity Results of sec-butyl isothiocyanate on *M. enterolobii*.

Extract	Period of treatment h	Linear equation y=ax+b	LC ₅₀ µg/mL	LC ₉₀ µg/mL	Correlation coefficient (R)
sec-butyl isothiocyanate	24	y=1.086x+0.049	0.902	13.642	0.936
	48	y=1.408x+0.152	0.780	6.346	0.949

Effects of sec-butyl isothiocyanate on the Hatching of Nematode Single-egg

The inhibitory effect of various concentrations of sec-butyl isothiocyanate on the hatching of nematode eggs in *M. enterolobii*. With the extension of treatment time and the increase of concentration, the inhibition rate of egg hatching also increases. Specifically, the concentration of 10µg/mL exhibited the most significant impact on nematode egg hatching, with the inhibition rate reaching 90.51% after 24 hours of treatment(Fig. 2-3).

Results of activity assay of *E.japonicum* extract geraniol against J2 *M. enterolobii* and Single-egg hatching

Activity of geraniol against J2 *M. enterolobii*

The outcomes of the activity assay indicated that the individual component, geraniol, exhibited substantial biological activity against J2 *M. enterolobii*, surpassing that of the negative control sterile water. Additionally, the inhibitory effect showed a tendency to escalate over time. The LC₅₀ was 0.547and 0.394µg/mL after 24 and 48 h of treatment, respectively. Moreover, the mortality rate of the nematodes reached 100% after 48 hours when exposed to 5µg/mL of geraniol(Table 2-7 and Table 2-8).

Table 2-7 Toxicity of different treatments against J2 *M. enterolobii*.

Treatment	Concentration µg/mL	Average mortality rate % at 24 h	Average mortality rate % at 48 h
Geraniol	5	85.57±3.05505a	100±0.00a
	2.5	70.13±1.52753b	82.21±2.08167b
	1.25	62.01±2.51661c	70.80±1.73205c
	0.625	50.33±1.52753d	58.05±1.52753e
	0.3125	43.29±1.52753e	50.34±1.15470f
90% Abamectin original powder	0.1mg/mL	63.33±1.527 c	68.33±1.527d
Sterile water	—	0.00	0.00

Note: Within the same column (a, b, ab), significant differences are denoted ($p<0.05$). The maximum value of the corrected mortality rate is designated as "a". For all other corrected mortality rates, if no significant difference is observed, they are labeled with the letter "a". Until a significantly different corrected mortality rate is identified, it is marked with the letter "b", and so on.

Table 2-8 Analysis of Toxicity Results of Geraniol on *M. enterolobii*.

Extract	Period of treatment h	Linear equation $y=ax+b$	LC ₅₀ μg/mL	LC ₉₀ μg/mL	Correlation coefficient (R)
Geraniol	24	$y=0.963x+0.253$	0.547	11.706	0.931
	48	$y=1.418x+0.574$	0.394	3.156	0.942

Effects of Geraniol on the Hatching of Nematode Single-egg

With the extension of treatment time and the increase of concentration, the inhibition rate of egg hatching also increases. The concentration of 5μg/mL has the inhibitory best effect on nematode egg hatching, and the inhibition rate reaches 82.04% at 24 h post-treatment(Fig. 2-4).

Conclusion

Cruciferae including *E.japonicum*, horseradish, kale, and mustard, have both edible and pharmacological values. Currently, the antibacterial activity of *E.japonicum* extracts has been reported, whereas the activity against root-knot nematodes has not been reported. In the present study, we found that the ethanolic extract of the rhizome of *E.japonicum* had good biological activity against J2 and Single-egg hatching of *M.enterolobii*. A total of 1210 peaks were obtained from the ethanolic extract of *E.japonicum* using GC-MS-VOCs technique for chemical compositional analysis. By reviewing the literature and other studies, 10 organosulfur compounds, lipids, organic heterocyclic compounds, and other substances with insecticidal and bacteriostatic effects were initially screened from the extract. Two of the compounds were selected for the determination of nematicidal activity, and it was found that sec-butyl isothiocyanate and geraniol had high inactivating activity against J2 and single egg hatching of *M. enterolobii*.

Discussion

In light of the environmental pollution resulting from the prolonged application of chemical pesticides, which not only disrupts the ecological equilibrium but also poses risks to human health, there is a global push towards the adoption of green pesticides to enhance both the quality of human habitation and food safety. Plant-derived pesticides align with this evolving trajectory, making them a preferred option for green pesticides in many of the latest pesticide formulations(Zhou et al.2013) .In recent years, researchers have identified over a hundred monomer constituents from medicinal plants, such as alkaloids(Kusakabe et al.2022), fatty acids(Zhang et al.2012), alcohols(Kim et al.2021), aldehydes(Caboni et al.2013), terpenoids(Kotsinis et al.2023),fatty acids(Zhang et al.2012), and others.

These components have demonstrated significant nematicidal properties. Isothiocyanate has been documented to exhibit inhibitory activity against J2 and single egg hatching of *Meloidogyne incognita* (Ntalli et al. 2017; Yu et al. 2005 and Zheng 2020). Geraniol, a monoterpene compound (Abdel et al. 2013), demonstrates a notable nematicidal effect against *Caenorhabditis elegans*. Additionally, furazone and its derivatives have been found to possess inhibitory activity against *M. incognita* (Luo et al. 2007 and Wang et al. 2018). In the present study, sec-butyl isothiocyanate and geraniol, which are mono-components present in the extract of *E. japonicum*, exhibited significant inactivating efficacy against *M. enterolobii*. According to research, cruciferous plants do not contain isothiocyanate compounds directly, but through the destruction of cells by cutting or chewing, etc. after the release of glucosidase and hydrolysis of isothiocyanate compounds, which are highly volatile and have a pungent odor (Zhang et al. 2022). Because of the presence of this substance, cruciferous plants have shown a wealth of antibacterial, anticancer, antioxidant and other pharmacological activities. Geraniols are natural monoterpenes that can be isolated from the essential oils of many aromatic plants such as balsam fir, etc. Geraniols have outstanding pharmacological properties, with the best antimicrobial ability of all monoterpene alcohols (Yu et al. 2019), and several studies have shown that geraniols are natural plant secondary metabolites that are easily volatilizable, have no side effects, and exert a wide range of activities mainly through the modulation of protein expression. In addition, geraniols have been approved by the U.S. Food and Drug Administration as a food additive to improve the taste of beverages and candies (Hadian et al. 2018). The results of this study further showed that the ethanol extract of rhizome of *E. japonicum* contained substances with good inhibitory effects on *M. enterolobii*, providing a basis for the development of phytogenic nematicidal substances from *E. japonicum*.

Plant-derived pesticides are usually composed of multiple components, and the mechanism of action is different from that of chemical pesticides, which does not directly kill pathogens or pests, but realizes prevention and control by regulating physiological metabolism in plants and enhancing disease resistance, so it is very difficult to generate drug resistance. Production, There have been some plant-derived pesticides successfully developed in China, such as ethylcin (organosulfur fungicide), developed in 1960., which inhibits the normal metabolism of fungi and bacteria through inhibiting the growth of microorganisms. Ethylcin can effectively control a variety of diseases such as cucumber gray mold, wilt, and rice blight, it can also be used as a new type of soil fumigant for its garlic odor and strong volatility, which has a good effect on the prevention and control of soil-borne pathogens and nematodes (Li 2023). Fumigants are a type of pesticide that utilizes the vapor generated during the volatilization of a substance to poison and kill harmful organisms. Soil fumigants are stable and highly effective, making them particularly suitable for the control of root-knot nematodes, are especially suitable for controlling rhizome-knot nematodes (Wang et al. 2023). This study shows that *E. japonicum* contains a large number of strong volatile substances, therefore, it has the potential to be developed as a fumigant. Furthermore, the extraction process of *E. japonicum* is simple and environmentally friendly, and its development and utilization as a botanical insecticide will bring about significant economic and ecological benefits.

Declarations

Author Contributions:

Huifang Wang and Baibi Zhu conceptualized the study and designed the research proposal; Xiaoli Dou implemented the research process, collected and organized the data, and wrote the paper; Yani Wu, Jiaojiao Lin and Xiaopeng Yin searched for relevant literature and purchased the experimental materials; Jiguang Luo obtained the research funding and designed the framework of the paper; Ying Wei and Zhiwen Li revised the paper. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement:

Not applicable.

Data Availability Statement:

Data from this study are depicted in the figures and tables included in this article.

Conflicts of Interest:

No potential conflicts of interest relevant to this article were disclosed.

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Figures



Figure 1

Fig. 1-1 rhizome of *E.japonicum*

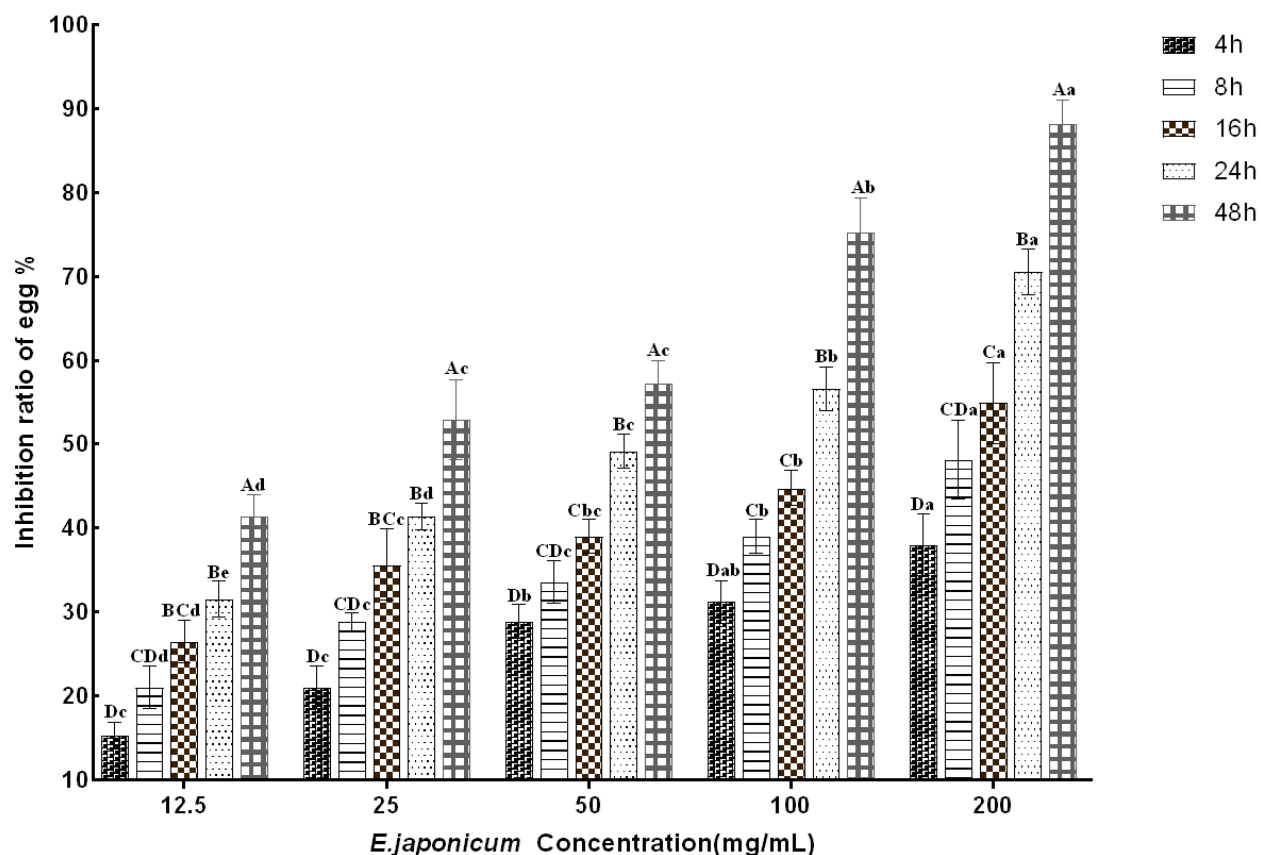


Figure 2

Fig.2-1: Effects of Rhizome of *E.japonicum* Extract on the Hatching of Nematode Single-egg

Note: Mean values labeled with different capital letters between different times at the same concentration indicate significant differences by Tukey's method ($P < 0.05$); mean values labeled with different lowercase letters between different concentrations at the same time indicate significant differences by Tukey's method ($P < 0.05$).

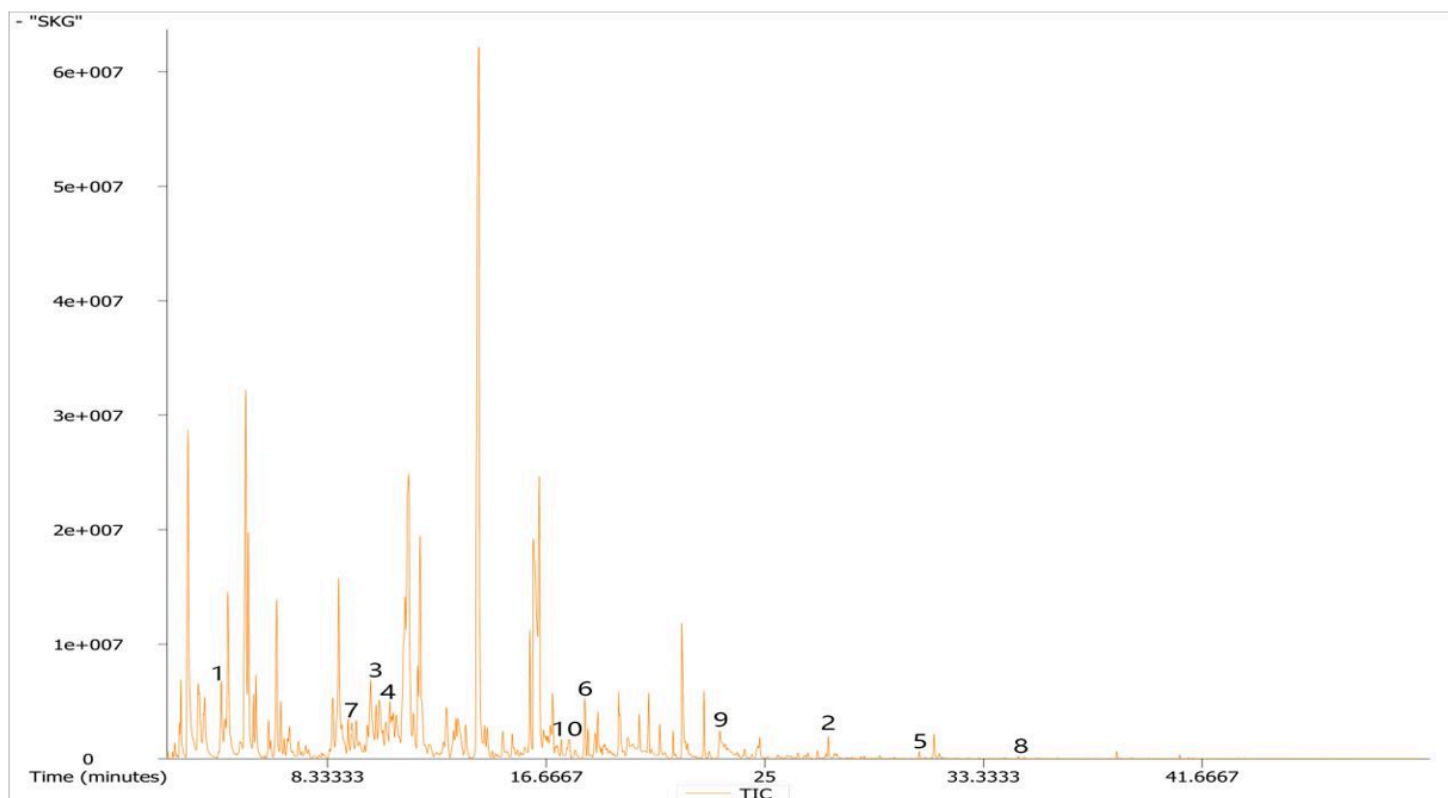


Figure 3

Fig.2-2 Total ion flow diagram of GC-MS-VOCs of the ethanolic extract from rhizome of *E.japonicum*

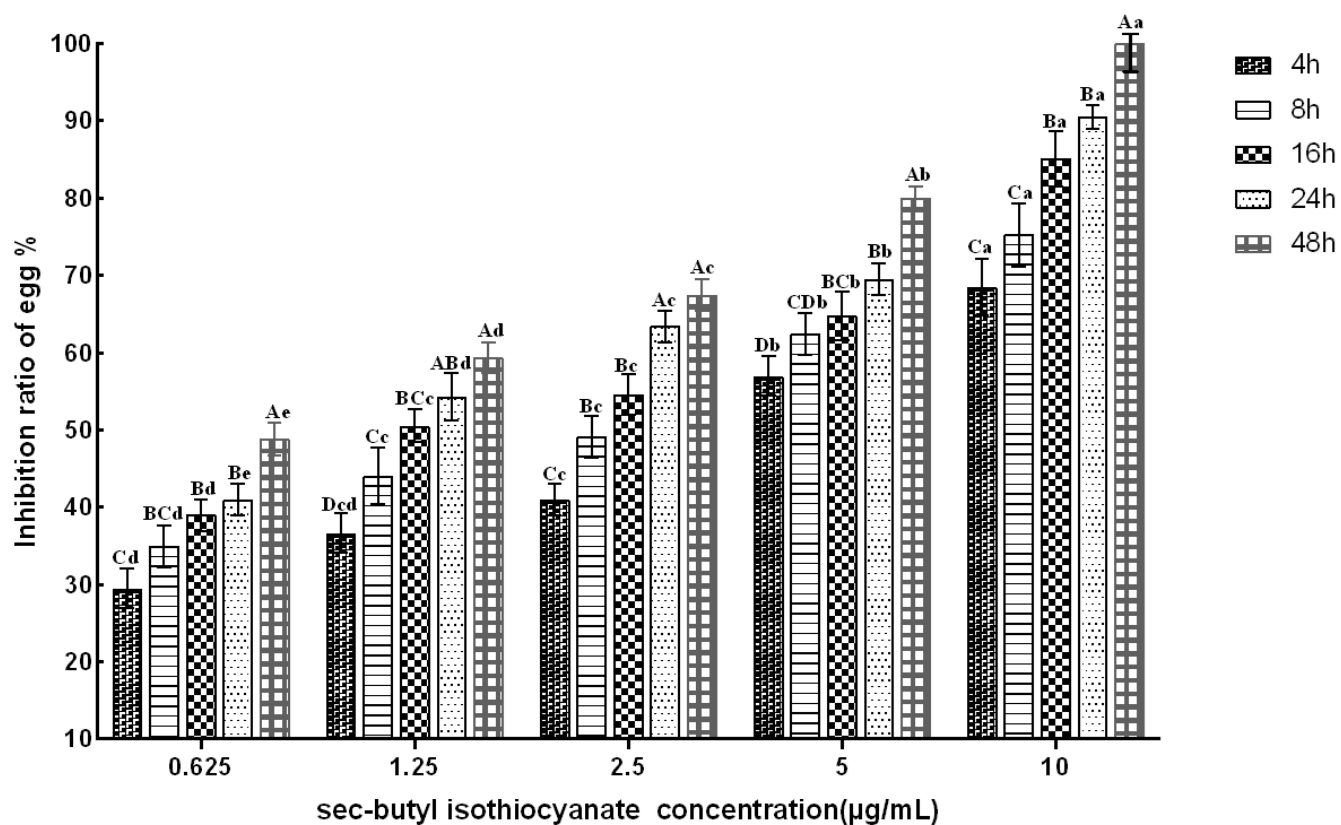


Figure 4

Fig. 2-3: Effects of sec-butyl isothiocyanate on the Hatching of Nematode Single-egg

Note: Mean values labeled with different capital letters between different times at the same concentration indicate significant differences by Tukey's method ($P < 0.05$); mean values labeled with different lowercase letters between different concentrations at the same time indicate significant differences by Tukey's method ($P < 0.05$).

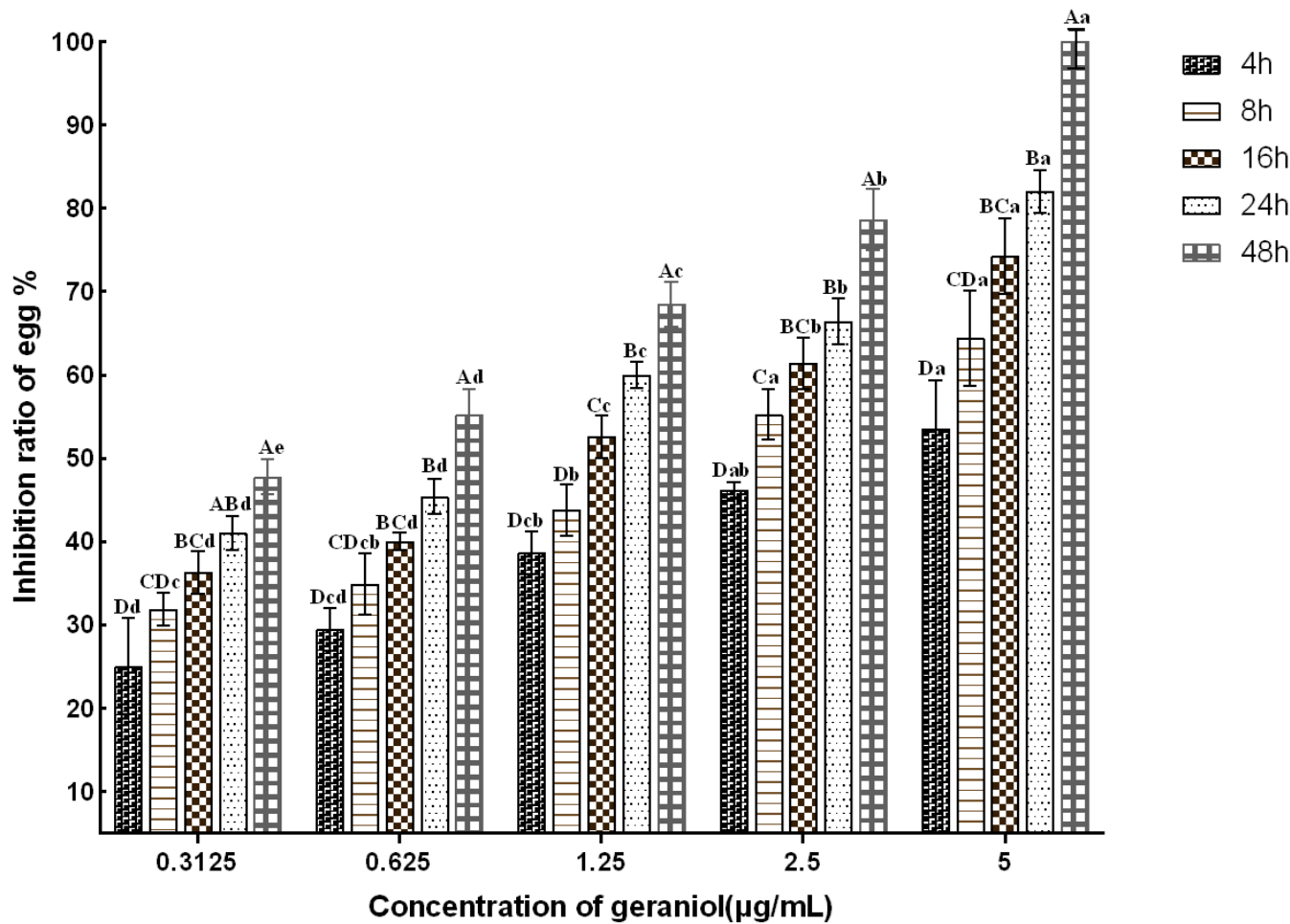


Figure 5

Fig. 2-4: Effects of geraniol on the Hatching of Nematode Single-egg

Note: Mean values labeled with different capital letters between different times at the same concentration indicate significant differences by Tukey's method ($P < 0.05$); mean values labeled with different lowercase letters between different concentrations at the same time indicate significant differences by Tukey's method ($P < 0.05$).