Using root aqueous extract of Asarum sieboldii to control Meloidogyne incognita in greenhouse tomatoes

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Research Article

Keywords: Asarum sieboldii, Meloidogyne incognita, soil nematode communities, greenhouse tomatoes

Posted Date: August 19th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7238657/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Journal of Pest Science on November 26th, 2025. See the published version at https://doi.org/10.1007/s10340-025-01978-z.

Abstract

Root-knot nematode (RKN, especially *Meloidogyne incognita*) is a growing threat to greenhouse tomatoes in China. However, control methods in the greenhouses in China are limited. The use of chemical pesticides such as fluopyram and thiazole phosphate to control *M. incognit*a is expensive and harmful to the environment. The Chinese herb *Asarum sieboldii* is commonly used and it shows potential as a plant-derived pesticide. We evaluated the effectiveness of *A. sieboldii* in regulating the soil nematode community and *M. incognita* in tomato greenhouses. Combining morphological identification with high-throughput sequencing techniques, we demonstrated that *A. sieboldii* root extract significantly reduced the relative abundance of plant-parasitic nematodes (PPN) while promoting the ecological and functional recovery of bacterivorous (BF) and fungivorous (FF) nematodes. The aqueous extract exhibited high toxicity against *M. incognita* J2s and reduced the number of root galls by 69.5% per gram of root, decreased the number of egg masses by 58.4% and enhanced shoot fresh weight by 53.6%. Through column chromatography and HPLC-MS analysis, methyl fumarate (MEF) and methyl gallate (MG) were identified as key active compounds, demonstrating LC50 values of 10.20 mg/L and 49.47mg/L with root-knot suppression rates reaching 64.8% and 61.3% under greenhouse conditions. This study presents the first evidence that *A. sieboldii* aqueous extract effectively controls *M. incognita* without compromising the ecological balance of soil, providing a theoretical foundation for developing environmentally-friendly botanical nematicides.

Introduction

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop in facility cultivation, offering high economic benefits. Continuous cultivation is common in solar greenhouses in Liaoning Province, China. As the area for protected vegetable cultivation expands, the incidence of nematode infections in vegetables has been increasing annually. This trend is primarily driven by the enclosed nature of protected cultivation areas, elevated temperature and humidity levels, and increasing replanting frequency (Li et al. 2024). Alterations in soil nematode populations, especially the accumulation of plant-parasitic nematodes, influence tomato yield and quality (Hamza et al. 2018). PPNs exert a substantial impact on global agricultural production, resulting in over $157 billion in annual economic losses. They are among the most critical pests and diseases affecting agricultural systems (Coyne et al. 2018).

Nematode species are abundant and widely distributed in the soil (Salamún et al. 2011; Mulder et al. 2005). Soil nematodes, key components of the soil biological network, significantly influence the soil environment and above-ground plants (Yeates 1999; van den Hoogen et al. 2019). Based on feeding habits and esophageal morphology, nematodes are classified into four main trophic groups: plant-parasites (PP), bacterivores (BF), fungivores (FF), and omnivores-predators (OP) (Yeates et al. 2003). Their community structure effectively reflects soil environment and health conditions (Gao et al. 2020). The ecological index of soil nematodes reflects changes in the structure and function of soil nematode communities during recovery from disturbances or across different ecosystems (Zhang et al. 2012). It is widely used in practice and serves as a key indicator of soil health (Lu et al. 2020). The abundance,

diversity, and other characteristics of soil nematode communities are closely related to the soil environment. Soil nematodes play a critical role in nutrient cycling, mineralization, and decomposition within the soil (Zasada 2019). The community structure of soil nematodes is influenced by various factors that affect the relative abundance and diversity of nematode trophic groups (Biswal 2022). The structure of soil nematode communities is sensitive to external environmental factors. The diversity of nematodes in ecosystems is commonly assessed using Shannon–Weiner index (*H'*), Simpson index (λ), evenness (J'), and abundance index (GR) (Yeates et al. 1999).

Root-knot nematodes (RKN, *Meloidogyne* spp.) are ranked among the top 10 most economically significant plant-parasitic nematodes (PPNs) globally (Jones et al. 2013). They are obligate endoparasitic nematodes that parasitize over 5,500 species of plants worldwide, including field and vegetable crops, causing significant annual economic losses (Abad et al. 2003; Jones et al. 2013). *M. incognita* is the dominant species in the tomato production region of Liaoning and primarily invades plant root tips through its second-stage juveniles (J2s), parasitizing the root cortex (Goverse and Smant 2014). The nematodes utilize a stylet to extract nutrients essential for their development and reproduction, resulting in an abnormal increase in underground root knots and stunted plant growth (Favery et al. 2016; Hewezi 2020). They ultimately cause significant damage to greenhouse vegetable cultivation, resulting in yield loss and, in severe cases, crop failure (Nguyen 2018). RKNs are difficult to control and often interact with other plant pathogens, forming complex infections (Li et al. 2020). In China, the management of RKNs predominantly relies on chemical methods (Turatto et al. 2018). Currently, RKN control mainly relies on nematicide chemicals such as flupyramide and thiazole phosphate. However, owing to their significant detrimental impacts on the environment and human health, the application of these highly toxic chemical nematicides is subject to stringent regulations in developed countries (Schneider et al. 2003). Given the potential health and environmental risks of synthetic pesticides, along with their nonselectivity and the development of pest resistance, a growing demand for safer, biodegradable pest control alternatives exists. Botanical pesticides present a promising alternative due to their low persistence, high selectivity, and low mammalian toxicity (Shao et al. 2020). In recent years, the development of plant-based pesticides has emerged as a crucial method for controlling PPN. More than 300 plant species spanning over 100 families are recognized for their nematicidal activity (Zhang et al. 2023). Notably, the early extraction of azadirachtin from plant sources facilitated the development of effective botanical insecticides, markedly advancing research in plant-based pesticides (Xuan et al. 2004). Furthermore, the application of ground chili peppers to soil has been shown to effectively suppress Meloidogyne incognita infestations, functioning as a biological fumigant. (Piedra Buena et al. 2007). The nematicidal activity of Mediterranean and humid subtropical plant metabolites against *Meloidogyne* species (Ntalli and Caboni 2012a, b). Rich plant resources in China provide a substantial advantage for researching and developing plant-derived pesticides. The nematicidal activity of 12 common plants was assessed using the Petri dish assay. The results indicated that extracts from all the tested plants could kill the *M. hapla* to varying degrees (Aydinli *et al.* 2010).

A. sieboldii is a perennial herb in the *Aristolochiaceae* family, with its medicinal components consisting of dried roots and rhizomes (Chinese Pharmacopoeia Commission 2020). It is mainly produced

domestically in the three northeastern provinces, particularly in Liaoning. It is produced in large quantities and readily available. People use sun-dried spices in the closet or granary to avoid pests. In recent years, the agricultural value of *A. sieboldii* has gradually increased. *Asarum* has excellent insecticidal properties(Wang et al. 2008; Liu et al. 2019), particularly against *Mythimna separata*, *Plutella xylostella* and *Culex pipiens pallens*(Li et al. 2014). β-asarone has insecticidal activity against *Sitophilus zeamais* (Qiu et al. 2014). *Asarum* essential oil inhibits the activity of amylase and lipase in *Ostrinia furnacalis* and *Mythimna separata*, resulting in their death(Ji et al. 2013). The ethanol and aqueous extracts of *A. sieboldii* inhibit the growth and acid production by *Streptococcus mutans* (Yu et al. 2006).

In this study, we investigated the toxic activity of *A. sieboldii* root aqueous extract against *M. incognita* and its effect in greenhouse on soil nematodes. Key active compounds were identified by HPLC-MS. Finally, the direct-contact nematicidal and pot experiments of the compounds against *M. incognita* were tested. Results of this study indicate that *A. sieboldii* root aqueous extract has a significant nematicidal activity on plant-parasitic nematodes without adversely affecting the soil ecosystem. Furthermore, two new compounds isolated from the aqueous extract exhibit nematicidal activity against *M. incognita*.

Materials and methods

Preparation of different concentrations of A. sieboldii root aqueous extract

A. sieboldii was purchased from a cultivation site in Benxi City, Liaoning Province. The roots of *A. sieboldii* were harvested, air-dried, crushed with a grinder, sieved through a 30-mesh sieve, and stored in sealed bags for future use. To aqueous extracts of *A. sieboldii* roots at five different concentrations (2%, 1%, 0.5%, 0.25%, and 0.125%), the appropriate amount of root powder was weighed and dissolved in 100 mL of sterile water at 50°C. The mixture was shaken at 200 rpm at room temperature (26℃) for 24 h. Subsequently, the extraction was filtered through filter paper (9 cm diameter, 50 μm pore size), and the final volume was adjusted to 100 mL (Rongai et al. 2017).

Soil sampling from tomato roots in the greenhouse

Soil samples were obtained from a greenhouse in the Taihe District, Jinzhou City, Liaoning Province, during the peak population period of the soil nematode community and high incidence of *M. incognita* (June to August summer season). The topsoil was removed and a 2.5cm-diameter soil drill was used to collect soil samples around the tomato roots in the greenhouse, using a five-point sampling method. Each sample covered an area of 4 m² in size, with a minimum distance of 10 m between samplings points. In each sample plot, ten soil samples were collected to a depth of 20 cm, combined into a composite sample, and immediately stored at 4°C. The samples were processed within one week and used for isolating soil nematodes and conducting subsequent pot experiments.

Extraction and identification of soil nematodes

Nematodes were separated isolated using the sucrose-centrifugation method, yielding clear soil nematode suspensions, which were collected in test tubes. These suspensions were then observed and counted using a stereomicroscope. The nematode counts were standardized per 100 g dry soil basis. After counting, the nematodes were euthanized in a 60°C water bath for 15 min, fixed in 4% formalin (TAF), and mounted on temporary slides(Nickle 1991). These slides were examined under a microscope for genus-level identification. Additionally, the identified nematodes were categorized into four trophic groups: bacterivores (BF), fungivores (FF), omnivores-predators (OP), and plant-parasites (PP) (Yeates et al. 1999).

In vitro toxicity of A. sieboldii root aqueous extract on nematodes

A nematode suspension and an equal volume of *A. sieboldii* root aqueous extract were incubated in a 9 cm Petri dish for 48 h in the dark at 24°C in a constant temperature incubator, with sterile water serving as the control group. After incubation, live nematodes were extracted using the Baermann funnel method (Tintori et al. 2022). The Baermann funnel apparatus consists of a funnel (10–15 cm in diameter) mounted on a rack, with a 10 cm section of hose attached to the lower end, and a water-stopping spring clip fitted to the hose opening. Due to hydrotropism and buoyancy, live nematodes swim in the water and eventually settle in the rubber tube at the end of the funnel, while dead nematodes remain in the funnel. The spring clip was then opened, and the liquid containing the live nematodes was collected using a test tube. Nematodes were counted and identified under a microscope (OLYMPUS DP80) according to nutritional groups and trophic classification. This experiment was repeated three times.

Effect of A. sieboldii root aqueous extract on tomato soil nematode communities in pot experiments

The tomato variety L-402 (developed by the Liaoning Academy of Agricultural Sciences) was surface-terilized, planted in pots filled with sterilized sandy loam soil (peat/vermiculite/sand = 2:1:1, vol/vol/vol), and cultivated until reaching the four-leaf stage. A 100 g soil sample from a greenhouse in Taihe District, Jinzhou City was placed in culture pots (9 × 9 × 10 cm), into which the four-leaf stage tomato seedlings were transplanted. One week after transplanting, 10 mL of *A. sieboldii* root aqueous extract was applied to the soil surrounding the root. Each treatment was conducted with six replicates, and the experiment was independently repeated three times. Soil samples were collected at 15 and 45 days after inoculation to isolate nematodes. The extracted nematodes were identified at the family and genus levels and counted using an OLYMPUS DP80 microscope. Several nematode ecological indices were employed to evaluate biodiversity and community structure. The Shannon-Weiner index(*H'*), Pielou uniformity index (*J'*), Wasilewsha index (WI), plant parasite index (PPI), and nematode maturity index (MI) were calculated according to the methods described by Yeates and Bongers (Yeates and Bongers 1999).

Total nematodes DNA extraction

Total nematode DNA was extracted from soil samples collected before inoculation and at 15 and 45 days of treatment with *A. sieboldii* root aqueous extract.The extraction was performed using the DNeasy Blood & Tissue Kit (Qiagen, Germany), and following the manufacturer's protocol. The extracts were

stored at -20°C for subsequent use (Du et al. 2020). The quality and concentration of the extracted genomic DNA were assessed via electrophoresis on a 1% agarose gel.

Gene amplification and Illumina sequencing

The V4 variable region of the 18S rDNA was amplified using the primers NF1F (5'-GGTGGTGACTGGCCGTTCTTAGTT-3') and 18S r2bR (5'-TAACAAGGGACGGGACGTAAT-3'). The total PCR amplification system was 25 μL (Long 2006; Porazinska et al. 2009). A nematode community sequencing library was constructed using PCR products and sequenced on the Illumina MiSeq. Sequencing was performed by Shanghai Majorbio Biotechnology using the Miseq PE300 platform (Illumina, SanDiego, USA), with a sequencing length of approximately 320 bp with an average of 75,000 clean reads per sample, and a minimum of 30,000 clean reads. Operational Taxonomic Unit (OTU) clustering was performed on high-quality sequences at a 97% similarity threshold (Edgar 2013).

1. **M. incognita extraction**

M. incognita was obtained from a pure population maintained on tomato plants in a greenhouse located in the Taihe District of Jinzhou City, Liaoning Province, for 60 days at a temperature of 26 ± 2°C. The nematodes were morphologically identified as *M. incognita*. To collect nematode eggs, egg masses were carefully removed from the infected roots and immersed in 0.5% NaClO for 30 seconds and rinsed five times with distilled water. The second-stage juveniles (J2s) were obtained by incubating the eggs in distilled water at 28°C in a beaker (Hussey and Barker 1973). J2s were collected daily to evaluate nematicidal activity and efficacy in pot experiments

Determination of the nematicidal activity (NA) of A. sieboldii root aqueous extract

To examine the NA of *A. sieboldii* root aqueous extract against *M. incognita*, *in vitro* experiments were conducted in Petri dishes (diameter: 1.5 cm). One milliliter of various concentrations of *A. sieboldii* root aqueous extract and 100 J2s of *M. incognita* were pipetted into each Petri dish. Sterilized water served as the negative control. The Petri dishes were then incubated at 26°C in the dark within an incubator. After 24 and 48 h of incubation, the number of dead juveniles was counted under a stereomicroscope. (Chen and Dickson 2000). Each treatment included 10 replicates, and the experiment was independently repeated three times. The mortality rate (MR) and corrected mortality rate (CMR) were calculated using the Schneider–Orelli formula (Zhang et al. 2016).

Efficacy of A. sieboldii root aqueous extract against M. incognita in pot experiments

Based on the results of the *in vitro* experiment, a 0.5% concentration of *A. sieboldii* root aqueous extract was selected, and its activity against *M. incognita* was further evaluated under greenhouse conditions. Tomato seeds (L402) were sown in pots filled with sterilized sandy loam and upon reaching the four-leaf stage seedlings were then transplanted into pots (approximately 800 cm^3) containing sterilized sandy loam. One week after transplanting, 20 mL of *A. sieboldii* root aqueous extract was inoculated into the soil surrounding the roots. Equal volumes of sterile water and 90 mg/L of Abamectin (Beijing

Zhongnongda Biotechnology Co.) was used as the negative and positive control, respectively. Each pot was inoculated with 1 mL of sterile water containing 1,000 J2s simultaneously. The tomato plants were maintained in a greenhouse at 26°C under a 16 h light and 8 h dark cycle photoperiod for 30 days, with irrigation provided every 2 days. After 30 days, plant physiological parameters were measured, and the number of galls and egg masses was visually assessed and quantified per gram of root. Each treatment included 10 replicates, and the experiment was repeated three times.

Isolation and purification of A. sieboldii root aqueous extract

The aqueous extract obtained from *A. sieboldii* roots was subjected to liquid-liquid partitioning with ethyl acetate. The resulting aqueous phase was collected, lyophilized under reduced pressure, and subsequently stored in a desiccator for downstream processing. Initial fractionation was performed using a HW-40C Toyopearl gel chromatography column with a methanol-water gradient, yielding seven primary fractions (Fr. 1-Fr. 7). Fr. 3 underwent further purification through silica gel column chromatography (200–300 mesh). A trichloromethane-methanol gradient system generated six subfractions (Fr. 3-1-Fr. 3–6). Structural characterization of Fr. 3 − 2 was achieved via high-performance liquid chromatography-mass spectrometry (HPLC-MS) using a ZORBAX SB-Aq column (4.6×250 mm, 5 μm) with acetonitrile-formic acid gradient elution at 0.5 mL/min.

Determination of the nematicidal activity of purified compounds from A. sieboldii root aqueous extract to M. incognita

Direct-contact NAs of the purified compounds identified from *A. sieboldii* root aqueous extract by HPLC-MS were compared with those of 2 commercially available compounds(Aladdin, China), namely Monoethyl fumarate (MEF) and Methyl gallate (MG). MEF and MG were prepared at concentrations ranging from 100 to 1000 mg/L. Subsequently, 1 mL aliquots of MEF and MG were transferred to a Petri dish (diameter: 1.5 cm), and 100 newly hatched J2s were pipetted to each well. MEF and MG were diluted in 50℃ sterilized water and wait for them allowed to return to room temperature. The same amount of sterilized water served as the control. These plates were incubated at 26°C in an incubator in the dark. After 48 h, the number of dead J2s was determined with a stereoscopic microscope (Olympus, Japan). Each treatment included 10 replicates, and the experiment was independently repeated three times.

Efficacy of purified compounds from A. sieboldii root aqueous extract to M. incognita in pot experiments

MEF and MG were selected and their activity against *M. incognita* was verified under greenhouse conditions. Tomato seeds were planted in pots containing sterilised sandy loam. Seedlings at the four-leaf stage were then transplanted into 800 cm^3 pots containing the same soil. Seven days after transplanting, a 10 mL sample of MEF and MG at a concentration of three times the LC50 was prepared and used to soak the plants. Equal volumes of sterile water were used as negative controls and equal volumes of abamectin (90 mg/L) were used as positive controls. Fertilizer was managed under normal

greenhouse conditions. At 30 days after inoculation, the number of galls and egg masses was recorded. Each treatment included 15 replicates, and the experiment was repeated three times.

Data analysis

The experiment was independently repeated three times, and the results represent a combined analysis of these three experiments. All analyses were conducted using Microsoft Excel 2007 and SPSS 25. ANOVA was used to compare the differences in soil nematode community characteristics across different treatments. Means differences were analyzed by Tukey's honest significant difference (HSD) tests ($P < 0.05$). Graphs were generated using GraphPad Prism 8.0 software. The HTS-related graphs in this study were analyzed on the online tool of Majorbio Cloud Platform (https://www.majorbio.com/tools)

Results

Effect of A. sieboldii root aqueous extract in vitro on soil nematode trophic groups under tomato greenhouse

Following isolation and morphological identification, a total of 24 genera were detected, with PP and BF identified as the dominant groups (Table 1). After 48 h of treatment with *A. sieboldii* root aqueous extract, the abundances of PP, BF, FF, and OP were statistically analyzed. The results indicated that *A. sieboldii* root aqueous extract exhibited significant toxicity toward plant nematodes, with the highest mortality rate of 66.73% observed in PPN (Fig. 1).

Table 1
Relative abundance of soil nematode genera under different treatments based on morphological identification and HTS

Nematode genus	Trophic group	c-p value	Relative abundance under each treatment/%					
			CK		AS-15 days		AS-45 days	
			I	II	I	II	I	II
Meloidogyne	PP	3	13.73 a	22.45 a	13.54 a	14.38 b	10.56 b	8.17 c
Helicotylenchus	PP	3	5.62a	5.86a	4.27b	4.90b	3.79c	4.54 b
Hoplolaiminae	PP	3	4.15a	2.82a	3.53b	1.97b	2.23c	**1.53 b**
Pararotylenchus	PP	3	2.25ab	3.91a	1.86b	3.88a	2.50a	3.06ab
Tylenchus	PP	1	4.86a	2.97b	4.48b	1.45b	2.68b	1.44 c
Zeatylenchus	PP	2	—	0.82a	—	0.16b	—	0.13 b
Filenchus	PP	2	3.46a	3.33a	2.97b	1.81b	2.76b	0.86 c
Psilenchus	PP	1	1.98b	1.72a	2.21ab	1.67ab	2.56a	1.53 b
Pratylenchus	PP	3	8.99a	11.17a	8.71ab	9.88b	5.60b	8.38 c
Tylenchorhunchus	PP	2	2.25a	—	1.84b	—	0.85c	—
Rotylenchulus	PP	2	—	0.08a	—	0.08a	—	0.09 a
Aulolaimus	PP	3	—	0.33a	—	0.32b	—	0.38 a
Scutylenchus	PP	3	—	0.16a	—	0.21a	—	0.17 a
Trichodorus	PP	4	—	0.31a	—	0.32a	—	0.19 b
Rhabditis	BF	1	2.25b	3.07a	2.78b	2.38a	5.91a	2.86 b
Protorhabditis	BF	2	1.21ab	1.78b	0.93b	1.91 b	1.45a	2.73 a
Diploseapter	BF	2	3.20b	1.48b	3.71b	2.22a	4.54a	1.87 ab
Panagrolaimus	BF	2	4.15a	2.23b	3.90b	2.22b	3.42b	4.06 a
Cephalobus	BF	2	2.76b	3.38c	2.59b	4.91b	4.27a	5.18 a
Eucephalobus	BF	2	3.89b	3.52a	4.26ab	3.69a	4.35a	3.67 a
Acrobeles	BF	3	9.59b	9.94 a	11.31 a	14.3 b	11.81 a	16.69 a

Note:I and II represent the morphological identification method and high-throughput sequencing method, respectively; "—" indicates that the nematode genus was not found. AS-15 days and AS-45 days:15 and 45 days of treatment with *A.sieboldii* root aqueous extract. Data marked with different letters in the same row indicate significant difference within different treatments at $P < 0.05$.

Nematode genus	Trophic group	c-p value	Relative abundance under each treatment/%					
			CK		AS-15 days		AS-45 days	
			I	II	I	II	I	II
Acrobeloides	BF	3	7.96b	12.33 c	8.15b	15.11b	11.24a	18.666a
Aphelenchoides	FF	2	3.99c	0.27a	4.26b	0.43a	5.25a	0.49 a
Aphelenchus	FF	2	2.95b	1.15b	3.14a	2.23a	3.04ab	1.60 b
Ditylwnchus	FF	2	2.08ab	1.12b	2.22a	1.86b	1.51b	2.72 a
Aporcelaimus	OP	5	1.99c	1.20a	3.70a	0.70b	2.75b	0.44 b
Dorylaimus	OP	4	1.99b	0.62b	3.16a	1.24 a	3.15a	1.24 a
Microdortlaimus	OP	4	1.37a	0.65c	1.67a	3.50b	1.31a	5.43 a
Mesodorylaimus	OP	2	—	0.11b	—	0.43a	—	0.49 a
Discolaimus	OP	2	—	0.08a	—	0.09a	—	0.10 a
Campydora	OP	2	—	0.08a	—	0.90a	—	1.27 a
Iotonchus	OP	5	1.04a	—	0.92ab	—	0.01b	—

Note:I and II represent the morphological identification method and high-throughput sequencing method, respectively; "—" indicates that the nematode genus was not found. AS-15 days and AS-45 days:15 and 45 days of treatment with *A.sieboldii* root aqueous extract. Data marked with different letters in the same row indicate significant difference within different treatments at $P < 0.05$.

Effect of A. sieboldii root aqueous extract in greenhouse on soil nematode communities and nematode ecological indices

Morphological and HTS identification indicated that the dominant trophic groups were PP and BF. HTS of soil nematodes generated 17,997, 10,798, and 65,918 high-quality nematode sequences across three treatments, respectively. Relative nematode abundances were calculated from these sequence data, with taxa below 1% relative abundance excluded (Table 1). In the PP group, the predominant genera were *Meloidogyne* and *Pratylenchus*, whereas *Acrobeles* and *Acrobeleides* were the dominant genera in the BF group. After 15 and 45 days of treatment with *A. sieboldii* root aqueous extract, the relative abundance of PPN decreased significantly, especially *Meloidogyne*, while the abundance of FF and BF nematodes increased significantly. The abundance of OP nematodes remained relatively unchanged. As time progressed, the relative abundance of *Meloidogyne*, *Helicotylenchus*, and *Pratylenchus* significantly decreased compared to the control group, with reductions of 3.14%, 1.63%, and 2.44% (Table 1). The OTU classification revealed that the top 30 genera were most abundant at this sampling site (Figs. 2 and 3). Significant differences in soil nematode communities were observed before and after 45 days.

Morphological and HTS analyses demonstrated that *A. sieboldii* root aqueous extract treatment significantly altered soil nematode diversity and community structure. With prolonged treatment duration, the indices of GR, WI, PPI, and MI exhibited significant changes at 15 and 45 days. W WI and MI increased markedly, whereas GR and PPI decreased significantly ($P < 0.05$). In contrast, *H'* and *J'* were not significantly changed. These changes were further validated by community analysis based on HTS. These changes suggest that *A. sieboldii* root aqueous extract may effectively reduce the relative abundance of PP without adversely affecting free-living nematodes (Table 2).

Table 2
Effect of *A. sieboldii* root aqueous extracts on nematode ecological indices using both morphological identification method and HTS

Treatment		*H'*	*J'*	*λ*	GR	WI	PPI	MI
II	CK	1.19 ± 0.01 a	0.86 ± 0.01 a	0.35 ± 0.02 a	17.86 ± 0.03 a	0.83 ± 0.01 c	2.45 ± 0.03 a	1.53 ± 0.02 b
	AS-15days	1.25 ± 0.01 a	0.90 ± 0.01 a	0.32 ± 0.07 a	15.57 ± 0.04 b	1.09 ± 0.07 b	2.17 ± 0.07 b	1.69 ± 0.04 ab
	AS-45days	1.21 ± 0.01 a	1.00 ± 0.02 a	0.33 ± 0.13 a	14.31 ± 0.20 b	1.82 ± 0.26 a	1.66 ± 0.15 c	1.99 ± 0.09 a
□	CK	0.82 ± 0.11 a	0.79 ± 0.08 a	0.48 ± 0.01 a	3.85 ± 0.13 b	0.75 ± 0.05 c	2.85 ± 0.71 a	1.28 ± 0.04 b
	AS-15days	0.96 ± 0.02 a	0.86 ± 0.02 a	0.43 ± 0.01 a	4.01 ± 0.14 a	0.96 ± 0.06 b	2.38 ± 0.07 b	1.57 ± 0.04 a
	AS-45days	0.96 ± 0.04 a	0.85 ± 0.03 a	0.43 ± 0.02 a	3.79 ± 0.05 b	1.24 ± 0.02 a	2.09 ± 0.06 c	1.74 ± 0.03 a

Note: I and II represent the morphological identification method and HTS.

Different lowercase letters indicate significant difference at the 0.05 level ($P < 0.05$).

Effects of A. sieboldii root extract on the mortality of J2s of M. incognita in vitro

The results indicated that 0.125–2% of *A. sieboldii* root aqueous extract exhibited strong toxicity against J2s of *M. incognita.* The corrected mortality rate (CMR) increased with both prolonged exposure time and higher extract concentrations. After 24 h of exposure to 0.5% *A. sieboldii* root aqueous extract, CMR of *M. incognita* exceeded 90%, while after 48 h, it surpassed 98% (Table 3). Statistical analysis revealed a highly significant effect of 0.5% and 0.25% *A. sieboldii* root aqueous extract on *M. incognita*. Therefore, the 0.5% concentration was selected for subsequent experiments.

Table 3
Effects of *A. sieboldii* root aqueous extract on J2s of *M.incognita*

Concentration (%)	24 h		48 h	
	MR (%)	CMR (%)	MR (%)	CMR (%)
2	95.9 ± 0.74 a	95.84	100 ± 0.00 a	100
1	95.67 ± 0.69 a	93.60	99.80 ± 0.13 a	99.79
0.5	92.20 ± 1.33 ab	92.59	97.80 ± 0.94 ab	98.64
0.25	55.32 ± 1.08 b	55.22	63.80 ± 0.76 b	62.68
0.125	34.70 ± 1.11 c	33.71	41.11 ± 1.5 c	39.28
sterile water	1.50 ± 0.21 d	-	3.00 ± 0.37 d	-

Note: The data are shown as the mean ± SE. The different letters indicate significant differences among the different treatments at 24 h and 48 h ($P < 0.05$). Results represent a joint analysis of three experiments.

Table 4
Effect of *A. sieboldii* root aqueous extract on tomato growth in pot experiment

Treatment	Shoot length(cm)	Root length(cm)	Shoot fresh weight(g)	Root fresh weight(g)
CK	21.10 ± 0.92 b	15.23 ± 2.99 b	2.44 ± 0.08 c	1.13 ± 0.09 a
AS	27.00 ± 0.34 a	19.09 ± 1.80 a	3.69 ± 0.48 a	1.09 ± 0.27 a
Avermectin	23.37 ± 0.85 ab	16.06 ± 0.34 ab	3.22 ± 0.05 b	1.24 ± 0.10 a

Note: The data are shown as the mean ± SE. The different letters indicate significant differences among the different treatments at 24 h and 48 h according to Tukey's HSD test ($P < 0.05$). Results represent a joint analysis of three experiments.

Evaluation of A. sieboldii root aqueous extract on M. incognita in pot experiments

Under greenhouse conditions, the application of *A. sieboldii* root aqueous extract significantly reduced the number of galls and egg masses in tomato roots compared to the negative control ($P < 0.05$). It reduced the number of root galls per gram and egg masses by 69.5% and 58.4%, respectively, compared to those of the negative control (Fig. 4). After treatment with *A. sieboldii* root aqueous extract, shoot length, root length, and shoot fresh weight were significantly higher than in plants treated with sterile water and Avermectin, with increase of by 32.15%, 28%, and 53.6%. *A. sieboldii* root aqueous extract had no significant effect on root fresh weight.

Assessment of the nematicidal activity (NA) of purified compounds from A. sieboldii root aqueous extract against J2s of M. incognita

The active components of the aqueous extract were separated and identified using gel and silica gel column chromatography and HPLC-MS(Fig. 5). We selected eight commercially available compounds for the nematicidal activity test; two of these, monoethyl fumarate (MEF) and methyl gallate (MG), were found to possess high nematicidal activity. The results showed that the LC50 values for components MEF and MG were 10.20 mg/L and 49.47 mg/L (Fig. 6).

Efficacy of purified compounds against M. incognita in pot experiments

Under greenhouse conditions, the number of galls and egg masses in tomato roots decreased significantly after the application of MEF and MG ($P < 0.05$). Specifically, MEF and MG reduced the number of root galls by 64.8% and 61.3%, respectively, compared with the negative control. MF had a similar effect to the positive control (Avermectin). MEF and MG reduced the number of egg masses on tomato roots by 58.3% and 50%, respectively, compared with the negative controls (Fig. 7).

Discussion

Due to the diverse reproductive patterns and strong adaptability of RKNs, it is difficult to control (Li et al. 2015). Therefore, exploring new biological control agents is crucial to ensuring both global food production and environmental safety (Schulz-Bohm et al. 2017). In this study, a novel, cost-effective, and environmentally friendly alternative to traditional chemical nematicide was identified to suppress *M. incognita* in greenhouse tomatoes. We used *in vitro* and pot experiments to evaluate that *A. sieboldii* root aqueous extract has a significant inhibitory effect on PPN without affecting soil ecological health, especially *M. incognita* and isolated the active compounds from *A. sieboldii* root aqueous extract that have potential NA to *M. incognita*.

According to in vitro experiments, *A. sieboldii* root aqueous extract had a significant toxic effect on PPN. The pot experiments analyzed and compared soil nematode communities in greenhouse tomatoes both before and after treatment, utilizing both morphological identification and HTS. Compared with morphological identification methods, HTS has greater advantages in detecting rare genera (relative abundance < 1%) (Du et al. 2020). The results indicated that, compared to the CK, the relative abundance of PPN decreased after 15 and 45 days of treatment with *A. sieboldii* root aqueous extract. In contrast, the relative abundance of BF. These nematodes belong to the opportunistic taxa that are highly resilient, reproduce efficiently, and exhibit resistance to environmental disturbance (DuPont et al. 2009; Walker 1992). Similarly, crushed plant material from the Cruciferae family, valued for its nematicidal properties, is incorporated into the topsoil as a biofumigant, effectively reducing soil nematode populations while enriching the soil for crop cultivation (Aires et al. 2009). Medicago sativa root suppressed root and soil population densities of both nematode species(D'Addabbo et al. 2009).

After 15 and 45 days of treatment, the diversity indices exhibited modifications, with significant increases in WI and MI and significant decreases in GR and PPI. Ecological indices calculated based on morphological and HTS methods showed similar patterns before and after treatment with *A. sieboldii* root aqueous extract. The diversity of nematodes in ecosystems is commonly assessed using H', λ,

evenness, and generic richness to evaluate species diversity in agroecosystems (Yeates et al. 1999). H' quantifies species diversity based on the relative abundance of species, independent of population density. The J' is a direct measure of the numerical uniformity of species within a community. The λ indicates population dominance; a lower λ value corresponds to greater species diversity among soil nematodes (Mulder et al. 2005). MI evaluates changes in soil ecosystem function following disturbance and recovery. A low MI indicates that the nematode community is in the early stages of succession, characterized by high soil disturbance, while a high MI indicates a late stage of succession with lower soil disturbance (Zhang et al. 2012). PPI is a specialized maturity index focused on PPN (Bongers 1990). WI reflects the composition of the soil nematode community and serves as an indicator of soil health (Yeates 2003; Li 2022).These finding imply that *A. sieboldii* root aqueous extract does not negatively impact soil ecology.

The application cost of *A. sieboldii* root aqueous extract is approximately $10–15 USD per hectare per treatment, significantly lower than that of thiazophos, which exceeds $130 USD per hectare. From an ecological perspective, the development and utilization of plant-derived pesticides compatible with human and the environments have become a major research focus in the life sciences in the 21st century (Singh et al. 2002). Plants produce a wide range of biologically active compounds with insecticidal and bacteriostatic properties that protect against pathogenic microorganisms and pests. These compounds can be harnessed to develop plant-derived pesticides that effectively inhibit harmful organisms while remaining safe for beneficial species (Wu 2002; Wang et al. 2018).Several studies have reported the effectiveness of plant-derived pesticides in controlling RKNs. For instance, extracts were prepared from various wild plant species on Okinawa Island, Japan, and demonstrated that the extracts from 11 wild plants exhibited nematicidal activity by eliminating and repelling J2s of *M. incognita* and inhibiting egg hatching (Taba et al. 2008). *Artemisia absinthium* L. had high nematicidal and antifungal activities in ethyl acetate extract of roots(Liu et al. 2019).

Direct contact of *A. sieboldii* root aqueous extract with J2s resulted in an increase in mortality and corrected mortality with increasing time and concentration. In the pot experiment, it not only reduces the number of root knots and egg sacs, but also promotes plant growth. To date, no reports have demonstrated the toxic effect of *A. sieboldii* root aqueous extract on *M. incognita*. Monoethyl fumarate (MEF) and Methyl gallate (MG), which were separated and identified by column chromatography and LC-MS, were found to be nematicidal components of the extract, with LC50 values of 10.20 mg/L and 49.47mg/L against J2s. As a derivative of a similar structure, Dimethyl fumarate (DMF), a structurally similar compound, is a known broad-spectrum antimicrobial agent with significant inhibitory effects on bacteria, yeast and molds. Methyl alginate fumarate (TMF) inhibits bacteria such as Escherichia coli, Bacillus subtilis, Bacillus thuringiensis and yeast (Zhang et al. 2010). This two compounds' nematicidal mechanisms will be the focus of further research in the future.

In conclusion, this study found that applying *A. sieboldii* root aqueous extract to the soil in a greenhouse containing tomatoes can significantly reduce the number of PPN, particularly *M. incognita*, without harming the soil ecological health. The aqueous extract and its compounds, namely MEF and MG, exhibit

targeted efficacy against *M. incognita*. The abundance of bioactive compounds found in *A. sieboldii*, combined with advances in extraction, purification and identification techniques, offers a variety of possibilities for nematode management.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Funding

This study was supported by the National Key R&D Program of China (2023YFD1400400), National Parasitic Resources Center (NPRC-2019-194-30) and China Agriculture Research System of MOF and MARA(CARS-04-PS13).

Author Contribution

All authors contributed to the study conception and design. Material preparation and data collection were performed by ZZ, , Y W, NY, HF, LC and YD. Analyses were performed by ZZ, XZ and XL. The first draft of the manuscript was written by ZZ, AS and YD. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors contributed to the study conception.

Data Availability

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

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Figures

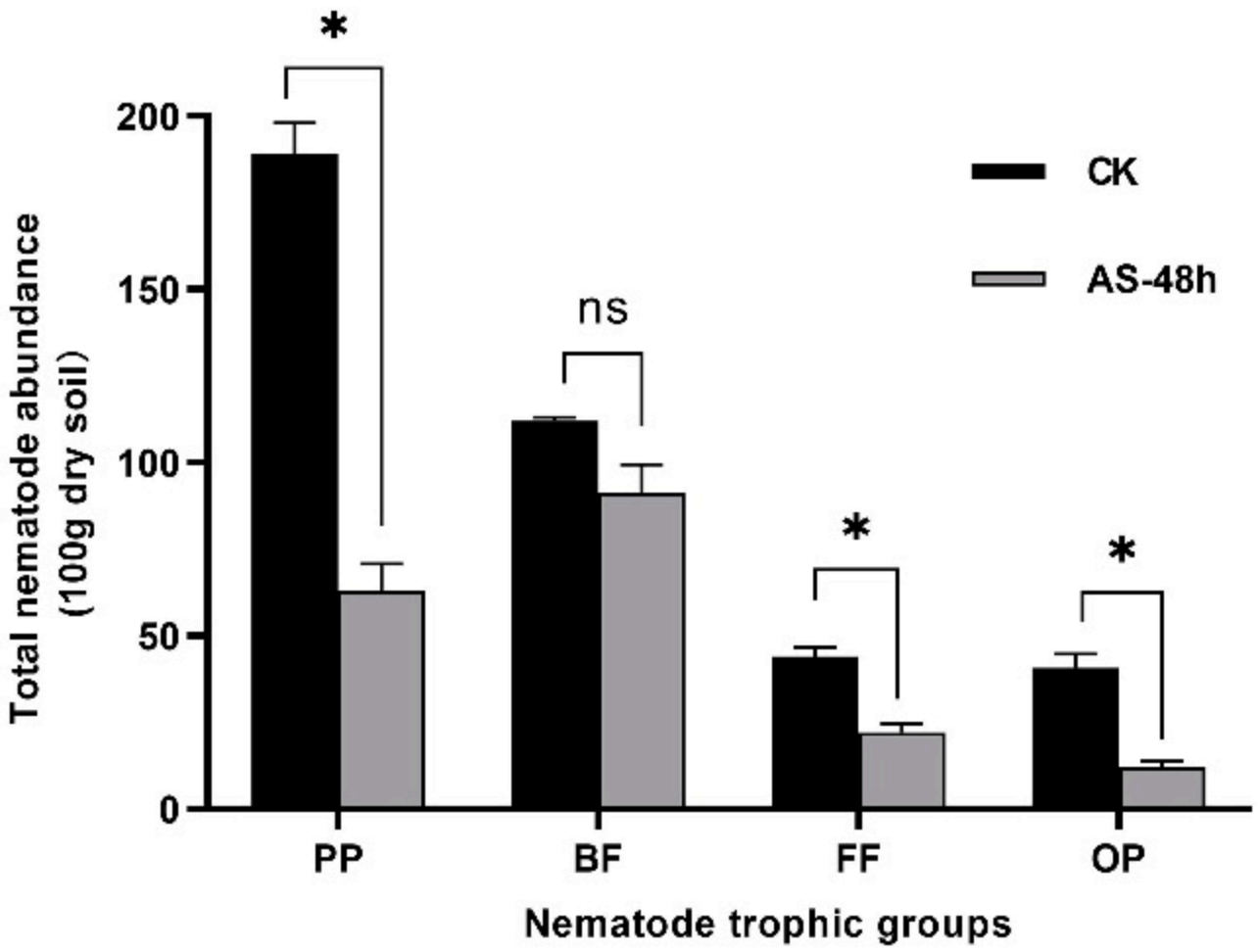

Figure 1

Effects of *A. sieboldii* root aqueous extract treatment on soil nematode trophic groups. The data are shown as the mean ± SE and indicate significant differences among the different treatments at 15 and 45days according to Tukey's HSD test ($P < 0.05$).

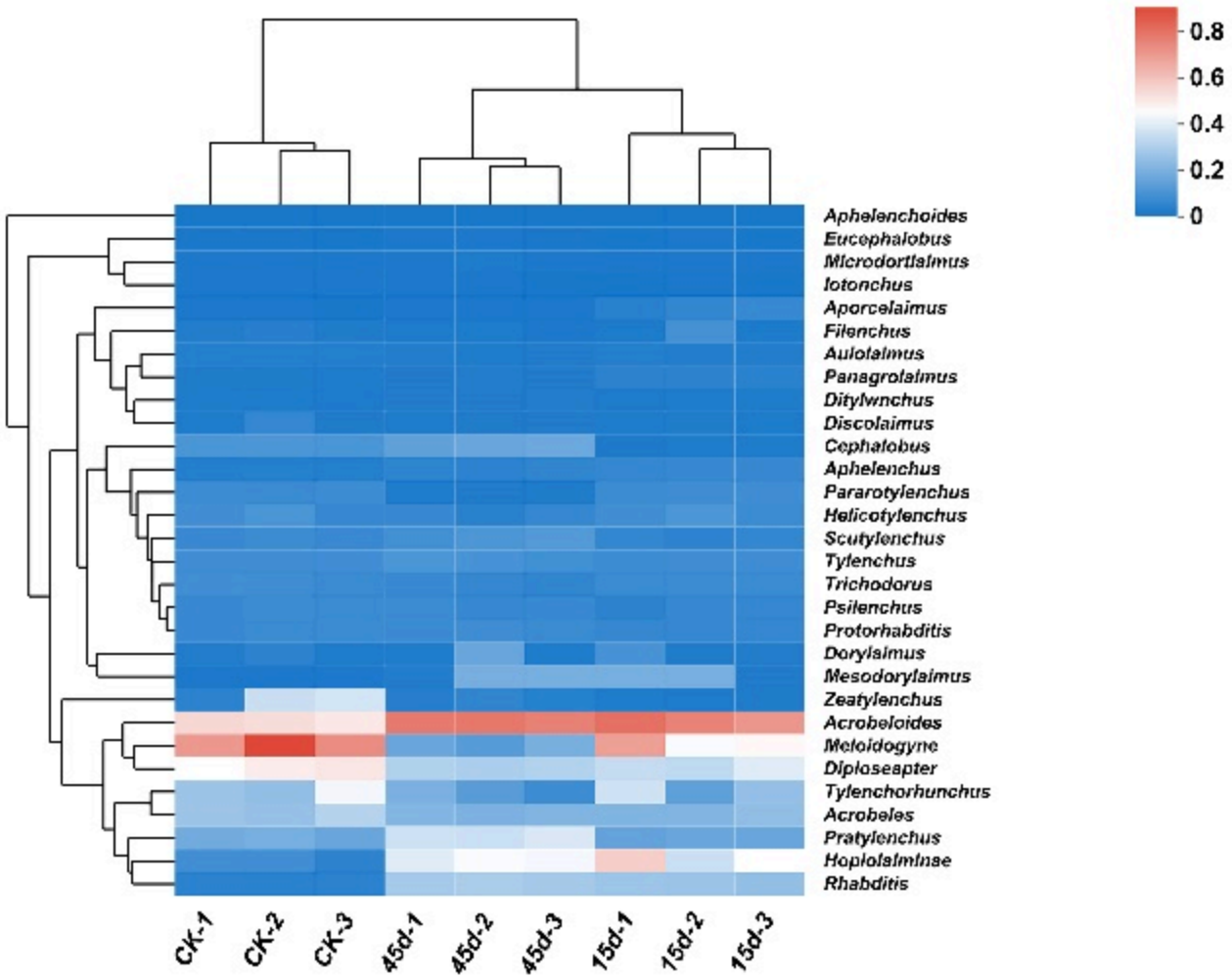

Figure 2

Heatmap of the top 30 genera of soil nematodes under different treatments using HTS.

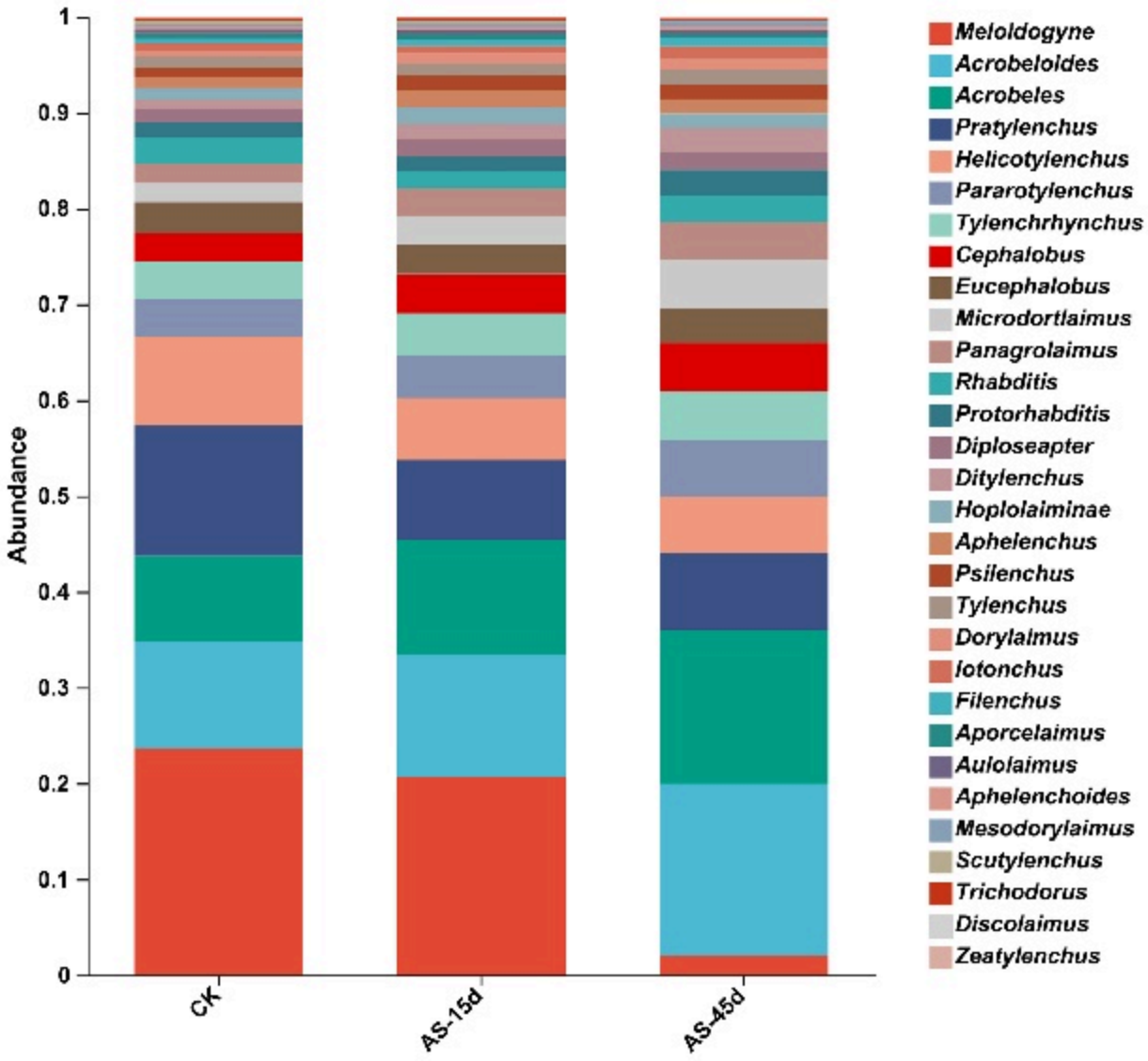

Figure 3

Relative abundance of nematodes at the genus level under different treatments using HTS

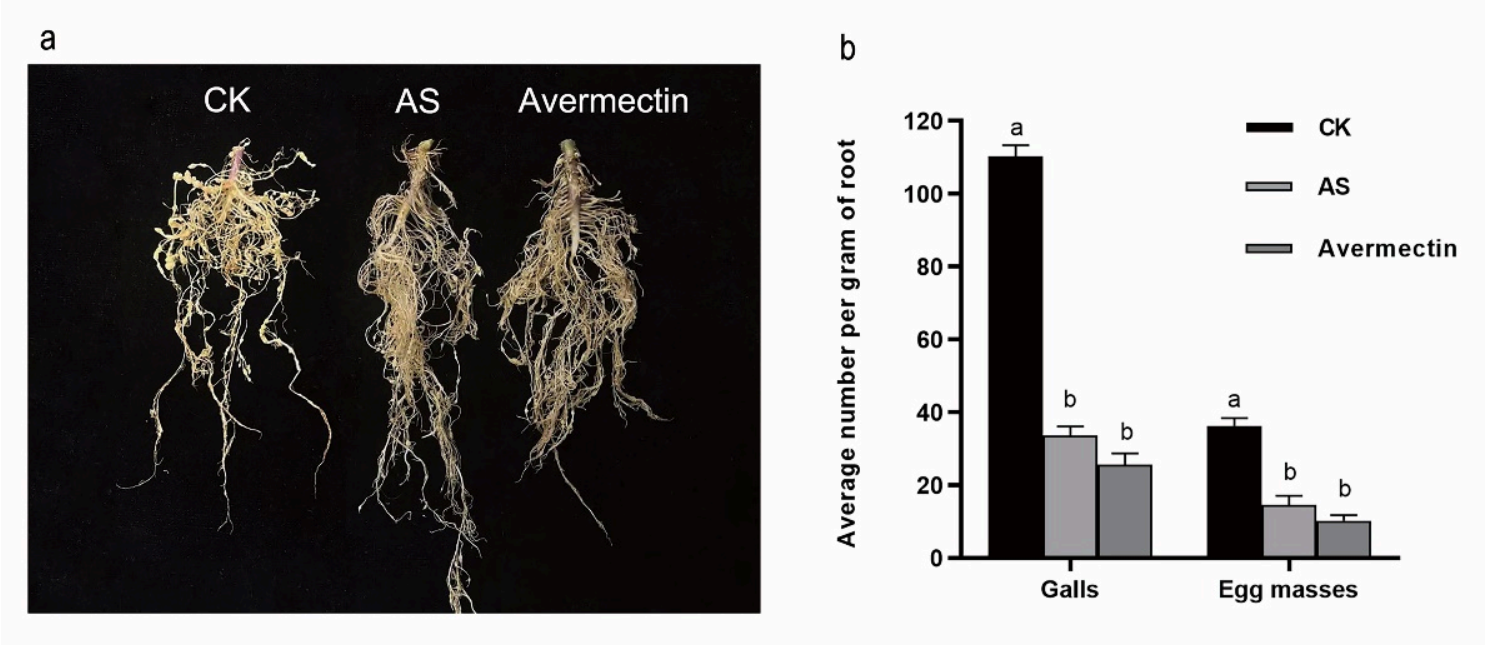

Figure 4

Efficacy of *A. sieboldii* root aqueous extract against *M. incognita* in pot experiments. Symptoms of tomato roots treated with water, *A. sieboldii* root aqueous extract, and Avermectin (**a**) and Number of root galls and egg masses per root system (**b**). Data are expressed as mean ± SE. The different letters indicate significant differences among the different treatments at 30 days according to Tukey's HSD test ($P < 0.05$). Results represent a joint analysis of three experiments.

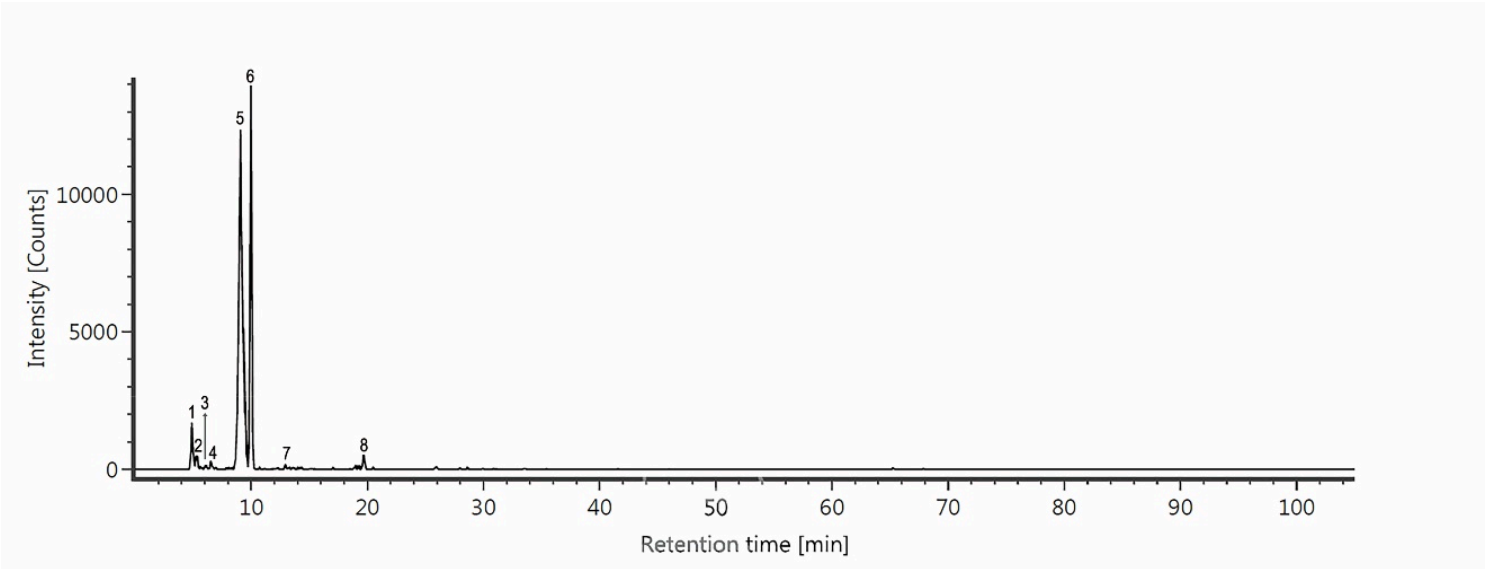

Figure 5

Total ion chromatogram (TIC) of Fr. 3-2 from *A. sieboldii* root aqueous extract by HPLC-MS.

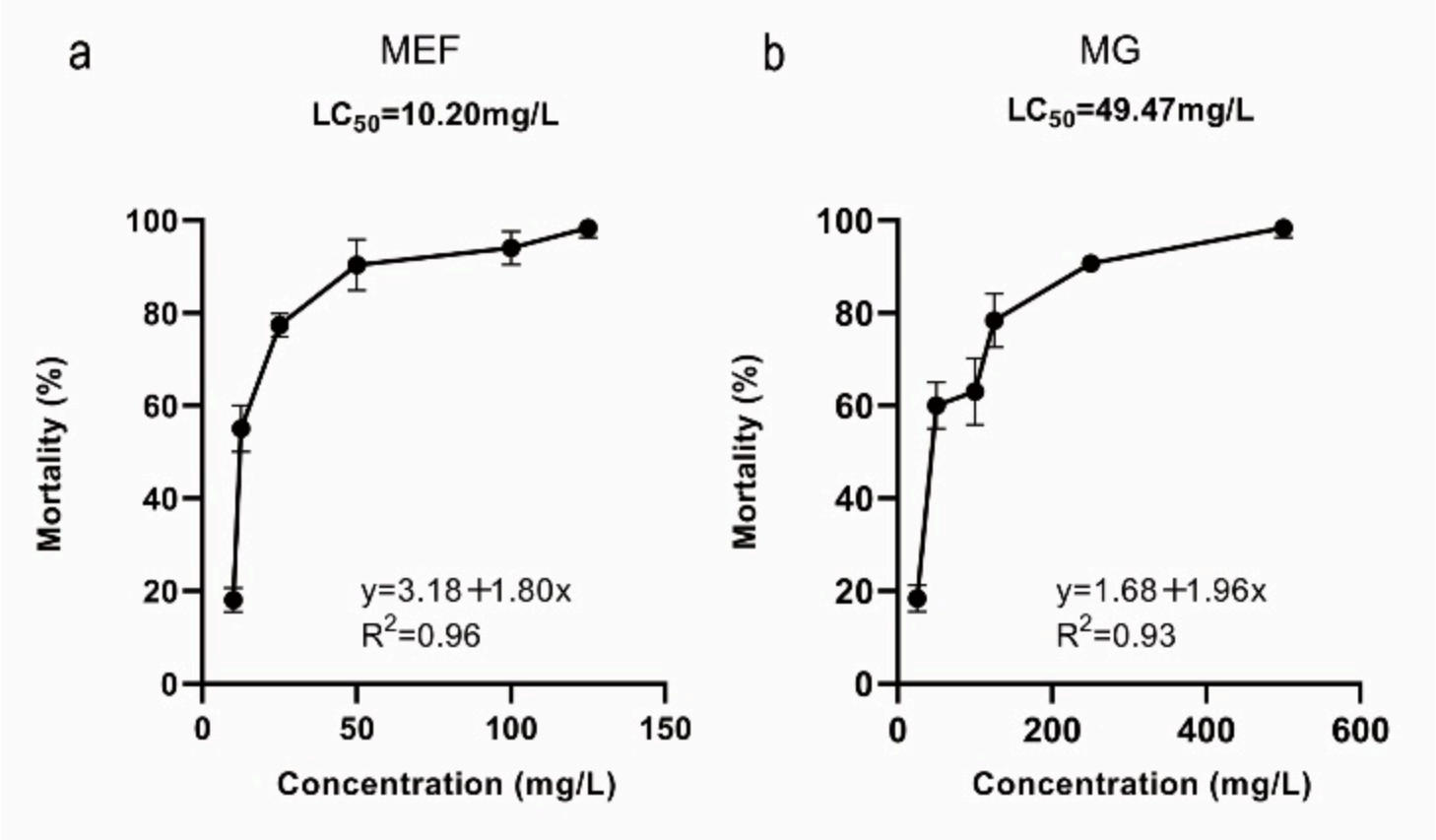

Figure 6

Regression curves of direct-contact activity against J2s of *Meloidogyne incognita* for MEF (**a**) and MG (**b**). The data are shown as the mean ± SE. Results represent a joint analysis of three experiments.

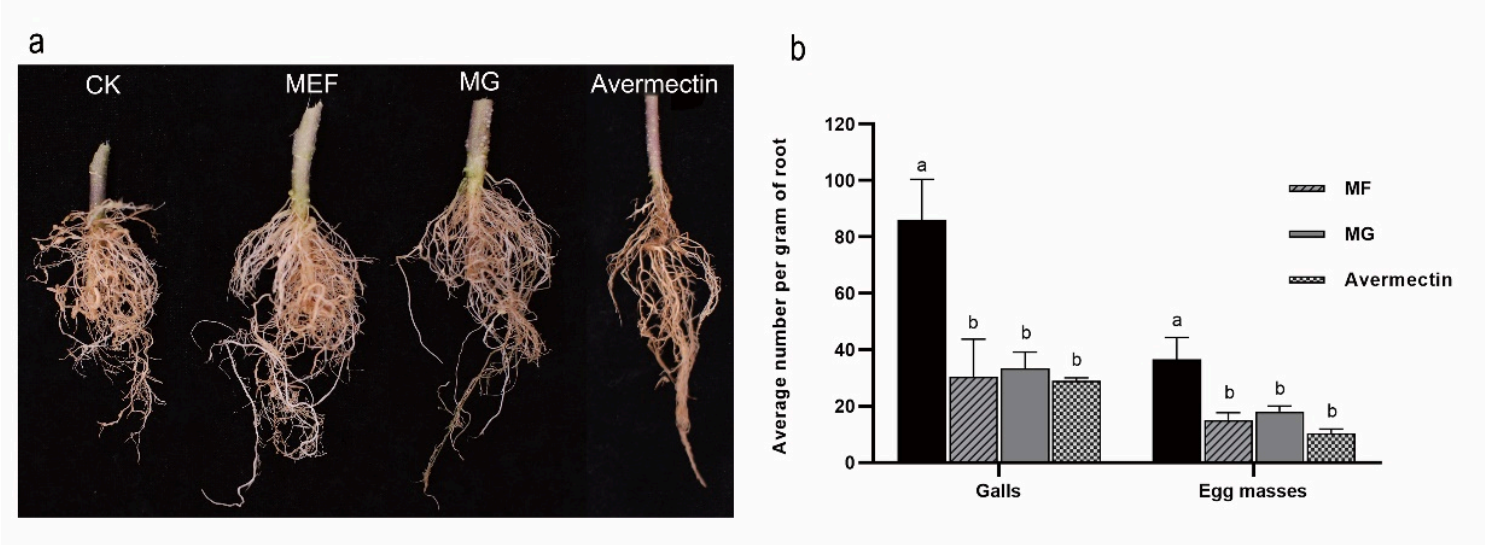

Figure 7

Efficacy of pure compounds against *M. incognita*in pot experiments. The data are shown as the mean ± SE. Symptoms of tomato roots treated with water, MEF, MG and Avermectin (**a**) and Number of root galls

and egg masses per root system (**b**). The different letters indicate significant differences among the different treatments at 30 days according to Tukey's HSD test ($P < 0.05$). Results represent a joint analysis of three experiments.