Effect of Earthworms on the Population Quantity of *Meloidogyne incognita* in Tomato Cultivation

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Abstract [**Objectives**] The paper was to further investigate the correlation between the presence of earthworms and the occurrence of *Meloidogyne incognita*. [**Methods**] The effect of earthworms on the population quantity of *M. incognita* and the growth of tomato plants was assessed through a potting experiment utilizing *Eisenia fetida* and *M. incognita* as the primary subjects of investigation. [**Results**] *E. fetida* exhibited an inhibitory effect on both the reproductive capacity and the prevalence of *M. incognita*. Additionally, it mitigated the adverse effects of *M. incognita* on the growth and development of tomato plants. [**Conclusions**] The findings of this study will establish a theoretical framework for utilizing earthworms and their metabolites to modulate the rhizosphere microecological environment, thereby facilitating the management of *M. incognita*.

Key words *Meloidogyne incognita*; Earthworm; Tomato; Population quantity

1 Introduction

In recent years, the area dedicated to the cultivation of facility vegetables has been increasing. However, the population of earthworms in the soil has been declining due to inappropriate fertilization practices and continuous cropping. Concurrently, the damage caused by root-knot nematodes has been exacerbating annually, posing a significant threat to the green, healthy, and sustainable development of agriculture. Research on effective strategies for the prevention and control of root-knot nematodes is of considerable importance for enhancing crop production and quality. Root-knot nematodes represent one of the most significant threats to plant health in global agricultural systems[1]. These nematodes possess a broad host range, capable of infesting over 3 000 plant species, including a majority of cultivated crops. Consequently, they can lead to yield reductions of approximately 10% to 20% on average, with potential losses exceeding 75% in severe infestations[2]. Over 90% of diseases associated with root-knot nematodes have been attributed to four specific species: *Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood, *M. hapla* Chitwood, and *M. javanica* (Treub) Chitwood[3]. Among these, *M. incognita* is responsible for the most significant damage. *M. incognita* is predominantly located in the soil at depths ranging from 5 to 30 cm[4]. This nematode compromises the structural integrity of the plant root system, depletes essential nutrients, and disrupts the normal metabolic processes of the plant by invading wounds and inducing the formation of gnarled root knots. Additionally, *M. incognita* can facilitate the co-infestation of other pathogens, leading to the weakening or potential mortality of the host plant, ultimately resulting in diminished crop yields or, in severe cases, the extinction of the crop[5].

Currently, the biological control of *M. incognita* primarily encompasses the screening of nematode-resistant varieties, the selection of biocontrol strains, and the identification of nematode-resistant genes, *etc.* Research has demonstrated that earthworms exert an inhibitory effect on the growth and reproduction of *M. incognita*[6]. If the mutualistic relationship between earthworms and *M. incognita* populations can be harnessed for biological control, it would present a more environmentally friendly, safer, and non-toxic alternative. Furthermore, this approach has the potential to enhance soil quality and increase biodiversity, thereby offering significant prospects for development[7].

The earthworm is classified within the class Oligochaeta and the order Opisthopora. Globally, there are approximately 4 000 species of terrestrial earthworms, categorized into 12 families and 181 genera. In China, a total of 306 species, 28 genera, and 9 families of earthworms have been documented[8]. Earthworms play a significant role in material cycling and energy transfer within soil ecosystems through various activities, including feeding, digestion, excretion (in the form of vermicompost), secretion (such as mucus), and burrowing[9]. The roles of earthworms within the ecosystem are primarily evident in the following areas: (i) their impact on essential processes such as the decomposition of soil organic matter and nutrient cycling; (ii) their effects on the physicochemical properties of soil; and (iii) their interactions with plants, microorganisms, and other animal species[10]. Earthworms, as significant macrofauna within terrestrial ecosystems, maintain a close symbiotic relationship with microorganisms and

Received: October 12, 2024 Accepted: November 18, 2024
Supported by Key R&D Project of Science & Technology Department of Ningxia Hui Autonomous Region (2021BBF02013); Post-doctoral Program of Hebei Province (2019003011); Hebei Province Innovation Ability Enhancement Plan Project (225676109H).
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other soil-dwelling organisms. Through their feeding activities and the secretion of specific chemical substances, earthworms can modulate the population quantity, activity levels, and functional roles of various organisms within the soil microcosm[11]. Raty *et al.* [12] demonstrated that earthworms significantly decrease nematode biomass in the soil. This reduction occurs primarily through direct predation on nematodes present in the soil and litter, as well as indirectly by modifying the nematode community structure via the deposition of vermicompost. Brown *et al.* [13] demonstrated that earthworms influenced nematode populations and community structure through both direct and indirect mechanisms. In laboratory microcell experiments utilizing forest soils, it was observed that the inoculation of earthworms led to a significant reduction in soil nematode quantity, as well as a notable alteration in their community structure.

The existing body of research concerning the interaction between earthworms and root-knot nematodes predominantly examines vermicompost, its fermentation broths, and the secretions generated by earthworms. These factors are investigated for their potential to mitigate nematode quantity in the soil by affecting the reproductive capacity of root-knot nematodes. Earthworms, recognized as significant macrofauna within terrestrial ecosystems, are often referred to as "ecosystem engineers". Earthworms maintain a significant symbiotic relationship with microorganisms and other soil organisms. They can affect the population quantity, activity, and functional roles of soil microorganisms through direct feeding behaviors and the secretion of their own biochemical compounds. In the current study, the earthworm *Eisenia fetida* and *M. incognita* were utilized as experimental subjects to investigate the interactions between their population quantities.

2 Materials and methods

2.1 Preparation of suspensions of eggs and second instar larvae of *M. incognita*

2.1.1 Preparation of egg suspension. *M. incognita* was cultured from tomato plants in a laboratory setting. Roots exhibiting a higher number of egg sacs were selected, subsequently cut into small sections measuring 0.5 to 1.0 cm. These sections were placed into a preservation box, to which an appropriate volume of 1% sodium hypochlorite (NaClO) solution was added. The box was then sealed and subjected to vigorous shaking for a duration of 5 min. The dregs and solution obtained after oscillation were poured into a combined mesh sieve with upper and lower sections of 280 – 500 mesh. This mixture was rinsed multiple times with a gentle stream of water. Subsequently, nematode eggs were collected on the 500 mesh sieve. Using a microscope, the number of eggs per 100 μL of the egg solution was quantified, and the solution was adjusted to achieve a suspension concentration of 2 000 eggs/mL[14].

2.1.2 Preparation of second instar larval suspension. The prepared oocysts were rinsed with distilled water and subsequently transferred into a small beaker containing sterile water. Following this, the oocysts were incubated in a thermostat maintained at 25 ℃ for a duration of 4 – 7 d. Newly hatched second instar larvae were collected at 24 h intervals. A microscope was employed to quantify the number of second instar larvae per 100 μL of the suspension, and a second instar larval suspension with a concentration of 2 000 larvae/mL was prepared.

2.2 *M. incognita* and earthworm inoculation After a 30 d cultivation period of tomato seedlings in a solar greenhouse, six experimental treatments were established: the blank control group consisting of normal tomato seedlings, the earthworms group with 5 earthworms per pot, the nematode second instar larvae group containing 5 000 second instar larvae per pot, the earthworms and nematode second instar larvae group comprising 5 000 second instar larvae and 5 earthworms per pot, the nematode eggs experimental group with 12 000 eggs per pot, and the earthworms and nematode eggs experimental group consisting of 12 000 eggs and 5 earthworms per pot.

2.3 Determination of growth and development of tomato The growth and development of tomato seedlings were assessed through the measurement of plant height, chlorophyll content, nitrogen content, and fresh weight. Plant height was measured using a tape measure, from the base of the plant stem to the apical point. Chlorophyll content was quantified with a chlorophyll meter, which was also utilized to assess nitrogen content. Fresh samples of the plants were weighed using an electronic balance to determine their fresh weight.

2.4 Determination of the infestation status of *M. incognita* The root system of the tomato plants was thoroughly rinsed with clean water, and the number of root knots present in each root system was subsequently counted. The root knot index was calculated using the following formula: Root knot percentage (%) = (Number of root knots / Fresh weight of roots). Disease index = Σ(Number of plants at each disease level × Representative value of the corresponding level)/(Total number of surveyed plants × Representative value of the highest level) × 100. The grading criteria for the disease index were established as follows: grade 0 signifies the absence of root knots throughout the entire root system; grade 1 denotes a slight infection of the root system, characterized by a percentage of root knots less than 10%; grade 2 indicates the presence of noticeable root knots, with the percentage of root knots ranging from 11% to 25%; grade 3 reflects a percentage of root knots between 26% and 50%; grade 4 corresponds to a percentage of root knots ranging from 51% to 75%; and grade 5 represents a percentage of root knots from 76% to 100%. The reproduction coefficient was determined using the formula $Rf = Pf/Pi$, where Pf denotes the total number of eggs and nematodes at the time of harvest, and Pi indicates the number of second instar larvae or eggs at the time of initial inoculation.

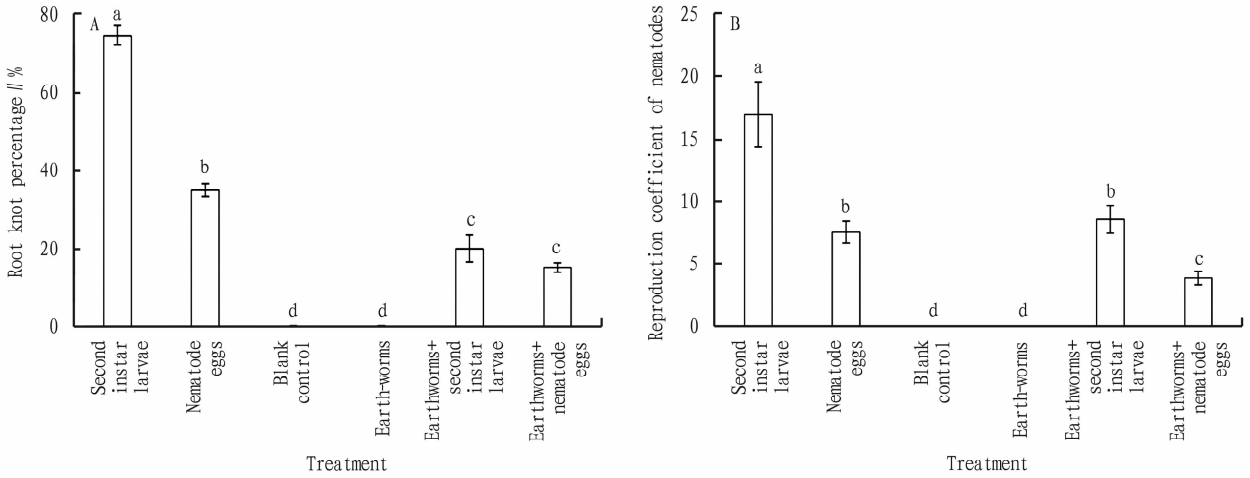
2.5 Data processing The SPSS software was employed to initially evaluate potential differences among subgroups regarding the changes in the aforementioned indicators through one-way ANOVA

analysis. Subsequently, the *LSD* method was utilized for conducting multiple comparisons between the groups.

3 Results and analysis

3.1 Effect of earthworms on *M. incognita* damage Following the introduction of *M. incognita* and earthworms into pots, the results comparing the differences in root knot percentage among various subgroups are presented in Fig. 1A. The findings indicated that all experimental groups exhibited a statistically significant increase in root knot percentage when compared to the control group ($P < 0.05$). There was no statistically significant difference in root knot percentage between the group comprising earthworms and nematode second instar larvae and the group consisting of earthworms and nematode eggs ($P = 0.295 > 0.05$). In contrast, the other groups exhibited statistically significant differences in root knot percentages when compared to one another ($P < 0.05$).

The variation in reproduction coefficients among the different subgroups is illustrated in Fig. 1B. The nematode reproduction coefficient for the second instar larvae group was recorded at 16.934, while the coefficient for the nematode eggs group was 7.525. Additionally, the earthworms and second instar larvae group exhibited a coefficient of 8.549, and the earthworms and nematode eggs group demonstrated a coefficient of 3.817. The results were generally consistent with the root knot percentage findings, indicating that the reproduction coefficients were significantly elevated in all experimental groups when compared to the control group ($P < 0.05$). In contrast to the results concerning root knot percentages, no statistically significant difference was observed in the reproduction coefficients between the nematode eggs group and the experimental group comprising earthworms and second instar larvae ($P = 0.161 > 0.05$). However, the reproduction coefficients of the earthworms and second instar larvae group were significantly higher than those of the earthworms and nematode eggs group ($P < 0.05$). A comparative analysis of the population quantity of root-knot nematodes in the experimental group containing earthworms and second instar larvae, as opposed to the experimental group comprising solely second instar larvae of nematodes, revealed that the population of *M. incognita* in the experimental group containing earthworms and second instar larvae was significantly lower than that observed in the nematode second instar larvae group. A comparison of the population quantity of root-knot nematodes in the experimental group containing earthworms and nematode eggs with that in the experimental group consisting solely of nematode eggs revealed that the population density of root-knot nematodes in the former group was significantly lower than in the latter group. This finding suggests that earthworms exert an inhibitory effect on the damage caused by *M. incognita*.



NOTE Different lowercase letters indicate statistically significant differences at the 0.05 level. The same below.

Fig. 1 Effect of earthworms on *Meloidogyne incognita* damage

3.2 Effect of earthworms and *M. incognita* on the growth of tomato The current experiment aimed to investigate the variation of specific indicators across various treatment groups, which included a blank control, earthworms, second instar larvae, nematode eggs, a combination of earthworms and second instar larvae, and a combination of earthworms and nematode eggs. The indicators evaluated in this study comprised growth rate, chlorophyll content, nitrogen content, root knot percentage, and reproduction coefficient. The results pertaining to the variations in the growth rates of plant height among different subgroups are illustrated in Fig. 2A. The heights of tomato seedlings in the blank control group, the second instar larvae group, the nematode eggs group, the earthworms and second instar larvae group, the earthworms and nematode eggs group, and the earthworms group were measured at 290, 237.5, 258.8, 246.3, 189.2, and 275 mm, respectively. It is evident that the growth rates of tomato seedlings in the experimental groups exhibited significant differences when compared to the control group ($P < 0.05$). However, no significant differences were observed in the growth rates of tomato seedlings among the various experimental groups ($P > 0.05$). The growth and development of tomato seedlings inoculated with second instar larvae or nematode eggs were inferior to those observed in the con-

trol group. Conversely, the growth and development of tomato seedlings in both the earthworms group and the combined earthworms and nematode eggs group were superior to those in the earthworms and second instar larvae group, yet still demonstrated poorer growth compared to the control group.

The analysis of chlorophyll variations among the different groups is presented in Fig. 2B. The results indicated a significant decrease in chlorophyll levels in the various types of larvae when compared to the control group ($P < 0.05$). The chlorophyll content in the second instar larvae group was significantly lower than that observed in the nematode eggs group, the earthworms and second instar larvae group, and the earthworms and nematode eggs group. The chlorophyll content in the nematode eggs group was significantly lower than that observed in the earthworms and nematode eggs group. In summary, the chlorophyll content in all groups containing second instar larvae was lower than that in the control group.

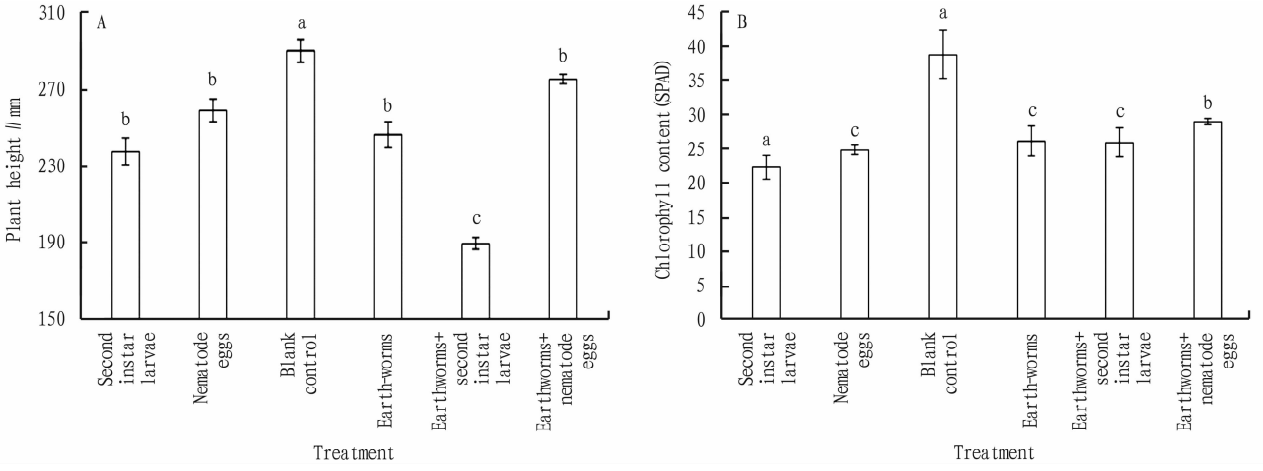


Fig. 2 Effect of earthworms and *Meloidogyne incognita* on the height (A) and chlorophyll content (B) of tomato seedlings

A comparison of the nitrogen content across various subgroups is presented in Fig. 3A. The results indicate that all subgroups exhibited significantly lower nitrogen content in comparison to the control group. In addition, the nitrogen content in the earthworms and second instar larvae group was significantly higher than that in the earthworms group, the second instar larvae group, and the nematode eggs group ($P = 0.026$, $P = 0.009$, $P = 0.018$). However, no significant differences were observed among the earthworms group, the second instar larvae group, and the nematode eggs group. A comparative analysis of the changes in fresh weight among various groups is presented in Fig. 3B. The fresh weights of the stems and leaves were recorded as 28.6, 17.3, 22.3, 23.8, 24, and 25.7 g, respectively, revealing significant differences across the treatments. Notably, the fresh weight of stems and leaves in the control group was significantly greater than that observed in the other treatment groups ($P < 0.05$). Conversely, the second instar larvae group exhibited the lowest fresh weight of stems and leaves. Furthermore, the fresh weights of the stems and leaves in the earthworms and second instar larvae group were significantly lower than those observed in the earthworms and nematode eggs group, as well as in the earthworms group. Conversely, the fresh weights of the stems and leaves in the earthworms group were significantly higher than those in the earthworms and nematode eggs group. However, no significant difference was found in the fresh weights of the stems and leaves between the nematode eggs group and the earthworms and second instar larvae group.

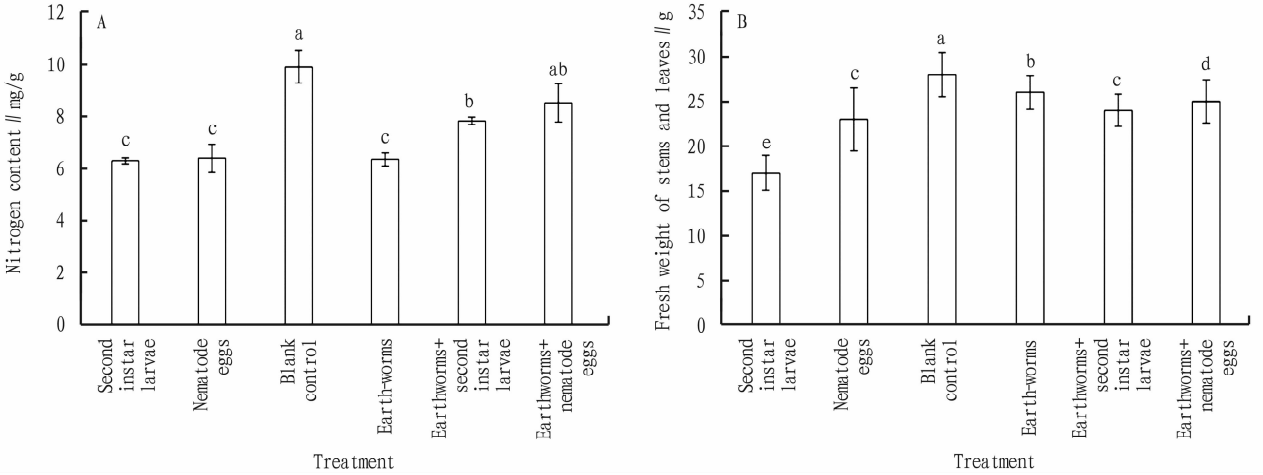


Fig. 3 Effect of earthworms and *Meloidogyne incognita* on the nitrogen content (A) and fresh weight (B) of tomato

4 Discussion

The findings of this study indicate that the introduction of *E. fetida* into the soil may effectively suppress southern root-knot nematode disease in tomato plants. In comparison to the groups containing second instar larvae and nematode eggs, a reduction in the number of root knots in the tomato roots was observed. Additionally, the physiological parameters of the aerial parts of the tomato plants were enhanced, and the reproduction coefficient of root-knot nematodes was diminished. The overall growth of tomatoes in the experimental group that included earthworms was inferior to that of the control group. This discrepancy may be attributed to the fact that the experiment was conducted in pots, which provided insufficient space for the earthworms to move freely. Consequently, the restricted movement of the earthworms likely hindered their ability to positively influence the growth and development of the tomato root system, thereby adversely affecting the overall growth and development of the tomatoes. The findings indicate that *E. fetida* may affect the population quantity of *M. incognita* and exert an inhibitory effect on the reproduction of root-knot nematodes.

Brown[15] has demonstrated that earthworms can affect nematode quantity and community structure through both direct and indirect mechanisms. Yeates *et al.*[16] discovered that earthworms were considered to be predators of nematodes, resulting in a reduction of nematode populations. The findings of this experimental study indicate that earthworms exert an inhibitory effect on the reproduction of *M. incognita*, leading to a reduction in the root knot percentage in tomato seedlings. Furthermore, they appear to mitigate the incidence of tomato root-knot nematode disease. It is hypothesized that the primary mechanism underlying this effect is the reduction of *M. incognita* quantity in the soil, which is achieved through the earthworms' feeding on the substrate, thereby decreasing the prevalence of the disease.

The study employed live earthworms placed in experimental pots, rather than utilizing earthworm secretions, to better simulate the conditions found in actual production environments. This approach aimed to yield more realistic experimental data and control effects, thereby providing valuable insights for the management of southern root-knot nematode disease in practical agricultural settings. The findings of the study indicated that earthworms exert a significant inhibitory effect on the reproduction of *M. incognita*. However, it is important to note that only one species of earthworm was examined, and no comprehensive investigations or experiments were conducted to assess the influence of different species or varying quantities of earthworms on the experimental outcomes. The selection of earthworm species and the appropriate population density required for the effective management of southern root-knot nematode disease, as well as the facilitation of optimal tomato growth, will be a focal point of future research in this study. Furthermore, the experiment solely examined the impact of earthworms on the population quantity of *M. incognita*, without delving into the underlying mechanisms responsible for the suppression of root-knot nematodes. In forthcoming experiments, the focus will be on analyzing the population dynamics between earthworms and *M. incognita*, as well as the interactions and mechanisms underlying the inhibition of *M. incognita* reproduction by earthworms. The findings of this study will establish a theoretical framework for utilizing earthworms and their metabolites to modulate the rhizosphere microecological environment, thereby aiding in the management of *M. incognita*. This research will contribute a scientifically grounded and effective theoretical basis for the biological control of *M. incognita* in practical agricultural applications.

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