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# Study on the Synergistic Treatment of Soil Fumigation and Biocontrol Bacteria for the Prevention and Control of Root-Knot Nematodes in Facility Soil

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**Abstract:** Years of continuous cropping have led to the inability to cure root-knot nematode disease. In this study, soil fumigation was used before tomato planting to study the decline rate and the effect of root-knot nematode population control after soil fumigation. During the experiment, "Wofengkang" organic sulfur soil fumigant was used. A constant temperature drying oven was used to artificially simulate the conventional greenhouse soil temperature to screen for optimal dosage of high-efficiency organic sulfur soil fumigant. The nematodes in each treatment were 0 and the reduction rate was 100% at the temperatures of 44 and55℃. To treat soil root-knot nematodes effectively, a dosage of 300-450 kg/hm$^2$ is recommended depending on the severity of the disease and the safe dosage of chemical fumigants. Tomatoes were planted after fumigation to measure the incidence rate, disease index, and population number of root-knot nematodes and to calculate the decline rate of nematodes. According to field experiments, the incidence rate, disease level, and disease index of root-knot nematodes were 64.37,3 and 60.18%, respectively, before and after fumigation. After the combination of the two, the three indicators were all 0 and reached a significant difference from that before treatment. There was a significant decline of 97.26% in the number of root-knot nematodes in 100g soil after fumigation compared to before fumigation. This technology can effectively prevent and control the root-knot nematode disease in greenhouse tomatoes.

**Keywords:** Greenhouse Tomatoes, Soil Fumigation, Root-Knot Nematodes, Reduction Rate, Prevention and Control Effects

## Introduction

Facility vegetables play an important role in agricultural production in Chifeng, Inner Mongolia, and are one of the pillar industries in Chifeng. Chifeng's facility agriculture area reached 1.7 million mu by the end of 2020, which was 53% of Inner Mongolia's planting area. Tomatoes, cucumbers, chili peppers, and eggplants are the main vegetables cultivated, which have made an important contribution to people's vegetable baskets and farmers' incomes in Chifeng. However, due to the characteristics of high investment, high output, and overloaded land use in the production of facility vegetables, coupled with the current low production conditions and management level of facility vegetables in Chifeng, the problem of continuous cropping obstacles is prominent. Multiple soil-borne disease pathogens accumulate in the soil and vegetable root diseases are becoming increasingly serious. Especially for plant root-knot nematode disease, due to the limited types of control agents, it is relatively difficult to control, which has become a limiting factor for the sustainable development of the facility vegetable industry in Chifeng. Chemical management measures are employed by most local farmers to prevent and manage root-knot nematode disease. Even though chemical pesticides have certain effects in a short period of time, they are toxic for adults and second-instar larvae, whereas they are not toxic for eggs and first-instar larvae. Moreover, chemical pesticides only influence nematodes in the soil layer within 20 cm, with nematodes reaching depths up to 1m underground. In addition, nematodes have a short life history and are mobile, and the long-term use of chemical pesticides has led to resistance. Therefore, no safe, effective, and ecological prevention and control methods have been found so far.

In recent years, with the increasing awareness of sustainable agricultural production food safety, and ecological environment protection, the use of biological control methods to control plant diseases and pests has received increasing attention and made significant progress (Yu, 2019; Dalla Pasqua *et al*., 2020). Different types of biopesticides, such as fungi (Atia *et al*., 2020), bacteria, and agricultural antibiotics, have played important roles in managing plant nematode diseases. Leij *et al*. (2015) used Verticillium chlamydosporium to control tomato root-knot nematodes, with a maximum control effect of 90%. Although biological control is relatively safe, green, and ecological, due to various factors such as soil conditions, field management, effectiveness, and adaptability of bacterial strains, the control effectiveness of different regions and sheds is unstable and varies greatly. At present, there are some studies that combine soil fumigants with biocontrol microorganisms to prevent soil-borne diseases. Ślusarski and Pietr (2009) used cotton wool and Trichoderma as-perellum to jointly control the compound soil-borne disease of chili peppers and the control effect was significant. Nie *et al*. (2016) reduced the incidence rate of the root-knot nematode by fumigation with Mianlong and Paecilomyces lilacinus. When the dosage of Mianlong was 35 g/m$^2$, the control effect reached 85.5%.

The above researches are only limited to potted plants, biocontrol bacterial colonization, or single technology experiments and there are few reports on field control experiments for root-knot nematode disease that have occurred in production practice. In this experiment, the incidence rate and disease condition of root-knot nematode were measured when the last crop of greenhouse tomato seedlings was pulled and the soil fumigant was used during the summer leisure period of local growers. After fumigation, the beneficial biocontrol bacteria such as "disease prevention microbial fertilizer" "Kexiansan powder" powder and "Paecilomyces lilacinus" water solution containing Paecilomyces lilacinus and Bacillus subtilis, to measure the population of root-knot nematodes before and after soil fumigation and during seedling pulling. Then the reduction rate and control effectiveness of nematodes are calculated, providing scientific basis and practical guidance for the effective prevention and control of root-knot nematode disease in facility vegetables. This study adopts a combination of chemical methods (soil fumigation) and biological control (biocontrol bacteria) measures, which not only have high control efficiency but are also environmentally friendly.

## Materials and Methods

### *Overview of the Experimental Site*

The experimental site was in Dachengzi (118°55′E, 42°14′N), Ningcheng County, Chifeng, Inner Mongolia. In this area, clay loam soils have an average annual temperature of 6.6°C, sunshine of 2800-3000, a frost-free period of 155-184 days, and 400 mL of precipitation per year. It is a temperate continental monsoon climate zone. The tested soil was 0-20 cm, with basic chemical properties of pH 7.38, EC value of 93.65 μS cm$^{-1}$, organic matter 3.59%, nitrate nitrogen 101.00 mg kg$^{-1}$, ammonium nitrogen 12.20 mg kg$^{-1}$, available phosphorus 210.00 mg kg$^{-1}$ and available potassium 534.00 mg kg$^{-1}$.

Test location: Wang JIE facility tomato shed in dachengzi, Ningcheng County, Chifeng City, Inner Mongolia.

### *Test Materials*

Ham 1 tomato variety: Provided by Chifeng Herun agricultural high tech industry development Co., Ltd.

The "Wofengkang" high-efficiency organic sulfur soil fumigant was provided by Beijing Qigao Biotechnology Co., Ltd., with a composition of 98% sweeping agent and molecular formula $C_5H_{10}N_2S_2$, which is efficient, safe, and residue-free. It is an R&D product of the 2060901 technology major special project (zdzx2018009). To guarantee that the agent was uniformly mixed into the tillage layer, mechanical rotary tillage-which controls the depth of the soil layer by 15-20 cm was performed after applying the proper amount of fine dirt and mixing it equally on the soil surface. Then the plastic film was covered immediately after watering. The soil was softened when the film was removed and it could be sowed or transplanted after five days if it had been sprayed beforehand.

Wofengkang bioorganic fertilizer: Provided by Beijing Qigao Biotechnology Co., Ltd., with effective viable bacteria ≥50 million/g, organic matter ≥45%, and containing medium and trace elements such as calcium, magnesium, sulfur, boron, zinc, molybdenum, copper and iron. 40 kg/bag, the dosage for facility fruits and vegetables is 2250-3000 kg/hm$^2$, as a base fertilizer for spraying application, hole application, and strip application. In this experiment, it was used as a base fertilizer for spraying.

Wofengkang microbial inoculum: Provided by Beijing Qigao Biotechnology Co., Ltd., and developed by the 2060901 science and technology major special project (zdzx2018009). Its effective viable bacterial count is ≥200 million/g and the dosage for facility fruits and vegetables is 2250-3000 kg/hm$^2$. It can be applied in ditches, in holes, or throughout the field, depending on whether the soil fertility for increasing or decreasing. Within this experiment, it served as the foundational fertilizer for spraying purposes.

Wofengkang genliqing: Provided by Beijing Qigao Biotechnology Co., Ltd., developed by the 2060901 science and technology major special project (zdzx2018009). Its effective viable bacterial count is ≥200 million/g it can be treated as base fertilizer, spread, strip, and hole application, 300-600 kg/hm$^2$, depending on soil fertility for increasing or reducing. In this experiment, it was used as a base fertilizer for spraying.

Wofengkang Kexian powder: Provided by Beijing Qigao Biotechnology Co., Ltd. It is a powder of Paecilomyces lilacinus, with an active live bacterial coun t ≥200 million/g. It is the result of the national high-tech R&D plan and the national bulk vegetable industry technology system construction project and is jointly developed with the Institute of Plant Protection of the Chinese Academy of Agricultural Sciences. The dosage is 30-60 kg/hm$^2$. In this experiment, it was used as a base fertilizer, root dipping, and spraying.

Kexianling: Provided by Beijing Qigao Biotechnology Co., Ltd., it is an upgraded product for the 2060901 technology major special project (zdzx2018009). It is a liquid agent, effective bacterial strain Paecilomyces lilacinus, effective live bacterial count ≥500 million/mL, and net content of 1000 mL/bottle. Its dosage is 15000-30000 mL/hm$^2$, used once every 1-2 months and applied as top application and drip irrigation. In this experiment, drip irrigation was applied with tomato planting water and slow seedling water and then was applied every 30 days thereafter.

Wofengkang "Wogenling: Provided by Beijing Qigao Biotechnology Co., Ltd., and developed by the 2060901 science and technology major special project (zdzx2018009). Its effective viable bacterial count is ≥2 billion/mL. After slowing down the seedlings of crops, it was used with pre-flowering water, 75 L/hm$^2$. According to the growth trend of the seedlings, they can be used 2-3 times during a growth period. In this experiment, it was used to irrigate tomatoes with water before flowering, promoting roots and preventing diseases.

"Wofengkang" Guangenbao: Its effective viable bacterial count is ≥1 billion/g. Before flowering and fruiting of vegetable crops, it is applied as 75 kg/hm$^2$ for drip irrigation or flushing application. In this experiment, it was used before the flowering and fruiting of tomatoes to promote roots and prevent diseases.

"Wofengkang" COVID-19: Provided by Beijing Qigao Biotechnology Co., Ltd., and developed by the 2060901 science and technology major special project (zdzx2018009). As a composite microbial agent, it is applied when the first ear of tomatoes is as large as a walnut, or used for the first fertilizer application when a bell pepper or eggplant is sitting, 75 kg/time/hm$^2$ for drip irrigation or flushing application, depending on the soil nutrient status for increasing or reducing. In the middle and late stages of crop growth, the combination of medium and trace element fertilizers and high potassium fertilizers can achieve better results. In this experiment, it was used when the first ear of tomatoes was as large as a walnut.

"Wofengkang" Guole: Provided by Beijing Qigao Biotechnology Co., Ltd., developed by the 2060901 science and technology major special project (zdzx2018009). It is a medium and trace element fertilizer, Ca+Mg ≥10%, N+$K_2O$ ≥32%, and $K_2O$ ≥20%. The usage and dosage are the same as those of chloramphenicol.

## *Experimental Design*

In this study, tomatoes with serious root-knot nematode disease in Chifeng were used as the research object, and soil fumigation was carried out in the summer before tomato planting. On July 20, 2020, the tomato seedlings were pulled to investigate the incidence and disease index of root-knot nematodes. The soil was collected and simulated at different soil temperatures in the laboratory to determine the control effect of soil fumigants on root-knot nematodes. The specific methods were as follows: The tomato rhizosphere soil of the above-mentioned facility where root-knot nematode disease occurred was collected and different dosages of soil fumigants were evenly mixed into the rhizosphere soil. The soil fumigant was applied at rates of 120, 300, 150, and 600 kg per 667 hm$^2$, respectively. 0 kg was not applied as the control. Total 5 treatments, each treatment was repeated 3 times and was cultured in a constant temperature drying oven. First, the conventional soil temperature of the summer shed was set to 38°C and the population of root-knot nematodes was measured after 15 days. Then the semi-lethal temperature of root-knot nematodes was set to 44°C and the population of root-knot nematodes was measured after 15 days. Finally, the lethal temperature of soil temperature was set to 55°C for 10 min, to measure the population of root-knot nematodes. The reduction rate of nematode was calculated to screen the dosage of soil fumigant.

Based on the above laboratory tests (Fig. 1), the field soil fumigation test was carried out with the optimal amount of soil fumigant. On August 23, 2020, the soil fumigation was carried out with "Wofengkang" a high-efficiency organic sulfur soil fumigant. The field dosage was 450kg/hm$^2$ and it was mixed with fine soil and spread evenly into the shed for ground tillage, watering, film covering, and keeping the shed closed for 15 days. Before soil fumigation, soil samples were taken to measure the population of root-knot nematodes in 100g soil. On September 10, the film was unveiled and released. On September 21 (after fumigation), soil samples were collected to determine the number of root-knot nematodes in 100 g of soil. On September 28, tomatoes were planted. Before planting, Wofengkang" bio-organic fertilizer 2400 kg/hm$^2$, microbial agent 300 kg/hm$^2$, Genliqing 300 kg/hm$^2$, and

Kexiansan 15 kg/hm$^2$ were applied for routine management, plowing, furrowing and planting. The planting water and slow seedling water were irrigated with "Wofengkang" Kexianling 15000 mL/hm$^2$. After 15 days of slowing down the seedlings, "Wofengkang" Wogenling 75 L/hm$^2$ was used for drip irrigation. Before flowering and fruiting, "Wofengkang" Guangenbao 75 kg/hm$^2$ was used for drip irrigation. When the first ear of fruit grew to the size of a walnut, "Wofengkang" Crownbacteryl 75 kg/hm$^2$ and Guole 75 kg/hm$^2$ were used 6 times each to pull seedlings. During this time, "Wofengkang" Kelining 15000 mL/hm$^2$ was applied with fertilizer drip irrigation. The site area was 25.50 m$^2$ and three cells were respectively set up in the front, middle, and back of the shed for sampling and determination of incidence and disease indexes. Root-knot nematodes were tracked and measured 7 days after tomato planting, before flowering and fruiting, at early fruit, full fruit, and seedling pulling stages. When pulling seedlings, the incidence rate and disease index were investigated.

(a)

(b)

(c)

**Fig. 1:** Field methods of soil fumigants; (a) Spraying soil fumigants in the field; (b) Drip irrigation; (c) Tight film covering

*Measurement Indicators and Methods*

*Collection Methods of Soil Samples*

The soil was taken from the 20 cm soil layer of the tomato rhizosphere using a "W" type 5-point sampling method, placed in a plastic bag, and transported to the laboratory. The count of root-knot nematodes was determined and the freshly collected soil samples were subjected to testing within a 3-day period.

*Soil Nematode Isolation Methods*

The Bayman shallow dish method was used:

(1) Place the sieve in a little pot and spread a layer of Kleenex over the sieve
(2) Crush and mix the soil well and take 100 g of soil and place it on the tissue paper
(3) Pour water through the gap between the sieve and the small basin, ensuring that the water covers the soil but not the tissues
(4) After 24-48 h at room temperature, pass the water in the small basin through a 500-mesh sieve. At this point, the nematodes remain on the screen. The nematodes are then washed into a glass dish with a small amount of water for 1-2 min and counted under a stereomicroscope (or microscope)

*Grading Method of Root-Knot Nematode Disease Index*

- Grade 0: Healthy roots without root knots
- Grade 1: Small root knots emerged, with an incidence rate of root knots recorded at less than 20%

- Grade 2: Several small adjacent root nodules on lateral roots join to form larger root nodules and the incidence of root nodules was 20-40%
- Grade 3: On certain taproots, large root knots were observed, with the incidence rate of root knots approximating 50%
- Grade 4: There was an underdeveloped lateral root system, large root knots appeared on the taproots and 60-80% of the taproots had root knots
- Grade 5: The entire root system was thickened, deformed, and rotten, without bearded roots and the incidence of root-knot was greater than 80%

Disease index =∑(diseased plants at all levels × representative value at all levels)/ (total surveyed plants × highest representative value) ×100;Prevention efficacy = (control disease index - treatment disease index)/ control disease index× 100%.

### *The Population of Root-Knot Nematodes Measured by Simulating Different Soil Temperatures*

Soil samples were taken from the previous crop of tomato seedlings, which were respectively from the front, middle, and back of the tomato greenhouse near the door, marking the location. Each location had an area of 60 $m^2$. "W" type multi-point sampling method was used, marking and bringing it back to the laboratory. In the laboratory, all soil samples were mixed to make a rectangular carton with a height of 20 cm, a length of 40 cm, and a width of 20 cm. The soil fumigant application amounts of 0, 120, 300, 150, and 600 kg/ 667 $hm^2$ were applied to the soil in the carton and each treatment was repeated 3 times. The temperature was set in a constant temperature drying oven according to the regular soil temperature, nematode semi-lethal temperature, and nematode lethal temperature within 20 cm of the summer stuffy shed. The population of root-knot nematodes in 100 g soil was sampled and the nematode reduction rate was calculated to screen the suitable soil fumigant application rat.

### *Data Processing*

Microsoft Excel 2007 software and SPSS26.0 software were used for data processing.

## Results

### *Effect of Soil Fumigants on Controlling Root-Knot Nematodes under Different Soil Temperature Treatments Simulated in the Laboratory*

The effect of soil fumigants on controlling root-knot nematodes under different soil temperature treatments simulated in the laboratory is shown in Table (1). Prior to soil fumigation treatment, the count of J2 root-knot nematodes in 100 g of soil was recorded as 8261.33. The conventional soil temperature of simulated summer soil fumigation in a constant temperature incubator was 38°C. After 15 days, the reduction rate of root-knot nematodes was 82.76-100.00% compared with that before the application of fumigant. The number of J2 root-knot nematodes treated with 600 kg/ 667 $hm^2$ in 100 g soil was 0 and the reduction rate was 100.00% compared with that before the application of fumigant. The semi-lethal temperature of nematodes in simulated soil was 44°C. After 15 days, the reduction rate of root-knot nematode in the four treated soils was 99.76-100.00% compared with that before the application of fumigant. The number of J2 root-knot nematodes in 100g soil was 0 and the reduction rate was 100.00% compared with that before the application of fumigant. The lethal temperature of nematodes in the simulated soil was 55°C for 10min and the reduction rate was 99.90-100.00% compared with that before the application of fumigant. The number of nematodes in the four treated soils was 0, which was 100.00% compared with that before the application of fumigant. The number of root-knot nematode J2 in the control 100 g soil was 8.

**Table 1:** Effect of soil fumigants on controlling root-knot nematodes under different soil temperature treatments simulated in the laboratory

| Soil fumigant application rate (kg/ $hm^2$) | Number of nematodes in the soil before the application of soil fumigant (J2/100 g) | Number of root-knot nematodes (J2/100 g, simulate 38℃, 15d) | Relative reduction rate before pre-application (%) | Number of root-knot nematodes (J2/100 g, simulate 44℃, 15d) | Relative reduction rate before pre-application (%) | Number of root-knot nematodes (J2/100 g, simulate 55℃ 10 min) | Relative reduction rate before pre-application (%) |
|---|---|---|---|---|---|---|---|
| 120 | 8261.33 | 20 | 99.76 | 0 | 100.00 | 0 | 100.00 |
| 300 | | 8 | 99.90 | 0 | 100.00 | 0 | 100.00 |
| 450 | | 4 | 99.95 | 0 | 100.00 | 0 | 100.00 |
| 600 | | 0 | 100.00 | 0 | 100.00 | 0 | 100.00 |
| Control | | 1424 | 82.76 | 20 | 99.76 | 8 | 99.90 |

## *Effect of Soil Fumigation and Biocontrol Bacteria on Root-Knot Nematode Number and Control Efficiency of Greenhouse Tomatoes*

### *Effect on the Incidence Rate of Root-Knot Nematodes*

The incidence rate of root-knot nematode was 64.37%, with a standard deviation of 16.30%. After soil fumigation, the incidence rate of root-knot nematode was investigated when pulling tomato seedlings. The results showed that root-knot nematode disease did not occur in all treatments, did not infect tomato roots, and did not form root knots. The t-test results of paired samples of the incidence of root-knot nematodes before and after soil fumigation showed that the t-value was 6.84 and P was 0.021 ($p<0.05$) (Figs. 2-3). The incidence of root-knot nematodes was significantly reduced before and after soil fumigation, reaching 64.37%. As shown in Table (2).

**Table 2:** Impact of soil fumigation on the incidence rate of root-knot nematode in tomatoes grown in greenhouses

| Different treatments | Incidence/ % | t | p | 95% confidence interval Lower limit | Upper limit |
|---|---|---|---|---|---|
| After soil fumigation | 0.00 | 6.84 | 0.021 | 23.88 | 104.85 |
| Before soil fumigation | 64.37 | | | | |

**Fig. 2:** Tomato roots that develop root-knot nematode disease before soil fumigation

**Fig. 3:** No root knot was formed in the tomato roots after the synergistic treatment of the two

### *Effect on Root-Knot Nematode Disease*

Table (3) shows that the average disease level of root-knot nematode disease measured before soil fumigation and during tomato seedling cultivation was 3.00, with a standard deviation of 0.83. After soil fumigation, when the previous tomato seedlings were pulled, the disease level of root-knot nematode disease was investigated. The results showed that root-knot nematode disease did not occur in all treatments, did not infect tomato roots, and did not form root knots. The t-test results of disease-level paired samples of root-knot nematode disease before and after soil fumigation showed that the t-value was 6.27 and p-0.025 ($p<0.05$). Prior to and following soil fumigation, a significant reduction in the disease level of the root-knot nematode was observed, with a reduction to 3.00.

The average disease index of root-knot nematode disease measured before soil fumigation and during tomato seedling cultivation was 60.18%, with a standard deviation of 7.94%. Post soil fumigation, an investigation was conducted into the disease index of root-knot nematodes during the cultivation of tomato seedlings (Fig. 4). The results showed that root-knot nematode disease did not occur in all treatments, did not infect tomato roots, and did not form root knots. The paired sample t-test results of the disease index of root-knot nematodes before and after soil fumigation showed that the t-value was 13.12 and the p-value was 0.006 ($p<0.05$). Before and after soil fumigation, the disease index of root-knot nematodes was significantly reduced by 60.18%.

### *Effect on the Decline Rate of Root-Knot Nematode Population*

Table (4) showed that the population of root-knot nematodes in 100 g soil before and after soil fumigation decreased significantly ($p<0.05$) and the reduction rate reached 97.26%.

**Fig. 4:** No root knot formation in the roots after the synergistic treatment of the tomato seedling stage

**Table 3:** Impact of soil fumigation on root-knot nematode disease of greenhouse tomatoes

| Different treatments | Disease grade | Disease index/ % | t-value | p-value | 95% confidence interval Lower limit | Upper limit |
|---|---|---|---|---|---|---|
| After soil fumigation | 0.00 | 0.00 | 6.27/13.12 | 0.025/0.006 | 0.94/40.45 | 5.07/79.92 |
| Before soil fumigation | 3.00 | 60.18 | | | | |

**Table 4:** Effects before and after soil fumigation on the population of root-knot nematodes of greenhouse tomatoes

| Different treatments | Number of root-knot nematodes (J2/100 g) | Nematodes decline rate/ % | t-value | p-value | 95% confidence interval Lower limit | Upper limit |
|---|---|---|---|---|---|---|
| After soil fumigation | 36.00 | 97.26 | 8.31 | 0.014 | 1940.10 | 616.57 |
| Before soil fumigation | 1314.33 | — | | | | |

## Discussion

Addressing the prevalent issues of soil acidification, compaction, secondary salinization, and the frequent occurrence of soil-borne diseases in China, numerous biological companies and scientific institutes have formulated and produced biocontrol agents with diverse functionalities. These agents are designed to control diseases, enhance yield and quality, and improve soil health by modulating the rhizosphere microbial flora to promote the dominance of beneficial bacteria (Jamali *et al*., 2020). The "Wofengkang" high-efficiency organic sulfur soil fumigant, bio-organic fertilizer, microbial inoculant, Kexiansan, Genliqing, Kelining, Wolgenling, Guangenbao, Coronary Fluoride, Guole and other products in this experiment were jointly developed by the institute of plant protection of the Chinese academy of agricultural sciences and Beijing Qigao biotechnology Co., Ltd. The effective strains were Bacillus subtilis, pink mucosis, Beauveria bassiana, and Penicillium lavender. High-efficiency organic sulfur soil fumigants, microbial inoculants, Genliqing, Wolganlin, Crowningham, Guole, and Kelinyl are newly developed and upgraded products of the 2060901 science and technology major project (ZDZX2018009). Among them, Purpureocillium lilacinum is an important group of parasitic fungi within root-knot nematodes (Shangguan *et al*., 2018). The application of Penicillium lavender to soil can reduce the number of southern root-knot nematodes by 45 and 69%, respectively (Zhao *et al*., 2019). Huang *et al*. (2016) found that the combination of racemoces and Paecilomyces lilacinus was the most effective by testing various biological control agents in Meloidogyne incognita. Studies on soil fumigation combined with biocontrol bacteria for root-knot nematode disease control in tomatoes (Wang *et al*., 2019; 2014; Liu *et al*., 2011), cucumber (Zhao *et al*., 2019), watermelon (Huang *et al*., 2016) and ginger Wang have been reported. Wang *et al*. (2019) used the combined application of cotton + biological fertilizer to reduce the incidence of the root-knot nematode by 15% and the effect of preventing and controlling root-knot nematode disease was 30.3%. Wang *et al*. (2014) reported that 60 days after tomato transplanting, the reduction rate of root-knot nematodes in 98% Mianlong soil fumigated with 450 kg/hm$^2$ application rate was 82.8%. Liu *et al*. (2011) presented that the reduction rate of tomato root-knot nematodes in soil was 88.6% after 90 days of fumigation with a 450 kg/hm$^2$ application rate of 98% mianlong. Nie *et al*. (2016) used the combined control of tomato root-knot nematode with Paecilomyces lilaci-nus and Paecilomyces lilaci-nus and the control efficacy was 79.5 and 63.96%, respectively. Zhang *et al*. (2019) used 98% Mianlong 225 kg/hm$^2$ application rate fumigation and 150 kg/hm$^2$ YB-04 biological fungus fertilizer furrow cucumber application. After 30, 60, and 90 days, the reduction rates of 2$^{nd}$ instar root-knot nematodes were 81.1, 81.7, and 89.1%, and the control effect was 87.8%. The control effect of deep tillage + cotton lone continuous cropping of watermelon root-knot nematode was more than 80% (Wu *et al*., 2016). Xiang *et al*. (2016) confirmed that the control efficacy of ginger root-knot nematode treated with 98% cotton long micro granules at commercial dosages of 40, 50, and 60 g/m$^2$ was greater than 85%. Plant parasitic nematode biocontrol bacteria have emerged in recent years and they have great application potential in biological control of nematodes and have gradually been recognized by growers.

## Conclusion

The laboratory simulation test results show that using different dosages of soil fumigants to fumigate the soil can effectively reduce the population of root-knot nematodes in 100 g soil. When the simulated temperature was 38℃, the number of nematodes treated with a soil fumigation dosage of 600 kg/hm$^2$ was 0 and the effect was significant. However, it is not recommended to use it in production, because the phenomenon of burning seedlings caused by toxic gases would appear. The results of later field experiments showed that the optimal dosage of soil fumigant was 450 kg/hm$^2$ in this experiment. It was found in later field experiments that some tomato plants had yellowed leaves, which may have been poisoned by the gas released by the fumigant. Upon reaching simulated nematode half-lethal and lethal temperatures of 44 and 55°C, respectively, the nematode count across various fumigant treatments was consistently zero, suggesting that the effectiveness of controlling root-knot nematodes is associated with soil temperature levels. In this experiment, the field dosage of soil fumigant was 450 kg/hm$^2$, which was the same as that of many scholars (Jamali *et al*., 2020; Shangguan *et al*., 2018). Before and after soil fumigation, the results of field nematode control experiments showed that the use of soil fumigants significantly reduced the field incidence, disease level, and disease index of root-knot nematodes. The incidence, disease level, and disease index of tomato during seedling pulling were 0 and no disease occurred. At the same time, the population of root-knot nematodes was greatly reduced and the reduction rate reached 97.26%. Therefore, for the facility vegetable soil where root-knot nematodes occur, the method of soil fumigation followed by beneficial bacteria reconstruction is effective. It can be popularized and applied in a small area in actual production, combined with the incidence of root-knot nematodes, soil conditions, and local management technology.

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## Author's Contributions

**Lijin Qin, Yingmin Hu and Jiahe Tian:** Designed and performed the experiments, analyzed the data, and prepared the paper.

**Chunyong Wang and Hao Zhang:** Participated to collect the materials related to the experiment.

**Xiangbin Yin and Han Qiu:** Designed the experiments and revised the manuscript.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

### *Conflict of Interest*

The authors declare that they have no competing interests.

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