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RESEARCH PAPER

Effectiveness of bionematicide from *Purpureocillium lilacinum* in controlling root-knot nematodes (*Meloidogyne* spp.)

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ABSTRACT

This research aimed to study the efficacy of the fungus *Purpureocillium lilacinum* as a bionematicide to control root-knot nematodes (RKN). Two steps of experiments were carried out in this study. The first experiment involved the application of various levels of bionematicide doses to control RKN on tomato plants. The second experiment tested the application of bionematicide (both as a single application and in combination with bromelain compost) to control RKN on guava cv. *Kristal*. A carbofuran nematicide was applied following the company's recommendation in this second experiment for comparison. The results of the first trial showed that the application of *P. lilacinum* bionematicide at doses ranging from 20–40 g per plant or 7–13 g per kg of soil was effective in reducing the J-2 RKN population in the soil and roots, as well as mitigating damage to plant roots. In the second experiment, it was shown that the application of *P. lilacinum* bionematicide, either alone or mixed with bromelain compost, was more effective than the application of carbofuran nematicide in reducing the J-2 RKN population in the soil and roots, as well as in minimizing root damage to guava seedlings. Additionally, the application of bionematicides mixed with compost proved more effective than their single application in reducing plant root damage. Furthermore, apart from being able to control nematode populations and plant damage, *P. lilacinum* bionematicide could stimulate plant growth.

Key words: bionematicide, guava, *P. lilacinum*, root-knot nematodes, tomato

INTRODUCTION

Root-knot nematode (RKN) (*Meloidogyne* spp.) is a significant plant pest organism in agriculture worldwide (Jones et al., 2013). In Indonesia, RKN is reported to attack and cause problems in various crops, including vegetable plants (Kurniawati et al., 2020; Mutala'liah et al., 2019; Supramana & Suastika, 2012), rice plants (Mirsam & Kurniawati, 2018; Nurjayadi et al., 2015), and guava plants (Nabilah et al., 2021). Controlling RKN is necessary to save production of vegetables, fruit, and rice on a global and national scale, especially in Indonesia, which has experienced significant losses due to attacks by these nematodes.

The use of chemical pesticides has been proven to have a negative impact on both public health and the environment (Gyawali, 2018). For instance, the application of 1,3-Dichloropropene nematicide has been found to negatively affect fungal free-living nematodes (Grabau et al., 2020; Watson & Deseager, 2019), while organophosphate and carbamate nematicides have been found to affect *Neoaplectana carpocapsae*, an insect-pathogenic nematode (Hara & Kaya, 1982). Therefore, it is crucial to develop nematode control technologies that are environmentally friendly and safe for public health, one of which is biological control technique.

Various types of nematode-antagonistic microorganisms can serve as biological control agents against nematodes. One of these microorganisms is the fungus *Purpureocillium lilacinum* fungus, formerly known as *Paecilomyces lilacinus* (Thom.) Samson. This fungus can be easily propagated using various media (Sundararaju & Cannayane, 2002; Bran et al., 2009). The *P. lilacinum* fungus is also reported to be effective as a biological control agent for root-knot nematodes (Singh et al., 2013; Grace et al., 2019; El-Ashry et al., 2021; Zhan et al., 2021). It has been formulated as a bionematicide and is marketed under various trade names (Lamovšek et al., 2013; Abd-Elgawad & Askary, 2018). The *P. lilacinum* fungus isolated from root-knot nematode eggs on guava plants in Lampung, Indonesia

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exhibited high pathogenicity in in-vitro tests (Swibawa et al., 2020). This fungus has been formulated as a bionematicide using cassava peel and banana tuber as ingredients; however, its effectiveness has not been reported. This study carried out two greenhouse-level experiments to evaluate the efficacy of *P. lilacinum* bionematicides in controlling *Meloidogyne* spp. This study aims to evaluate the efficacy of *P. lilacinum* bionematicide in controlling *Meloidogyne* spp. through two greenhouse-level experiments.

MATERIALS AND METHODS

Research Site. This study consisted two sets of greenhouse-level experiments. Experiment I was carried out in the Greenhouse of the Faculty of Agriculture, Universitas Lampung, from February to June 2019. Experiment II was conducted in the experimental field of Hajimena Village, Natar, South Lampung, from January to June 2020. The laboratory processes for both experiments were performed at the Plant Pest Science Laboratory, Faculty of Agriculture, Universitas Lampung.

Bionematicide Preparation. The bionematicide is made from a mixture of dried banana tubers, dried cassava peels, rice, and dried shrimp shells. The banana tubers, cassava peels, and shrimp shells were dried in an oven at 60 ^{o}C for 48 hours, then pounded and sieved with a 2 mm sieve to produce powder. Rice is used as a growing medium for the fungus *P. lilacinum* isolates B01TG. The bionematicide, weighing 400 g, is composed of 180 g of banana tuber powder, 180 g of cassava peels powders, 38 g of rice covered with *P. lilacinum* fungus, and 2 g of shrimp shells powders. The bionematicide contains approximately 10^8 conidia per g.

Experiment I: The application of various levels of bionematicide doses to control Root Knot Nematode on tomato plants

Experimental Design. Experiment I was arranged in a Completely Randomized Design consisting of five doses of *P. lilacinum* bionematicide: 0, 5, 10, 20, and 40 g per 3 kg of plant media soil, with five replications.

Materials. Tomatoes cv. 'Victory' were planted in polybags with a capacity of 3 kg, each containing sterile soil and sand mixture (3:1) as the soil medium, with one plant per polybag. Three days before planting, bionematicide was sprinkled into 10 cm planting holes. RKN (*Meloidogyne* spp.) eggs, collected from the roots of guava cv. *Kristal* infected with RKN at PT GGP PG 4, East Lampung, were infested on plants seven days after planting (DAP). Each plant was infested with 2000 eggs, extracted using a 1% chlorine (NaOCl) solution. The plants were watered daily. Additionally, NPK fertilizer was applied at a dose of 10 g per plant twice, at 35 and 56 DAP.

Observations of nematode populations were conducted when the plants were 98 DAP. Nematodes were extracted from 5 g of roots using a modified Baermann method, while those from 300 cc of soil were extracted using the centrifugation method with a sugar solution. The modified Baermann funnel is made from a 2 mm filter in a 15 cm diameter bowl lined with tissue paper.

A total of 300 cc of soil was added to 2 L of water, then stirred until homogenized and left for 1 minute before filtered through a 1 mm sieve. The soil suspension was collected in a bucket and left for 3 minutes, then filtered using a 53 µm sieve. Soil particles stuck to the filter were collected in a beaker glass. The filtrate was filtered with a 38 µm sieve, and the soil attached to the filter was added and mixed with the soil from the second filter. This soil suspension was centrifuged at 3500 rpm for 3 minutes. The supernatant was discarded, and the soil precipitate was mixed with a sugar solution (500 g sucrose in 1000 mL water) in an amount twice the volume of the precipitate, then stirred until mixed. The mixture was centrifuged again at 1500 rpm for 1.5 minutes. The supernatant, now a suspension of nematodes in a sugar solution, was rinsed with running water using a 38 µm sieve. The nematode suspension was then collected in a suspension jar.

A total of 5 g of washed roots was cut into 1.5 cm pieces, then macerated in a blender for 10–15 minutes. The macerated root pieces were placed on a sieve lined with tissue paper and soaked in water for 48 hours. The nematodes collected in the bowl were then filtered using a 38 µm sieve, and the nematode suspension was collected in a jar.

The nematodes were killed using hot water (60 ^{o}C) and fixed with golden X solution (Hooper et al., 2005). Juvenile-2 (J-2) nematodes (see Figure 1) were counted using a binocular stereo microscope (Leica-EZ40 HD, Switzerland).

Root damage in the form of root galls was scored according to a scale of 0–10 (Zeck, 1971): Score 0 = root system without galls; 1= very few small galls were detected (2%) with careful observation; 2 = numerous visible small galls formed (4%); 3 = many small galls,

some merging into larger galls without affecting root function; 4 = numerous small and several large galls, with most roots are still functional; 5 = approximately 50% of root system is non-functional due to severe galls; 6 = enlarged galls on main root and surrounding roots; 7 = 75% of root system non-functional due to severe galls; 8= no healthy roots, disrupted shoot growth, but plant still green; 9 = root system and galls rotting, plant dying; and 10 = plants dead or dying. The intensity of root damage was calculated using the disease severity formula (Barker, 1985).

$$Ds = \frac{(\sum vi \times ni)}{(N \times V)} \times 100\%$$

Ds = Disease severity intensity;
vi = Root galls score (0–10);
ni = i^{th} plant;
V = The highest root gall score, namely 10;
N = Number of plants measured.

Tomato plant growth was assessed based on the dry weight of the shoot and root. The shoots and roots of each plant were oven-dried at 60 °C for 48 hours, then weighed on a digital scale (Max 2100 g, d= 0.1 g, SCALTEC SPO 61, Germany).

Experiment II: Application of bionematicide to control RKN on guava cv. *Kristal*

Experimental Design. Experiment II utilized a Completely Randomized Design with five treatments, each replicated five times. The treatments included a control, *P. lilacinum* bionematicide at a dose of 100 g per plant (equivalent to 14 g per kg of soil), *P. lilacinum* bionematicide at 100 g per plants combined with 100 g of bromelain compost, and carbofuran nematicide at a rate of 10 g per plant. Each plant was grown in a planting medium of soil + sterile sand (3:1), totaling 7 kg per plant. The *P. lilacinum* bionematicide used was the same as that used in Experiment I.

Materials. Grafted guava cv. *Kristal* originating from PT. GGP PG4 East Lampung were planted in 10-kg-polybags filled with 7 kg of sterile soil and sand (3:1). The bionematicide dose used was equivalent to 40 g per tomato plant with 3 kg of soil, as in experiment I. Application to the planting hole was conducted three days prior to transplanting the guava seedlings. A total of 5000 RKN eggs were infested per plant seven days after planting, obtained from the roots of guava plants (as in Experiment I). Plants were watered daily, and NPK fertilizer was applied twice at a dose of 15 g per plant 90 and 120 DAP.

Observations were made 120 days after planting. Nematodes were extracted from the soil and roots and counted as in Experiment I. Root damage in the form of galls on guava plants was assessed using a scoring system ranging from 0 to 5 (Barker, 1985), where a score of 0= no galls formed, 1= 1–10% galls observed, 2= 11–20% galls observed, 3= 21–55% galls observed, 4= 56–80% galls observed, and 5= 81–100% galls observed. The intensity of root damage was calculated using the disease severity formula (Barker, 1985).

$$Ds = \frac{(\sum vi \times ni)}{(N \times V)} \times 100\%$$

Ds = Disease severity intensity;
vi = Root galls score (0-5);
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V = The highest root galls score, namely 10;
N = Number of plants measured.

Data Analysis. The observation data from these Experiment were analyzed using analysis of variance (ANOVA) and tested using the Least Significant

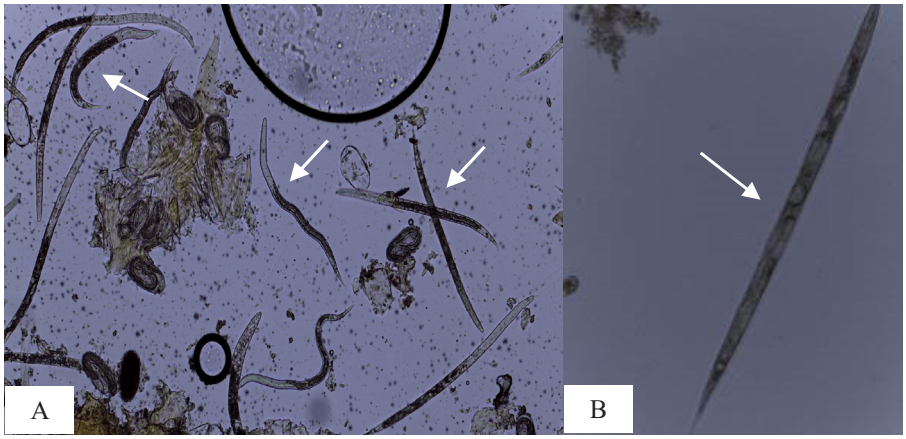

Figure 1. *Meloidogyne* spp. A. Several juvenile (J-2) nematodes lived at 60× magnification; B. One juvenile (J-2) nematode died at 100× magnification (B).

Difference (LSD) test at a significance level of 5%, with the assistance of the R Statistical Software for Windows 4 (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria) (R Core Team, 2021).

RESULTS AND DISCUSSION

Experiment I: Application of various levels of bionematicide doses to control Root Knot Nematode on tomato plants. Observation results revealed that tomato plants were attacked by nematodes, displaying symptoms of root galls and sparse fibrous roots. Significant root galls were observed in plants that were not treated with *P. lilacinum* bionematicide, to the extent that the roots resembled tuberous roots (Figure 2B). In contrast, plants treated with *P. lilacinum* bionematicide displayed healthy roots with either no or little galls (Figure 2A).

The juvenile 2 (J-2) root-knot nematode population in the soil, nematode population in the roots, and intensity of root damage in tomato plants treated with bionematicide are presented in Table 1. Analysis of variance indicated that bionematicide dosage significantly affected the population of juvenile nematodes in both soil and roots. Plants treated with higher doses of bionematicides displayed lower nematode populations in both soil and roots. Specifically, the nematode population in the soil and roots of plants treated with *P. lilacinum* fungal bionematicide at a dose of 40 g was significantly lower than in plants treated with doses of 5–10 g. The nematode population in the soil of control reached 1340 individuals per 300 cc of soil, while in the roots, it reached 3299 individuals per 5 g of roots.

The intensity of root damage in plants treated with 40 g of *P. lilacinum* bionematicide was lower than that in plants treated with bionematicide dose ranging from 5 to 20 g per plant, with 3 kg of soil as the planting medium. The intensity of root damage in control plants reached 70% (Table 1).

The biomass of tomato plants infested with *Meloidogyne* spp. was influenced by the treatment with *P. lilacinum* bionematicide. The shoot dry weight of control plants was lower than that of plants treated with bionematicide, whereas the opposite trend observed for root dry weight. Control plants has a shoot dry weight only 12.83 g, while the plants treated with 40 g bionematicide reached 44.51 g. Conversely, the dry weight of roots in control plants was 3.19 g, higher than that in plants treated with 40 g bionematicide, which was 1.25 g. However, the shoot/root dry weight ratio of plants treated with 40 g *P. lilacinum* bionematicide was higher than that of control plants (Table 2). Plant biomass was used in this reserach to assess plant growth.

Experiment II: Application of bionematicide to control RKN on guava cv. *Kristal.* Similar to the observations in Experiment I, guava seedlings attacked by *Meloidogyne* spp. showed knotted roots (root galls) with fewer fibrous roots. However, in plants infested with nematodes but treated with bionematicide, root galls did not form, whereas in control plants without bionematicide treatment, large root galls were formed (Figure 3).

Similar to the results of Experiment I, nematode populations and root damage were influenced by bionematicide treatment. The nematode population in the soil and roots of guava seedlings treated with bionematicide was significantly lower than that in control plants and plants treated with carbofuran. There was no significant difference in nematode population between plants treated with bionematicide alone and those treated with bionematicide plus compost.

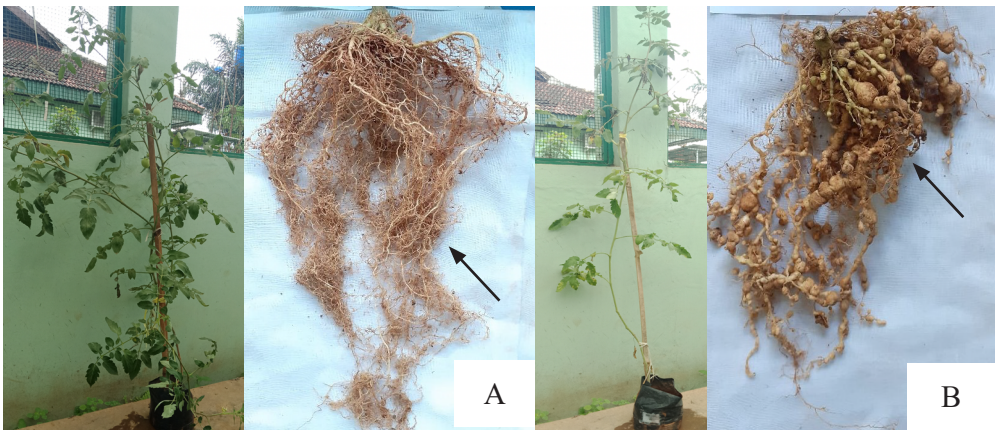

Figure 2. Healthy tomato plants and roots as well as plants attacked by RKN and knotted roots. A. Healthy; B. Knotted roots (root galls) caused by *Meloidogyne* spp.

Similarly, there was no difference in nematode population between control plants and plants treated with the chemical nematicide carbofuran. The intensity of root damage in plants treated with bionematicide plus compost was only 20%, whereas the intensity of root damage in control plants reached 88% (Table 3).

In Experiments I and II, the bionematicide containing the active ingredient of *P. lilacinum*, using cassava peel and banana stem as carrier, was found to be effective in controlling root-knot nematodes (*Meloidogyne* spp.). In Experiment I, the application of the *P. lilacinum* bionematicide at a dose of 20–40 g was more effective than doses 5–10 g. The population of J-2 root-knot nematodes in the soil and roots in plants treated with a dose of 20–40 g was lower than in control plants and those treated with bionematicide at doses of 5–10 g (Table 1). The results of Experiment I revealed that the effective dose of the *P. lilacinum* bionematicide in controlling root-knot nematodes in tomato plants was 20–40 g per plant, with 3 kg of soil as the planting medium, equivalent to 7–14 g of bionematicide per 1 kg of soil. Abbas et al. (2011) reported that a suspension of the *P. lilacinum* fungus, previously called *Paecilomyces lilacinus*, was effective in controlling root-knot nematodes in eggplant plants. Meanwhile, Yankova et al. (2014) reported that the *P.*

Table 1. The population of juvenile (J-2) Root-Knot Nematodes (RKN) in soil and roots and the intensity of root damage in tomato plants treated with bionematicide

Doses of bionematicide (g per plant)	Population of (J-2) RKN		
	In the soil (individual per 300 cc of soil)	In the roots (individual per 5 g of root)	Intensity of root damage (%)
0	1340.00 a	3299.80 a	70.00
5	1230.60 b	2762.00 b	56.00
10	1133.20 c	2460.00 c	46.00
20	965.80 d	2141.00 d	30.00
40	904.20 e	1601.00 e	18.00

Means followed by the same letters in the same column are not significantly different according to LSD test at 5%.

Table 2. Shoot dry weight, root dry weight, and shoot/root dry weight ratio of tomato plants infested with *Meloidogyne* spp. as affected by various bionematicide doses

Doses of bionematicide (g per tanaman)	Shoot dry weight (g)	Root dry weight (g)	Shoot/root dry weight ratio
0	12.83 e	3.19 a	4.15 d
5	16.90 d	3.02 a	5.65 d
10	24.57 c	2.12 b	11.79 c
20	32.76 b	1.65 bc	21.14 b
40	44.15 a	1.25 c	35.88 a

Means followed by the same letters in the same column are not significantly different according to LSD test at 5%.

Figure 3. Healthy guava seedling cv. "*Kristal*" and roots as well as guava seedling attacked by RKN and knotted roots. A. Healthy roots; B. Knotted roots (root galls) caused by *Meloidogyne* spp.

lilacinus bionematicide with a spore density of 1 x 10^{10} per g was effective in controlling root-knot nematodes on cucumber plants. The application of the *P. lilacinum* bionematicide with a concentration of 4 x 10^{9} cfu per mL using the watering method at a dose of 5 L per ha was effective in controlling potato cyst nematodes *Globodera* spp. (Seenivasan, 2017).

The effectiveness of this bionematicide was also demonstrated in Experiment II. The results of Experiment II showed that bionematicide applied alone or mixed with compost was more effective than the chemical nematicide (Carbofuran). The population of J-2 root-knot nematodes on guava seedlings treated with the bionematicide alone and in combination with bromelain compost was lower than the nematode population on plants treated with the chemical nematicide (Carbofuran) and control plants (Table 3). These result was consistent with those of Grace et al. (2019), who reported that the application of *P. lilacinum* bionematicide through seed treatment, combined with vermicompost fertilizer enriched with *P. lilacinum*, was effective in controlling the nematode *Meloidogyne incognita* on tuberose flowers (*Polianthes tuberosa*). In Experiment II, the application of bionematicide plus compost was more effective than Carbofuran in controlling root-knot nematodes. This contrasts with the finding of Dawabah et al. (2019), who reported that the effectiveness of *P. lilacinum* bionematicide mixed with *Pasteuria penetrans* bacteria and fertilizer (chicken or cow manure) showed similar effectiveness compared to Carbofuran 10 G.

The effectiveness of *P. lilacinum* bionematicide in controlling root-knot nematodes (*Meloidogyne* spp.) was also demonstrated by reducing plant root damage. The results of Experiment I showed that the intensity of root damage in tomato plants infested with root-knot nematodes, at a rate 2000 eggs per plant and treated with *P. lilacinum* bionematicide at a dose of 40 g, was only 18%, while root damage in control plants reached 70% (Table 1). Similar results were observed in Experiment II, where the intensity of damage to guava seedlings infested with 5000 root-knot nematode eggs and treated with *P. lilacinum* bionematicide mixed with bromelain compost was only 20%, compared to 88% in control plants without bionematicide. Experiment II also demonstrated that applying compost along with the bionematicide could reduce the root damage.

The growth of plants infested with root-knot nematodes and treated with *P. lilacinum* bionematicide remained robust. In Experiment I, the shoot dry weight of plants treated with the bionematicide was four times higher than that of control plants. This finding aligns with Sharma & Pendy (2009) report, which indicated that biological control agents like *P. lilacinum* increased plant growth, as measured by the dry weight and fresh weight of shoots and roots, as well as plant height. Fiandani et al. (2021) also found that applying *P. lilacinum* bionematicide to nematode-infested plants resulted in higher shoot fresh weight and production to control plants. In Experiment I, the dry weight of the roots of the control plants was higher than that of plants treated with bionematicide, resulting in a smaller shoot/root dry weight ratio (Table 2). This was due to the presence of many large root galls in control plants, leading to a higher value of root dry weight (Figure 1). Galled roots may not function optimally in absorbing water and nutrients, thereby disrupting plant growth.

Based on the results of this research, the fungal bionematicide made from *P. lilacinum*, which used cassava peel and banana stem as carriers, is not only effective in controlling nematodes but also in promoting plant growth. The enhanced plant growth is attributed to the carrier materials (cassava peel and banana stem), which serve as sources of organic material. Additionally, *P. lilacinum* has been reported to act as a plant growth promoter by increasing the availability of nitrogen and phosphorus as plant nutrients (Baron et al., 2020).

Table 3. Population of Juvenile 2 (J-2) Root-Knot Nematodes (RKN) in the soil and roots and the intensity of root damage in guava plants treated with nematicide

Treatments	Population of J-2 RKN		
	In the soil (individual per 300 cc soil)	In the roots (individual per 5 g roots)	Intensity of root damage (%)
Control	2276.80 a	2340.80 a	88.00
Chemical Nematicide (Carbofuran)	2656.20 a	2512.00 a	76.00
P. lilacinum Bionematicide	955.80 b	439.30 b	40.00
P. lilacinum Bionematicide + compost	773.60 b	599.80 b	20.00

Means followed by the same letters in the same column are not significantly different according to LSD test at 5%.

CONCLUSION

The *Purpureocillium lilacinum* fungal bionematicide, made from cassava peel and banana stem, was effective in controlling root-knot nematodes (*Meloidogyne* spp.). The population of root-knot nematodes in the soil and roots, as well as root damage in the plants treated with the bionematicide, were lower than in control plants and those treated with Carbofuran. Overall, the results of this research demonstrated that *P. lilacinum* fungal bionematicide, when mixed with compost fertilizer, is effective in controlling nematode populations, reducing plant damage, and promoting plant growth. *P. lilacinum* shows promosing potenstial for development as a bionematicide.

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AUTHORS' CONTRIBUTIONS

IGS and YF planned and carried out the experiment. AF and SB maintained the plants in the greenhouse. RS isolated and mass-produced the fungus *P. lilacinum*. Sol and Pur produced the bionematicides. FXS analyzed the data.

COMPETING INTEREST

The authors declare that there is no competing interest regarding the publication of manuscripts.

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