Bangladesh J. Bot. **48**(3): 845-851, 2019 (September) Special

EVALUATION OF VERMICOMPOST AND EXTRACTS ON TOMATO ROOT-KNOT NEMATODE

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Keywords: Vermicompost, Extract, Tomato root-knot nematode, Biocontrol, Control effect

Abstract

A laboratory assay and pot experiment were conducted to explore the influence of different concentrations of vermicompost and its extracts against root-knot nematode in tomato. The results showed that the hatchability of eggs significantly decreased with increasing concentrations of vermicompost extract. The knockdown rate and mortality of *Meloidogyne incognita* J2s significantly increased with increasing concentrations of vermicompost extract and treatment time, and the mortality rates reached up to 88.46% at a concentration of 90% compared to that of the untreated control. Pot experiments showed that the number of root-knots reduced and the relative control effect was improved.

Introduction

Many types of organic wastes, such as straw residue, fungus chaff, sesame residue, biogas slurry, livestock manure, green manure, rice bran, and grain processing by-products, have effects of improving soil environments, alleviating continuous cropping barriers, preventing and controlling soil-borne diseases and promoting plant growth. Because this type of material is cheap, simple and easy to obtain and has a wide range of sources, more and more research has been conducted on this type of material as the matrix of new crop cultivation and disease prevention. Vermicompost is the main product of the biodegradation of organic waste by earthworms, and contains some antagonistic microorganisms. It has a good control of the occurrence of soil-borne pathogens of crops (Hu *et al*. 2004, Wang *et al*. 2010). As a biological organic fertilizer, vermicompost plays an important role in the field of applied research to control soil-borne diseases, and the use of vermicompost added to fertilizer or cultivation matrix for the control of soil-borne diseases has been reported. Suppression of *Plasmodiophora brassicae*, *Phytophthora nicotianae* and *Fusarium lycopersici* by vermicomposts has been reported (Nakamura 1996, Szczech 1999). It was demonstrated that fungal diseases of gerbera plants, such as *Rhizoctonia solani*, *Phytophthora drechsleri* and *Fusarium oxysporum*, were generally suppressed by the incorporation of vermicompost into the growth media (Rodriguez *et al*. 2000). Sporulation reduction of the pathogen *Phytophthora cryptogea* after treatment with vermicomposts was described (Orlikowski 1999). Previous studies showed that aqueous extracts of vermicomposts inhibited the mycelial growth of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Corticium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* (Nakasone *et al*. 1999). Adding a proper amount of vermicompost to the cultivation matrix had some preventive effects on few common soil-borne diseases such as damping-off and wilt (Edwards *et al*. 2004). It was reported that addition of organic additives such as green manure, barnyard manure and compost was effective for improving soil conditions, such as by the release of nitrogen compounds, organic acids, or other compounds that had adverse effects

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on nematodes (Thoden *et al*. 2011). Hu found that vermicompost could inhibit damping-off caused by *Rhizoctonia solani* and wilt caused by *Fusarium oxysporum* at the seedling stage in cucumber (Hu *et al*. 2002). In this study, vermicompost was used to control tomato root-knot nematode, which could lay a theoretical foundation for the study of new and green methods for the management of root-knot nematode.

Materials and Methods

Vermicompost provided by Harbin Liangshun Biotechnology Development Company was obtained from fresh cow dung digested by earthworms. The tested tomato variety was Yingfen No. 8 susceptible to root-knot nematode. The nematode *Meloidogyne incognita* was cultured on a susceptible tomato seedlings in the greenhouses.

The infected roots and galls were separated and gently washed under running water. The mature egg masses were collected and sterilized in 0.5% NaClO for 3 min, and then flushed with sterilized water 3 times. The prepared egg masses were put in a small beaker with sterilized water for 4 days at 25°C. The freshly hatched second-stage juveniles (J2s) were collected every 24 hrs. The roots with many egg masses were selected, cut into small pieces of 0.5 to 1 cm, put them in a fresh-keeping box with an appropriate amount of 1% NaClO, and shook them for 5 min. The scum and solution were poured into a 280 to 500 mesh screen, and rinsed repeatedly with purified water. The eggs were collected on a 500 mesh screen, and the NaClO was washed off. The egg liquid was transferred into a small beaker, and the number of eggs per 100 µl was counted using a microscope (Liu 1995).

The vermicompost extract was made according to the method of Xu Dabing (Xu *et al*. 2009). The vermicompost was fully dried and crushed with a 20 mesh screen. Then, 20 g of vermicompost was placed into a 250 ml conical flask, and 100 ml of deionized water was added. It was sealed for 3 days at 25°C on a rocker. The oscillating turbid liquid was rocked evenly, first filtering out the larger residue with gauze, then filtering the obtained filtrate with 2 layers of filter paper 3 times, and finally filtering the filtrate into the vacuum filter funnel. When the filtrate was almost free of granular material, it was transferred to the cone bottle, containing the vermicompost extract. The vermicompost extract was diluted with sterilized water to concentrations of 10, 30, 50, 70 and 90%.

Three similar (fresh yellow) and identical egg masses were added to a sterilized flat 24-well cell culture plate. Then, 1 ml of different concentrations of vermicompost extract was added to the well with a pipette, and 1 ml of sterilized water treatment was used as the untreated control. Each treatment was replicated 3 times and placed in a 26°C incubator. The number of freshly hatched juveniles was counted after 7 days, and the relative inhibition ratio was calculated.

Two hundred µl of egg liquid (approximately 100 eggs) was added to a sterilized flat 24-well cell culture plate, and then 0.8 ml of different concentrations of vermicompost extract was added to each well with a pipette. The untreated control consisted of 0.8 ml of sterilized water. Each treatment was replicated 3 times and cultured in a 26°C incubator. The number of newly hatched juveniles and unhatched eggs was observed after 7 days, and the hatching rate was calculated.

One hundred second-stage juveniles (J2s) were added to a sterilized flat 24-well cell culture plate, and 0.8 ml of different concentrations of vermicompost extract was added to each hole with a pipette, 0.8 ml of sterilized water treatment was used as an untreated control. Each treatment was replicated 3 times and cultured in a 26°C incubator. The shape of J2s was observed with a stereoscopic microscope after 24, 48 and 72 hrs. If the J2s did not respond to the external vibration when the 24-well cell culture plate was lightly touched and still maintained a needle-like, stiff shape, they were considered to be knocked down. The NaOH stimulation method (Denilson *et al.*

2009) was used to determine whether the J2s were alive. The J2s were treated with 1% NaOH, after which the activities of the J2s were observed. When the body of a J2 remained static after treatment for 3 min, it was considered dead; the mortality rate of J2s was calculated after 72 hrs.

Tomato seedlings grew in the seedling tray and were transplanted when they grew 5 leaves. The soil was sieved through a 5 mm diameter mesh and then sterilized by heat. The total 1 kg mixture of vermicompost and soil with five different proportions (V/V) of 1 : 0 (T1), 3 : 1 (T2), 1 : 1 (T3), 1 : 3 (T4) and 0 : 1 (T5) was used as the medium for seedling growth. Tomato seedlings were transplanted to plastic pots, with one plant per pot, and were inoculated with 1000 J2s of *M. incognita*. All the treatments were replicated 5 times with random block arrangement and maintained for 35 days under greenhouse conditions. Upon termination of the experiment, the plants were carefully uprooted, and observations were recorded for the growth characteristics of the host plant and the number of root-knots and egg masses on the root. The relative control effect was then calculated according to Wang *et al*. (2015).

The data of this experiment were analysed by NPS software, and Duncan's new repolarization difference method was used for multiple comparisons.

Results and Discussion

The effect of vermicompost extract on the hatchability of egg masses is shown in Fig. 1. When the concentration of vermicompost extract was 10%, the hatchability of egg masses was inhibited, and the relative inhibition rate was 27.25%. As the concentration of the vermicompost extract increased, the relative inhibition rate increased as well, and the relative inhibition rate reached 71.63% when the concentration was 90%.

The effect of vermicompost extract on the hatchability of eggs is shown in Fig. 2. When the concentration of vermicompost extract was 10%, the hatchability of the *M. incognita* eggs decreased by 14.6%. When the concentration of the extract was 30%, the hatchability of the eggs decreased significantly up to 38.85%. As the concentration of vermicompost extract increased, the hatchability of eggs decreased. When the concentration of the extract was 90%, only 11.88% of eggs hatched. The hatchability of eggs under different concentrations was significantly different.

The knockdown rate of the J2s increased with increasing concentrations of vermicompost extract and treatment time. The knockdown rate of the J2s was significantly different at different concentrations of vermicompost extract. When the concentration was 10%, the knockdown rate of J2s was 16.35% after treatment for 24 and 48 hrs, whereas the knockdown rate was 17.35% for 72 hrs, which was 14% higher than that of the control. When the concentration was 50%, the knockdown rate was notably improved. After treatment for 24 hrs, the knockdown rate with 70 and 90% concentrations reached 59.65 and 77.88%, and after 72 hrs, it reached 68.27 and 88.46%, respectively (Fig. 3).

The mortality rate of the J2s increased with increasing concentrations of vermicompost extract. When the concentration of vermicompost extract was 10%, the mortality rate of the J2s was 18.27%, which was 15.39% higher than that of the control. When the concentration was 30%, the mortality of J2s was not significantly different from that of 10%. However, the mortality rate significantly increased to 52.88 and 75.96% when the concentration was 50 and 70%, respectively. The mortality rate reached 88.46% when the concentration was 90% (Fig. 4).

The control effects of vermicompost on tomato root-knot are provided in Table 1. The data suggested that vermicompost inhibited tomato root-knot disease, but the inhibitory effects did not increase with the increase in the proportion of vermicompost. The control effect of T2 (25% vermicompost) reached 36.30%, which was better than that of T3 (50% vermicompost). The number of galls was considerably reduced, and the control effect increased to 70 for T4 (75%

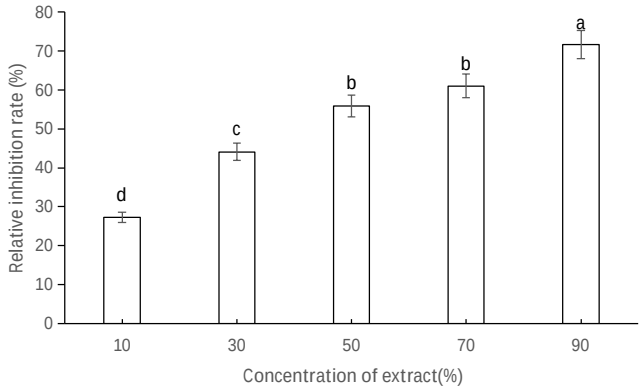

Fig. 1. Effect of vermicompost extract on the hatchability of *Meloidogyne incognita* egg masses.

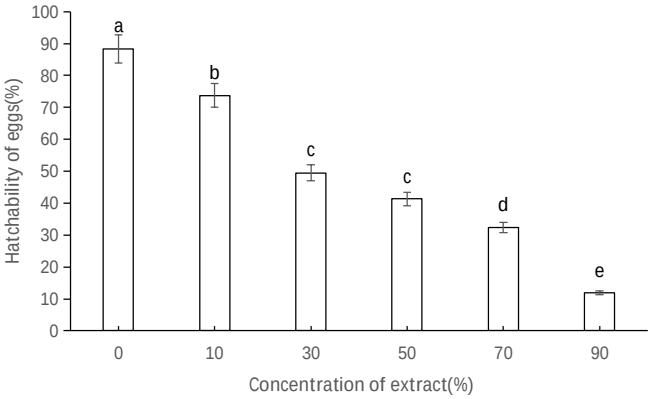

Fig. 2. Effect of vermicompost extract on the hatchability of *Meloidogyne incognita* eggs.

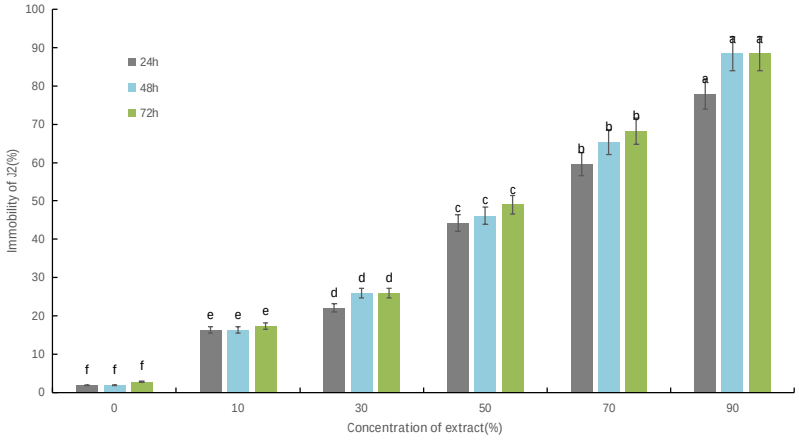

Fig. 3. Effect of vermicompost extract on the knockdown rate of *Meloidogyne incognita* (J2s).

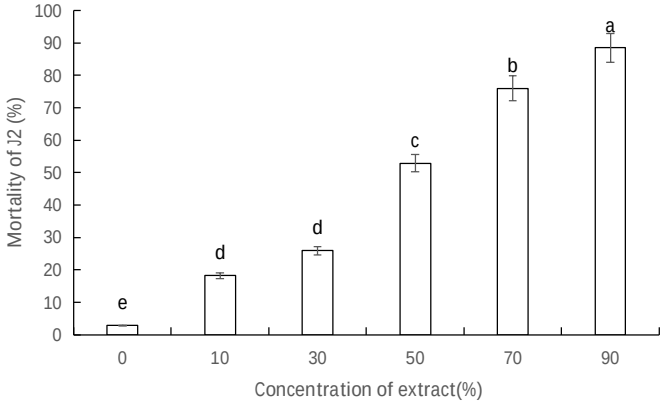

Fig. 4. Effect of vermicompost extract on the mortality rate of *Meloidogyne incognita* (J2s).

Table 1. Effect of vermicompost on the growth parameters of tomato and the number of root-knot symptom.

Treatment	Plant height (cm)	Fresh weight of aerial part (g)	Number of root-knot per plant	Control efficacy (%)
T1	59.00 ± 1.58 a	225.00 ± 3.54	104.67 ± 1.13 a	–
T2	61.33 ± 1.22 a	241.65 ± 1.54	66.67 ± 2.95 ab	36.30 b
T3	55.33 ± 2.18 ab	220.00 ± 3.16	76.00 ± 2.35 ab	27.39 a
T4	47.33 ± 1.84 bc	84.15 ± 1.25	25.67 ± 1.18 b	75.48 c
T5	43.00 ± 2.24 c	90.85 ± 1.17	29.00 ± 1.58 b	72.29 c

Air-dried soil : earthworm feces (v/v) were 1 : 0 (T1), 3 : 1 (T2), 1 : 1 (T3), 1 : 3 (T4), 0 : 1 (T5); lowercase letters in the same column indicated significant difference at $p < 0.05$ level.

vermicompost) and T5 (100% vermicompost). However, the tomato plants were shorter, at only 47.33 and 43 cm, respectively, and the fresh weight of the upper ground was also lower than that of other treatments, at only 84.15 and 90.85 g, respectively. With T2, the height of the tomato plant was 61.33 cm, and the fresh weight was 241.65 g, which was higher than that of T1.

This study demonstrated that vermicompost had great potential to decrease the amount of chemical pesticides and fertilizers on crops, which could reduce the costs of food production (Yurdagul 2010). Previous studies on solid vermicompost applications for the control of plant nematodes have been conducted, and the results demonstrated significant success in the suppression of plant parasitic nematodes (Arancon *et al.* 2002). Vermicompost fortification treatment resulted in reduced nematode infection on the host of cluster bean, and increased growth characteristics. It was confirmed that vermicompost was an excellent growth promoter (Kumar *et al*. 2011). Vermicompost decreased the number of galls on both resistant and susceptible tomato roots and increased the concentration of root defence metabolites and the expression of defence-related genes (Xiao *et al.* 2016). The results of the laboratory assays indicated that the extract of vermicompost could significantly inhibit the hatchability of egg masses and eggs and had obvious knockdown and lethal effects on the J2s. The effect increased with increasing concentration, which was the same as the inhibitory effect of Eupolyphaga frass on *Meloidogyne*

incognita. It is suggested that vermicompost extract could be applied to the cultivation matrix in the process of tomato production, and the specific concentration and dosage could be selected according to the physical and chemical properties and fertility of the soil to ensure normal growth of the plant. The application of the formula should control the root-knot nematode but should not affect the normal growth of tomatoes, which was considered an important problem. It was observed that vermicompost could induce systemic resistance in cucumber, which was related to the active substance in vermicompost; perhaps the microorganisms in vermicompost played an important role in the induced resistance (Hu *et al*. 2004). Proposed disease suppression mechanisms for controlling plant pathogens via application of conventional composts were defined as general or specific suppression mechanisms that include microbial antagonism, nutrient release, induced host resistance, and abiotic inhibitory factors of disease suppression (Yurdagul 2010). It was proven that there were two mechanisms of suppression, one dependent on systemic plant resistance and the other mediated by microbial competition, antibiosis and hyper-parasitism (Hoitink and Grebus 1977). It was suggested that the general suppression mechanism would be the more predominant case for vermicomposts because vermicomposting enhanced both biodiversity and the number of microorganisms (Edwards *et al*. 2004). The control effect of vermicompost and its extract on *Meloidogyne incognita* was investigated in this study. However, the control mechanisms for root-knot nematode have not been further explored. The present study will focus on analysing the species, activities and mechanisms of microorganisms in vermicompost that are antagonistic to *Meloidogyne incognita* in subsequent investigations.

Acknowledgements

The authors are grateful to the Natural Science Foundation of Heilongjiang Province (LH2019C034) for financial support for this research.

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(Manuscript received on 9 September, 2019; revised on 22 September, 2019)