

## Effects of Arugula Vermicompost on the Root-Knot Nematode (*Meloidogyne javanica*) and the Promotion of Resistance Genes in Tomato Plants

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Root-knot nematodes are the most important plant-parasitic nematodes worldwide. Many efforts have been made to find non-chemical, risk-free, and environmentally friendly methods for nematode control. In this study, the effects of compost and vermicompost of arugula (*Eruca sativa*) on *Meloidogyne javanica* were investigated in three glasshouse experiments. In addition, the expression of the defense-related genes *non-expressor of pathogenesis-related 1 (NPR1)* and *lipoxygenase 1 (LOXI)* was detected in tomato plants treated with vermicompost of arugula at 0, 2, 7, and 14 days after nematode inoculation. The result showed that the vermicompost of arugula significantly reduced the reproduction factor of the nematode by 54.4% to 70.5% in the three experiments and increased the dry weight of shoots of infected tomato plants. Gene expression analysis showed that *LOXI* expression increased on the second and seventh day after nematode inoculation, while *NPR1* expression decreased. The vermicompost of arugula showed stronger nematode inhibitory potential than the vermicompost of animal manure. The ver-

micompost of arugula is superior to arugula compost in suppressing the activity of *M. javanica* and reducing its impact. It manipulates the expression of resistance genes and could induce systemic resistance against root-knot nematodes.

**Keywords :** control, *Eruca sativa*, induced systemic resistance, plant-parasitic nematode, vermicompost

Root-knot nematodes (RKNs; *Meloidogyne* spp.) are the most important plant-parasitic nematodes (PPNs) worldwide. They are high on the list of plant pathogens affecting crop production (Sasser, 1977). Nematodes are traditionally controlled with chemical nematicides (Sikora et al., 2008), which are expensive and pose a high risk to the environment and human health (Dong and Zhang, 2006). Many attempts have been made to find suitable alternatives to chemical nematicides and several methods have been introduced (Kerry, 2000).

A variety of *Brassicaceae* species have been reported as hosts or associated with a wide range of economically important PPNs, including RKNs. On the other hand, they can reduce the destructive effects of the most harmful nematodes (Fourie et al., 2016). *Eruca sativa* Mill. (arugula, rocket) is a winter annual plant in the *Brassicaceae* family and is a poor host for *M. hapla*, *M. incognita*, and *M. javanica*. As a cover or trap crop, it has the potential to control RKNs (Curto et al., 2005; Daneel et al., 2018; Edwards and Ploeg, 2014; Melakeberhan et al., 2006). It is a source of glucosinolates that have suppressive effects against PPNs (Sarikamis et al., 2017). Compounds such as erucin, pentyl isothiocyanate, hexyl isothiocyanate, (E)-2-hexenal, 2-ethylfuran, and methyl thiocyanate present in *E. sativa*

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were toxic to *M. incognita* (Aissani et al., 2015). Growing arugula as a winter crop before sowing the susceptible main crop reduced damage by *M. hapla* and *M. arenaria* (Aydinli and Mennan, 2018; Melakeberhan et al., 2006). In addition, incorporation of above-ground parts of *E. sativa* cv. Nemat significantly reduced the population densities of *M. incognita* and *M. javanica* in tomato roots (Daneel et al., 2018). The use of arugula for soil amendment significantly reduced the number of *M. incognita* females and galls in tomato roots and second-stage juveniles (J2s) in soil (Ntalli et al., 2019).

RKNs are obligate sedentary parasites that manipulate plant defense mechanisms to establish permanent feeding sites, the multinucleate giant cells. Strong suppression of hormone pathways associated with plant defense, particularly salicylic acid (SA) and ethylene (ET) pathways, has been observed in giant cells and galls at the early stage of nematode infection (Kyndt et al., 2013). SA is an essential molecule of systemic acquired resistance (SAR) (An and Mou, 2011). Suppression of SA signaling in the plant helps in nematode infection (Molinari et al., 2014). The protein nonexpressor of pathogenesis-related 1 (NPR1) is a receptor of SA. The binding of SA to NPR1 promotes the expression of downstream defense genes (Wu et al., 2012; Zhang and Li, 2019). NPR1 is a major component of the complex network of regulatory interactions between the SA and jasmonic acid (JA)/ET responses, a positive regulator of the SAR pathway and the major regulator of plant defense signaling (Backer et al., 2019).

JA and its derivatives are involved in the induced systemic resistance (ISR) pathway (Ghasemi Pirbalouti et al., 2014). Lipoxygenases (LOXs) present in eukaryotes play an important role in JA biosynthesis and in plant response to various biotic and abiotic stresses, including nematodes. The JA pathway has been reported to play a key role in the immunity of rice roots to *M. graminicola*. Blocking its biosynthesis by the application of LOX inhibitors increases the infection and susceptibility of rice to the nematode (Nahar et al., 2011). On the other hand, artificially increasing the concentrations of JA impairs the ability of nematodes to suppress JA biosynthesis and signal transduction, significantly reducing the number of developing females (Kammerhofer et al., 2015). It was shown that the mutant tomato plants overexpressing JA had lower infection with *M. incognita* than the control plants (Wubie and Temesgen, 2019). Similarly, application of methyl-JA to *Arabidopsis* leaves reduced infection with *Heterodera schachtii*, while infection of two mutants deficient in JA biosynthesis *lox6* and *dde2* (*delayed dehiscence 2*) enhanced female development compared to wild type (Kammerhofer et al., 2015).

Lipoxygenase 1 (LOX1) synthesizes the JA in *Arabidopsis* during leaf senescence (Viswanath et al., 2020). The level of *LOX1* expression was down-regulated in tomato roots inoculated with the fungus *Trichoderma atroviride*, the RKN *M. javanica*, or both, 4 and 6 days after treatment (de Medeiros et al., 2017). On the other hand, a number of JA-, ET- and SA-biosynthetic or dependent genes were over-expressed 7 days after inoculation with *M. incognita*, and the nematode juveniles and eggs increased in the roots of maize *lox3-4* mutants (Gao et al., 2008).

Combining the nematicidal properties of arugula with those of vermicompost is likely to increase their efficacy. As far as we know, there is no report on the nematicidal activity of arugula vermicompost and its effects on plant response to nematode infection. Therefore, this study investigated the effects of compost and vermicompost of arugula on the RKN *M. javanica* in tomato roots under glasshouse conditions. In addition, the expression of defense-related genes *NPR1* and *LOX1* was analyzed in nematode-infected tomato plants treated with arugula vermicompost.

## Materials and Methods

**Collection and inoculum of *Meloidogyne javanica*.** Cucumber roots heavily infected with the RKN *M. javanica* were collected from an infested greenhouse. The single egg mass technique was used to grow the RKN on tomato roots (*Solanum lycopersicum* L. cv. Early Urbana). Specific primers for *M. javanica*, *M. incognita*, and *M. arenaria* were used to confirm morphological identification of the nematode species (Dong et al., 2001). The eggs of *M. javanica* were extracted from infected roots using sodium hypochlorite (Hussey and Barker, 1973).

**Preparation of compost and vermicompost.** Arugula seeds (local cultivar) were sown at the end of summer. The plants were harvested in spring at flowering time and used to make compost and vermicompost. To prepare arugula compost, the above-ground plant parts were cut into small pieces and 10 kg of the shredded plants were poured into a 20-liter plastic container with some holes at the bottom. The plant material was mixed every week with a garden fork to aerate it, stored for three months and watered as needed.

Arugula and common vermicomposts were prepared by adding red worm (*Eisenia fetida*) to the shredded plants and cow manure in separate containers. The mesh basket containing the vermicompost with  $30 \pm 5$  red worm was placed on top of the containers. The worms gradually penetrated and fed on the plant tissue and manure. After three

months, the vermicompost was sieved and used for the experiments.

**Chemical analysis of compost and vermicompost of arugula.** The chemical properties of the compost and vermicompost of arugula were determined by analyzing a sample of 200 g each. The pH was measured using a pH meter in an aqueous suspension ratio of 1:10 (w/v). Total nitrogen was determined by the Kjeldahl method (Bremner and Mulvaney, 1982), phosphorus (P) by the Vanadate/molybdate method (yellow method) (Chapman and Pratt, 1961). Cationic elements (Zn, Fe, Cu, Mn, Ca, and Mg) were measured using an atomic absorption spectrophotometer (AA-670, Shimadzu Scientific Instruments, Kyoto, Japan) (Hoenig, 2005).

**Glasshouse experiments.** Three experiments were conducted to investigate the effects of compost and vermicompost of arugula, and common vermicompost on nematode indices of *M. javanica* and growth parameters of infected tomato plants (cv. Early Urbana). In the first experiment, the effects of compost and vermicompost of arugula were compared with the common vermicompost in two trials. Then, the best treatment was selected and used in the second experiment, and its effect on the expression of defense-related genes *LOX1* and *NPR1* was evaluated in the third experiment.

#### Effects of arugula compost and vermicompost on *Meloidogyne javanica* and infected tomato plants.

**First trial:** tomato seeds (cv. Early Urbana) were sown in plastic pots (19 cm diameter) filled with 3 kg of pasteurized mixed soil (field soil and river sand in a ratio of 1:2; N = 0.10%, P = 7 mg/kg, K = 420 mg/kg; pH = 6.98). Based on soil analysis, 40 mg P/(kg soil)/(250 mg triple superphosphate (kg soil)) and 60 mg N/(kg soil)/(360 mg potassium nitrate (kg soil)) were thoroughly mixed with the soil before sowing. The treatments were arugula compost, arugula vermicompost, common vermicompost, and soil without organic matter as control. Sixty grams (2% of potting soil) of the compost or vermicompost was thoroughly mixed with the soil before sowing. Tomato plants in half of the treated pots were inoculated at the four-leaf stage by pouring a suspension containing about 6,000 *M. javanica* eggs (two eggs/gram of soil) into the soil at a depth of 5 cm around the plant roots. The experiment was laid out on a glasshouse bench in a completely randomized design with four replicates. The pots were observed daily and watered as needed. During the experiment, the average values of minimum and maximum air temperatures in the glasshouse

were 25°C and 30°C, respectively.

Sixty days after nematode inoculation, the plant in each pot was harvested and its shoots were dried in an oven at  $65 \pm 3^\circ\text{C}$  for 48 h and weighed. The J2s of RKN in 100 g of mixed soil of each pot were extracted by tray method (Whitehead and Hemming, 1965) and counted, and the number of J2s in the potting soil was calculated. In addition, the number of galls and egg masses in one gram of tomato roots in each pot was counted after staining with fuchsin acid (Coyne et al., 2007). Then the final population (Pf) and the reproduction factor (Rf) of *M. javanica* were calculated ( $Rf = Pf/Pi$ ).

**Second trial:** the second trial of the experiment was conducted similarly to the first, but there were eight replicates and the average values of the minimum and maximum air temperatures of the glasshouse were 26°C and 32°C, respectively.

#### The effect of arugula vermicompost on nematode activity.

The second experiment was conducted to confirm the effect of the best treatment from the first experiment. The vermicompost of arugula, which proved to be the most effective treatment against *M. javanica* in the first experiment, was used in the second experiment, while the soil without amendment served as control. Sixty grams of vermicompost of arugula was added to 3 kg of soil in each plastic pot and mixed thoroughly. Tomato seeds (cv. Early Urbana) were sown and the plants were inoculated at the four-leaf stage by pouring a suspension of about 6,000 *M. javanica* eggs (two eggs/gram of soil) into the soil at a depth of 5 cm around the roots of each plant. Glasshouse conditions were the same as in the first experiment. The experiment was laid out in a completely randomized design with four replicates. The plants were kept up to 60 days after inoculation, and then the dry weight of the shoots of the tomato plants, as well as the reproduction factor (Rf) and the final population of the nematode were determined.

#### Expression of defense-related genes *LOX1* and *NPR1*.

This experiment was conducted to evaluate the expression of *LOX1* and *NPR1* in tomato plants 0, 2, 7, and 14 days after nematode inoculation. The treatments were soil with arugula vermicompost and soil without organic material as control. The experiment was conducted in 1 kg plastic pots (12 cm in diameter) filled with pasteurized mixed soil (field soil and river sand in a ratio of 1:2). Vermicompost of arugula (20 g; 2% of soil pot) was mixed with the pasteurized soil before sowing. Then tomato seeds (cv. Early Urbana) were sown in each pot. The experiment was laid out on the glasshouse bench in a completely randomized design

with three replicates for each time point. At the four-leaf stage, the roots of tomato plants were inoculated with a suspension of approximately 2,000 *M. javanica* eggs. Pots were observed daily, watered as needed, and maintained in the glasshouse at 25–30°C. To evaluate the expression of defense genes, leaf samples were collected from each treatment 0, 2, 7, and 14 days after nematode inoculation and stored at –80°C, and their dry weight was measured at the same time points.

Stored leaf samples were ground in liquid nitrogen using a mortar and pestle. RNA was extracted from the leaves of each plant. Total RNA was extracted using Topazol plus Top RNA purification kit (Topazgene, Karaj, Iran) according to the manufacturer's protocol and used for first-strand cDNA synthesis with oligo dT primers and the M-MuLV reverse Transcriptase cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA) from DNase-treated (Thermo Scientific) total RNA extracts. PCR reactions were performed using Bioneer Real-Time Quantitative PCR (Daejeon, Korea) and Top 2× Real-Time PCR Master Mix (Topazgene) in a total volume of 12.5 µl with reactions containing 20 ng cDNA and 10 µM of each primer. The cDNA from three biological replicates and two technical replicates was used and analyzed for each sample. All reaction conditions were 94°C for 2 min, 40 cycles of 94°C for 20 s, 57°C for the 20 s, and 72°C for 20 s. Threshold cycle (Ct) values were calculated and transcript abundance was calculated in Microsoft Excel from Ct values and normalized to  $\beta$ -*Tubulin* gene signal. Relative expression values were calculated using  $2^{-\Delta\Delta CT}$  (Livak and Schmittgen, 2001). Expression of the desired genes in tomato plants treated with arugula vermicompost and *M. javanica*, and in control plants (sown in soil with *M. Javanica*) was measured at 0, 2, 7, and 14 days after nematode inoculation. The *LOXI* and *NPRI* genes were selected as representative defense pathway SA (*NPRI*) and JA (*LOXI*). The primers and their corresponding sequences are listed in Table 1 (de Medeiros et al., 2017; Rubio et al., 2014).

**Statistical analysis.** SAS statistical software was used for data analysis (SAS ver. 9.1, SAS Institute Inc., Cary, NC, USA). The analysis of parametric indices (plant indices) was performed using the Proc ANOVA method and the

**Table 1.** Sequences of the primer sets used for quantitative real-time PCR (*LOXI*, *NPRI*, and  $\beta$ -*Tubulin* genes)

Gene name	Primer sequence
<i>LOX</i> For	GCCTCTCTTCTTGATGGA
<i>LOX</i> Rev	GTAGTGAGCCACTTCTCCAA
<i>NPRI</i> For	GGCGGACAACCTGCGTCAAC
<i>NPRI</i> Rev	GCTCTCGTGGTCTGGCAAGC
<i>Tubulin</i> For	GAATATCAACAATACCAGGATGC
<i>Tubulin</i> Rev	AGGATTGGTATTGATCATCAGCA

PCR, polymerase chain reaction; *LOXI*, lipoxygenase 1; *NPRI*, nonexpressor of pathogenesis-related 1.

non-parametric indices (nematode indices) were performed using the Friedman rank test method. Mean values were compared using a posthoc Tukey honestly significant difference test ( $P < 0.05$ ). In addition, the means of the treatments in the second experiment were compared using a *t*-test.

## Results

**Chemical properties of compost and vermicompost of arugula.** The chemical properties of compost and vermicompost of arugula are shown in Table 2. The results show that the pH of vermicompost of arugula was lower but the total nitrogen, P, Fe, and Mg contents were higher than that of compost.

**Effects of compost and vermicompost of arugula on the growth of *Meloidogyne javanica* and infected tomato plants (first and second experiment).** In the first and second trials of the first experiment, both the common and arugula vermicomposts significantly ( $P < 0.05$ ) increased the dry weight of the healthy and infected tomato plants, but the arugula vermicompost showed the greatest effect on the dry infected plants (Tables 3 and 4). The arugula vermicompost showed the same effect in the second experiment, increased the dry weight of the infected tomato plants by more than 2.8 times compared to the control (Table 5).

The results of the first experiment showed that the treatments had different effects on nematode indices. In the first trial, all treatments significantly reduced the number of egg

**Table 2.** Chemical properties of compost and vermicompost of arugula

Type of compost	pH	Total N (%)	P (mg/kg)	Zn (g/kg)	Fe (g/kg)	Cu (g/kg)	Mg (mg/kg)	Ca (mg/kg)	Mn (g/kg)
Arugula compost	8.3	2.76	5,250	67.9	191.8	61.5	11,732	3,736	242.5
Arugula vermicompost	7.1	3.48	7,500	58.8	219.8	44.9	12,657	3,389	199.8

**Table 3.** Effects of common vermicompost, compost and vermicompost of arugula on shoot dry weight and nematode indices of *Meloidogyne javanica* in infected tomato roots, under greenhouse conditions (first trial of the first experiment)

Treatment	Shoot dry weight (g)		Egg masses/ root	Eggs/ root	J2s/ pot soil	Final population <sup>a</sup>	Reproduction factor (Rf)	Rf reduction (%) <sup>b</sup>
	With nematode	Without nematode						
Arugula compost	1.1 d	3.3 c	67 b	22,075 bc	4,300 c	26,471 bc	6.6 bc	65.2
Arugula vermicompost	5.3 b	8.6 a	66 b	18,706 c	3,650 c	22,449 c	5.6 c	70.5
Ordinary vermicompost	2.7 c	4.8 b	75 b	33,325 ab	8,140 b	41,573 ab	10.3 ab	45.3
Control (without organic matter)	1 d	2.6 c	138 a	60,779 a	15,200 a	76,141 a	19.0 a	-

Data are means of four replicates. Means within a column with the same letter are not significantly different ( $P < 0.05$ ).

<sup>a</sup>Eggs + J2s + females.

<sup>b</sup>Reduction percent of nematode reproduction factor compared to the control.

**Table 4.** Effects of common vermicompost, compost and vermicompost of arugula on shoot dry weight and nematode indices of *Meloidogyne javanica* in infected tomato roots, under greenhouse conditions (second trial of the first experiment)

Treatment	Shoot dry weight (g)		Egg masses/ root	Eggs/ egg mass	Eggs/ root	J2s/ pot soil	Final population <sup>a</sup>	Reproduction factor (Rf)	Rf reduction (%) <sup>b</sup>
	With nematode	Without nematode							
Arugula compost	18 bcd	18.62 bc	609 b	80.25 a	33,509 a	3,984 b	39,525 a	6.58 a	32.54
Arugula vermicompost	22.12 ab	23 a	918 ab	30.5 b	19,531 b	2,531 c	24,510 b	4.08 b	58.17
Ordinary vermicompost	15.87 cd	19.1 abc	1,180 a	41.88 ab	41,861 a	5,203 b	49,608 a	8.26 a	15.33
Control (without organic matter)	10.4e	14.41 d	1,399 a	46 ab	46,991 a	9,375 a	58,596 a	9.76 a	-

Data are means of eight replicates. Means within a column with the same letter are not significantly different ( $P \leq 0.05$ ).

<sup>a</sup>Eggs + J2s + females.

<sup>b</sup>Reduction percent of nematode reproduction factor compared to the control.

masses and J2s in the soil. Vermicompost and compost of arugula reduced eggs in the root system and the final population and reproduction factor of *M. javanica*, but the number of galls/root and eggs/egg mass were similar to the control in all treatments (Table 3). In the second trial, the arugula compost significantly reduced the number of egg masses on the root system, and the arugula vermicompost reduced the number of eggs in the egg masses and roots. All treatments significantly reduced the number of J2s in the soil. Among them, the arugula vermicompost was the most effective and reduced the reproduction factor by 70.5 and 58.17% in the first and second trials, respectively,

compared to the control (Tables 3 and 4). In the second experiment also, the arugula vermicompost reduced the reproduction factor by 54.36% (Table 5).

**Expression of defense genes (third experiment).** The expression of *NPR1* and *LOX1* genes in tomato plants was studied to better understand the response of plants to RKN infection and treatment with arugula vermicompost. In this experiment, arugula vermicompost increased the shoot dry weight of infected plants on all days studied (Fig. 1), but it was higher on days 7 and 14 (Fig. 2).

Gene expression in the tomato plants treated with aru-

**Table 5.** Effects of vermicompost of arugula on nematode indices and growth parameters of tomato plants infected with *Meloidogyne javanica* (second experiment)

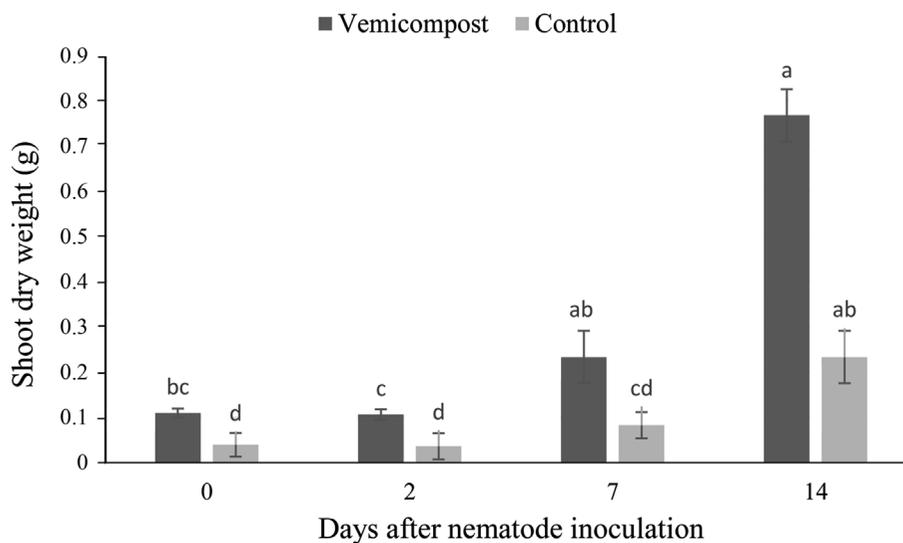
Treatment	Shoot dry weight (g)	Final population	Reproduction factor (Rf) <sup>a</sup>	Rf reduction (%) <sup>b</sup>
Arugula vermicompost	9.5**	49,947*	8.3*	54.36
Control (without organic matter)	3.3	109,447	18.2	-

Data are means of four replicates.

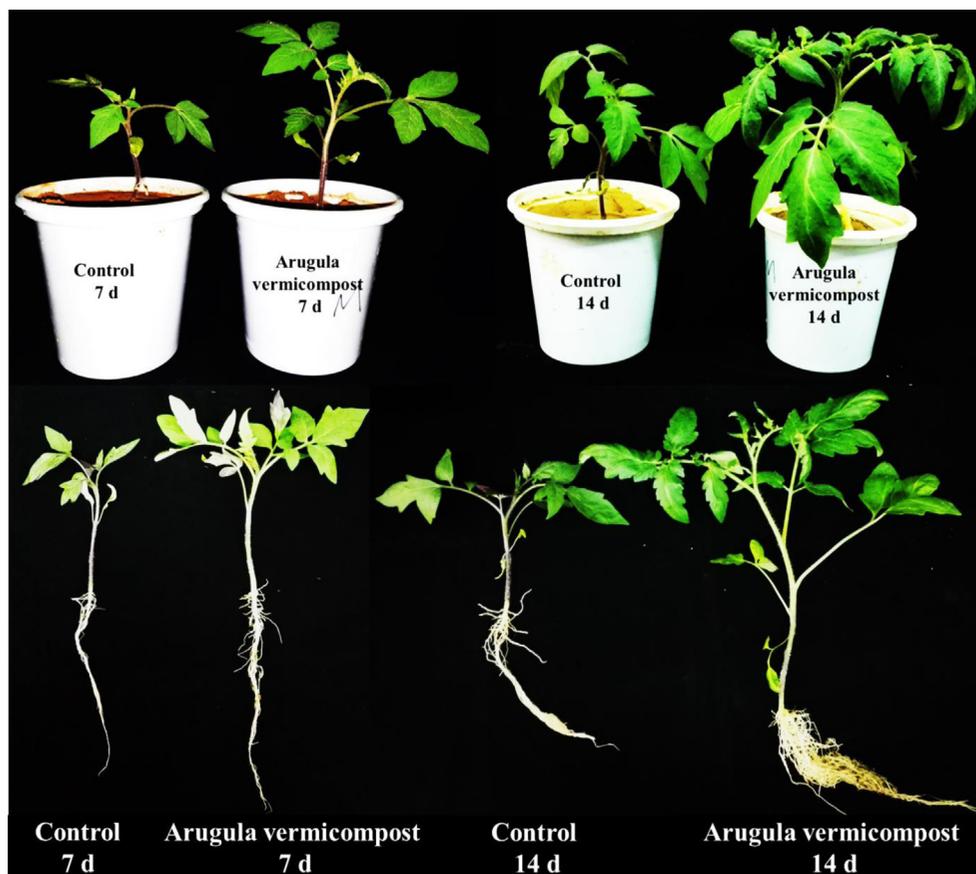
\*, \*\* show significant difference from control at  $P < 0.05$  and  $P < 0.01$  respectively as determined by *t*-test.

<sup>a</sup>Eggs + J2s + females.

<sup>b</sup>Reduction percentage of nematode reproduction factor compared to the control.



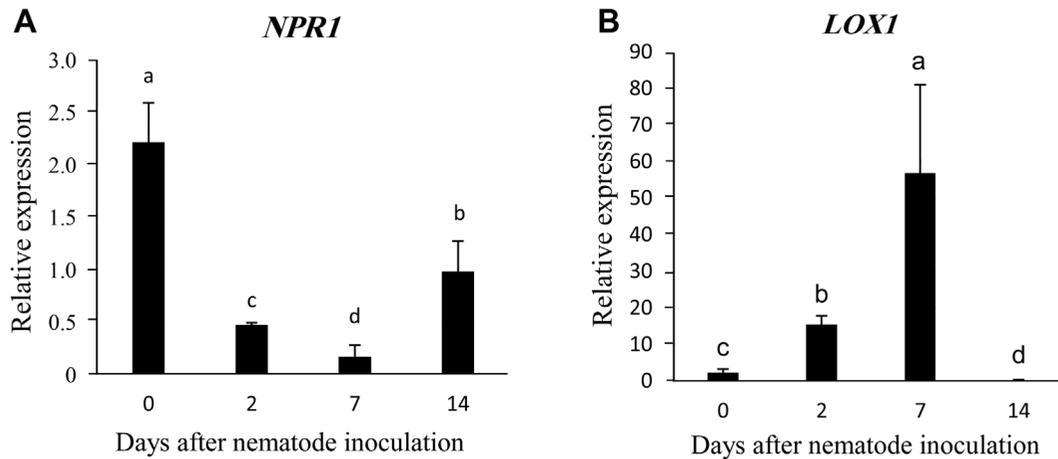
**Fig. 1.** Effect of arugula vermicompost on shoot dry weights of tomato plants 0, 2, 7, and 14 days after inoculation with *Meloidogyne javanica*. Data are the means of three replicates. Bars with the same letters are not significantly different ( $P < 0.05$ ).



**Fig. 2.** Effect of arugula vermicompost on growth of tomato plants 7 and 14 days after inoculation with *Meloidogyne javanica*.

gula vermicompost and control plants is shown in Fig. 3. The results showed that the expression of *NPRI* and *LOXI* genes were almost the same at the beginning of nematode

inoculation, but later changed differently. The expression of *NPRI* in the treated plants was down-regulated at 2 and 7 days, and then increased slightly 14 days after inoculation



**Fig. 3.** Relative expression of *nonexpressor of pathogenesis-related 1* (*NPR1*) (A) and *lipoxygenase 1* (*LOXI*) (B) genes in leaves of tomato plants treated with arugula vermicompost at 0, 2, 7, and 14 days after inoculation with *Meloidogyne javanica*. Expression of the desired genes in the plants was measured relative to the control (plants treated with *M. javanica* only; without vermicompost) ( $2^{-\Delta\Delta CT}$  method). Data are means of three replicates. Bars with the same letters are not significantly different ( $P < 0.05$ ).

(Fig. 3A). On the other hand, the expression of *LOXI* was increased at 2 and 7 days, and then decreased significantly at 14 days (Fig. 3B).

## Discussion

The present study showed the inhibitory effect of arugula vermicompost against RKN. Arugula vermicompost was superior to arugula compost and common vermicompost in reducing the population of *M. javanica*. Two experiments confirmed that arugula vermicompost was more effective in increasing the shoot dry weight of nematode-infected plants and reducing the final population and reproduction factor of *M. javanica*. The growth of tomato plants in the second trial of the first experiment was better than the first. The first experiment was conducted in winter and the second in spring with longer days. Previous studies reported that vermicompost suppressed the population of PPNs in a field experiment (Arancon et al., 2002). Application of vermicompost in a pot experiment against *Meloidogyne* spp. reduced the number of galls and improved relative control efficacy (Liu et al., 2019; Rostami et al., 2014). Our results suggest that the chemical properties of vermicompost of arugula differ from those of its compost and that earthworms improve the nematicidal properties of arugula. The vermicomposting process increases the levels of N, P, K, Ca, and Mg in the worm manure, while decreasing the C:N ratio, pH, and total organic carbon (Raphael and Velmourougan, 2011). Earthworm activity on organic material alters the chemical, physical and biological properties of these materials. Garg et al. (2006) recorded an increase EC,

which could be related to the production of ions and minerals during vermicomposting. Mineralization of N and P, decomposition of organic matter and  $CO_2$  production lead to a decrease in pH in vermicompost (Pathma and Sakthivel, 2012). The N content in worm manure increases due to the mucus, hormones, and enzymes of earthworms (Tripathi and Bhardwaj, 2004). Carbon loss is higher than N loss due to  $CO_2$  biooxidation during decomposition of the materials (Lazcano et al., 2008). The increase in total P is probably related to the phosphatase activity of the microorganisms in the vermicomposting process. In addition, vermicomposting causes a significant increase in  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $K^{2+}$  in the decomposed material (Pathma and Sakthivel, 2012).

The vermicompost is effective in improving plant growth and resistance to agricultural pests. It increases the size of the microbial population in the soil, adds various nutrients including macronutrients, micronutrients and humus, and improves the physical properties of the soil (Adhikary, 2012; Tognetti et al., 2005). In addition, vermicompost is rich in microbial activity that can suppress nematode infection. It includes fungi such as *Trichoderma* spp., chitinolytic bacteria, *Pseudomonas* spp., and predatory mites (Edwards et al., 2011; Pathma and Sakthivel, 2012). Microbial activity induces systemic resistance (ISR) to RKNs (Adam et al., 2014; Siddiqui and Shaikat, 2002).

Arugula plants belong to the family *Brassicaceae*. The effectiveness of some plant species of this family in reducing damage by RKNs is due to the non-glucosinolate and glucosinolate compounds, which are toxic to nematodes (Matthiessen and Kirkegaard, 2006). The oil of arugula inhibited the root-knot development and reduced the nema-

tode population (Akhtar and Mahmood, 1993). Isothiocyanates are prominent compounds that have nematocidal activity and are derived from glucosinolates (Ntalli and Caboni, 2017). Leaf tissue of *Eruca* species contains glucosativin (4-mercaptobutyl), glucoerucin (4-methylthiobutyl), and glucoraphanin (4-methylsulfinylbutyl), which are kinds of glucosinolates (Pasini et al., 2011; Villatoro-Pulido et al., 2013), could suppress RKNs.

Vermicompost treatment caused the highest expression of polyphenol oxidase D (*PPOD*) and flavonol synthase (*FLS*) genes, and increased the content of phenols and flavonoids in the roots of tomato plants inoculated with *M. incognita*. The *PPOD* gene, which plays a role in polyphenol metabolism, protects plants from pathogens and environmental stress (Xiao et al., 2016). A number of plant growth-promoting rhizobacteria (PGPR) such as *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Azotobacter* in vermicompost induce plant resistance (Gopinath and Prakash, 2014; Hoitink and Grebus, 1997). The physicochemical properties of vermicompost have led to its introduction as a suitable substrate for plant growth (Singh et al., 2020). Moreover, earthworms have the potential to stimulate the root growth of an *Arabidopsis thaliana* mutant with impaired auxin transport. Regulation of gene expression in the presence of earthworms is known to be responsive to biotic and abiotic stresses or the application of exogenous hormones, and has strong analogies with systemic resistance induced by signaling molecules released by PGPR and elicitors by non-virulent pathogens (Puga-Freitas et al., 2012).

In our study, plants treated with arugula vermicompost and *M. javanica* showed increased expression of *LOXI*. It has been suggested that LOXs may play a role in the interaction between pea root-*Heterodera goettingiana* and in resistance mechanisms (Leone et al., 2001). *LOXI* encodes LOX, which is probably the first enzyme to activate JA synthesis (Melan et al., 1993). LOXs are involved in wound response and pest resistance in plants and increase volatile compounds. *LOX* products such as hexanal have a negative effect on plant pathogens (Hildebrand, 1989). *Pseudomonas putida* induced resistance of tomato plants to *Botrytis cinerea* by stimulating the LOX pathway (Akram et al., 2008). In agreement with the present study, it was shown that the expression of *LOX3* in maize gradually increased and peaked 7 days after inoculation with the RKN *M. incognita*, indicating that *LOX3* is required for normal plant development (Gao et al., 2008). Considering the increase in *LOXI* expression in the present study and the role of *LOXI* in the defense system JA, it can be concluded that vermicompost of arugula may play a role in the induction

of ISR. Vermicompost is rich in PGPRs and several reports suggest that they induce systemic resistance after infestation by PPNs (Burkett-Cadena et al., 2008; Ramamoorthy et al., 2001).

Expression of *NPRI*, which has been evaluated as a representative of the SA pathway, was decreased in infected tomato plants treated with arugula vermicompost. *NPRI* is the major protein of the SA signaling pathway. *NPRI* expressed in some cotton lines exhibits resistance to various fungal, bacterial, and nematode pathogens (Parkhi et al., 2010). This gene regulates the SA defense pathway against *H. schachtii* (Wubben et al., 2008). Expression of *AtNPRI* increased the resistance of transgenic tobacco plants to the RKN *M. incognita*, which was associated with a reduction in the number of galls and egg-masses numbers in the transgenic plant compared to the wild type (Priya et al., 2011). Downregulation of *NPRI* showed that vermicompost of arugula did have no effect on the SA pathway. Our result is consistent with the use of vermicompost tea against *M. incognita* on cucumber. It showed that the nematode penetration into the plant roots treated by vermicompost tea was lower than that in the control. Moreover, the expression of defense-related genes such as *CHIT-1*, *PAL-1*, and *LOXI* was up-regulated, while the expression of *PR-1* decreased (Mishra et al., 2018).

The relative expression of both genes in this study was almost the same before nematode incubation, but the comparison of *LOXI* and *NPRI* expression at other time points after nematode incubation showed that the increased expression of one gene coincided with the decreased expression of the other gene. Antagonism between the ET/JA- and the SA-defense pathways causes mutual inhibition, demonstrating crosstalk between SA and the ET/JA signaling pathway. JA-biosynthesis genes are repressed by SA, and activation of the ET/JA-pathway also represses the SA-response (Li et al., 2019).

In conclusion, vermicompost of arugula (*Eruca sativa*) is superior to arugula compost in suppressing *Meloidogyne javanica* activity and reducing its impact. It manipulates the expression of resistance genes and could induce ISR against RKNs. Arugula is a suitable earthworm food to produce suppressive vermicompost for nematode suppression.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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The authors declare that the present article is part of the result of the first author's doctoral dissertation project approved by the Vice-Chancellor of Education and Post-graduate Studies, Shiraz University. Furthermore, they declare that all relevant ethical issues have been taken into consideration in conducting the research and writing the manuscript.

## References

- Adam, M., Heuer, H. and Hallmann, J. 2014. Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. *PLoS ONE* 9:e90402.
- Adhikary, S. 2012. Vermicompost, the story of organic gold: a review. *Agric. Sci.* 3:905-917.
- Aissani, N., Urgeghe, P. P., Oplos, C., Saba, M., Tocco, G., Petroitto, G. L., Eloh, K., Menkissoglu-Spiroudi, U., Ntalli, N. and Caboni, P. 2015. Nematicidal activity of the volatilome of *Eruca sativa* on *Meloidogyne incognita*. *J. Agric. Food Chem.* 63:6120-6125.
- Akhtar, M. and Mahmood, I. 1993. Control of plant-parasitic nematodes with "Nimin" and some plant oils by bare-root dip treatment. *Nematol. Mediterr.* 21:89-92.
- Akram, A., Ongena, M., Duby, F., Dommes, J. and Thonart, P. 2008. Systemic resistance and lipoxygenase-related defence response induced in tomato by *Pseudomonas putida* strain BTP1. *BMC Plant Biol.* 8:113.
- An, C. and Mou, Z. 2011. Salicylic acid and its function in plant immunity. *J. Integr. Plant Biol.* 53:412-428.
- Arancon, N. Q., Edwards, C. A., Lee, S. S. and Yardim, E. 2002. Management of plant parasitic nematode populations by use of vermicomposts. In: *Proceedings of brighton crop protection conference-pests and diseases*, pp. 705-710. British Crop Protection Council, Farnham, UK.
- Aydinli, G. and Mennan, S. 2018. Biofumigation studies by using *Raphanus sativus* and *Eruca sativa* as a winter cycle crops to control root-knot nematodes. *Braz. Arch. Biol. Technol.* 61:e18180249.
- Backer, R., Naidoo, S. and van den Berg, N. 2019. The non-expressor of pathogenesis-related genes 1 (NPR1) and related family: mechanistic insights in plant disease resistance. *Front. Plant Sci.* 10:102.
- Bremner, J. M. and Mulvaney, C. S. 1982. Nitrogen-total. In: *Methods of soil analysis, Part 2: chemical and microbiological properties*, eds. by A. L. Page, R. H. Miller and D. R. Keeney, pp. 595-624. American Society of Agronomy, Madison, WI, USA.
- Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K. S., van Santen, E. and Kloepper, J. W. 2008. Suppressiveness of root-knot nematodes mediated by rhizobacteria. *Biol. Control* 47:55-59.
- Chapman, H. D. and Pratt, P. F. 1961. *Methods of analysis for soils, plants and waters*. University of California, Berkeley, CA, USA. 309 pp.
- Coyne, D. L., Nicol, J. M. and Claudius-Cole, B. 2007. *Practical plant nematology: a field and laboratory guide*. International Institute of Tropical Agriculture, Cotonou, Benin. 91 pp.
- Curto, G., Dallavalle, E. and Lazzeri, L. 2005. Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinolate content. *Nematology* 7:203-212.
- Daneel, M., Engelbrecht, E., Fourie, H. and Ahuja, P. 2018. The host status of Brassicaceae to *Meloidogyne* and their effects as cover and biofumigant crops on root-knot nematode populations associated with potato and tomato under South African field conditions. *Crop Prot.* 110:198-206.
- de Medeiros, H. A., de Araújo Filho, J. V., de Freitas, L. G., Castillo, P., Rubio, M. B., Hermosa, R. and Monte, E. 2017. Tomato progeny inherit resistance to the nematode *Meloidogyne javanica* linked to plant growth induced by the biocontrol fungus *Trichoderma atroviride*. *Sci. Rep.* 7:40216.
- Dong, K., Dean, R. A., Fortnum, B. A. and Lewis, S. A. 2001. Development of PCR primers to identify species of root-knot nematodes: *Meloidogyne arenaria*, *M. hapla*, *M. incognita* and *M. javanica*. *Nematropica* 31:271-280.
- Dong, L. Q. and Zhang, K. Q. 2006. Microbial control of plant-parasitic nematodes: a five-party interaction. *Plant Soil* 288:31-45.
- Edwards, C. A., Arancon, N. Q. and Sherman, R. L. 2011. *Vermiculture technology: earthworms, organic wastes, and environmental management*. CRC Press, Boca Raton, FL, USA. 624 pp.
- Edwards, S. and Ploeg, A. 2014. Evaluation of 31 potential biofumigant brassicaceous plants as hosts for three *Meloidogyne* species. *J. Nematol.* 46:287-295.
- Fourie, H., Ahuja, P., Lammers, J. and Daneel, M. 2016. Brassicaceae-based management strategies as an alternative to combat nematode pests: a synopsis. *Crop Prot.* 80:21-41.
- Gao, X., Starr, J., Göbel, C., Engelberth, J., Feussner, I., Tumlinson, J. and Kolomiets, M. 2008. Maize 9-lipoxygenase ZmLOX3 controls development, root-specific expression of defense genes, and resistance to root-knot nematodes. *Mol. Plant-Microbe Interact.* 21:98-109.
- Garg, P., Gupta, A. and Satya, S. 2006. Vermicomposting of different types of waste using *Eisenia foetida*: a comparative study. *Bioresour. Technol.* 97:391-395.
- Ghasemi Pirbalouti, A., Sajjadi, S. E. and Parang, K. 2014. A review (research and patents) on jasmonic acid and its derivatives. *Arch. Pharm.* 347:229-239.
- Gopinath, R. and Prakash, M. 2014. Isolation of plant growth promoting rhizobacteria (PGPR) from vermicompost and ef-

- fect on growth of green gram (*Vigna radiata* L.). *Int. J. Curr. Microbiol. Appl. Sci.* 3:1072-1081.
- Hildebrand, D. F. 1989. Lipoxxygenases. *Physiol. Plant.* 76:249-253.
- Hoening, M. 2005. Sample dissolution for elemental analysis: dry ashing. In: *Encyclopedia of analytical science*, 2nd ed., eds. by P. Worsfold, A. Townshend and C. Poole, pp. 131-136. Elsevier Academic Press, Amsterdam, Netherlands.
- Hoitink, H. A. J. and Grebus, M. E. 1997. Composts and the control of plant diseases. In: *Humic substances, peats and sludges: health and environmental aspects*, eds. by M. H. B. Hayes and W. S. Wilson, pp. 359-366. The Royal Society of Chemistry, Cambridge, UK.
- Hussey, R. S. and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025-1028.
- Kammerhofer, N., Radakovic, Z., Regis, J. M. A., Dobrev, P., Vankova, R., Grundler, F. M. W., Siddique, S., Hofmann, J. and Wieczorek, K. 2015. Role of stress-related hormones in plant defence during early infection of the cyst nematode *Heterodera schachtii* in Arabidopsis. *New Phytol.* 207:778-789.
- Kerry, B. R. 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annu. Rev. Phytopathol.* 38:423-441.
- Kyndt, T., Vieira, P., Gheysen, G. and de Almeida-Engler, J. 2013. Nematode feeding sites: unique organs in plant roots. *Planta* 238:807-818.
- Lazcano, C., Gómez-Brandón, M. and Domínguez, J. 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* 72:1013-1019.
- Leone, A., Melillo, M. T. and Bleve-Zacheo, T. 2001. Lipoxxygenase in pea roots subjected to biotic stress. *Plant Sci.* 161:703-717.
- Li, N., Han, X., Feng, D., Yuan, D. and Huang, L.-J. 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: do we understand what they are whispering? *Int. J. Mol. Sci.* 20:671.
- Liu, D., Han, W., Zhang, Y. and Jiang, Y. 2019. Evaluation of vermicompost and extracts on tomato root-knot nematode. *Bangladesh J. Bot.* 48:845-851.
- Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta CT}$  method. *Methods* 25:402-408.
- Matthiessen, J. N. and Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Crit. Rev. Plant Sci.* 25:235-265.
- Melakeberhan, H., Xu, A., Kravchenko, A., Mennan, S. and Riga, E. 2006. Potential use of arugula (*Eruca sativa* L.) as a trap crop for *Meloidogyne hapla*. *Nematology* 8:793-799.
- Melan, M. A., Dong, X., Endara, M. E., Davis, K. R., Ausubel, F. M. and Peterman, T. K. 1993. An Arabidopsis thaliana lipoxxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. *Plant Physiol.* 101:441-450.
- Mishra, S., Wang, K.-H., Sipes, B. S. and Tian, M. 2018. Induction of host-plant resistance in cucumber by vermicompost tea against root-knot nematode. *Nematropica* 48:164-171.
- Molinari, S., Fanelli, E. and Leonetti, P. 2014. Expression of tomato salicylic acid (SA)-responsive pathogenesis-related genes in *Mi-1*-mediated and SA-induced resistance to root-knot nematodes. *Mol. Plant Pathol.* 15:255-264.
- Nahar, K., Kyndt, T., De Vleeschauwer, D., Höfte, M. and Gheysen, G. 2011. The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. *Plant Physiol.* 157:305-316.
- Ntalli, N. and Caboni, P. 2017. A review of isothiocyanates biofumigation activity on plant parasitic nematodes. *Phytochem. Rev.* 16:827-834.
- Ntalli, N., Zioga, D., Argyropoulou, D. M., Papatheodorou, M. E., Menkissoglu-Spiroudi, U. and Monokrousos, N. 2019. Anise, parsley and rocket as nematicidal soil amendments and their impact on non-target soil organisms. *Appl. Soil Ecol.* 143:17-25.
- Parkhi, V., Kumar, V., Campbell, L. M., Bell, A. A., Shah, J. and Rathore, K. S. 2010. Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing Arabidopsis *NPR1*. *Transgenic Res.* 19:959-975.
- Pasini, F., Verardo, V., Cerretani, L., Caboni, M. F. and D'Antuono, L. F. 2011. Rocket salad (*Diplotaxis* and *Eruca* spp.) sensory analysis and relation with glucosinolate and phenolic content. *J. Sci. Food Agric.* 91:2858-2864.
- Pathma, J. and Sakthivel, N. 2012. Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus* 1:26.
- Priya, D. B., Somasekhar, N., Prasad, J. S. and Kirti, P. B. 2011. Transgenic tobacco plants constitutively expressing Arabidopsis *NPR1* show enhanced resistance to root-knot nematode, *Meloidogyne incognita*. *BMC Res. Notes* 4:231.
- Puga-Freitas, R., Barot, S., Taconnat, L., Renou, J.-P. and Blouin, M. 2012. Signal molecules mediate the impact of the earthworm *Aporrectodea caliginosa* on growth, development and defence of the plant *Arabidopsis thaliana*. *PLoS ONE* 7:e49504.
- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot.* 20:1-11.
- Raphael, K. and Velmourougan, K. 2011. Chemical and microbiological changes during vermicomposting of coffee pulp using exotic (*Eudrilus eugeniae*) and native earthworm (*Perionyx ceylanensis*) species. *Biodegradation* 22:497-507.
- Rostami, M., Olia, M. and Arabi, M. 2014. Evaluation of the effects of earthworm *Eisenia fetida*-based products on the pathogenicity of root-knot nematode (*Meloidogyne javanica*) infecting cucumber. *Int. J. Recycl. Org. Waste Agric.* 3:58.
- Rubio, M. B., Quijada, N. M., Pérez, E., Domínguez, S., Monte, E. and Hermosa, R. 2014. Identifying beneficial qualities of

- Trichoderma parareesei* for plants. *Appl. Environ. Microbiol.* 80:1864-1873.
- Sarikamis, G., Aydınli, G. and Mennan, S. 2017. Glucosinolates in some brassica species as sources of bioactive compounds against root knot nematodes. *Int. J. Adv. Res.* 5:271-278.
- Sasser, J. N. 1977. Worldwide dissemination and importance of the root-knot nematodes, *Meloidogyne* spp. *J. Nematol.* 9:26-29
- Siddiqui, I. A. and Shaukat, S. S. 2002. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. *J. Phytopathol.* 150:469-473.
- Sikora, R. A., Pocasangre, L., zum Felde, A., Niere, B., Vu, T. T. and Dababat, A. A. 2008. Mutualistic endophytic fungi and *in-planta* suppressiveness to plant parasitic nematodes. *Biol. Control* 46:15-23.
- Singh, A., Karmegam, N., Singh, G. S., Bhadauria, T., Chang, S. W., Awasthi, M. K., Sudhakar, S., Arunachalam, K. D., Biruntha, M. and Ravindran, B. 2020. Earthworms and vermicompost: an eco-friendly approach for repaying nature's debt. *Environ. Geochem. Health* 42:1617-1642.
- Tognetti, C., Laos, F., Mazzarino, M. J. and Hernández, M. T. 2005. Composting vs. vermicomposting: a comparison of end product quality. *Compost Sci. Util.* 13:6-13.
- Tripathi, G. and Bhardwaj, P. 2004. Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). *Bioresour. Technol.* 92:275-283.
- Villatoro-Pulido, M., Priego-Capote, F., Álvarez-Sánchez, B., Saha, S., Philo, M., Obregón-Cano, S., De Haro-Bailón, A., Font, R. and Del Río-Celestino, M. 2013. An approach to the phytochemical profiling of rocket [*Eruca sativa* (Mill.) Thell]. *J. Sci. Food Agric.* 93:3809-3819.
- Viswanath, K. K., Varakumar, P., Pamuru, R. R., Basha, S. J., Mehta, S. and Rao, A. D. 2020. Plant lipoxygenases and their role in plant physiology. *J. Plant Biol.* 63:83-95.
- Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* 55:25-38.
- Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., De Luca, V. and Després, C. 2012. The *Arabidopsis* NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell Rep.* 1:639-647.
- Wubben, M. J. E., Jin, J. and Baum, T. J. 2008. Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. *Mol. Plant-Microbe Interact.* 21:424-432.
- Wubie, M. and Temesgen, Z. 2019. Resistance mechanisms of tomato (*Solanum lycopersicum*) to root-knot nematodes (*Meloidogyne* species). *J. Plant Breed. Crop Sci.* 11:33-40.
- Xiao, Z., Liu, M., Jiang, L., Chen, X., Griffiths, B. S., Li, H. and Hu, F. 2016. Vermicompost increases defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants. *Appl. Soil Ecol.* 105:177-186.
- Zhang, Y. and Li, X. 2019. Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Curr. Opin. Plant Biol.* 50:29-36.