BIODIVERSITAS
Volume 22, Number 6, June 2021
Pages: 3256-3264

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220630

Cost-effective bacteria-based bionematicide formula to control root-knot nematode *Meloidogyne* spp. in tomato plants

IIS NUR ASYIAH[1,♥], JEKTI PRIHATIN[1], AHDA DWI HASTUTI[1], SUGENG WINARSO[2], LENNY WIDJAYANTHI[3], DWI NUGROHO[4], KURNIAWAN FIRMANSYAH[5], ANKARDIANSYAH PANDU PRADANA[6]

[1]Biology Education Program, Faculty of Teacher Training and Education, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia. Tel.: +62-331-330738, ♥email: iisnaza.fkip@unej.ac.id
[2]Soil Science Program, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[3]Agricultural Extension Program, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[4]Indonesian Coffee and Cocoa Research Institute. Jl. PB. Sudirman 90, Jember 68118, East Java, Indonesia
[5]Tiga Kreasi Bersama Limited Partnership (*Commanditaire Vennootschap*). Jl Sukowono No 144, Bondowoso 68263, East Java, Indonesia
[6]Plant Protection Program, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia

Manuscript received: 20 April 2021. Revision accepted: 21 May 2021.

Abstract. *Asyiah IN, Prihatin J, Hastuti AD, Winarso S, Widjayanthi L, Nugroho D, Firmansyah K, Pradana AP. 2021. Cost-effective bacteria-based bionematicide formula to control root-knot nematode* Meloidogyne *spp. in tomato plants. Biodiversitas 22: 3256-3264.* The root-knot nematode, *Meloidogyne* spp. can infect and cause loss production in various horticultural plants, including tomatoes. In the previous study, we found 3 endophytic bacteria isolates and 1 rhizobacterium isolate that could control several plant-parasitic nematodes. In this study, we formulated these bionematicide isolates with cheap and environmentally friendly organic materials. The formula was fortified using several organic matters, vitamin sources, protein sources, and sugar sources. The research was conducted in an experimental land with a history of severe root-knot nematode infection. The analysis showed that there were 63.7 J2 *Meloidogyne* spp. per 100 ml of soil on the experimental land. The application was given at a time interval of 2 weeks at the concentration of 0.5%, 1%, 1.5%, and 2%, with a dose of 100 ml per plant. As a negative control, the plant did not give any treatments, and as a positive control, the plant was given 5 g carbofuran per plant. The results revealed that treatment with 2% bionematicide formula concentration showed the best consistent result. This treatment increased canopy wet weight by 38.63% and root dry weight by 106.97% compared to negative control. The P4 treatment was also found effective to increase fruit weight by 33.61% and fruit diameter by 26.16% as compared to negative control. Increased plant growth in P4 treatment was closely related to the total of root-knot suppression and root damage intensity. In the P4 treatment, the total of root-knots and root damage intensities was 44.83% and 32.66%, respectively, compared to the negative control. This suppression also occurred in the nematode population and nematode eggs. In the P4 treatment, the total of *Meloidogyne* spp. J2 in soil and root was lower by 60.74% and 66.24%, respectively, compared to the negative control. A similar phenomenon also occurred in the total of eggs, which was 79.40% lower than the total of eggs in the negative control. This study provides the latest information about a cost-effective bacteria-based bionematicide formula, which is effective in suppressing *Meloidogyne* spp. infection in tomato, and promotes the growth and development tomato plant.

Keywords: *Bacillus*, bionematicide, endophyte, field, *Pseudomonas dimunita*, rhizobacteria, tomato

INTRODUCTION

The root-knot nematode, *Meloidogyne* spp., is known to be a cosmopolite nematode that infects more than 2000 plant species (Subedi et al. 2020). In Indonesia, the root-knot nematodes have been reported to infect food, spice, plantation, and horticulture plants in various regions. Kurniawati et al. (2020) reported that *M. incognita* was found in celery plants in Bogor District, West Java, Indonesia. Moreover, Igensius and Syaifudin (2019) reported that *M. javanica* was identified to infect tomato plants in Samarinda, East Kalimantan, Indonesia. Tomato plants infected with *Meloidogyne* spp. showed a symptom of knots occurrence in the root. Knots are formed due to the physiological disruption in plants induced by root-knot nematode (Philbrick et al. 2020). The root that is the feeding site of *Meloidogyne* spp. can modify its cell, thereby increasing the cell size. The enlarged size is then called the giant cell (Olmo et al. 2019). The occurrence of plant root-knot infected with *Meloidogyne* spp. causes the disruption of nutrient and water absorption systems from the soil. This phenomenon causes disrupted plant growth, followed by other symptoms, such as withering, stunting, and decreased yield (Collett et al. 2021). The yield loss due to the root-knot nematode infection is reported to vary depending on the nematode species that infected the host (Kayani and Mukhtar 2018). Different species and host varieties infected with *Meloidogyne* spp. can provide different yield losses. Mukhtar (2018) reported that the yield loss due to *Meloidogyne* spp. infection in tomato plant reaches up to 40%, while the severe infection leads to higher yield loss. The root-knot nematode infection can even cause total yield loss or mortality in host plants if it is followed by secondary infection, such as fungi or pathogenic bacteria (Beyan et al. 2019). Kumar et al. (2017) reported that the *Meloidogyne* spp. and *Fusarium*

oxysporum infections in tomato plants cause host mortality. A similar condition was also reported by Furusawa et al. (2019), who stated that the *Meloidogyne* spp. and *Ralstonia solanacearum* infections in tomatoes cause a total yield loss.

Various attempts have been made to control the root-knot nematodes in tomato plants. Nevertheless, *Meloidogyne* spp. infection in tomato land, mainly in conventional land owned by the farmer, has still become a problem that cannot be handled completely. Further. It is necessary to discover cheap, applicable, effective, and efficient solutions to overcome this problem. An effort that can be done is to develop a bionematicide formula with the active materials of antagonistic bacteria (Viljoen et al. 2019; Mosahaneh et al. 2021). In previous study, we found 8 bacterial isolates including 6 endophytic bacteria and 2 rhizobacteria. These bacteria were reported to be capable of controlling coffee plant root-lesion nematodes either in single-use (Asyiah et al. 2015; Asyiah et al. 2018) or in consortium form (Asyiah et al. 2020). The characterization results showed that the isolates produced protease and chitinase extracellular enzymes. In another way, eight isolates were also able to fix nitrogen and dissolve phosphate (Asyiah et al. 2015; Asyiah et al. 2018). Endophytic bacteria are defined as bacteria whose life cycle is partially or entirely exists in plant tissue without causing symptoms for their host plant (Latha et al. 2019). Meanwhile, rhizobacteria are bacteria that live in the plant rooting region (Goswami and Suresh 2020). Both endophytic bacteria and rhizobacteria are reported to have a mutualism symbiosis with their host plant (Singh 2018). The host plant provides nutrients and niche for bacteria, while endophytic bacteria and rhizobacteria produce secondary metabolites to protect the plant (Oleńska et al. 2020). Protease enzyme produced by bacteria has a vital role in controlling the root-knot nematodes. Protease can lyse the nematode body surface and cause mortality in nematodes (De Souza Gouveia et al. 2017). Moreover, chitinase enzyme also has a similar mechanism to that of protease because chitinase enzyme can cause mortality in nematodes (Soliman et al. 2019). The capability of endophytic bacteria and rhizobacteria in nitrogen fixation and phosphate dissolution is also promoted growth in tomato plants. Yavarian et al. (2021) reported that nitrogen fixating bacteria in tomatoes effectively increased plant growth. In a separate report, Zhang et al. (2017) reported that phosphate dissolving bacteria from *Pseudomonas* sp. and *Bacillus* sp. genera could also promote tomato plant growth either in the greenhouse or in field.

The bacteria discovered are required to be formulated to simplify the application in the field by farmers, increase the preservation period, and to maintain their effectiveness. The endophytic bacteria and rhizobacteria formulation can be done in liquid form or in compost-enriched bacteria (Patel et al. 2021). Soumare et al. (2020) reported that the essential condition in antagonistic bacteria formulation was sufficient nutrient availability during the preservation process and formula capability to maintain the viability of the bacterial cells during the preservation period. In general, bacteria will provide a higher preservation capability in liquid formulation. The availability of abundant water can be additional nutritious material and a cell protectant that is more abundant and evenly. Moreover, a formula in liquid form is also reported to be more sustainable against temperature stress during the preservation period or distribution process (Fasusi et al. 2021). In a separate report, Chaudhary et al. (2020) stated that the antagonistic bacteria formula should fulfill several criteria, such as (i) providing a suitable micro-environment for microbes; (ii) having physical and chemical characteristics to support during the preservation period; (iii) organizable pH carrying media; (iv) stable during the preservation period; (v) allowing rapid and controlled release of bacteria; (vi) economical and environmentally friendly. The carrying materials that may be used in the antagonistic bacteria formulation are peat, coal, clays, lignite, inorganic soil, charcoal, composts, plant waste materials, and other organic materials (Çakmakçı 2019). The effectiveness of bacteria formula as bio nematicides have been reported by several researchers. Pradana (2016) reported that compost-enriched endophytic bacteria were effective in controlling the *Meloidogyne* spp. root-knot nematode in the tomato plants. Nagachandrabose (2018) also reported that the liquid formula composed of molasses mixture and antagonistic bacteria effectively controlled root-knot nematodes in carrot plants. In this study, we formulated endophytic bacteria and rhizobacteria that were previously isolated, identified, and characterized using liquid carrying material fortified with the organic materials. The formula was then tested for its effectiveness in controlling the *Meloidogyne* spp. in tomato field.

MATERIALS AND METHODS

Study area and research period

The study was performed from October 2020 to January 2021 in tomato infected root-knot nematodes land owned by the farmers in Sumber Ketempah Village, Jember District, East Java, Indonesia.

Bacterial isolates

The bacterial isolates used were: 3 endophytic bacteria and 1 rhizobacteria belong to two genera, *Pseudomonas* and *Bacillus* (Table 1). All bacteria were identified and characterized in the previous study. Each isolate was tested for its compatibility, finding that all isolates were compatible to be combined in a consortium (Asyiah et al. 2015; Asyiah et al. 2018).

Table 1. Bacterial isolates used in the study

Isolate code	Bacterial species	Status	Reference
SK07	*Bacillus* sp.	Endophyte	(Asyiah et al. 2015; 2018)
SK14	*Bacillus* sp.	Endophyte	
KB14	*Bacillus* sp.	Endophyte	
PD01	*Pseudomonas dimunita*	Rhizobacteria	

Table 2. Physical characteristics of the experimental land

Fraction	Diameter (µm)	Percentage (%)	Total percentage (%)
Sand	>1000	18.65	62.1
	500-1000	6.25	
	200-500	19.36	
	100-200	12.44	
	50-100	5.40	
Dust	20-50	6.35	26.83
	10-20	3.81	
	2-10	16.67	
Clay	0.05-2	3.20	11.07
	0-0.05	7.87	

Cost-effective bionematicide formulation

The bacterial consortium suspension was produced using Bean Sprout Extract Broth (BSEB). 200 g of bean sprout was boiled in 1000 ml of aquadest to make BSEB broth. The suspension was then filtered by separating the bean sprout from its extract. The bean sprout extract was mixed with 20 g sugar and sterilized with an autoclave (Ilmi et al. 2019).

One Ose from each isolate was cultured in 250 ml Bean Sprout Extract Broth (BSEB) in Erlenmeyer flask for 48 hours at 30˚C and rotated at 300 rpm. The total of Erlenmeyer flask used was 30 flasks, therefore obtained 7500 ml bacterial consortium suspension.

As carrying materials, wet cow manure, vitamins, amino acids, and sugar were used with the detailed composition, which was the confidential trading condition of *Tiga Kreasi Bersama Limited Partnership* (*Commanditaire Vennootschap*), Indonesia. All materials were then mixed with 1000 L water equipped with a filtered air pump to prevent anaerobic conditions. The 7500 ml bacterial consortium suspension was then mixed with 1000 L carrying materials. After mixing, the bacterial consortium was then incubated in the following formula for 30 days before being used. The suspension formed after 30 days of incubation was then called a bionematicide.

Experimental land condition

The land used for the experiment was analyzed for its physical, chemical, and biological characteristics. The texture of 10 fractions was observed on the initial experiment using the pipetting and soil fraction percentage calculation methods. Based on the fraction percentage analysis, physical characteristics of soil is presented in Table 2.

The chemical characteristics of soil observed in the initial experiment were organic-C, total N, available P, available K, and pH H_2O levels. The chemical characteristics of soil are presented in Table 3.

Furthermore, biological characteristic of soil was observed by the population of root-knot nematode J2. From 20 sampling points, it was recorded that 63.7 of *Meloidogyne* spp. J2 root-knot nematode averagely existed per 100 ml soil in the land used for the experiment.

Field experiment

The SL 283 tomato variety was used in the experiment. This variety was mainly planted by farmers in Jember District, East Java, Indonesia, and was reported to be susceptible to the root-knot nematode infection. The tomato seeds with 4 true leaves were taken into the land and planted on beds covered with plastic mulch. Planting was performed in a randomized design with 6 treatments and 5 replications. Each replication contained 16 experimental plants, therefore, total 480 whole tomatoes were planted in the experiment. Carbofuran active compound-based nematicide was used as positive control and no nematicide materials were used in negative control (Liu et al. 2020). In detail, the treatments used in this study are presented in Table 4.

The K+ treatment was only applied once on the initial planting, while the P1, P2, P3, and P4 treatments were applied once in 2 weeks for 3 months. The dose applied for P1 to P4 treatments was 100 ml per plant. Moreover, tomato plants in all treatments were fertilizer based on the dose recommended for tomato plant fertilization. After being planted, the tomato plants were maintained for 3 months until bearing fruit.

Variables observed were plant height, canopy wet weight, root wet weight, canopy dry weight, root dry weight, total of knots in roots, root damage intensity, total of *Meloidogyne* spp. J2 in soil and root, total of *Meloidogyne* spp. eggs, weight per tomato fruit, and total of fruits per plant. All variables were observed in the final study. The root damage intensity was calculated based on the root damage scale according to Zeck scale (Giné and Sorribas 2017). Furthermore, the total of *Meloidogyne* spp. J2 was calculated by extracting all nematodes with white head-tray method in soil and condensation method in the root (Bell and Watson 2001). The extracted nematodes were then calculated their population and the occurrence percentage of *Meloidogyne* spp. J2. The calculation result was then used to convert the total of J2 in soil and in the root. In addition, the extraction of nematode eggs from roots was performed using 2% sodium hypochlorite following the protocols described by Kayani and Mukhtar (2018).

Table 3. Chemical characteristics of soil in experimental land

Characteristics	Value
Organic-C	0.89 g 100 g^{-1}
Nitrogen	0.1 g 100 g^{-1}
C/N Ratio	9
P_2O_5	56 mg 1000 g^{-1}
Morgan K_2O	529 ppm
pH H_2O	6.4

Table 4. Treatments used in field experiment

Treatment code	Note
K-	Without additional materials
K+	5 g carbofuran per plant on the initial planting
P1	0.5% bionematicide formula
P2	1% bionematicide formula
P3	1.5% bionematicide formula
P4	2% bionematicide formula

Data analysis

Data were analyzed using Analysis of Variance (ANOVA), if there was a difference, the analysis was continued using a continuous test following the Duncan Multiple Range Test (DMRT) method with 95% degree of confidence. The analysis was performed using the *DSAASTAT version 1.101* program (Munif et al. 2019).

RESULTS AND DISCUSSIONS

Tomato plant growth

The result exhibited that tomato plants treated with various concentrations of cost-effective bacteria-based bionematicides, showed varied growth. Plants treated with the bionematicide formula showed 5.66% to 6.58% higher plant height compared to K-, which was statistically insignificant. In the canopy wet weight variable, P4 treatment showed the best performance. The average weight of wet canopy in P4 treatment was 101.9 g, which was 38.63% higher than that of K-. Statistically, only P4 treatment showed a significant difference compared to the K-and other treatments. The observation of canopy dry weight variable showed that there was an insignificant difference among treatments. Although the canopy dry weight treated with the bionematicide formulas notified the value of 8.66% to 45.11% higher than the K-, and had statistically insignificant differences.

Furthermore, in root wet weight, all bionematicide formulas had insignificant differences. Although the bionematicide formula application generally increases the root wet weight, these treatments were statistically insignificant. The root wet weight showed an insignificant performance, the application of bionematicide formula could significantly increase the root dry weight. The root dry weight of P1, P2, P3, and P4 treatments were 2.26 g, 2.26 g, 2.488 g and 2.67 g, respectively. Whereas in K-and K+ treatment the average root dry weight was 1.29 g and 1.39 g, respectively. The higher root dry weight was noted as 75.19% each in (P1 and P2 treatments) and 92.24% in P3 treatment and 106.97% in P4 treatment than K-. The average data of tomato plant growth in various treatments are presented in Table 5.

Tomato plant production

Tomato production in plants treated with the various concentrations of bionematicide formula showed different results. The K+, P1, P2, and P3 treatments were insignificantly different from K-treatment in the fruit weight variable. Plants treated with the P4 treatment showed significantly different fruit weight than K-and K+ treatments. The average fruit weight in plants treated with the P4 treatment was 34.54 g, which indicates a difference of 33.61% compared to K-.

The fruit diameter variable also had a similar pattern to the fruit weight. Plants with K+, P1, P2, and P3 treatments were insignificantly different from K-. In P4 treated plants, the average diameter of fruit was 4.34 cm, which was 26.16% higher than K-. The P4 treatment was the only treatment that obtained a significantly different value compared to K-.

All treatments showed significant difference in total fruit per plant. In P1 to P4 treatments, the total fruit difference was 3.57% to 31.25% higher than the K-that had statistically insignificant difference. Moreover, the average fruit weight, fruit diameter, and total of fruit per plant is presented in Table 6.

Table 5. Average of tomato plant growth

Treatments	Plant height (cm)	Canopy wet weight (g)	Canopy dry weight (g)	Root wet weight (g)	Root dry weight (g)
K-	$61.80^{a} \pm 4.29$	$73.50^{a} \pm 11.83$	$7.27^{a} \pm 0.67$	$30.50^{a} \pm 10.44$	$1.29^{a} \pm 0.53$
K+	$62.63^{a} \pm 9.63$	$73.22^{a} \pm 14.39$	$7.26^{a} \pm 2.02$	$30.64^{a} \pm 3.51$	$1.39^{a} \pm 0.31$
P1	$65.30^{a} \pm 14.74$	$73.58^{a} \pm 10.11$	$7.90^{a} \pm 1.72$	$30.01^{a} \pm 5.71$	$2.26^{b} \pm 0.67$
P2	$65.75^{a} \pm 11.34$	$73.64^{a} \pm 12.43$	$8.81^{a} \pm 1.50$	$33.84^{a} \pm 8.05$	$2.26^{b} \pm 0.59$
P3	$65.42^{a} \pm 7.59$	$73.77^{a} \pm 9.04$	$10.40^{a} \pm 3.47$	$33.33^{a} \pm 6.93$	$2.48^{b} \pm 0.55$
P4	$65.87^{a} \pm 13.60$	$101.90^{b} \pm 30.59$	$10.55^{a} \pm 4.57$	$36.57^{a} \pm 1.61$	$2.67^{b} \pm 0.85$

Note: Numbers in the column followed by the same letter were insignificantly different at the p-value of 0.05 (Duncan Multiple Range Test).

Table 6. Average of tomato plant production

Treatments	Fruit weight (g)	Fruit diameter (cm)	Total of fruits per plant
K-	$25.85^{a} \pm 2.57$	$3.44^{a} \pm 0.32$	$22.40^{a} \pm 4.04$
K+	$25.84^{a} \pm 4.11$	$3.55^{a} \pm 0.49$	$22.80^{a} \pm 4.97$
P1	$27.31^{a} \pm 3.65$	$3.77^{ab} \pm 0.30$	$23.20^{a} \pm 3.96$
P2	$27.51^{a} \pm 1.89$	$3.83^{ab} \pm 0.49$	$25.00^{a} \pm 5.83$
P3	$29.21^{ab} \pm 7.60$	$3.83^{ab} \pm 0.41$	$26.40^{a} \pm 5.37$
P4	$34.54^{b} \pm 0.92$	$4.34^{b} \pm 0.38$	$29.40^{a} \pm 5.37$

Note: Numbers in the column followed by the same letter were insignificantly different at p-value of 0.05 (Duncan Multiple Range Test).

Nematode infection and population

The application of bionematicide formula in tomato plants showed various responses in the pathological variable. The bionematicide formula application with 2% concentration (P4) suppressed the total of root-knots significantly. In P1 to P3 treatments, averages of root-knots decreased from 14.30% to 39.72% compared to K-, had a statistically insignificant difference. In the P4 treatment, the average of root-knots was 61.36, recorded 44.83% lower than K-treatment.

A similar pattern was also shown in the root damage intensity variable. In root damage intensity, K+, P1, P2, and P3 treatments had insignificant difference compared to K-. The bionematicide formula treatment with 0.5% to 1.5% doses exhibited lower damage intensity values than K-and statistically insignificant. In P4 treatment, the average root damage intensity value was 2.02, and 32.66% lower than K-. The P4 treatment became the only treatment that obtained a significant difference to K-in the root damage intensity value. The average of the total root-knots and root damage intensity in each treatment is presented in Table 7.

In the total of *Meloidogyne* spp. J2 in soil, P3 and P4 treatments significantly decrease J2 population in soil compared to K-. The K+, P1, and P2 treatments showed an insignificant difference compared to K-. In P3 treatment, the average population of *Meloidogyne* spp. in soil was 23.94, while in P4 treatment it was 21.42. Compared to K-(54.56), the total of nematodes was found to be lower in P3 and P4 56.12% and 60.74%, respectively.

In the total of *Meloidogyne* spp. J2 observation in the root, K+ and P1 treatments were insignificantly from K-. Treatments that were significantly different from the K-(31.11) were the P2 (24.33), P3 (17.17), and P4 (10.50). In P2, P3, and P4 treatments, the average of *Meloidogyne* spp. J2 population in plant root was lower 22.14%, 44.80%, and 66.24% than K-, respectively. Moreover, results of total *Meloidogyne* spp. eggs per 5 g tomato root showed that K+ and P1 treatments were insignificantly different from K-. The P2 treatment was significantly different from K-, but insignificantly different from K+. Treatments that showed a significantly different result from K-and K+ were P3 and P4. If compared to K-(141.67), the average of nematode eggs in P3 (28.67) and P4 (29.17) treatments were lower at 79.76% and 79.40%. Furthermore, data related to the average total of *Meloidogyne* spp. J2 in soil and in the root, and the average total of *Meloidogyne* spp. eggs in each treatment are presented in Figure 1.

Table 7. Total of knots and root damage intensity in various treatments

Treatments	Total of root-knots	Root damage intensity
K-	$111.22^{b} \pm 15.21$	$3.00^{b} \pm 0.63$
K+	$112.12^{b} \pm 30.22$	$2.93^{b} \pm 0.86$
P1	$95.31^{ab} \pm 33.06$	$2.74^{ab} \pm 0.91$
P2	$96.47^{ab} \pm 54.10$	$2.45^{ab} \pm 0.61$
P3	$67.04^{ab} \pm 17.42$	$2.26^{ab} \pm 0.84$
P4	$61.63^{a} \pm 15.86$	$2.02^{a} \pm 0.64$

Note: Numbers in the column followed by the same letter were insignificantly different at p-value of 0.05 (Duncan Multiple Range Test).

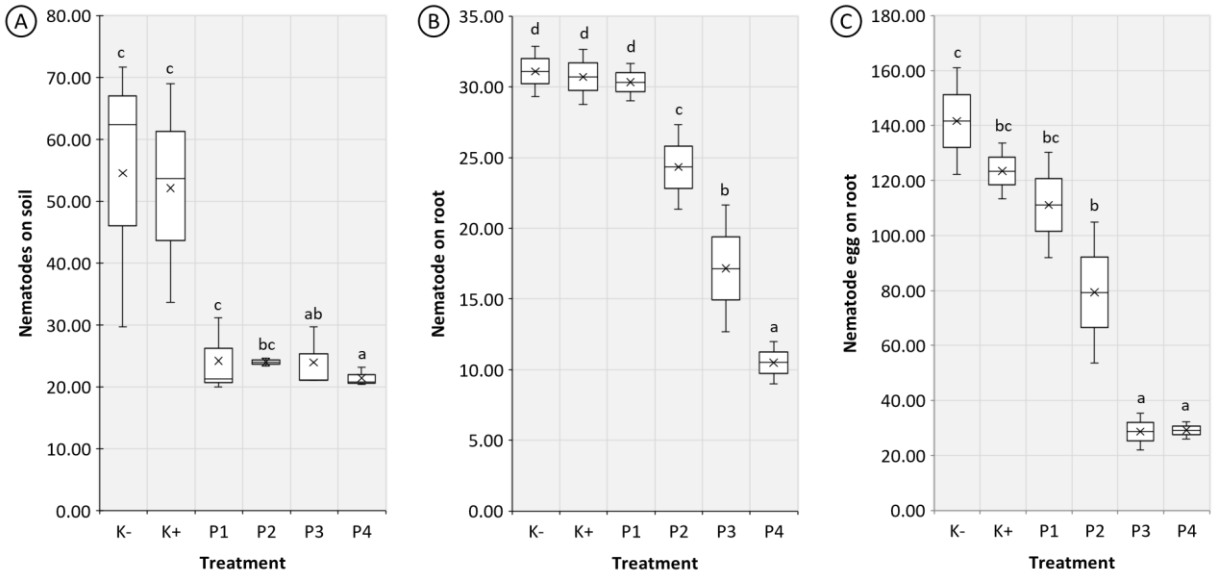

Figure 1. Average of (A) J2 *Meloidogyne* spp. in soil; (B) *Meloidogyne* spp. in root; and (C) *Meloidogyne* spp. eggs in root

Discussion

Plant growth is commonly affected by internal and external factors. One of the external factors that mainly contribute to plant growth is nutrient availability (Davidson and Gu 2012). The plant height, canopy dry weight, and root wet weight variables showed an insignificant result. This phenomenon is thought to be due to the influence of chemical fertilizers application. The application of chemical fertilizers causes the plant's nutritional needs to be met so that the influence of bacteria in increasing plant growth is not well expressed (Timsina 2018). The synthetic chemical fertilizer provides nutrients that the plants could use directly (Kopittke et al. 2019). Nevertheless, treatments in the present study recorded a significant increase in canopy wet weight and root dry weight. This increase was due to the physiological activity of bacteria in the bionematicide formula (Mehmood et al. 2018).

Tariq et al. (2017) reported that rhizobacteria had dual roles as plant protector agent and plant growth promoter. In a separate study, Afzal et al. (2019) also reported that endophytic bacteria could induce plant growth. Both rhizobacteria and endophytic bacteria had similar mechanisms in promoting plant growth. Both were reported to produce Indole-acetic acid (IAA), or commonly known as auxin. The auxin hormone plays an essential role in plant growth (Li et al. 2016). Kunkel and Harper (2018) reported that auxin phytohormone could induce root growth, regulate cell enlargement, promote plant cell elongation, increase apical dominance and xylem differentiation. Auxin is primarily found in seed embryos and meristematic tissue that grow actively, such as plant sprouts, root tips, and twig/leaf tip (Casanova-Sáez et al. 2021). In a separate study, Wagi and Ahmed (2019) reported that bacteria from the *Bacillus* sp. group could produce auxin and promote plant growth. Besides, bacteria from *Pseudomonas* sp. and *Serratia* sp. groups were also reported to produce IAA (Kudoyarova et al. 2017). The IAA production by bacteria was reported in various numbers, depending on the bacteria types and their environment (Çakmakçı et al. 2020).

In this study, the application of bionematicide formula with the active ingredients of rhizobacteria and endophytic bacteria was also able to enhance the results. The fruit weight and diameter in plants treated with P4 treatment showed the best and significantly different results than other treatments. Liu et al. (2017) reported that the biological agent from the bacteria group had several mechanisms to increase plant growth. *Bacillus* sp. and *Pseudomonas* sp. were reported as bacteria with good nitrogen fixation capability (Santoyo et al. 2016) . Nitrogen is an essential nutrient element required by plants for growth and development (Leghari et al. 2016). Also, the biological agent can release P bound with Al and Fe in soil. In some cases, P occurs in soil but cannot be utilized by plants due to binding to other elements (Redel et al. 2016). The biological agent was reported to produce a phosphatase enzyme that could release P bound from other elements (Vejan et al. 2016; Divjot et al. 2021). P element is one of the essential nutrient elements that contribute to flowering and fruit formation in plants. Plants lacking P element were reported to have reduced fruit production, or the fruit production was far from the genetic potential (Kapoor et al. 2004). Increased tomato production in plants treated with bionematicide formula was closely related to the infection level in the root. Plants treated with bionematicide formula application at 2% dose showed a lower total of root-knots and root damage intensity than the control plant. Berendsen et al. (2012) reported that the health rooting system was a critical success in plant production. A similar condition was also reported by Munif et al. (2019), who stated that tomato plants with health rooting systems showed 32% to 78% higher results than the plant infected by *Meloidogyne* spp. The nutrient element and water absorptions in healthy plant roots allow the plant to have better metabolism, producing more fruits (Na et al. 2017).

The suppression of total root-knots and root damage intensity in plants is closely related to the suppression of the nematode population in soil and roots. The present investigation revealed that the rhizosphere and plant roots treated with bionematicide formula resulted in a lower total of nematodes in soil and root than the control plants. Several studies reported that suppression of total nematodes in soil and root was closely related to the physiological activity of bacteria (Tran et al. 2019). Rhizobacteria and endophytic bacteria can directly or indirectly suppress the nematode population. Directly, both bacteria can produce extracellular enzymes, such as protease and chitinase (Wiratno et al. 2019). Chitinase is an enzyme that catalyzes chitin hydrolytic degradation as a linear polymer composed of β-1,4-N-acetile-D-glucosamine (GlcNAc) monomers that are widely distributed in nature. This enzyme was reported to be capable of lysing the outer nematode structure composed of chitin (Jha and Modi 2018). Chitinase is involved in inducing plant resistance against plant pathogen attack. The chitinase enzyme activity in suppressing the plant-parasitic nematodes has been reported by Gupta et al. (2017) and Kassab et al. (2017). Besides chitinase, another enzyme that contributes to suppress *Meloidogyne* spp. population in the soil is protease. Protease enzyme was reported to be capable of degrading root-knot nematode cuticle and eggs that are composed of proteins (De Souza Gouveia et al. 2017; Gomes et al. 2019). Safni et al. (2018) reported that bacteria with the capability of producing chitinase and protease enzymes could *in vitro* lyse *Meloidogyne* spp. nematode stylet.

Liu et al. (2020) stated that bacteria from *Bacillus* genus effectively suppressed root-knot nematodes in soil in the greenhouse experiment. Nishantha et al. (2018) reported that *Pseudomonas fluoroscens* effectively suppressed total of root-knots and root damage intensity due to *Meloidogyne* spp. infection. Another study reported that the biological agent from rhizobacteria and endophytic bacteria groups could suppress the total of nematode eggs in tomato plant rooting system (Mardhiana et al. 2017; Viljoen et al. 2019).

This study concluded that the application of cost-effective bacteria-based bionematicide formula is effective at 2% concentration and 100 ml per plant dose on once in 2 weeks interval, which can suppress the population of root-

knot nematode in soil and root and total of root-knot nematode eggs. This suppression depends on the total of knots formed and root damage intensity in tomato plants. Decreased pathological variables are correlated with the increased growth, as presented from the increased canopy wet weight, root dry weight, fruit weight, and fruit diameter.

ACKNOWLEDGEMENTS

This work was supported by Indonesia Endowment Fund for Education, Indonesian Ministry of Finance (*LPDP Kemenkeu RI*) through the *RISPRO LPDP* grant scheme with the contract number of PRJ-38/LPDP/2020.

REFERENCES

Afzal I, Shinwari ZK, Sikandar S, Shahzad S. 2019. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res 221: 36-49. DOI: 10.1016/j.micres.2019.02.001.

Asyiah I, Wiryadiputra S, Fauzi I, Harni R. 2015. Populasi *Pratylenchus coffeae* (Z) dan pertumbuhan bibit kopi Arabika akibat inokulasi *Pseudomonas diminuta* L. dan *Bacillus subtilis* (C.). Pelita Perkebunan 31 (1): 30-40. DOI: 10.22302/iccri.jur.pelitaperkebunan.v31i1.77. [Indonesian]

Asyiah IN, Mudakir I, Hoesain M, Pradana AP, Djunaidy A, Sari RF. 2020. Consortium of endophytic bacteria and rhizobacteria effectively suppresses the population of *Pratylenchus coffeae* and promotes the growth of Robusta coffee. Biodiversitas 21 (10): 4702-4708. DOI: 10.13057/biodiv/d211032.

Asyiah IN, Soekarto S, Hoesain M, Iqbal M, Hindersah R, Narulita E, Mudakir I. 2018. The endophytic bacteria isolation as biological control agent of *Pratylenchus coffeae*. Asian J Microbiol Biotechnol Environ Sci 20 (1): 165-171.

Bell N, Watson R. 2001. Optimising the whitehead and hemming tray method to extract plant-parasitic and other nematodes from two soils under pasture. Nematol 3 (2): 179-185. DOI: 10.1163/156854101750236312.

Berendsen RL, Pieterse CM, Bakker PA. 2012. The rhizosphere microbiome and plant health. Trends Plant Sci 17 (8): 478-486. DOI: 10.1016/j.tplants.2012.04.001.

Beyan A, Seid A, Shifa H. 2019. Response of tomato genotypes to *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* co-infestation under glasshouse conditions. Pak J Nematol 37: 63-82. DOI: 10.18681/pjn.v37.i01.p63-82.

Çakmakçı R. 2019. A review of biological fertilizers current use, new approaches, and future perspectives. Int J Innov Sci Eng Technol 5 (7): 83-92.

Çakmakçı R, Mosber G, Milton AH, Alatürk F, Ali B. 2020. The effect of auxin and auxin-producing bacteria on the growth, essential oil yield, and composition in medicinal and aromatic plants. Cur Microbiol 77 (4): 564-577. DOI: 10.1007/s00284-020-01917-4.

Casanova-Sáez R, Mateo-Bonmatí E, Ljung K. 2021. Auxin metabolism in plants. Cold Spring Harb Perspect Biol 13 (3): a039867. DOI: 10.1101/cshperspect.a039867.

Chaudhary T, Dixit M, Gera R, Shukla AK, Prakash A, Gupta G, Shukla P. 2020. Techniques for improving formulations of bioinoculants. 3 Biotech 10 (5): 1-9. DOI: 10.1007/s13205-020-02182-9.

Collett RL, Marais M, Daneel M, Rashidifard M, Fourie H. 2021. *Meloidogyne enterolobii*, a threat to crop production with particular reference to sub-Saharan Africa: an extensive, critical and updated review. Nematol 23 (3): 247-285. DOI: 10.1163/15685411-bja10076.

Davidson D, Gu FX. 2012. Materials for sustained and controlled release of nutrients and molecules to support plant growth. J Agri Food Chem 60 (4): 870-876. DOI: 10.1021/jf204092h.

de Souza Gouveia A, de Freitas Soares FE, Morgan T, Sufiate BL, Tavares GP, Braga FR, Monteiro TSA, Geniêr HLA, de Freitas LG, de Queiroz JH. 2017. Enhanced production of *Monacrosporium thaumasium* protease and destruction action on root-knot nematode *Meloidogyne javanica* eggs. Rhizosphere 3: 13-15. DOI: 10.1016/j.rhisph.2016.12.001.

Divjot K, Rana KL, Tanvir K, Yadav N, Yadav AN, Kumar M, Kumar V, Dhaliwal HS, Saxena AK. 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and-mobilizing microbes: a review. Pedosphere 31 (1): 43-75. DOI: 10.1016/S1002-0160(20)60057-1.

Fasusi OA, Cruz C, Babalola OO. 2021. Agricultural sustainability: microbial biofertilizers in rhizosphere management. Agriculture 11 (2): 163. DOI: 10.3390/agriculture11020163.

Furusawa A, Uehara T, Ikeda K, Sakai H, Tateishi Y, Sakai M, Nakaho K. 2019. *Ralstonia solanacearum* colonization of tomato roots infected by *Meloidogyne incognita*. J Phytopathol 167 (6): 338-343. DOI: 10.1111/jph.12804.

Giné A, Sorribas FJ. 2017. Effect of plant resistance and BioAct WG (*Purpureocillium lilacinum* strain 251) on *Meloidogyne incognita* in a tomato-cucumber rotation in a greenhouse. Pest Manag Sci 73 (5): 880-887. DOI: 10.1002/ps.4357.

Gomes E, Soares F, Souza D, Lima L, Sufiate B, Ferreira T, Queiroz J. 2019. Role of *Synadenium grantii* latex proteases in nematicidal activity on *Meloidogyne incognita* and *Panagrellus redivivus*. Braz J Biol 79 (4): 665-668. DOI: 10.1590/1519-6984.188129.

Goswami M, Suresh D. 2020. Plant growth-promoting rhizobacteria—alleviators of abiotic stresses in soil: a review. Pedosphere 30 (1): 40-61. DOI: 10.1016/S1002-0160(19)60839-8.

Gupta R, Singh A, Ajayakumar P, Pandey R. 2017. Chitinolytic microbes confer *Meloidogyne incognita* resistance and augment secondary metabolites in *Bacopa monnieri* (L.) Pennell. Arch Phytopathol Pflanzenschutz 50 (3-4): 178-196. DOI: 10.1080/03235408.2017.1285522.

Igensius B, Syaifudin EA. 2019. Identifikasi spesies nematoda *Meloidogyne* sp pada tanaman tomat (*Solanum lycopersicum* L) dan seledri (*Apium graveolens* L) di Samarinda. Jurnal Agroekoteknologi Tropika Lembab 1 (2): 136-143. DOI: 10.35941/jatl.1.2.2019.1980.136-143. [Indonesian]

Ilmi M, Putri L, Muhamad A, Cholishoh A, Ardiansyah S. 2019. Use of mung bean sprout (tauge) as alternative fungal growth medium. J Phys: Conf Ser 1241 (1): 012015. DOI: 10.1088/1742-6596/1241/1/012015.

Jha S, Modi H. 2018. Statistical optimization of chitinase production by *Streptomyces rubiginosus* SP24 and efficacy of purified chitinase to control root-knot nematode infection in *Vigna radiata* under controlled conditions. Chem Biol Technol Agric 5 (1): 1-13. DOI: 10.1186/s40538-018-0133-0.

Kapoor R, Giri B, Mukerji KG. 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* mill on mycorrhizal inoculation supplemented with P-fertilizer. Bioresour Technol 93 (3): 307-311. DOI: 10.1016/j.biortech.2003.10.028.

Kassab S, Eissa M, Badr U, Ismail A, Abdel Razik A, Soliman G. 2017. Nematicidal effect of a wild type of *Serratia marcescens* and its mutants against *Meloidogyne incognita* juveniles. Egypt J Agronematol 16 (2): 95-114. DOI: 10.21608/ejaj.2017.56204.

Kayani MZ, Mukhtar T. 2018. Reproductivity of *Meloidogyne incognita* on fifteen Cucumber cultivars. Pak J Zool 50 (4). DOI: 10.17582/journal.pjz/2018.50.5.1717.1722.

Kopittke PM, Lombi E, Wang P, Schjoerring JK, Husted S. 2019. Nanomaterials as fertilizers for improving plant mineral nutrition and environmental outcomes. Environ Sci Nano 6 (12): 3513-3524. DOI: 10.1039/C9EN00971J.

Kudoyarova GR, Vysotskaya LB, Arkhipova TN, Kuzmina LY, Galimsyanova NF, Sidorova LV, Gabbasova IM, Melentiev AI, Veselov SY. 2017. Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. Acta Physiol Plant 39 (11): 1-8. DOI: 10.1007/s11738-017-2556-9.

Kumar N, Bhatt J, Sharma RL. 2017. Interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lycopersici* on Tomato. Int J Curr Microbiol Appl Sci 6: 1770-1776. DOI: 10.20546/ijcmas.2017.608.209.

Kunkel BN, Harper CP. 2018. The roles of auxin during interactions between bacterial plant pathogens and their hosts. J Exp Bot 69 (2): 245-254. DOI: 10.1093/jxb/erx447.

Kurniawati F, Nursipa NT, Munif A. 2020. Nematoda parasit pada seledri (*Apium graveolens* L.) dan pengendaliannya menggunakan bakteri

endofit secara in vitro. Agrovigor 13 (1): 70-81. DOI: 10.21107/agrovigor.v13i1.6304. [Indonesian]

Latha P, Karthikeyan M, Rajeswari E. 2019. Endophytic bacteria: prospects and applications for the plant disease management. In Plant Health Under Biotic Stress, Springer. DOI: 10.1007/978-981-13-6040-41.

Leghari SJ, Wahocho NA, Laghari GM, HafeezLaghari A, MustafaBhabhan G, HussainTalpur K, Bhutto TA, Wahocho SA, Lashari AA. 2016. Role of nitrogen for plant growth and development: a review. Adv Environ Biol 10 (9): 209-219.

Li S-B, Xie Z-Z, Hu C-G, Zhang J-Z. 2016. A review of auxin response factors (ARFs) in plants. Front Plant Sci 7: 47. DOI: 10.3389/fpls.2016.00047.

Liu G, Lin X, Xu S, Liu G, Liu F, Mu W. 2020. Screening, identification and application of soil bacteria with nematicidal activity against root-knot nematode (*Meloidogyne incognita*) on tomato. Pest Manag Sci 76 (6): 2217-2224. DOI: 10.1002/ps.5759.

Liu H, Carvalhais LC, Crawford M, Singh E, Dennis PG, Pieterse CM, Schenk PM. 2017. Inner plant values: diversity, colonization and benefits from endophytic bacteria. Front Microbiol 8: 2552. DOI: 10.3389/fmicb.2017.02552.

Mardhiana M, Pradana AP, Adiwena M, Santoso D, Wijaya R, Murtilaksono A. 2017. Use of endophytic bacteria from roots of *Cyperus rotundus* for biocontrol of *Meloidogyne incognita*. Biodiversitas 18 (4): 1308-1315. DOI: 10.13057/biodiv/d180404.

Mehmood U, Inam-ul-Haq M, Saeed M, Altaf A, Azam F, Hayat S. 2018. A brief review on plant growth promoting rhizobacteria (PGPR): a key role in plant growth promotion. Plant Prot 2 (2): 77-82.

Mosahaneh L, Charehgani H, Abdollahi M, Rezaei R. 2021. Biological control agents in the management of different initial population densities of *Meloidogyne javanica* in tomato. Acta Phytopathol Entomol Hung 55 (2): 151-159. DOI: 10.1556/038.55.2020.016.

Mukhtar T. 2018. Management of root-knot nematode, *Meloidogyne incognita*, in tomato with two *Trichoderma* species. Pak J Zool 50 (4): 1589-1592. DOI: 10.17582/journal.pjz/2018.50.4.sc15.

Munif A, Supramana S, Herliyana EN, Pradana AP. 2019. Endophytic bacterial consortium originated from forestry plant roots and their nematicidal activity against *Meloidogyne incognita* infestation in greenhouse. Acta Univ Agric et Silvic Mendelianae Brun 67 (5): 1171-1182. DOI: 10.11118/actaun201967051171.

Na J, Hui X, Li W-j, Wang X-y, Qian L, Liu S-s, Pei L, Zhao J-l, Heng J. 2017. Field evaluation of *Streptomyces rubrogriseus* HDZ-9-47 for biocontrol of *Meloidogyne incognita* on tomato. J Integr Agric 16 (6): 1347-1357. DOI: 10.1016/S2095-3119(16)61553-8.

Nagachandrabose S. 2018. Liquid bioformulations for the management of root-knot nematode, *Meloidogyne hapla* that infects carrot. Crop Prot 114: 155-161. DOI: 10.1016/j.cropro.2018.08.022.

Nishantha K, Jayasiri H, Herath H, Nugaliyadde M, Dissanayake M, De Costa DM. 2018. Antagonistic effect of eight Sri Lankan isolates of *Pseudomonas fluorescens* on, *Meloidogyne incognita* in tomato, *Lycopersicon esculentum*. Int J Environ Agric Biotech 3 (6): 1980-1986. DOI: 10.22161/ijeab/3.6.4.

Oleńska E, Małek W, Wójcik M, Swiecicka I, Thijs S, Vangronsveld J. 2020. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: a methodical review. Sci Total Environ 734: 140682. DOI: 10.1016/j.scitotenv.2020.140682.

Olmo R, Cabrera J, Fenoll C, Escobar C. 2019. A role for ALF4 during gall and giant cell development in the biotic interaction between *Arabidopsis* and *Meloidogyne* spp. Physiol Plant 165 (1): 17-28. DOI: 10.1111/ppl.12734.

Patel JS, Kumar G, Bajpai R, Teli B, Rashid M, Sarma BK. 2021. PGPR formulations and application in the management of pulse crop health. In *Biofertilizers*. Elsevier. DOI: 10.1016/B978-0-12-821667-5.00012-9.

Philbrick AN, Adhikari TB, Louws FJ, Gorny AM. 2020. *Meloidogyne enterolobii*, a major threat to tomato production: current status and future prospects for its management. Front Plant Sci 11 :606395. DOI: 10.3389/fpls.2020.606395.

Pradana A. 2016. Konsorsium bakteri endofit sebagai agens biokontrol nematoda puru akar *Meloidogye incognita* pada tomat. [Thesis]. IPB University, Bogor. [Indonesian]

Redel Y, Cartes P, Demanet R, Velásquez G, Poblete-Grant P, Bol R, Mora M. 2016. Assessment of phosphorus status influenced by Al and Fe compounds in volcanic grassland soils. J Soil Sci Plant Nutr 16 (2): 490-506. DOI: 10.4067/S0718-95162016005000041.

Safni I, Lubis K, Tantawi AR, Murthi S. 2018. Isolation and characterization of rhizobacteria for biological control of root-knot nematodes in Indonesia. J ISSAAS 24: 67-81.

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR. 2016. Plant growth-promoting bacterial endophytes. Microbiol Res 183: 92-99. DOI: 10.1016/j.micres.2015.11.008.

Singh I. 2018. Plant growth promoting rhizobacteria (PGPR) and their various mechanisms for plant growth enhancement in stressful conditions: a review. Eur J Biol Res 8 (4): 191-213. DOI: 10.5281/zenodo.1455995.

Soliman GM, Ameen HH, Abdel-Aziz SM, El-Sayed GM. 2019. In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. Bull Natl Res Cent 43 (1): 1-7. DOI: 10.1186/s42269-019-0200-0.

Soumare A, Boubekri K, Lyamlouli K, Hafidi M, Ouhdouch Y, Kouisni L. 2020. From isolation of phosphate solubilizing microbes to their formulation and use as biofertilizers: status and needs. Front Bioeng Biotechnol 7: 425. DOI: 10.3389/fbioe.2019.00425.

Subedi S, Thapa B, Shrestha J. 2020. Root-knot nematode (*Meloidogyne incognita*) and its management: a review. J Agric Nat Resour 3 (2): 21-31. DOI: 10.3126/janr.v3i2.32298.

Tariq M, Noman M, Ahmed T, Hameed A, Manzoor N, Zafar M. 2017. Antagonistic features displayed by plant growth promoting rhizobacteria (PGPR): a review. J Plant Sci Phytopathol 1: 38-43. DOI: 10.29328/journal.jpsp.1001004.

Timsina J. 2018. Can organic sources of nutrients increase crop yields to meet global food demand?. Agronomy 8 (10): 214. DOI: 10.3390/agronomy8100214.

Tran TPH, Wang S-L, Nguyen VB, Tran DM, Nguyen DS, Nguyen AD. 2019. Study of novel endophytic bacteria for biocontrol of black pepper root-knot nematodes in the central highlands of Vietnam. Agronomy 9 (11): 714. DOI: 10.3390/agronomy9110714.

Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq Boyce A. 2016. Role of plant growth-promoting rhizobacteria in agricultural sustainability—a review. Molecules 21 (5): 573. DOI: 10.3390/molecules21050573.

Viljoen JJ, Labuschagne N, Fourie H, Sikora RA. 2019. Biological control of the root-knot nematode *Meloidogyne incognita* on tomatoes and carrots by plant growth-promoting rhizobacteria. Trop Plant Pathol 44 (3): 284-291. DOI: 10.1007/s40858-019-00283-2.

Wagi S, Ahmed A. 2019. *Bacillus* spp.: potent microfactories of bacterial IAA. PeerJ 7: e7258. DOI: 10.7717/peerj.7258.

Wiratno W, Syakir M, Sucipto I, Pradana AP. 2019. Isolation and characterization of endophytic bacteria from roots of *Piper nigrum* and their activities against *Fusarium oxysporum* and *Meloidogyne incognita*. Biodiversitas 20 (3): 682-687. DOI: 10.13057/biodiv/d200310.

Yavarian S, Jafari P, Akbari N, Feizabadi MM. 2021. Selective screening and characterization of plant growth-promoting bacteria for growth enhancement of tomato, *Lycopersicon esculentum*. Iranian J Microbiol 13 (1): 121-129. DOI: 10.18502/ijm.v13i1.5502.

Zhang J, Wang P, Fang L, Zhang Q-A, Yan C, Chen J. 2017. Isolation and characterization of phosphate-solubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. Polish J Microbiol 66 (1): 57-65. DOI: 10.5604/17331331.1234993.