Consortium of endophytic bacteria and rhizobacteria effectively suppresses the population of *Pratylenchus coffeae* and promotes the growth of Robusta coffee

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Abstract. *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: 4702-4708.* Pratylenchus coffeae *as the coffee-parasitic nematode can cause a yield loss from 28.7% to 78.4% on Robusta coffee (*Coffea canephora *A. Froehner). Environmentally friendly and sustainable efforts can be made to control this nematode by applying the antagonistic agents from the bacterial group. The application of antagonistic agents in a consortium has a better effectivity than a single application. This study was aimed to identify the effectivity of the consortium of endophytic bacteria and rhizobacteria formulated on a cost-effective molasse medium against the* P. coffeae *population and growth of Robusta coffee in a greenhouse. A total of nine bacterial isolates of genus* Bacillus *and* Pseudomonas *were used during experiment. The formulation was prepared by mixing the bacterial suspension and 2% molasses with a ratio of 1:1 (v/v). The greenhouse assay was performed following the completely randomized block design pattern with five treatments and five replications. Robusta coffee plant clone BP 936 was treated with a 10 mL (10^9 CFU mL^{-1}) bacterial consortium mixture while control was treated with 10 mL sterilized water. Each plant was inoculated with* P. coffeae *50 J2 after a week of bacterial consortium application. The result showed that all bacterial consortiums were effective in suppressing the population of* P. coffeae *in soil and root of Robusta coffee. All consortiums gave a better result than control in suppressing the population of* P. coffeae*. The best suppression of nematodes was shown by the Cst 02 consortium with the suppression value of 62.5% in the root and 76.1% in the coffee plant rhizosphere. The application of bacterial consortium increases the plant height (28.02-44.51%), number of leaves (127.27-168.18%), and leaf area (278.8-425.57%). In this study, suppression of* P. coffeae *population by bacterial consortium showed a positive correlation with plant growth. This study presents the information of endophytic bacteria and rhizobacteria consortium from* Bacillus *and* Pseudomonas *group that is effective in suppressing the population* P. coffeae *and improves the growth of Robusta coffee.*

Keywords: *Bacillus*, greenhouse, molasse, *Pratylenchus coffeae*, *Pseudomonas*, Robusta coffee

INTRODUCTION

The plant-parasitic nematode is one limiting factor in coffee production. Three nematode species have been reported to be the primary pathogen on the coffee plant worldwide, namely *Meloidogyne incognita*, *Radopholus similis*, and *Pratylenchus coffeae* (Trinh et al. 2012; Tuyet et al. 2012; Santos et al. 2018). Of those three species, *P. coffeae* is the most prominent nematode due to its distribution and the yield loss of its cause. *P. coffeae* is reportedly found in all coffee-producing provinces of Indonesia (Indarti and Putra 2018). Low coffee production due to *P. coffeae* infection is about 28.7% to 78.4% in Robusta coffee (*Coffea canephora* A. Froehner). In a separate study, Senthilkumar et al. (2013) reported that Robusta coffee infected by *P. coffeae* could still produce up to five years, but Arabica coffee could only produce up to two years. Arabica coffee had a low-value production of over 95% (Villain et al. 2000).

The symptoms of *P. coffeae* infection were lesions and necrosis on the root and eventually, lateral root rotted.

(Senthilkumar and Deivamani 2016). Damaged roots disrupt the process of absorption of water and nutrients from soil to the entire plant. Chlorosis occurs in plants due to disruption in the transport of water and nutrients, the symptom (yellowing) begins from the nearest leaves to the stem, then the main branches grow slightly, and the stem is easily shaken due to the depleted root, finally, the plant dies (Villain et al. 2000).

Efforts to control *P. coffeae* can be made in some ways, namely technical culture, resistant variety use, agrochemical use, and biological control. Although all efforts have been made, the infection of *P. coffeae* s has become an unsolved problem until now. Various studies revealed that one of the promising control for *P. coffeae* is biological control (Munif and Giyanto 2015; Gunasekaran et al. 2018). Prospective biological agents used as biological control of *P. coffeae* generally come from the endophytic bacteria and rhizobacteria (Oliveira et al. 2019).

Endophytic bacteria are those bacteria that can live in plant tissue for their half or whole life cycle without causing symptoms. The interaction occurs between plants,

and endophytic bacteria are a mutualism interaction (Afzal et al. 2019; Firdous et al. 2019). Previously, six *Bacillus* sp. of endophytic bacteria have been isolated from Arabica and Robusta coffee. Based on the greenhouse assay, these six isolates of *Bacillus* sp. could suppress nematode penetration up to 85% of the coffee plant. In addition, two rhizobacteria i.e. *Pseudomonas dimunita* and *Bacillus subtilis* were isolated from coffee plant. These rhizobacterial isolates obtained could also suppress *P. coffeae* population up to 50% in the Arabica coffee plant (Yulitaasary et al. 2017). The endophytic bacteria and rhizobacteria can protect the coffee plant from *P. coffeae* infection through two mechanisms, i.e. directly and indirectly (Abd-Elgawad 2016). Bacteria can directly produce metabolic compounds as anti-nematodes, such as extracellular enzymes and volatile compounds. The extracellular enzymes reported to suppress the plant-parasitic nematodes are protease and chitinase enzyme (Abdel-Salam et al. 2018). The volatile compound as anti-nematode is hydrogen cyanide (HCN) (Abd El-Rahman et al. 2019). Indirectly, the endophytic bacteria and rhizobacteria can increase plant resistance through induced systemic resistance (ISR) mechanism. ISR is a plant induced resistant phenomenon due to the biological agent stimulation (Mhlongo et al. 2018; Nguvo and Gao 2019). The endophytic bacteria and rhizobacteria can also stimulate plants to produce PR-protein as a resistant compound-complex. Moreover, some biological agents have the ability to fix nitrogen from the environment. Biological agents can also dissolve a bound nutrient elements such as P available to plants (Pham et al. 2017; Bendaha and Belaouni 2020).

The application of biological agent consortium to control plant-parasitic nematodes is generally more effective than a single application. Biological agent consortium is a combination of several biological agents that is mutually compatible and synergetic. The advantage of biological agent consortium is a more diverse mode of actions from the consortium formula made (Munif et al. 2019). Therefore, the endophytic bacteria and rhizobacteria consortium as biological control agents of *P. coffeae* have a great chance to succeed. This study was aimed to identify the effect of endophytic bacteria and rhizobacteria consortium against *P. coffeae* population and to observe the growth of Robusta coffee infected with *P. coffeae*.

MATERIALS AND METHODS

Isolation of endophytic bacteria and rhizobacteria

The nine isolates used in this study were previously studied and identified. The detail of these nine isolates is presented in Table 1.

Endophytic bacteria and rhizobacteria consortium formulation on cost-effective molasse medium

The endophytic bacteria and rhizobacteria were grown on Trypticase Soy Agar (TSA) media for 24 hours at 37°C. After 24 hours, three loops were taken from each isolate to be used in the formulation stage. The formulation was made by growing the bacteria simultaneously on 100 mL Trypticase Soy Broth (TSB), then incubated at 37 °C for 48 hours at 100 rpm. After 48 hours, the bacterial suspension grown on TSB media was mixed with a 2% molasse at the ratio of 1:1 (v/v). This mixture was then called as a bacterial consortium. The bacterial consortium was incubated for 72 hours at 100 rpm, after this stage, the bacterial consortium was ready to be used on the next assay (Lobo et al. 2019). The detail of the bacteria used on each consortium is presented in Table 2.

Experimental study

The study was performed in a greenhouse of Biological Education Study Program, Faculty of Educational Sciences, University of Jember. The plants used were Robusta coffee clone BP 936, with a height of 15 cm and the number of leaves was three, obtained from the Indonesian Coffee and Cocoa Research Center. Robusta coffee plants were planted on a mixture of soil, sand, and organic matter with the ratio of 1:1:1 (v/v/v). The soil characteristic used in this study is presented in Table 3.

The assay plant was planted on a pot with a diameter of 15.4 cm. An assay was performed following a completely randomized block design pattern with five treatments and five replications, (i) coffee plants infected with nematode and given Cst01 consortium; (ii) coffee plants infected with nematode and given Cst02 consortium; (iii) coffee plants infected with nematode and given Cst03 consortium; (iv) coffee plants infected with nematode and given Cst04 consortium; (v) coffee plants infected with nematode and without bacterial consortium treatment (control).

Table 1. Name of endophytic bacteria and rhizobacteria isolates

Isolate code	Bacterial species	Status	Reference
SK07	*Bacillus* sp.	Endophyte	
SK14	*Bacillus* sp.	Endophyte	
SK15	*Bacillus* sp.	Endophyte	
KB11	*Bacillus antrachis*	Endophyte	Asyiah et al. (2015, 2018)
KB14	*Bacillus* sp.	Endophyte	
KB63	*Bacillus* sp.	Endophyte	
PD01	*Pseudomonas dimunita*	Rhizobacteria	
BS01	*Bacillus subtilis*	Rhizobacteria	

Table 2. Composition of bacterial consortium

Consortium code	Bacterial isolate composition
Cst01	SK07 + SK14 + KB11
Cst02	SK07 + SK14 + KB14 + PD01
Cst03	SK07 + SK15 + KB11 + KB14 + KB63 + PD01 + BS01
Cst04	SK15 + KB11 + KB14 + KB15 + KB63 + PD 01 + BS01

Table 3. Soil characteristic for greenhouse assay

Total-N	Total-P	Available-P	Available-K	Organic C	C/N ratio
0.24%	16.70 mg 100 mg^{-1}	14.65 ppm	79.82 ppm	2.39%	9.95

One week after planting, the assay plant was given a treatment of bacterial consortium. The application of bacterial consortium was performed by watering 10 mL (10^9 CFU mL^{-1}) of bacterial consortium on the rooting area of the coffee plant. As a control, the plant was treated with 10 mL sterilized water. One week after bacterial consortium application, the plant was infested with *P. coffeae* 50 J2. The treated plants were maintained for 16 weeks (Senthilkumar and Deivamani 2016).

Pathological parameters

These parameters were used to measure the effectiveness of bacterial consortium as a biocontrol agent against *P. coffeae*. The pathological parameters observed were the number of nematodes in coffee plant roots and soil. Nematode extraction from soil was performed using a White Head Tray method, while the nematode extraction from the root was performed using a mist chamber. The observations were recorded at the 16th week after plant infested with *P. coffeae* 50 J2.

Plant growth parameters

These parameters were used to measure the effectiveness of bacterial consortium as a plant growth promoter of the Robusta coffee plant infected with *P. coffeae*. The parameters observed were plant height (cm), number of leaves, and leaf area (cm^2). The observations were noted at the 16th week after plant infested with *P. coffeae* 50 J2.

Statistical analysis

Data were analyzed using an analysis of variance (ANOVA) when a significant difference occurred among treatments; the analysis was continued with Duncan's Multiple Range Test (DMRT) with 95% degrees of confidence. A program used for analysis was IBM SPSS Statistic 24.0 (Senthilkumar and Deivamani 2016).

RESULTS AND DISCUSSION

Anti-nematode activity of bacterial consortium

Bacterial consortium showed better results in the pot assay experiment. The number of nematodes in root or soil on a plant treated with bacterial consortium was significantly different from the control plant (without bacterial consortium treatment) (Table 4). The results showed that the average nematode population on coffee roots in each treatment was 217.6 (Cst 03), 192.6 (Cst 01), 139.4 (Cst 04), 104.4 (Cst 02), and in control it was 371.8. The best performance to suppress the number of *P. coffeae* in root when compared to control was shown by the Cst02 consortium (71.9%), followed by Cst04 (62.5%), Cst01

(48.2%), dan Cst03 (41.5%). The bacterial consortium also showed a better performance in suppressing the *P. coffeae* population on the Robusta coffee rhizosphere. The average nematode population in the soil was 49 (Cst 01), 25.8 (Cst 02), 37.8 (Cst 03), 27.4 (Cst 04), and 108 (Control). The suppression of nematode population in soil when compared to control was 76.1% (Cst02) followed by 74.6% (Cst04), 65% (Cst03), and 54.6% (Cst01). The percentage of the nematode population suppression is presented in Table 6.

Based on the analysis above, all bacterial consortiums were found to be effective in suppressing the *P. coffeae* population either in the coffee root or rhizosphere. Effectiveness was indicated by different superscripts on the Duncan's Multiple Range test result at P-value ≤ 0.05. Although all consortiums were effective in suppressing the *P. coffeae* population, the Cst02 consortium showed the stable and best performance among all bacterial consortiums.

Table 4. Effect of bacterial consortium against *Pratylenchus coffeae* population after 16 weeks of treatment

Treatments	*P. coffeae* population	
	Root	Soil
Cst 01	192.6^a ± 10.50	49.0^a ± 3.80^a
Cst 02	104.4^b ± 15.56	25.8^b ± 3.70^b
Cst 03	217.6^a ± 04.56	37.8^a ± 4.08^a
Cst 04	139.4^c ± 06.42	27.4^b ± 4.56^b
Control	371.8^d ± 55.25	108.0^c ± 21.77^c

Note: Values followed by different superscript letters are significant at P ≤ 0.05 over control

Table 5. Effect of bacterial consortium on the growth of Robusta coffee infected *Pratylenchus coffeae*

Treatments	Plant height (cm)	Number of leaves	Leaf area (cm^2)
Cst 01	23.4^a ± 5.80	10.7^a ± 2.66	416.3^a ± 224.9
Cst 02	26.3^a ± 4.37	11.3^a ± 2.92	577.6^a ± 260.0
Cst 03	23.3^a ± 2.05	10.0^a ± 0.79	200.6^{ab} ± 103.6
Cst 04	25.9^a ± 2.74	11.8^a ± 4.69	345.6^{ab} ± 190.6
Control	18.2^b ± 2.97	4.4^b ± 2.30	109.9^b ± 81.5

Note: Values followed by different superscript letters are significant at P ≤ 0.05 over control

Tabel 6. Suppression of nematode populations by consortium of endophytic bacteria compared to control plants

Treatments	Population suppression (%)	
	Nematodes in root	Nematodes in soil
Cst 01	48.2	54.6
Cst 02	71.9	76.1
Cst 03	41.5	65
Cst 04	62.5	74.6
Control	0	0

Tabel 7. Correlation coefficient between growth variables

	Nematodes in root	Nematodes in soil	Plant height	Number of leaves	Leaf area
Nematodes on roots	1				
Nematodes on soil	0.960[**]	1			
Plant height	-0.994[**]	-0.974[**]	1		
Number of leaf	-0.961[**]	-0.976[**]	0.964[**]	1	
Leaf area	-0.865	-0.708	0.809	0.746	1

Note: **. Correlation is significant at the 0.01 level (2-tailed)

Growth promoter activity on Robusta coffee infected *Pratylenchus coffeae*

All assay plants were in a similar condition and insignificantly different on the initial assay. Plant height, number of leaves, and leaf area were in identical condition in the initial assay. The bacterial consortium showed positive effect on the growth of coffee plants. A significant difference was observed on several growth variables, namely plant height, number of leaves, leaf area, and canopy dry weight. The application of bacterial consortium had a significant effect on plant height compared to the control plant. The best performance to increase plant height was found in the Cst02 bacterial consortium (26.3 cm), followed by Cst 04 (25.9 cm), Cst 01 (23.4 cm), dan Cst 03 (23.3 cm) while in control it was 18.2 cm. The above bacterial consortiums could increase the plant height by 44.51% (Cst02), 42.31% (Cst 04), 28.57% (Cst 01), and 28.02% (Cst 03). Although all bacterial consortiums gave a significant influence when compared to the control treatment, the plant height among bacterial consortium treatments was not different. This condition indicated that all consortiums had a relatively similar effectivity to increase the plant height.

The application of bacterial consortium also showed positive effect on the number of leaves in Robusta coffee. Based on the observations, plants treated with bacterial consortium had a significantly different number of leaves with the control plants. The bacterial consortium that showed the best performance to increase the number of leaves was Cst 04 (11.8), followed by Cst 02 (11.3), Cst 01 (10.7), and Cst 03 (10) as compared to control where it was 4.4. When compared to the control plant, the above bacterial consortiums could increase the number of leaves by 168.18% (Cst 04), 156.82% (Cst 02), 143.18% (Cst 01), and 127.27% (Cst 03). The results of the analysis indicated that all treatments were significantly different from the control plant but insignificantly different among treatments. The effectiveness of the endophytic bacteria consortium as plant growth promoter can also be seen from its ability to increase the leaves area of Robusta coffee plants. The results indicated that the two bacterial consortiums i.e. Cst02 and Cst01 showed a significantly different leaf area with control plants. The average leaf area in Cst02 was 577.6 cm^2 and in Cst01 it was 416.3 cm^2 while the average leaf area in the control was 109.9 cm^2. The higher leaf area was recorded in the bacterial consortium of Cst02 (425.57%) and Cst01 (278.8%), respectively. The other two consortiums, namely Cst 03 and Cst04, had higher leaf area than the control, these treatments were insignificantly different on the DMRT with 95% degrees of confidence. Moreover, plant height, number of leaves, and leaf area are presented in Table 5.

Variables correlation

The number of nematodes in the roots and the number of nematodes in the soil were found to have a correlation with several growth variables. The correlation test result showed a significantly negative correlation between the number of nematodes in soil and root with the plant height. The higher number of nematodes in soil and root, the lower plant height occurred. Based on the correlation test, the number of nematodes in root and soil had a correlation coefficient of (-0.996 and -0.974) with plant height, respectively. These coefficient values indicated that the number of nematodes in soil and root had a significant difference in plant height; however, the number of nematodes in root had a greater effect on the plant height.

The number of nematodes in soil and root was negatively correlated with the number of leaves. Due to higher number of nematodes in soil and root, fewer numbers of leaves were occurred. Based on the correlation test, the number of nematodes in root and soil had a correlation coefficient of -0.961 and -0.976 with the number of leaves, respectively. These values indicated that the number of nematodes in soil and root significantly influenced the number of leaves in Robusta coffee; however, the number of nematodes in the root had a more significant effect. Furthermore, the number of nematodes in soil and root were insignificantly correlated with the leaf area. Although high nematode population in soil and root cause low leaf area, this kind of influence was insignificantly different. The correlation among variables is presented in Table 7 and Figure 1.
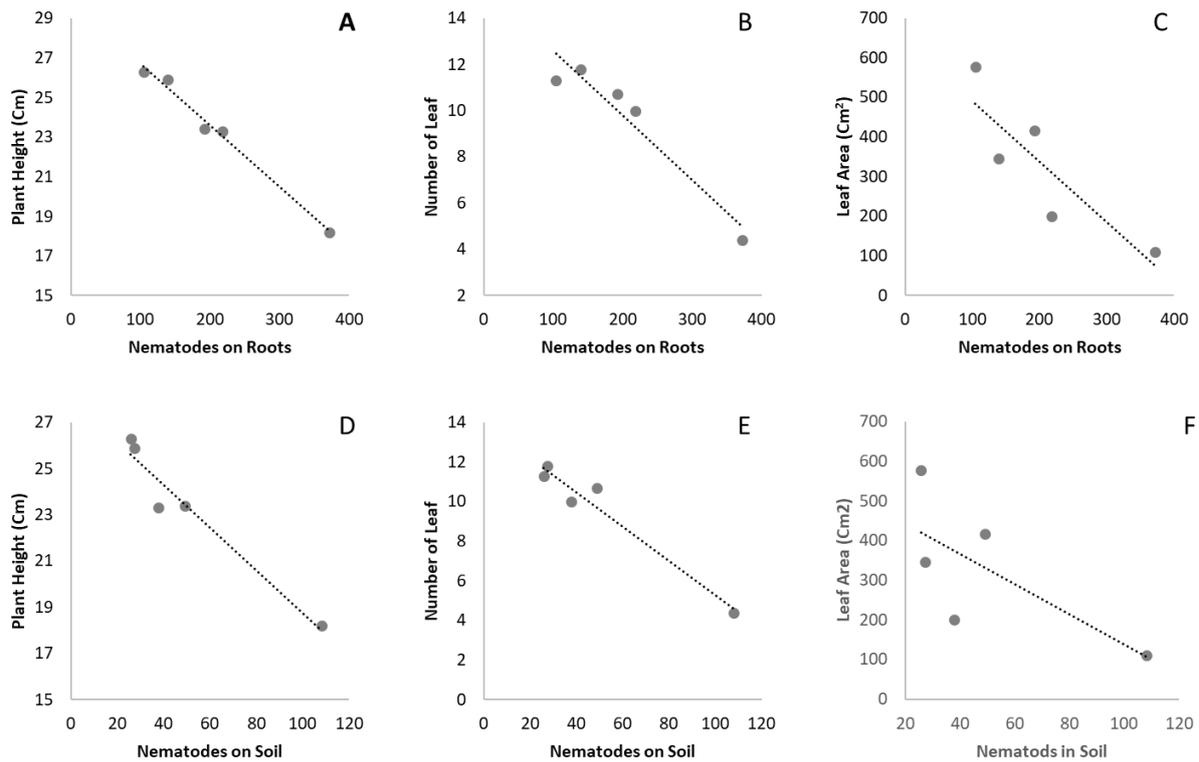
Figure 1. Correlation between the number of nematodes in root and soil with plant growth variables

Discussion

The suppression of the *P. coffeae* population in soil and root by bacterial consortium can occur directly and indirectly. Rhizobacteria and endophytic bacteria produced secondary metabolites that are directly toxic for nematodes (Thom et al. 2013; Jha and Saraf 2015). The metabolites that are commonly secreted by either rhizobacteria or endophytic bacteria are protease and chitinase extracellular enzymes (Kaur et al. 2013). Protease enzymes can hydrolyze proteins into simple compounds. The hydrolysis result is commonly in the form of peptides or amino acids (Contesini et al. 2018). Several previous researchers have reported the effectiveness of protease enzyme to suppress nematode population and egg hatching. The application of endophytic bacteria and rhizobacteria has been reported to be able to suppress the growth of *Meloidogyne incognita* (Thongkaewyuan and Chairin 2018), *Radopholus similis* (Chen et al. 2018), *Globodera rostochiensis* (López-Lima et al. 2013), and *Pratylenchus coffeae* nematode (Giribabu et al. 2019). Similarly, protease enzyme, and chitinase enzyme secreted by endophytic bacteria and rhizobacteria also acts as lyase for plant-parasitic nematode cells (Ha et al. 2014). Safni et al. (2018) reported that *Pratylenchus* spp. of nematode soaked with chitinolytic bacterial filtrate culture, causing damage to the stylet part. The nematode stylet was reported to be abnormal, and some of which were disrupted. Besides extracellular enzymes, endophytic bacteria and rhizobacteria have also been reported to be able to suppress the plant-parasitic nematodes by producing a volatile hydrogen cyanide (HCN) compound (Mardhiana

et al. 2017; Munif et al. 2019). The HCN compound is reported to have a nematicidal activity against many plant-parasitic nematode types, including *Pratylenchus coffeae* (Munif and Giyanto 2015).

Endophytic bacteria and rhizobacteria could indirectly induce the plant resistance through an induced systemic resistance (ISR) mechanism. ISR is one of the resistant mechanisms with a broad spectrum. The induced plant produced more resistant compounds than the normal condition (Mhlongo et al. 2018). The resistant compounds commonly produced by plants are phenolic and pathogenesis-related protein (PR-protein) compounds. PR-proteins are specific proteins that act as plant resistant compounds from pathogen attack or invasion (I Finkina et al. 2017). In a separate study, Zaynab et al. (2019) reported that plant producing high PR-protein had a higher resistant capability against plant-parasitic nematode infections.

The application of endophytic bacteria and rhizobacteria was also reported to increase coffee plant growth. Bacteria can fix nitrogen from the environment and release it into a form that can be directly utilized by plants. Furthermore, the endophytic bacteria and rhizobacteria can also dissolve phosphate element (P) bonded in soil. Phosphate in the soil is commonly bonded in the form of P stone or other deposits that are unable to be absorbed directly by plants. Several bacterial groups reportedly capable of fixing N and dissolving P are *Pseudomonas*, *Bacillus*, *Serratia*, *Paenibacillus*, and *Micrococcus* (Kenneth et al. 2019).

Endophytic bacteria and rhizobacteria consortium was reported to have a positive effect than the single bacterial application. Bacterial consortiums when applied together increase their effectiveness by more than 30% compared to individual use (Munif et al. 2019). This statement has been confirmed by Munif and Giyanto (2015), who has compared the application of single bacteria and consortium on the coffee plant. Bacterial consortium gave a better result to suppress the *P. coffeae* population and enhance e coffee plant growth. The bacterial consortium formulation as biofertilizer and biopesticide with molasse media has also been reported to be effective in the previous studies (Hanudin et al. 2016). Kumari et al. (2019) reported that molasse could be used as cheap formulation media, easily obtained, environmentally safe, capable of supporting bacterial life during the preservation period.

The effectivity of bacterial consortium formula to suppress the nematode population is closely related to the plant growth. Lower nematode population in soil and root can reduce the root damage. Root health is the primary key to plant fertility during the cultivation period. A healthy root can give more optimal results on the nutrient elements and water absorption. Similar results were seen by Avelino et al. (2009) who stated that a higher root damage level by *P. coffeae* caused a more inhibited plant growth. On the other hand, Panneerselvam et al. (2008) reported that the number of *P. coffeae* in the rhizosphere of the coffee plant was positively correlated with the level of coffee roots damage. This study shares the information that endophytic bacteria and rhizobacteria consortium formulated into a cost-effective medium with molasse, which is effective in suppressing the *P. coffeae* population in soil and root of Robusta coffee plant.

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