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Biocontrol of root-knot nematode *Meloidogyne incognita* in arabica coffee seedling by using fortified bacterial consortium

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ABSTRACT

The damage caused by the southern root-knot nematode (*Meloidogyne incognita*) in coffee plants has been reported in various countries, including Indonesia. The measures to control the nematodes on coffee seedlings and fields depend on synthetic nematicides. Addressing this issue requires not only a more environmentally friendly and cheaper technology but also equal effectiveness comparable to synthetic nematicides. This study aimed to test the effectiveness of fortified bacterial consortium (FBC) involving a combination of liquid organic fertilizer (LOF), botanical pesticide, and a bacterial consortium to control *M. incognita* infection in *Coffea arabica* seedlings. The treatments in this study consisted of control, synthetic nematicide with the active ingredient fluopyram, and various FBC concentrations ranging from 20%, 40%, 60%, 80%, and 100%. The results demonstrated that the application of 60% to 100% FBC increased plant growth. The most effective and efficient treatment for increasing plant growth was the application of 60% FBC. When compared with control plants, 60% FBC treatment resulted in 6.8% longer root, 9.5% higher plant, 5.3% heavier plant fresh weight, and 4.8% heavier root fresh weight. We also found that the application of 60% to 100% FBC increased the amount of chlorophyll in leaves. FBC application also reduced the number of nematodes in the soil up to 60.6%, the number of galls up to 286.4%, and the severity of root damage up to 118.2%. This study indicates that the application of 60% FBC is the most effective and efficient in controlling *M. incognit*a and stimulating the growth of *C. arabica* seedlings.

Key words: *Bacillus*; *pseudomonas*; endophyte; rhizobacteria; fluopyram.

1 INTRODUCTION

Coffee is one of the leading commodities currently under massive development in various countries. This is strongly intertwined with the increasing global demand for quality coffee (Ibrahim; Zailani, 2010). The facts show that improving the quality and quantity of coffee is not without challenges, such as nematode infection. Several nematodes such as *Pratylenchus coffeae, Radopholus similis*, and root-knot nematodes (*Meloidogyne* spp.) are known to threaten coffee production (Barros et al., 2014; Trinh et al., 2009).

Root-knot nematodes (RKNs) are a cosmopolitan pathogen that parasitizes more than 2000 plant species. Its fast life cycle (± 30-60 days) requires imperative control measures to prevent epidemics of RKNs infection (Elling, 2013; Subedi; Thapa; Shrestha, 2020). Several RKNs species reported to infect coffee plants all over the world, including Indonesia, are *M. incognita, M, javanica, M. coffeicola,* and *M. exigua* (Halimah; Supramana; Suastika, 2013; Kurniawati; Supramana; Adnan, 2017; Taher; Supramana; Suastika, 2012). Previous reports stated that RKN infection has declined coffee production up to 45% in Brazil (Barbosa et al., 2004). The potential loss is reported to be even more substantial when it infects *Coffea arabica* plants (Barbosa; Souza; Vieira, 2010). This is in line with Anzueto et al. (2001) who argue that *Coffea arabica* plant is more susceptible to infection due to various pathogens compared to *Coffea canephora* var. Robusta and *Coffea liberica*. RKNs infection in coffee plants generally begins in the seedling phase, although in some cases infection is also evident when the plants have started to mature (Barros et al., 2011).

Coffee plants infected by RKNs will show general symptoms as the infection on other plants. The symptoms below the soil surface include the development of root galls and stunted roots. Above ground level, the symptoms include wilted plant leaves, yellowish-green leaves, stunted growth, and an unpleasant look of the plant. Symptoms above the soil surface occur because the root system is disturbed, interfering with water distribution from the soil to all plant parts (Albuquerque et al., 2010; Goulart et al., 2019; Muniz et al., 2009).

Some measures to control RKNs can be performed by the use of biological agents, botanical pesticides, and synthetic chemical nematicides. However, to date, the problems associated with RKNs infection in coffee still prevail. The abovementioned measures are deemed ineffective because each control is carried out independently. Halimah, Munif and Giyanto (2015) reported that using a single isolate of endophytic bacteria to control *P. coffeae* was not effective. However, the control involving a bacterial consortium has demonstrated an infection suppression rate of up to 87%. Munif, Sukarno and Gusmaini (2021) also reported that the control of *M. incognita* using a consortium of bacteria was more effective than using a single isolate.

Panpatte et al. (2021) reported a new method for controlling RKNs using the fortified bacterial consortium (FBC). The fortified bacterial consortium is defined as the combination of the bacterial consortium with other mutually compatible materials to increase shelf life and antagonists. In this study, a consortium of bacteria (*Providencia vermicola* AAU PR1, *Pseudomonas putida* AAU PR2, and *Pseudomonas fluorescens* AAU PR3) was fortified using a botanical pesticide made from aqueous extracts of *Azadirachta indica, Ipomoea carnea,* and *Brassica juncea*. These extracts were chosen because they were reported to have nematicidal activity. The control of *M. incognita* using FBC was reported to be 83% more effective compared to using a bacterial consortium without fortification. However, the effectiveness of FBC in controlling the nematodes in coffee remains underexplored.

Controlling *M. incognita* using FBC is more effective because FBC contains more active ingredients that can suppress *Meloidogyne* spp. (Panpatte et al., 2021). Previous studies state that bacterial antagonists from the rhizobacteria group and endophytic bacteria can produce extracellular protease and chitinase enzymes (Tian; Yang; Zhang, 2007). Protease and chitinase enzymes can nullify nematodes, inhibit the hatching of *M. incognita* eggs, and destroy the stylet of nematodes. Another research also states that the bacteria from the genera *Pseudomonas, Bacillus,* and *Serratia* can produce nematicidal HCN (Beneduzi; Ambrosini; Passaglia, 2012; Lodewyckx et al., 2002). Furthermore, the aqueous extract of *A. indica* contains several compounds that are toxic to nematodes (Javed et al., 2007).

Previous researchers have succeeded in isolating, selecting, and characterizing a consortium of bacteria to control plant-parasitic nematodes (Asyiah et al., 2020; Asyiah et al., 2021; Asyiah et al., 2018). The bacterial consortium can be fortified to increase its effectiveness in controlling *M. incognita* infection in *C. arabica* plant. Despite the reports of the fortified bacterial consortium using botanical pesticides, the fortification of the bacterial consortium using botanical pesticides and liquid organic fertilizers only receives scant attention. The addition of liquid organic fertilizer has the potential to extend the shelf life of bacteria and increase plant growth. Yuan et al. (2013) state that liquid organic fertilizer contains nutrients essential for rhizobacteria. In addition, liquid organic fertilizer (LOF) can increase the growth of coffee seedlings by up to 67% compared to control plants (Ormeño Diaz; Ovalle; Rey, 2018).

This study aims to examine the effectiveness of the fortified bacterial consortium involving bacterial consortium, botanical pesticide, and liquid organic fertilizer as an agent for controlling *M. incognita* and promoting the growth of *C. arabica* seedlings.

2 MATERIAL AND METHODS

2.1 Site and Time

The research was carried out at the Plant Disease Laboratory and Greenhouse in Plant Protection Study Program, Faculty of Agriculture, University of Jember – Indonesia from June to December 2021.

2.2 Source of Bacterial Isolates

The bacterial isolates were three endophytic bacteria and one rhizobacterium which had been isolated, identified, and characterized in previous studies (Table 1). Each isolate was tested for compatibility, and these were found compatible to be combined in a consortium (Asyiah et al., 2020).

Table 1: Bacterial isolates in this study.

Isolate codes	Bacterial species	Status	References
SK07	Bacillus sp.	Endophyte	(Asyiah et al., 2018; Asyiah et al., 2015)
SK14	Bacillus sp.	Endophyte	
KB14	Bacillus sp.	Endophyte	
PD01	Pseudomonas dimunita	Rhizobacteria	

2.3 The Production of Bacterial Consortium

The bacterial consortium was prepared on Tryptone Soya Broth (TSB) medium (HiMedia, India). A total of 6 g of TSB media was dissolved in 200 ml of distilled water and then sterilized using an autoclave. One oose from each bacterial isolate was put into sterile media. Subsequently, incubation was carried out at 28° C for 48 hours while shaking at 150 rpm, before further testing (Munif et al., 2019).

2.4 The Production of Botanical Pesticide

The botanical pesticide was prepared through an aqueous extraction technique following Azad et al. (2013). The raw materials were leaves of *A. indica* and *Aglaia odorata* which were reported to have nematicidal activity. The leaves were dried in an oven at 40° C until the water content reached ± 13%-15%. When dried, the leaves were chopped and filtered using a 100-mesh sieve. The resultant powder was used for extraction.

A total of 50 g of *A. indica* powder and 50 g of *A. odorata* powder were soaked with distilled water until the volume reached 1000 ml. This was carried out for 24 hours, and during the immersion, the suspension was shaken using a shaker at 200 rpm. After 24 hours of immersion, the suspension formed was filtered using a 300-mesh sieve. The filtered suspension was then referred to as the botanical pesticide for further testing.

2.5 The Production of Liquid Organic Fertilizer

Liquid organic fertilizer was made from coffee husk waste, cow dung waste, and coconut water. The LOF consisted of 10 kg of coffee husk waste, 10 kg of cow dung waste, 10 L of coconut water waste, 3 L of molasses, and 100 L mixture including 1 L of commercial effective microorganisms and water. The mixture was fermented using a fermenter for 30 days. After 30 days, the LOF suspension was ready for use. LOF was filtered using a filter cloth to separate coarse particles (García Molano; Parra Alba; Páez Guevara, 2021).

LOF then evaluated its characteristics. The organic-C content was analyzed using the Walkley & Black method according to the protocol outlined by Jha et al. (2014). In addition, foreign matter analysis was conducted using the sorting and sieving. The content of the heavy metals As, Hg, Pb, Cd, Ni, and Cr was analyzed using the wet oxidation method according to the method specified by Buurman, Van Lagen dan Velthorst (1996). The LOF pH was measured with a pH meter. The organic-N content and total N were evaluated using the Kjeldahl methods (Nahm, 2003). P_2O_5, K_2O, total Fe, Mn, Cu, Zn, B, and Mo were analyzed using the wet oxidation method (Junaidi; Windari; Aini, 2022). Following the method described by Rhodes and Kator (1988), *Escherichia coli* and *Salmonella* spp. were also looked for in LOF.

2.6 Compatibility Test of Bacterial Consortium and Botanical Pesticide (In Vitro Experiment)

This test aimed to ensure that the botanical pesticide did not inhibit the growth of the bacterial consortium. The test was carried out using the paper disk diffusion method following Hoesain et al. (2021). A total of 100 µl of a consortium suspension of endophytic bacteria was spread on Tryptic Soya Agar (TSA, HiMedia, India) media in a petri dish with a diameter of 9 cm. After the bacterial suspension was flattened, four sterile paper disks were placed on top of the media. Next, 30 µl of botanical pesticide which had been sterilized using a millipore syringe filter with Ø 12.25 mm and pore size of 0.2 µm was dripped on top of the paper disk. This bacterial consortium was incubated for 24 hours. As a negative control, the bacterial consortium was dripped using sterile distilled water, while the positive control used chloramphenicol, 1000 ppm. Compatibility was indicated by the absence of a clear zone around the paper disk.

2.7 The Production of Fortified Bacterial Consortium

The FBC was produced by combining bacterial consortium with botanical pesticides and liquid organic fertilizers according to the modified protocol (Panpatte et al., 2021). Bacterial isolates were grown on a TSB medium for 48 hours to make the bacterial consortium. A total of 150 ml of the bacterial consortium was mixed with 150 ml of botanical pesticide and 700 ml of liquid organic fertilizer which had been diluted with water (1:1, v/v).

2.8 Greenhouse Experiment

The coffee seedlings used in this test were 3 months-old AS2K clones of *C. arabica* obtained from the Plant Protection Study Program – University of Jember. This clone was quite susceptible to root-knot nematodes. Coffee seeds were planted in pots with a diameter of 20 cm and height of 15 cm. This was filled with a mixture of soil, sand, and compost (1:1:1), each of which had been sterilized. After one week, coffee seedlings were treated using 100 ml of nematicide and FBC per plant at various concentrations. The treatments included water control, 3% synthetic nematicide (500 g L^{-1} fluopyram), 20%, 40%, 60%, 80%, and 100% FBC.

Three days after the coffee seedlings had been planted, 750 J2 *M. incognita* per pot was inoculated according to Silva et al. (2020) protocol for testing coffee resistance to RKNs. J2 *M. incognita* were obtained from a greenhouse rearing. Tomato plants (Tantyna variety) cultivated in sterile soil and inoculated with a single egg mass of *M. incognita* were used for rearing. *M. incognita* eggs were then extracted from tomato plant roots by using 2% NaOCl. The extracted nematode eggs were washed with distilled water and kept in a dark environment for 7 days until they hatched.

Coffee seedlings were then reared to determine the effect of FBC on *M. incognita* infection. FBC was applied once a week for 12 weeks. This test applied a randomized complete block design (RCBD) with 7 treatments and 4 replications with 5 test plants in each replication. Thirteen weeks after J2 *M. incognita* inoculation, agronomic and pathological variables were observed.

The agronomic variables consisted of root length, plant height, crown fresh weight, root fresh weight, amount of chlorophyll a and b. A tape measure was used to measure the length of the longest roots from its base to its end. Weighing the plant's crown devoid of roots provided the measurement of the crown's fresh weight. After the roots had been cleared of any soil particles, their fresh weight was measured. The chlorophyll was observed using a spectrophotometric method by following Fabrowska et al. (2018). A total of 1 g of leaves were crushed and then macerated using 100 mL of 80% acetone. The solution was then homogenized until the chlorophyll dissolved. The suspension was then filtered using Whatmann filter paper. The filtrate was put in a cuvette and measured with a spectrophotometer at a wavelength of 646 mm for chlorophyll a and 663 mm for chlorophyll b.

The pathological variables included the number of RKN in the soil, the number of nematodes in the roots, the number of root gall, and the scale of root damage. The nematodes were extracted from the soil using the white head tray method following Bell and Watson (2001), while the extraction of nematodes from the roots was carried out using a mist chamber (Crow; Habteweld; Bean, 2020). Root damage was observed using the scale determined by Zeck (1971).

The data were analyzed for variance using the DSAASTAT version 1.101. When a difference was identified, the Tukey test with a 95% confidence interval would be performed.

3 RESULTS

3.1 The Characteristics of the Bacterial Consortium

All bacteria in this study were compatible to be combined, as evidenced by the absence of a clear zone in the dual culture test. The density of the endophytic bacterial consortium was marked at 4.3 × 109 CFU mL^{-1}.

3.2 The Characteristics of Liquid Organic Fertilizer

The liquid organic fertilizer in this study had a pH of 5.25. Organic-C content was marked at 10.23%, while organic-N was marked at 0.18% (Table 2). The fertilizer also contained N, P_2O_5, K_2O, Mn, Cu, Zn, B, and Mo, at different rates. The results of microbial contaminant analysis showed that the fertilizer did not contain *Escherichia coli* and *Salmonella* sp.

3.3 The Compatibility between Bacterial Isolate and Botanical Pesticide

The bacterial consortium was compatible with the botanical pesticide. This was indicated by the absence of a clear zone around the bacterial colonies on petri dishes. This was also confirmed by the results of the negative control which did not show any clear zone around the bacterial colonies. Furthermore, in the positive control using antibiotics, a clear zone was formed around the bacterial colonies, indicating an incompatible reaction.

3.4 The Effects of Fortified Bacterial Consortium on Plant Growth

Fortified bacterial consortium exerts various effects on plant growth (Figure 1). Although no statistical difference was marked in the root length between treatments using 60%, 80% and 100% FBC, treatment using 60% FBC generated the most efficient results. When compared with control and synthetic nematicide treatments, 60% FBC was found to produce longer roots by 6.8% and 6.9%, respectively.

Table 2: The Properties of Liquid Organic Fertilizer.

No	Parameters	Units	Results	Methods
1	Organic-C	%	10.23	Walkley & Black
2	Foreign matter	%	-	Sorting and Sieving
3	Heavy Metals:			
	As	ppm	0.00	Wet Oxidation, HNO_3 + $HclO_4$, AAS – Hydride Cold Vapour
	Hg	ppm	0.00	Wet Oxidation, HNO_3 + $HclO_4$, AAS – Hydride
	Pb	ppm	0.68	Wet Oxidation, HNO_3 + $HclO_4$, AAS
	Cd	ppm	0.00	Wet Oxidation, HNO_3 + $HclO_4$, AAS
	Ni	ppm	0.18	Wet Oxidation, HNO_3 + $HclO_4$, AAS
	Cr	ppm	0.43	Wet Oxidation, HNO_3 + $HclO_4$, AAS
4	pH	-	5.35	Electrometry, pH meter, (1:5)
5	Organic-N	%	0.18	Kjeldahl, Titrimetry
6	Macro Nutrients:			
	N	%	0.21	Kjeldahl, Titrimetry
	P_2O_5	%	0.16	Wet Oxidation, HNO_3 + $HclO_4$, Molybdovanadat, Spectrophotometry
	K_2O	%	0.32	Wet Oxidation, HNO_3 + $HclO_4$, AAS - Flamephotometry
7	Micro Nutrients:			
	Total-Fe	ppm	0.00	Wet Oxidation, HNO_3 + $HclO_4$, AAS
	Mn	ppm	10.32	Wet Oxidation, HNO_3 + $HclO_4$, Spectrophotometry
	Cu	ppm	6.27	Wet Oxidation, HNO_3 + $HclO_4$, AAS
	Zn	ppm	3,46	Wet Oxidation, HNO_3 + $HclO_4$, AAS
	B	ppm	1.24	Wet Oxidation, HNO_3 + $HclO_4$, Azomethine-H, Spectrophotometry
	Mo	ppm	2,47	Wet Oxidation, HNO_3 + $HclO_4$, Spectrophotometry
8	Microbial Contaminants:			
	Escherichia coli	MPN/ml	Negative	Most Probable Number (MPN)
	Salmonella sp.	MPN/ml	Negative	Most Probable Number (MPN)

The observation of plant height marked similar results to that of root length. The treatment associated with the highest plant was 60% FBC. Administering FBC at concentrations of 20% and 40% did not significantly affect plant height when compared with control plants and plants with synthetic nematicide. When compared with control and synthetic nematicide treatments, the plant height in 60% FBC was 9.5% higher.

Similar results were also documented in terms of the fresh weight of plant canopy and root fresh weight. In these two variables, there was no significant difference among the treatments of 60% to 100% FBC, but mean canopy weight treated with 60% FBC was heaviest.

The lowest chlorophyll content was found in control plants with 2 mg g^{-1} of chlorophyll, followed by 20% FBC (2.3 mg g^{-1}), 40% FBC (2.4 mg g^{-1}), synthetic nematicide (2.9 mg g^{-1}), and 60%, 80%, and 100% FBC with 3.1 mg g^{-1} chlorophyll each. The data regarding the total chlorophyll load are presented in Table 3.

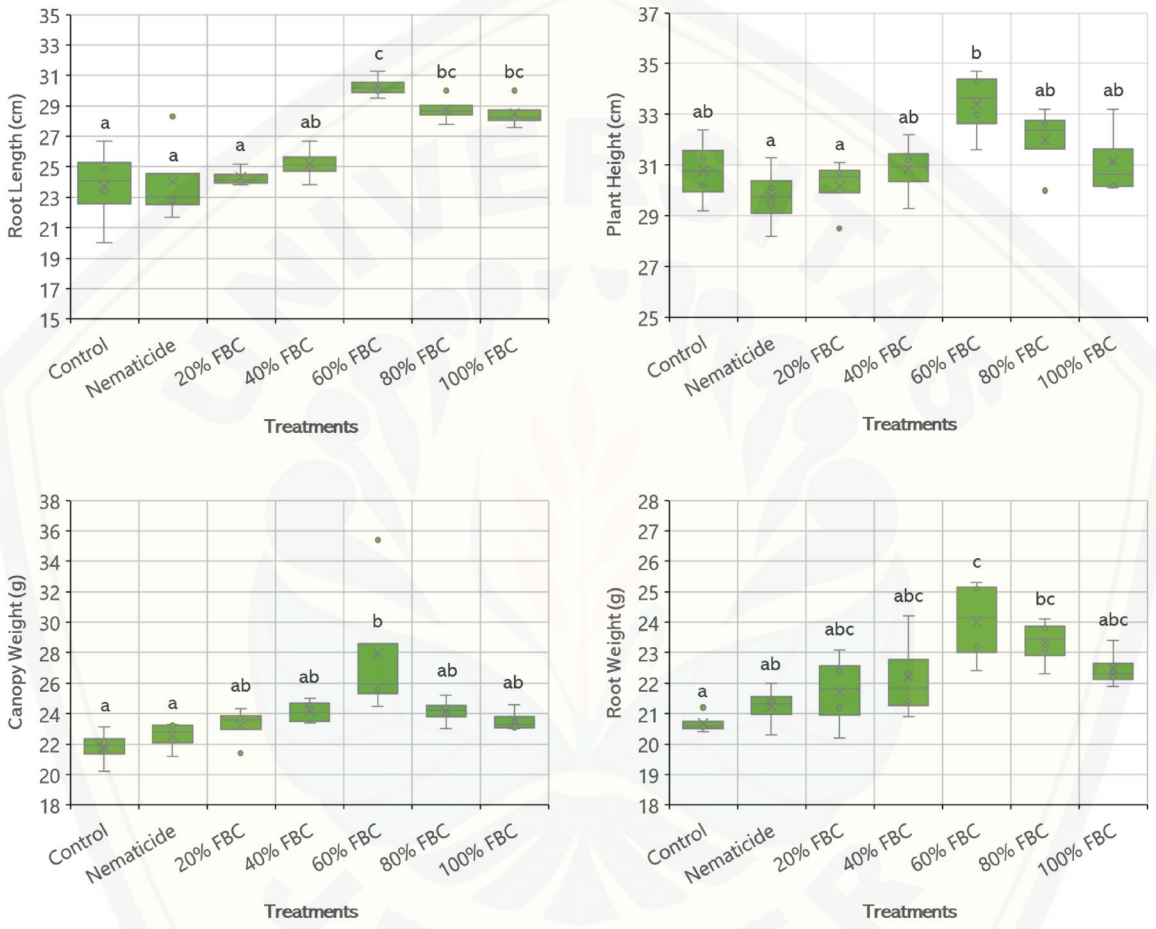

Figure 1: The effects of different FBC concentrations on the growth of *C. arabica* seedlings. Note: the different letters above the bar graph indicate a significant difference in the Tukey HSD test with a 95% confidence interval.

Table 3: The effects of synthetic nematicide treatment and various concentrations of FBC on chlorophyll of *C. arabica* seedlings

Treatments	Chlorophyll A (mg g^{-1} fresh leaf)	Chlorophyll B (mg g^{-1} fresh leaf)	Total Chlorophyll (mg g^{-1} fresh leaf)
Control	1.7	0.3	2.0
Synthetic nematicide	2.1	0.8	2.9
20% FBC	1.8	0.5	2.3
40% FBC	1.8	0.6	2.4
60% FBC	2.3	0.8	3.1
80% FBC	2.3	0.8	3.1
100% FBC	2.4	0.7	3.1

3.5 The Effectiveness of Fortified Bacterial Consortium in Suppressing *Meloidogyne incognita* Infection in Coffee

The results of our study showed that there was no significant difference in the nematode number in post-treatment between the applications of FBC and synthetic nematicides. This is not the case in the average nematode in roots. The control plants had an average of 226.5 nematodes per 20 g of roots, which was not significantly different from the average number of nematodes in the roots of the 20% and 40% FBC treatments. Significant differences were identified in plants treated with synthetic nematicide and those with 60%, 80%, and 100% FBC. Statistically, the administration of FBC with a concentration of 60-100% generates the same effect as synthetic chemical nematicides.

Concerning the number of galls, FBC treatments at all concentrations could suppress the number of galls on coffee roots. However, when compared with the application of synthetic nematicides, administering FBC at concentrations of 60% to 100% was found the most effective. Furthermore, considering the severity of root damage, control plants had the highest severity with an average damage scale of 6. This value was not significantly different from FBC treatment at concentrations of 20% and 40%. However, treatment using FBC at concentrations of 60%, 80%, and 100% generated significantly different results compared with control plants, but these three treatments were not significantly different from synthetic nematicide treatment. The data on the effectiveness of FBC in suppressing root-knot nematodes in *C. arabica* are presented in Figure 2.

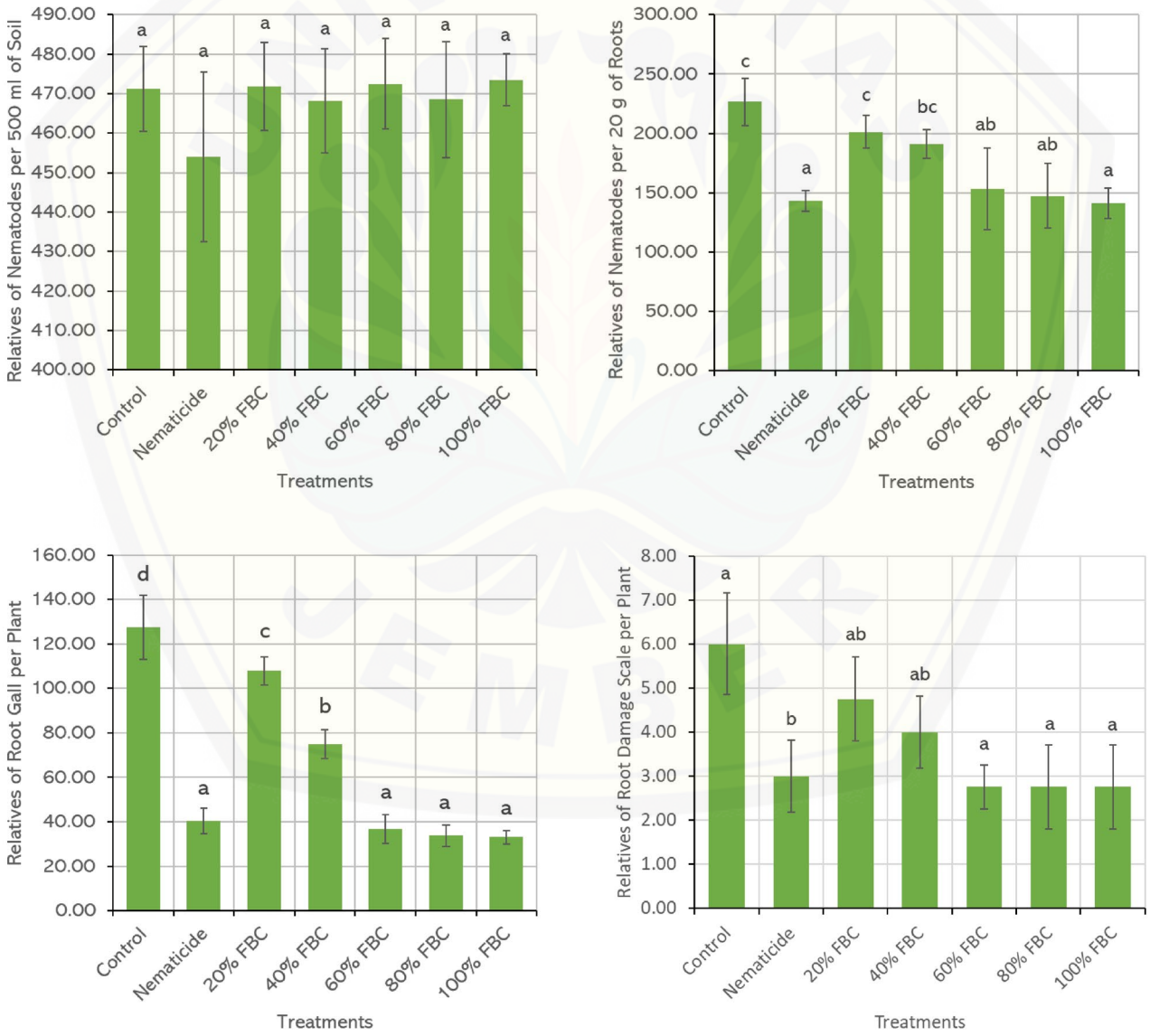

Figure 2: The effects of different concentrations of FBC on *Meloidogyne incognita* in *C. arabica* seedlings. Note: the different letters above the bar graph indicate a significant difference in the follow-up Tukey HSD test with a 95% confidence interval.

4 DISCUSSION

Plant-parasitic nematodes, including root-knot nematodes, often cause considerable losses to coffee production (Barbosa et al., 2004; Zinger et al., 2021). Nematode control in coffee plantations can be carried out using synthetic nematicides, but it is fairly expensive. As such, coffee plantations require alternative measures that are more environmentally friendly while maintaining comparable effectiveness to synthetic nematicides. This study discovered a formula of a fortified bacterial consortium that is equally effective as synthetic nematicides.

The application of fortified bacterial consortium generates higher effectiveness in controlling plant-parasitic nematodes, compared with that involving a single bacterium isolate. However, the bacterial consortium needs to involve mutually compatible bacteria (Halimah; Munif; Giyanto, 2015). Compatibility indicates that the metabolites secreted by one bacterium do not suppress the growth of other bacteria. Compatibility is not only limited to the effects between bacteria, but also between bacteria and other substances (Hoesain et al., 2021). In this study, the bacterial consortium was compatible with botanical pesticides. Panpatte et al. (2021) and Hoesain et al. (2021) have reported on the compatibility of plant growth-promoting bacteria with botanical pesticides. Botanical pesticide contains compounds that can control plant-disturbing organisms. However, generally, these compounds are not broad spectrum. Some microbes can survive exposure to active compounds within botanical pesticides (Akila et al., 2011).

In this study, FBC was made from of liquid organic fertilizer, botanical pesticide, and a consortium of bacteria. This combination enables different treatments for coffee seeds, than the three substances applied independently. Liquid organic fertilizer boosts plant growth by providing essential nutrients for coffee (Ormeño Diaz; Ovalle Silva; Rey, 2018). The organic matter in the fertilizer can also provide nutrients needed by antagonistic bacteria to thrive (Moridi et al., 2019). This finding is coherent with Nurhayati, Nurahmi dan Marziah (2019) who contends that coffee seedlings with liquid organic fertilizer have 23% longer root lengths and 13% higher plant height compared to control plants.

Plant growth can also be influenced by the bacterial activity associated with FBC. In this direction, Dorjey, Dolkar dan Sharma (2017) and Gutiérrez-Mañero et al. (2001) report that *Bacillus* sp. and *Pseudomonas* sp. can stimulate plant growth through several mechanisms. Bacteria can fix nitrogen (N) from the environment and convert it into an essential substance for plants (Basu et al., 2021). Also, these bacteria can dissolve phosphate (P) and make it available to plants (Billah et al., 2019). In nature, phosphate is available in large enough quantities in the soil, but the amount is often bound by other elements, such as Al and Fe. Plant growth-promoting bacteria can nullify these bonds through the production of phosphatase enzymes (Bargaz et al., 2021).

The results of this study indicate that the application of FBC can increase plant growth and chlorophyll production. Compared with control plants, FBC treatments at concentrations of 60% to 100% are found to be the most effective to support plant growth. The administration of FBC at a concentration of 60% is more effective than that at a concentration of 80% and 100%. The concentrations at 80% and 100% cannot lead to maximum growth, assumedly, due to excessive liquid concentration. Plant growth is closely related to the nutrient content in FBC. The test results of liquid organic fertilizer quality have shown that the fertilizer in this study contains sufficient macro and micro nutrients essential for plant growth, therefore improving chlorophyll production in leaves (Amujoyegbe; Opabode; Olayinka, 2007).

The plant growth in this study is associated with decreased nematode infection in the roots. The lower the severity of nematode infection in the roots, the more they can absorb nutrients and water (Asyiah et al., 2021). Root-knot nematode infection often damages root tissue and interferes with root function (Elling, 2013). In this study, nematode infection has been successfully suppressed by applying FBC, which consists of a consortium of bacteria, botanical pesticides, and LOF. In harmony, Khan et al. (2019) reported the use of *A. indica* to control RKNs in vitro and greenhouse study. Also, Lengai, Muthomi and Mbega (2020) states that the extract of *Aglaia odorata* have a good potential as botanical pesticide. The leaf extract of *A. indica* contains active compounds such as β-sitosterol, hyperoside, nimbolide, quercetin, quercitrin, rutin, azadirachtin, and nimbine (Sarkar; Singh; Bhattacharya, 2021). Some of these compounds have nematicidal activity against *Meloidogyne incognita* (Hernández-Carlos; Gamboa-Angulo, 2019).

A consortium of endophytic bacteria has been proven effective in controlling RKNs infection in various plants (Asyiah et al., 2020; Halimah; Munif; Giyanto, 2015; Munif et al., 2019). Antagonistic bacteria can control RKNs either directly or indirectly. Bacteria can directly produce protease and chitinase enzymes as secondary metabolites. Both enzymes can lyse proteins and chitins which are important to nematode cell walls and nematode eggs (Khan; Williams; Nevalainen, 2004; Soliman et al., 2019). In addition, antagonistic bacteria can produce HCN, a volatile compound that is toxic to nematodes (Maheshwari; Bhutani; Suneja, 2019). Bacteria can indirectly increase plant resistance to parasitic nematode infections through a mechanism called induced systemic resistance (ISR). The mechanism stimulates plants to produce resistance compounds, such as tannins, saponins, glycosides, and pathogenesis-related protein (PR-Protein) (Kloepper; Ryu, 2006; Pieterse et al., 2014). Munif et al. (2019) confirm that the application of a bacterial consortium can suppress root-knot nematode infection in tomatoes while increasing plant growth.

5 CONCLUSION

The application of fortified bacterial consortium consisting of liquid organic fertilizer, botanical pesticide, and bacterial consortium at a concentration of 60% is proven the most effective and efficient in suppressing root-knot nematode infection in *C. arabica* seedlings. The effectiveness is comparable to that of the synthetic nematicide fluopyram. Compared to plants treated with the synthetic nematicide fluopyram, those treated with 60% FBC had 9.3% fewer root galls and a lower level of root damage. The increase in root length, canopy fresh weight, and root fresh weight is only identified in treatment involving FBC at concentrations ranging from 60% to 100%, yet this potential growth is absent in treatment involving the synthetic nematicide.

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7 AUTHORS' CONTRIBUTION

APP and AB wrote the manuscript and performed the experiment, MH and INA supervised the experiment and co-worked the manuscript, MA and AIAY conducted all statistical analyses.

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