Application of bio-silica and citronella oil nanoemulsion, neem oil, and endophytic bacteria formula for the java tea root-knot nematode population suppression

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Abstract. Root-knot nematode is a potential obstacle to sustainable cultivation for the medicinal java tea plant (*Orthosiphon aristatus*). Some botanical/bio-pesticide formula (citronella oil and bio-silica nanoemulsion, neem oil, and endophytic bacterial *Pseudomonas fluorescens* and *Burkholderia cepacia*) were evaluated for their effectiveness in root-knot nematode control. The botanical/bio-pesticides were drenched on nematode inoculated-seedling plant (var. Orsina 3 Agribun) roots in polybags, twice with 14 days interval. The experiments were randomized block design with two factors, with six treatments for the main plot and two factors as the co-plot. The treatments were replicated six times with ten plants in each replication. Three months after treatment, from the root-knot nematode population, plant growth, and leaves dry weight analysis, were indicated that all formula tested effectively suppressed the root-knot nematode population in the root, except endophytic bacterial formula. However, the synergistic nematicidal effect of the endophytic bacteria formula (1.5%) against root-knot nematode was provided by the root incorporation of bamboo charcoal compost as much as 89.68%. Citronella-oil (1%) and bio-silica (2%) nanoemulsion, and neem oil formulas (1%) effectively suppressed nematode populations (99.33% and 97.65%, and 99.66%, respectively), increasing the sinensetin content/production in comparison with control; and could be evaluated further in the plantation in the field.

Keywords: java tea plant, root-knot nematode, botanical-/bio-pesticide, eco-friendly nematode control.

1. Introduction
Java tea/ kidney tea plant (*Orthosiphon aristatus* Blume Miq.) is one of the medicinal plants prioritized for hypertension adjuvants in the 2020 National Research Priority flagship for medicinal plants; it requires a guarantee of product safety and quality of raw materials. The raw materials for this herbal are not only efficacious but also must be free from hazardous chemical residues (including pesticides). Root-knot nematodes could be the potential obstacles to the sustainable production of raw materials for the plant, especially if the plant were cultivated continuously in the same field. However, environmentally friendly medicinal plant pest control techniques are still very limited. Plant-parasitic
nematodes could be managed through several environmentally friendly approaches such as using natural enemies, applying botanical pesticides, enhancing cultural practices, and cultivating resistant cultivars.

Botanical pesticide such as neem oil and essential oil has long been known effective in controlling plant pests and diseases, especially in countries that have used herbs in their daily lives [1]. Most of the phytochemical compounds contained in some plants have pesticidal bioactivity, but less persistent than synthetic pesticides, then reduce the negative impact on human health and the environment and the natural enemy. Eugenol in clove essential oil is toxic to insects, nematodes, golden snails, and plant pathogenic fungi, while citronellal is insect repellent with EC50, 5-10 times lower than synthetic pesticides [2]. Eugenol and citronellal in clove and citronella grass oil are effectively controlled the root-knot nematode on ginger and black pepper and several important black pepper pests [2, 3].

The use of endophytic bacteria to control nematodes in plantation crops such as coffee, black pepper, and patchouli has been reported [4, 5, 6, 7, 8]. Mekete et al. (2009), used the endophytic bacteria Bacillus pumilus and B. mycoides to control the nematode M. incognita on coffee plants [4]. Those bacteria were able to suppress the population and number of root-knots 33 and 39%, respectively. The use of endophytic bacteria can suppress the nematode population of Pratylenchus brachyurus by 54.8-70.6% and increase patchouli plant fresh weight by 23.62-57.48% [9]. Endophytic bacteria are bacteria that live in plant tissues, but do not harm the host plant tissue [10]. The use of endophytic bacteria as a nematode biological agent provides more advantages because it has the same niche as the nematode/pathogen [11], where they both colonize root tissue; so that the endophytic bacteria could be able to reach the nematode target faster. The mechanism of endophytic bacteria in controlling nematodes in roots is to induce resistance, space competition and produce anti-nematode metabolites [12, 13, 14].

Silicates are known to increase plant resistance to diseases caused by fungi and bacteria [15]. Research on rice plants, applying silicate (Si) was able to suppress the intensity of leaf spot incidence [16]. Recently, some research groups investigated the potential of silica as a pesticide. Nanosilica has been reported to provide insecticide activity on its own through the desiccation of insects’ cuticles (http://www.nanopool.eu/english/news.htm).

Some botanical-/ bio-pesticides have been formulated by conventional and or nanotechnology methods by research groups, such as citronella-oil and bio-silica nanoemulsion, neem oil, and endophytic bacterial formula. Those formulae were evaluated for their effectiveness in controlling the root-knot nematode population development on the java tea plant.

2. Materials and methods
2.1. Preparation of chemical and the botanical-/bio-pesticide formula
Four botanical-/ bio-pesticides formula, i.e., citronella oil and bio-silica nanoemulsion, neem oil, and endophytic bacterial bioagent (Pseudomonas fluorescens and Burkholderia cepacia), were observed their effectiveness in controlling the root-knot nematode population development in java tea roots. Formulas of citronella grass oil nanoemulsion, neem oil, and endophytic bacterial bioagent were formulated in ISMCR, Bogor, and bio-silica nanoemulsion formula was prepared by ICAPRD, Bogor. Biofertilizer bamboo charcoal compost, which was also used in this experiment to evaluate their synergist integration with biopesticides formula in nematode population suppression and in increasing plant growth, was prepared by IFPRD, Bogor. Nematicide carbofuran was used as a chemical comparison of the effectiveness of botanical-/ bio-pesticides (Table 1).
Table 1. Several botanical-/ bio-pesticides formula were tested and or their integration with biofertilizer to evaluate their effectiveness against nematode population suppression on java tea plants.

| No. | Botanical-/bio-pesticide and or biofertilizer treatment application | Concentration/ dosage | Application method |
|-----|---------------------------------------------------------------|------------------------|--------------------|
| 1.  | citronella oil nanoemulsion (citronellal)                     | 1% (according to the recommended concentration on the package) | Drenching on the soil surface around roots area |
| 2.  | neem oil (azadirachtin and nimbin compound)                   | 1% (preliminary concentration evaluation in laboratory) | Drenching on the soil surface around roots area |
| 3.  | endophytic bacteria formula (*Pseudomonas fluorescens* and *Burkholderia cepacia)* | 1,5% (formulator concentration recommendation) | Drenching on the soil surface around roots area |
| 4.  | bio-silica nanoemulsion (at least 10% natural silica nanoemulsion solution dissolved as SiO2) | 2% (formulator concentration recommendation) | Drenching on the soil surface near the roots after the application of botanical-/ bio-pesticide |
| 5.  | carbofuran                                                   | 25 g (according to the recommended dosage on the package) | Sprinkled on the soil surface near the roots before the application of botanical-/bio-pesticides |

| Biofertilizer | Bamboo charcoal compost (lignocellulolytic microbe and charcoal) | 200 g (formulator dosage recommendation) | Sprinkled on the soil surface near the roots before the application of botanical-/bio-pesticides |

2.2. Experimental java tea plants preparation

The java tea cultivar “Orsina 3 Agribun” (white flower with brown stem clone) was used in the study. The cultivar was susceptible to root-knot nematode *Meloidogyne* spp. The java tea plants were cultivated in 40x40 cm²-polybags (8 kg soils). The experimental polybags contained unsterilized nematode-infested field soils: sand: cow manure = 2: 1:1 media and the polybags were arranged in the field. Seedlings aged four weeks were transplanted. Four weeks after transplanting, the seedlings were fertilized with Urea (N), KCl (K), and SP-16 (P) as SOP, followed by nematode inoculation and botanical-/bio-pesticides application by pouring 200 ml of the formula solution containing the desired concentration per plant.

2.3. Root-knot nematode inoculum preparation

Nematode inoculum was obtained by extraction of nematode larvae from the nematode infested java tea plant roots. The extracted nematode larvae were then made into a suspension by adding 100 ml of sterile water containing 25-50 nematode larvae for each experimental plant. Extraction of nematodes from plant roots was carried out using a modified Baermann funnel method [17].

2.4. Experimental designs

The experiments were designed as randomized block design. The treatment designs were two factors with six treatments (biopesticide application) for the main plot and two factors (biofertilizer) for the co-plot. The treatments were replicated six times with ten plants in each replication. (Table 2). Two months after planting, the plants were treated with the formula twice by drenching 200 ml of the desired concentration/ dosage of each formula around the plant root system area, then replicated in the next 14 days. The granule formula of chemical carbofuran were spread around the plant roots area (Table 2).
Table 2. Treatment design.

| Botanical/Bio-pesticide formula | Without Bamboo Charcoal Compost | With Bamboo Charcoal compost |
|--------------------------------|---------------------------------|-----------------------------|
| Control (aquadest)             | K0P0= Aquadest                  | K0P1= aquadest + BC compost |
| Citronella-oil nanoemulsion (CO NE) | K1P0= CO NE                    | K1P1= CONE + BC compost     |
| Neem oil (NMO)                 | K2P0= NMO                      | K2P2= NMO + BC compost      |
| Endophytic bacteria (BA)       | K3P0= EB                       | K3P3= EB + BC compost       |
| Bio-silica nanoemulsion (BIOS NE) | K4P0= BIOS NE                | K4P4= BIOS NE + BC compost |
| Carbofuran (C)                 | K5P0= C                        | K5P5= C + BC compost        |

Nematode inoculation was carried out by pouring 100-150 ml of a solution containing 25-50 nematode larvae of *Meloidogyne* spp. in the root area of two months-old seedlings. The application of botanical/bio-pesticides was carried out twice with two weeks intervals, by the "drenching" method, pouring about 200 ml of the formula solution with a concentration according to the treatment, one month after root-knot nematode inoculation.

2.5. Response designs
Approximately four months after planting, and after nematode larvae infestation, and after botanical/biopesticide application, the plants were uprooted, and the fresh plant weight, plant leaves dry weight, nematode population in the roots, and the percentage of sinensetin compound content levels, were analyzed. Furthermore, the data of nematode population in roots were used to calculate the percentage of nematode population suppression using the following equation; where N1 was nematode population on control plants and N2 was nematode population on biopesticide-treated plants (1):

\[
\text{The nematode population suppression percentage} = \frac{N1 - N2}{N1} \times 100\% \tag{1}
\]

For dry leaves weight and sinensetin content level analysis, the plants were dried (withered) at room temperature for 2-3 days, until the moisture content reached 8-10%. Then the dried leaves were weighed to analyze their dry leaf weight and sinensetin content in Test Laboratory of ISMECRI, Bogor.

2.6. Statistical data analysis
The data were analyzed by Analysis of Variance (ANOVA), and treatments were compared by Duncan’s Multiple Range Test (DMRT) at 5% level (α 0.5). Differences between means were considered significant at P< 0.05.

3. Results and discussions
3.1. The effect of botanical/bio-pesticide formula toward nematode population suppression
From the results of statistical analysis (results of contrast analysis), generally most of the botanical/bio-pesticides formula tested (citronella oil and bio-silica nanoemulsion, and neem oil) were significantly suppressed the root-knot nematode populations (about 91.27% and 87.65%, and 99.66%, respectively), except on the treatment of endophytic-bacteria (Table 4). However, the bamboo charcoal (BC) compost root incorporation increased the effectiveness of endophytic bacteria application toward nematode population suppression (80.63%) (Table 4, Figure 1/K3P1). The treatment combination of endophytic bacterial formula and BC compost application significantly decreased the nematode population down to 370.67 vermiforms (from 3,593.00 vermiforms nematode larva on endophytic bacteria application alone) (Figure 1), which the formula effectiveness was as same as chemical carbofuran (93.96%) (Table 4).
From the results of contrast analysis, the average of effectiveness value between the single application of biopesticide (without-BC compost) and the combined application of biopesticide (with BC compost) toward nematode population suppression was not relatively different. Only on the single application of endophytic bacterial formula (without-BC compost) and the combined application of biopesticide (with BC compost) toward nematode population suppression was significantly different; where the combined application of endophytic bacteria and BC compost application shown synergistic interaction in the nematode population decrease/suppression (Table 3).

![Figure 1. Nematode population level in Java tea plant root based on botanical-/bio-pesticides formula treatment and their combined with bamboo charcoal compost application.](image)

**Table 3.** Contrast analysis results of botanical-/bio-pesticide treatment against the root-knot nematode population suppression.

| Contrast                        | DF  | JK     | KT     | F Hitung | Pr>F  |
|--------------------------------|-----|--------|--------|----------|-------|
| Control vs. other all treatment| 1   | 6510926.422 | 6510926.422 | 4.53* | 0.0411 |
| Without compost vs. with compost| 1   | 2282896.333  | 2282896.333  | 1.59 ns | 0.2167 |
| Control vs. Biopesticide       | 1   | 8885488.889  | 8885488.889  | 6.18* | 0.0183 |
| Control vs. Endophytic bacteria | 1   | 43.556    | 43.556    | 0.00 ns | 0.9956 |
| Control vs. BIOS               | 1   | 7372800.000 | 7372800.000 | 5.13* | 0.0304 |
| Control vs. carbofuran         | 1   | 7424088.889 | 7424088.889 | 5.17* | 0.0299 |

Note:

- K0P0 = control
- K0P1 = bamboo charcoal (BC) compost
- K1P0 = citronella-oil nanoemulsion
- K1P1 = citronella oil nanoemulsion + BC compost
- K2P0 = Neem oil formula
- K2P1 = Neem oil + BC compost
- K3P0 = Endophytic bacteria
- K3P1 = Endophytic bacteria + BC compost
- K4P0 = Bio-silica nanoemulsion
- K4P1 = Bio-silica nanoemulsion + BC compost
- K5P0 = carbofuran
- K5P1 = carbofuran + BC compost
Table 4. The effect of botanical-/bio-pesticides formula and their combined bamboo charcoal (BC) compost root incorporation toward the root-knot nematode populations suppression percentage.

| Biopesticide formula (K) | Nematode population suppression (%) based on biopesticide treatment without BC Compost compared with control (P0) | Nematode population suppression (%) based on biopesticide treatment combined with BC Compost compared with control (P1) |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Control (K0)            | 0.00 b                                                                                                                     | 0.00 b                                                                                                                     |
| Citronella-oil nanoemulsion (K1) | 91.27 a                                                                  | 97.91 a                                                                  |
| Neem oil (K2)           | 99.66 a                                                                                                                   | 95.12 a                                                                                                                   |
| Endophytic bacteria (K3) | 0.00 b                                                                                                                   | 80.63 a                                                                                                                   |
| Bio-silica nanoemulsion (K4) | 97.65 a                                                                                                                  | 95.47 a                                                                                                                  |
| Carbofuran (K5)         | 93.96 a                                                                                                                   | 100.00 a                                                                                                                  |

Note: Averages in the same column followed by the same letter are not significantly different by DMRT at α=0.05. BC = bamboo charcoal; The table represents the average of 30 plants (3 replicates, each replication consisted of 10 plants); Plants treated with biopesticides were Java tea plants inoculated previously with nematodes (approximately 25 larva-2 (L2) per plant).

3.2. The effect of botanical-/bio-pesticide formula application toward plant growth

The effect of botanical-/bio-pesticide treatment on nematode-inoculated java tea plant was indicated that all biopesticide treatment generally caused a positive effect on plant growth as measured with plant and leave fresh weight compared with control plant; except on endophytic bacteria treatment, where there was a slightly decreased fresh plant and leaf weight (Table 5). The best plant growth was shown by java tea plant treated with neem oil formula (246.18 g and 102.04 g), followed by bio-silica nanoemulsion (237.86 g and 106.15 g), and then citronella oil nanoemulsion (180.80 g and 86.83 g). Neem oil and nano-biosilica formulas shown the same good effect as the comparison nematicide carbofuran on fresh plant and leave weight (236.75 g and 105,097); compared to control plant (175.26 g and 77.76 g) (Table 5).

Table 5. The effect of botanical-/bio-pesticide formula toward plant growth level (fresh plant and leaf weight).

| Botanical-/Bio-pesticides treatment | Fresh plant weight average (g) | Fresh leaf weight average (g) |
|------------------------------------|--------------------------------|-------------------------------|
| Control                            | 175.26 b                       | 77.76 abc                     |
| Citronella oil nanoemulsion        | 180.80 ab                      | 86.83 ab                      |
| Neem oil formula                   | 246.18 a                       | 102.04 a                      |
| Endophytic bacteria formula        | 161.9* b                       | 84.05 ab                      |
| Bio-silica nanoemulsion            | 237.86 a                       | 106.15 a                      |
| Carbofuran                         | 236.75 a                       | 105.097 a                     |

Note: Averages in the same column followed by the same letter are not significantly different by DMRT at α=0.05. The table represents the average of 30 plants (3 replicates, each replication consisted of 10 plants); Plants treated with biopesticides were Java tea plants inoculated previously with nematodes (approximately 25 larva-2 (L2) per plant). *) incidence of plant growth suppression.
3.3. The effect of botanical-/bio-pesticide formula application on yield and sinensetin content level

In Indonesia, the Java tea plant yield are generally in the product of dried leaves. On dry leaf weight, the effect of bio-silica-nanoemulsion treatment showed the highest yield (43.00 g), followed by neem oil formula treatment (40.33 g), and showed the same good effect as the chemical nematicide carbofuran (37.00 g), in compared with control plants (30.33 g) (Table 6).

Based on the analysis of the percentage of sinensetin levels in dry leaf simplicia, it was shown that the effect of botanical-/bio-pesticide treatment on nematode infested-Java tea plant were generally higher than the control plants; except for neem oil formula which appeared to be slightly lower (0.12%) than the control plant (0.14%) (Table 6). However, the levels of sinensetin content in the leaves yield which treated with neem oil, were still in line with and higher than the herbal pharmacopeia standard (0.10%). The highest sinensetin content was shown in the treatment of citronella oil nanoemulsion (0.18%), followed by endophytic bacterial formula (0.17%), and bio-silica nanoemulsion (0.15%), and carbofuran (0.15%) (Table 6).

For the sinensetin production, the highest value was derived from plants with the treatment of bio-silica nanoemulsion (1.93 g), followed by carbofuran (1.67), neem-oil formula (1.45g), and citronella-oil nanoemulsion (1.35); which higher than sinensetin production of control plant (1.27) (Table 6). Sinensetin production is influenced by leaf production and sinensetin content levels, so even though sinensetin levels are high, and leaf production is low, the sinensetin production will be low as well, and vice versa (Darwati 2021, personal communication), in comparison with sinensetin production of control plants.

Table 6. The effect of botanical-/bio-pesticide formula on yield (dry leaf weight, total dry leaf weight), yield, and sinensetin content/production.

| Biopesticides formula treatment | Dry leaf weight (g)/plant | Total dry leaf weight (g) | Sinensetin content (%) | Sinensetin production (g) |
|-------------------------------|--------------------------|--------------------------|------------------------|---------------------------|
| Control                       | 30.33 ab                 | 909.9                    | 0.14                   | 1.27                      |
| Citronella oil nanoemulsion   | 25.0 ab                  | 750                      | 0.18                   | 1.35                      |
| Neem oil formula              | 40.33 a                  | 1,209                    | 0.12                   | 1.45                      |
| Endophytic bacterial formula  | 23.00 ab                 | 690                      | 0.17                   | 1.17                      |
| Bio-silica nanoemulsion       | 43.00 a                  | 1,290                    | 0.15                   | 1.93                      |
| carbofuran                    | 37.0 a                   | 1,110                    | 0.15                   | 1.67                      |

Note: Averages in the same column followed by the same letter are not significantly different by DMRT at α=0.05. The table represents the average of 30 plants (3 replicates, each replication consisted of 10 plants); Plants treated with botanical-/bio-pesticides were Java tea plants inoculated previously with nematodes (approximately 25 larva-2 (L2) per plant).

The hypothesis, which effective botanical-/bio-pesticides formula application directly positively correlated with and affected toward nematode population suppression in plant roots, thereby reducing the damage of plant roots, so that it increased plant growth as measured by the increase of fresh plant weight, consequently toward the increase of yield as measured by the increase of the dry leaf weight, and finally to the increase of the sinensetin content and or sinensetin production level. Compared to control, this hypothesis is in line with the effective application of bio-silica nanoemulsion formula and nematicide carbofuran. The hypothesis was not in line to the effective application of the citronella-oil nanoemulsion formula, and endophytic bacteria formula (combined with bamboo charcoal compost) toward the yield (dry leaf weight), and to the application of neem oil formula toward the sinensetin content. However, the sinensetin content of all effective biopesticide formula application in this experiment were still in line with the pharmacopeia herbal standard (0.10%). It means that yield (dry leaves weight and sinensetin content in plants) were not affected by the level of nematode infection in the roots, but were more affected by the type of biopesticide formula applied. Concentration application of 1.0% of citronella oil nanoemulsion in this experiment was probably a little bit phytotoxic (toward dry leaf weight). During the experiment lasting, some experimental plants were attacked by leaf pests.
which caused considerable leaves loss, so that resulted in low leaf yield level even though under low nematode populations infestation on some effective botanical-/ bio-pesticide application treatments.

The neem oil formula application effectively suppressed the growth of nematode populations on the plant roots, and increasing plant growth and yield and sinensetin production. For decades, the effectiveness of neem and its various products has been extensively investigated and used in controlling root-knot nematodes. The bioactive ingredients nimbidin and thionimone isolated from neem extract were reported to play a role in killing and inhibiting the growth of the nematode larvae of *Hoplolaimus indicus*, *Rotylenchulus reniformis*, and *Meloidogyne incognita* [18]. Applications of neem leaves, neem root exudates, and extracts of neem oil refining wastewater are toxic to the root-knot nematodes *M. incognita* and *Helicotylenchus indicus*, so that they can kill and inhibit the development of nematode larvae [19, 20, 21]. The suppression of nematode population development in roots will increase plant growth indirectly.

The citronella oil nanoemulsion application effectively suppressed the nematode population development, as effective as the chemical nematicide carbofuran. In a preliminary *in Vitro* study in the laboratory, the citronella oil nanoemulsion has effectively killed the larvae of *Meloidogyne* sp. as much as 100% at concentrations of 0.5%, 1.0%, and 1.5% [22]. In this experiment, citronella-oil nanoemulsion formula in 1% concentration application twice, with intervals every 14 days, significantly reduced the nematode population (91.227%, but a little bit suppressed yield (dry leaf weight). However, this application increased the sinensetin content. It is necessary to study further the effectiveness of citronella-oil nanoemulsion in lower concentration levels for suppressing nematode populations but not inhibiting plant growth.

The application of bio-silica nanoemulsion to the roots can suppress nematode populations and increase plant growth and yield, but the suppression mechanism is unknown. Preliminary evaluation in *in Vitro* test had not been carried out in the laboratory. Application in the field was carried out with 2% concentrations recommended by the formulator based on the results on rice plants. The biosilica used in this experiment is a natural silica nanoemulsion solution dissolved at least 10% as SiO$_2$ (Hoerudin 2020, personal communication). Silicates (Si) are known to increase plant resistance to diseases caused by fungi and bacteria [15]. Research on rice plants, application of silicate on leaves was able to suppress the intensity of leaf spot incidence [16]. In rice plants, silicate treatment in rice leaves can induce the formation of phytoalexin compounds in rice leaves to become more resistant to leaf spot disease caused by *Pyricularia grisea* [23]. Silicates play a role in phenol metabolism and lignin biosynthesis in plant cell walls [24]. Secondary metabolites such as lignin and phenol have an important role in plant resistance. In other research experiments, nano-silica has been reported to provide insecticide activity on its own, through the desiccation of insects’ cuticles. It has also been successfully applied as a thin film to boost cereal germination and decrease fungal growth (http://www.nanopool.eu/english/news.htm).

On the endophytic bacteria formula application was effective toward nematode population suppression if it combined with the bamboo charcoal application. The synergistic mechanism of its combination application has not been yet known. Bioagent formula use in this experiment contained endophytic bacteria *Pseudomonas fluorescens* dan *Burkholderia cepacia*. This endophytic bacteria formula in nematode control in this experiment was the preliminary evaluation without *in Vitro* evaluation in the laboratory test before. *P. fluorescens* dan *B. cepacia* were two of several endophytic bacterial that have anti-nematode bioactivity and were effective in controlling root-knot nematodes [25, 26, 11]. The use of endophytic bacteria suppressed the incidence of yellow disease symptoms and the nematode population of *Meloidogyne* sp.; and increased the number of flowers per stem segment and the fresh weight of black pepper berries [6]. Siddiqui and Shaukat used *Pseudomonas aeruginosa* and *P. fluorescens* to control *M. javanica* in tomato and soybean plants [27]. The mechanism of endophytic bacteria in controlling the nematodes in roots is to induce resistance, space competition, and produce anti-nematode metabolites [12, 13, 14]. Endophytic bacteria such as *Bacillus* sp. and *Pseudomonas* sp. showed their physiological character with proteolytic activity, cellulolytic, fluorescence, chitinolytic, HCN (cyanic acid), and phosphate solubilization [28]. *P. fluorescens* induced the systemic resistance
to root-knot nematode infection (*Meloidogyne* spp.) through independent signal transduction of salicylic acid accumulated in roots. Siddiqui and Shaukat reported the role of salicylic acid biosynthesis in enhancing defense mechanisms against parasitic nematodes [29]. *P. fluorescens* in culture also produces 2,4 diacetyl phloroglucinol which can kill L2 of java root-knot nematode *M. javanica* [27]. The important factors in applying bioagent formulas in the field include how bioagent can survive and multiply well to establish their population in the field ecosystem for performing nematicidal bioactivity optimally. Indigenous rhizobacteria that are formulated must have long life span and maximum biological activity, so that they can survive optimally in long shipping and storage conditions [30] and are suitable for agronomic practices [31]. The appropriate formula will provide a habitat that can protect microorganisms and is more tolerant of fluctuations in temperature, humidity, and chemical pesticides so that the potential for life and colonization is increased properly. In this experiment, bamboo charcoal compost (contained lignocellulolytic microbes) incorporation probably provide a habitat that can protect endophytic bacteria and is more tolerant of fluctuations in temperature, and humidity so that the potential for life and colonization is increased properly. Further research on the synergistic effect mechanism provided by bamboo charcoal compost application on endophytic bacterial *P. fluorescens* and *B. cepacia* formula and other bioagents formulas is the analyzation of endophytic bacteria population in the soil around roots between single application and combined application of endophytic bacteria and bamboo charcoal compost.

4. Conclusion
Bio-silica nanoemulsion (2% solution), citronella oil nanoemulsion (1% solution), and neem oil emulsion (1% solution) formula were significantly suppressed the nematode population in the java tea roots (99.33%, 97.65%, and 99.66%, respectively). Endophytic bacterial *P. fluorescens* and *B. cepacia* formula (1.5% solution) were effectively suppressed the nematode population in combined application with bamboo charcoal compost as much as 89.68%. Accordingly, botanical-/ bio-pesticides formula based on natural compounds from bio-silica and citronella oil nanoemulsion, and neem oil emulsion, was expected to be a possible alternative botanical-/ bio-pesticide for preventing the nematode population build-up in sustainable java tea plant cultivation field. However, further studies need to be carried out were, to evaluate the lower concentration application of the botanical-/ bio-pesticide formula for the efficient field application, and the mode of action of synergistic action of combined application of endophytic bacteria bioagent and bamboo charcoal compost toward nematode population suppression.

Author’s contribution
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