Effectiveness of some clove and citronella oil based-pesticide formulas against root-knot nematode on ginger

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Abstract. Three clove and citronella oil based-pesticide formulas were evaluated their effectivity against root-knot nematode *Meloidogyne* spp. on ginger in two experimental fields. Three botanical pesticide formulas were mixture formula of: 1) clove and citronella oil, 2) citronella oil and salycilic acid, and 3) clove and java turmeric oil. Chemical carbofuran was used as control chemical pesticide. The experiments were arranged in Randomized Block Design with 3-4 replications (each replication consisted of 10 to 15 plants). Two months after planting, the plants were treated with the formula by drenching the 200 ml of 0.5-2.0% of each formula solution around the plant root system area, then repeated in 14 days interval. At 5-months aged, the plants uprooted, and parameters value were analyzed. Among the three formulas, mixture of citronella oil and salycilic acid at 1.5 and 2.0% concentration effectively reduced the penetration rate of the nematode into roots and rhizome. The formula suppressed the nematode population development in roots as much as 93.75% and the percentage of rhizome-knot symptom as much as 71.60% and 73.17% in two locations consistently. The effectivity of citronella oil and salycilic acid mixture formula towards *Meloidogyne* sp. could be studied further in ginger plantation.

1. Introduction

Ginger (*Zingiber officinale* Rosch.) is one of important medicinal and spice crops and export commodity for Indonesia. One of major constraints in ginger cultivation was root-knot nematode *Meloidogyne incognita*, affecting the quantity and quality of the ginger production/yield [1, 2]. Those nematodes commonly were seed borne contaminant. *Meloidogyne* spp (38 species) fulfilled one or more of the criteria to be considered to present a phytosanitary risk, and infestation of the nematode on ginger rhizome could be implied in export rejection [3, 4]. *Meloidogyne* sp. infected roots and rhizome tissue, where the influence of the nematode feeding caused the giant cell formation, caused hypertrophy and hyperplasia then inducing galls on root and rhizome surface. The nematode infection caused wounds to physiological predispose the entry of bacterial wilt *Ralstonia solanacearum* which might be their principal economic hazard.

Plant parasitic nematodes could be managed through several approach such as using natural enemies, enhancing cultural practices, cultivating resistant cultivars, and applying pesticides. A common problem among agricultural workers is too high exposure to synthetic pesticides which could have serious implications not only for their own health, but also for the health of the consumers, live stocks and the environment [5]. Natural plant products such as essential oils have gained interest for use in pest control because of their low environmental impact [6]. EOs are well-known for their antimicrobial, antiviral,

antioxidant, antifungal, anti-bacterial, antinematode; and insecticidal properties; have shown potential and promising as new nematicide source [5, 7-10]. Special regulatory status combined with the wide availability of essential oils from the flavor and fragrance industries, has made it possible to fast track commercialization of essential oil-based pesticides [8]. For these reasons, the Indonesian government is stimulating efforts to reduce the dependency on synthetic pesticides by stimulating alternative methods including the development of formulations of botanical pesticides to control pests [5]. Some clove and citronella oil based-formula were effectively suppressed the population of root-knot nematode *Meloidogyne* sp. on ginger in greenhouse experiment >50% [7]. Those formulas were needed to be evaluated further in the field experiment to see the consistency performance of formula effectivity against nematode *Meloidogyne* spp.

2. Materials and methods

The experiments were carried out in two (2) locations, in experimental fields of ISMECRI in Cimanggu, Bogor and Cicurug, Sukabumi, West Java, Indonesia.

2.1. Preparation of chemicals and the EOs formula

The EOs (clove oil, citronella grass oil, java turmeric oil) formula used in this experiment were formulated in Plant Protection Laboratory, ISMECRI, Bogor as emulsiable concentrate formula. The EOs were produced in post-harvest laboratory of ISMECRI, Bogor. Chemical carbofuran was used as control chemical [11]. Three clove and citronella oil formula with their concentration/ dosage and their application method used in this experiment were shown in Table 1.

Table 1. Clove oil and citronella oil-based pesticide formula, concentration and its application method used in efficacy test against root-knot nematode *Meloidogyne* spp. on ginger in Experimental fields of ISMECRI in Cimanggu, Bogor and Cicurug, Sukabumi

Clove and Citronella Oil-Based Pesticide Formula Treatment	Concentration of Formula Solution and Dosage of Control Chemical		Application Method
	Cimanggu	Cicurug	
Control/ without treatment	0.0%	0.0%	300 ml of water were drenched into soil around roots area
Clove oil + citronella oil formula (CloveO+CitronellaO) (emulsiable concentrate/ EC)	2.0%	1.0%	300 ml of formula solution were drenched into soil around roots area
Citronella oil + salycilic acid (CitronellaO+salycilicA) (EC)	2.0%	1.5%	300 ml of formula solution were drenched into soil around roots area
Clove oil + java turmeric oil formula (cloveO+javatumericO) (EC)	2.0%	1.5%	300 ml of formula solution were drenched into soil around roots area
Carbofuran (granule)	10.0 g	10.0 g	The chemical was spread out on soil around the plant roots area

2.2. Production of experimental ginger plants

The ginger cultivar used in the study was big white ginger. The cultivar was susceptible to root-knot nematode *Meloidogyne* spp. In Bogor, the ginger plants were cultivated in 40×40 cm^2-polybags (8 kg soil), and in Sukabumi in 60×60 cm^2 polybags (10 kg soil). The experimental polybags soils contained unsterilized nematode-infested field soils and the polybags were arranged in the field.

Rhizome seeds were washed from soil debris and air dried, then cut into 30-40 g pieces, then each was sown in polybags contained field soil. One month after planting, the seedlings were fertilized with Urea (N), KCL (K) and SP-16 (P) as SOP. The EOs formula were applied 2 months after planting by

pouring 300 ml of EOs formula solution containing the desired concentration. At the 5-month aged, the plants were sampled/ up rooted, and the dry weight of ginger rhizome, nematode population in the roots and the percentage of rhizome-knots symptom incidence were analyzed.

2.3. Experimental design

The EOs formula was tested twice in 2 locations of experimental fields of ISMECRI in Cimanggu, Bogor and in Cicurug, Sukabumi, West Java, Indonesia. The experiments were designed as randomized block design. The treatment designs were single factor with 5 treatments. The treatments were replicated 4 times with 10 plants in each replication in Bogor, and 3 times with 15 plants in each replication in Sukabumi (Table 1). Two months after planting, the plants were treated twice with the tested formula by drenching 300 ml of 1.0-1.5% concentration of each formula solution around the plant root system area, then replicated in the next 14 days. The granule formula of chemical carbofuran wee spread around the plant roots area (Table 1).

2.4. The effect of EOs formula treatment on the nematode infection rat.

At the 5-month aged, the plants were sampled/ up rooted, nematode population development in roots and infection severity of the nematode were analyzed.

2.4.1. Nematode population development.

Nematode population were calculated based on the number of 2nd stage-larva and eggs per gram roots by method of Hooper [12]. The roots were clean washed from soil debris, weighted, and cut into 0.5-1.0 cm^2 pieces. The root cuts were put into flask contained 1.05% sodium hypochlorite ($NaOCl_2$) solution, then shake by hand for 4 minutes then washed into serial sieves of 200 mesh (75 µm pore size), 325 mesh (45 µm pore size), dan 400 mesh (38 µm pore size). The nematodes extracted from sieving were counted under 100 times magnification of compound microscope.

2.4.2. Infection severity.

Infection severity of *Meloidogyne* spp. on rhizome was analyzed by the percentage of rhizome-knot symptom incidence, based on the formula by Djiwanti and Supriadi [7]:

$$I = \frac{n}{N} \times 100\%$$

I= Incidence of rhizome-knot symptoms; n = number of rhizome branch shown the rhizome-knot symptom; N= number of all rhizome branches observed.

2.5. Observation on the effect of botanical pesticide formula treatment on the plant growth

Observation on the effect of EOs formula treatment on plant growth was analyzed based on rhizome dry weight and plant height, four and five months after planting. Rhizome dry weight was rhizome weight after air dried for two months in room temperature. Plant growth inhibition was also observed to see whether the concentration of the formula and its application interval caused the phytotoxic effect or not.

2.6. *Data analysis*

The effectivity of EOs formula was expressed by significant level of rhizome-knot symptom percentage and nematode population suppression, and whether the application of the formula caused phytotoxic effect such as plant yellowing or plant growth inhibition incidence. The data were analyzed by ANOVA, and treatments were compared by Duncan's Multiple Range Test at 5% level (α 0.5).

3. Results and discussions

All the three formulas tested suppressed the rhizome-knot symptom percentage compared to control in two field locations (88.46% and 58.80% on cloveO+citronellagrassO, 71.60% and 73.17% on citronellagrassO+salycilicA, 80.36% and 48.82% on cloveO+javaturmericO). Similar results were

shown on chemical carbofuran application (79.25 and 46.70% in Cimanggu, Bogor and Cicurug, Sukabumi, respectively) (Table 2).

Table 2. The average of rhizome-knot symptom percentage and suppression percentage after pesticide formula application in two experimental field locations

Clove and Citronella Oil-Based Pesticide Formula Treatment	Rhizome-Knot percentage (Suppression Percentage) *)	
	Cimanggu, Bogor	Cicurug, Sukabumi
Control/ without treatment	25.21[b]	61.65[c]
CloveO+CitronellaO (emulsiable concentrate/ EC)	2.91[a] (88.46)	25.40[ab] (58.80)
CitronellaO+salycilicA (EC)	7.16[a] (71.60)	16.54[a] (73.17)
CloveO+javatumericO (EC)	4.95[a] (80.36)	31.55[b] (48.82)
Carbofuran (granule)	5.23[a] (79.25)	32.86[b] (46.70)

*) Averages in the same column followed by the same letter are not significantly different by DMRT at α=0.05

The observation on the effect of the EOs pesticide formula application on nematode population in roots was carried out in Cicurug, Sukabumi only. The result shown that all formulas tested significantly suppressed the nematode population in compared to control (99.03% on cloveO+citronellagrassO formula, 93.75% on citronellagrassO+salycilicA formula, and 100.00% on cloveO+java turmericO formula). The effectivity of those formula relatively like that of chemical carbofuran (85.78%) (Figure 1).

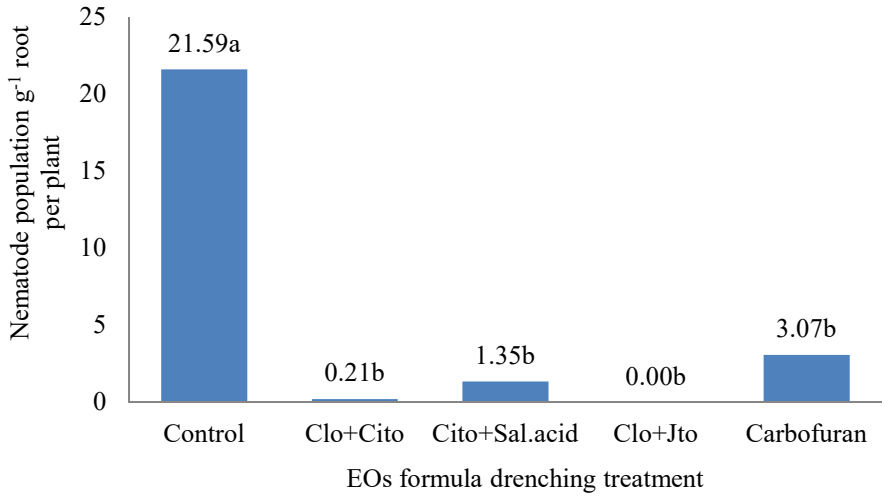

Figure 1. Average of root-knot nematode (*Meloidogyne* sp.) population g^{-1} root of each ginger plants based on essential oil formula treatments (control= without treatment, Cl+Cit= cloveO+CitronellagrassO mixture formula, Cit+Sal.acid= citronellagrassO+salycilicA mixture formula, Cl+Jtum= cloveO+javaturmericO mixture formula, carbofuran= chemical nematicide), five months after planting in non-sterile soil in polybags (10 kg) in Cicurug, Sukabumi

Observation on rhizome dry weight in two experimental locations shown that there were no rhizome dry weight decrease/suppression on citronellagrassO+salycilicA formula application, even there were rhizome dry weight increase/enhancement in Cimanggu, Bogor and Cicurug, Sukabumi by 9.42 and 23.70%, respectively. The similar result shown in chemical carbofuran application, where the

enhancement of rhizome dry weight in two locations by 33.35 and 35.31%, respectively. On cloveO+javaturmericO formula application, the rhizome dry weight decrease was shown only in Cimanggu, Bogor (-46.25%), contrary to rhizome dry weight enhancement evidence (31.91%) in Cicurug. While that of on cloveO+citronellagrassO formula application, the rhizome dry weight suppression was shown in 2 experimental locations, in Cimanggu and Cicurug, respectively -10.99% and -26.9% (Table 3).

Table 3. The rhizome dry weight average of ginger yield, 4 months after planting in Cimanggu, Bogor and 5 months after planting in Cicurug, Sukabumi

Botanical Pesticide Treatment [a)]	Rhizome Dry Weight (G) [B)] (Rhizome Dry Weight Inhibition Percentage) c)	
	Cimanggu, Bogor	Cicurug, Sukabumi
Control	13.73	107.93
CloveO+citronellagrassO formula (EC)[a)]	12.22 (-10.99)[c)]	79.12 (-26.69)
CitronellagrassO+salycilicA formula (EC)	15.2 (9.42)	141.46 (23.70)
CloveO+java turmericO formula (EC)	7.38 (-46.25)	158.51 (31.91)
Carbofuran (G)	20.6 (33.35)	166.84 (35.31)

[a)] EC= emulsiable concentrate, G= granule

[b)] Data in table were average value of rhizome dry weight per plant of 40 experimental plant in Cimanggu, and 75 experimental plants in Cicurug

[c)] – (negative) = plant growth inhibition evidenced in term of rhizome dry weight inhibition percentage evidenced

Observation on plant growth revealed that the cloveO+citronellagrassO formula application inhibited the plant height, in compared to citronellagrassO+salycilicA mixture formula and chemical carbofuran application, and to control plant (Figure 2).

Basically, all three botanical pesticide formula tested effectively in suppressed the nematode infection rate on ginger rhizome and in roots. However, among the three EOs pesticide formulas tested, there were some plant growth inhibition effect incidence/evidence (in term of rhizome dry weight decrease in compared with control) after the formula application on cloveO+citronellagrassO formula in two locations and on cloveO+java turmericO formula in Bogor only. There was no plant growth inhibition effect evidence on citronellagrassO+salycilicA formula application in two locations, as consistent as chemical carbofuran 3% (Table 3).

Based on the consistent result on the penetration suppression rate of *Meloidogyne* sp. into roots (nematode population g^{-1}) and rhizome (rhizome-knot symptom incidence level) without any evidence of plant growth inhibition in two experimental locations, indicated that citronellagrassO+salycilicA formula had stable performance in nematode suppression efficacious. The performance was similar with chemical carbofuran. Essential oils have fumigant and contact toxicity to crop pests [13], bacterial pathogen [14-16], root-knot nematode *Meloidogyne* sp. [7], soil-borne fungus [7, 17, 18], and viral pathogen [19]. The weakness of essential oil utilization was evaporation of the volatile ingredient. The addition of salicylic acid into citronella grass oil formula might prolong the performance of the formula. Salicylic acid (SA), a plant hormone plays an important role in induction of plant defense against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms. According to Maia and Moore [20], to prolong the performance of citronella grass oil-based formula effectivity, could be overcome with i.e. mixed the citronella oil with vanillin 5%, nano technology engineering and encapsulation. Currently, commercial plant protection products comprising proprietary mixtures of terpenes as active compound are available [21]. Creating EOs synergistic combination is to reduce the dose of potentially polluting substances and reduce the risk of developing resistance [22].

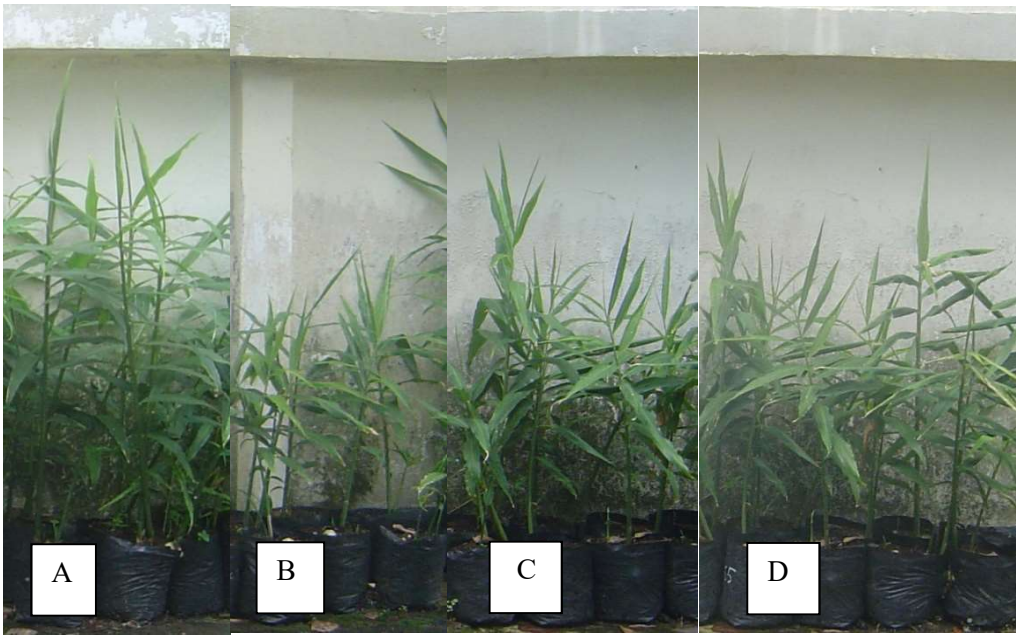

Figure 2. Plant growth of ginger plants four months after planting in Cimanggu, Bogor, indicated that treatment of cloveO+citronellagrassO mixture formula caused plant height inhibition (B), in compared to control plant (A), and plants with treatment of citronellagrassO+salycilicA mixture formula (C), and plants with treatment of chemical carbofuran (D).

From some observation in experimental field evaluation of pesticide effectivity (including botanical pesticide), application of the botanical pesticide formula was often followed by plant growth inhibition effect, especially in soil drenching application or seed treatment application method [7] (figure 1, data were not shown). Dadang *et al.* [23] observed the application of *A. odorata* extract caused phytotoxicity effect to cabbage and inhibited plant growth.

Actually, mixing the plant material in pest control can reduce the dependence on one of the plant materials compared to single usage. Mixing two of them in certain ratio were expected to provide higher biological action, resulted in synergistic effect [13, 24]. However, in this experiment, application of cloveO+citronellagrassO formula might be toxic to plant. In *in vitro* test, clove oil or citronella grass oil alone was highly toxic to nematode larvae, causing mortality as much as 100% in 1% solution in 1 (one) hour [7]. This result indicated that to evaluate the mixture formula effectiveness, it is necessary also to evaluate their phytotoxic effect on plants despite their efficacious effect on pest insect and disease pathogen. There are possibilities that mixing composition of clove oil and citronella grass oil resulted in more toxic pesticidal solution in compared to clove oil or citronella grass oil solution only. This clove and citronella grass oil mixture formula application as soil drenching toward nematode control on ginger should be evaluated further their concentration levels and interval application in the field.

4. Conclusions

CitronellagrassO+salycilicA formula was the potential formula to be developed as botanical pesticide toward root-knot nematode control on ginger. The formula could offer a promising strategy to control nematode *Meloidogyne* sp. in organic ginger plantation.

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