Antifungal effectiveness of some essential oils and their mixtures against *Fusarium oxysporum f.sp cubense* that causes fusarium wilt disease of banana plants

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Abstract. Antifungal effectiveness of several essential oil botanical pesticides and their mixtures against the fungus *Fusarium oxysporum f.sp. cubense* (Foc) which causes fusarium wilt disease of banana plants in vitro, was conducted from March to July 2023. The aim of the study was to obtain an effective botanical pesticide formulation and the right concentration for controlling the Foc causing Fusarium wilt disease in banana plants. The study was arranged in a completely randomized design in factorials with three replications. The experiment was carried out using two methods: a) Suppressing colony diameter using Potato Dextrose Agar (PDA) medium, b). Suppressing colony biomass using Potato Dextrose Broth (PDB) medium. The treatment was the formulation of essential oil (F) botanical pesticides as the first factor; F1 (*Cymbopogon flexuosus* oils), F2 (*C. nardus* essential oils), F3 (*Eugenia aromatica* leaf essential oils), F4 (*Cinnamomum burmanii* leaf oils), F5 (F1+F2), F6 (F1+F3), F7 (F1+F4) and F8 (F2 +F3), F9 (F2+F4) and F10 (F3+F4) The level of concentration (C) as a second factor C1(1000 ppm) and C2(2000 ppm). The results showed that the F3 (*E. aromatica* leaf oils) treatment had the best antifungal activity against Foc. At a concentration of 2000 ppm inhibition of the diameter growth and colony biomass reached 100%, followed by the F1 treatment (*C. flexuosus* oils) up to 89.73% and 95.75%, the F2 treatment (*C. n*ardus oils) reach 84.58% and 89.67% and the lowest antifungal activity is F4 (*C. burmanii* leaf oils) which only 77.08% and 84.50%. The best mixed formulations were F5(F1+ F3) and F8(F2 + F3), at the same concentration suppression of colony growth reaching 100%. In general, mixed essential oils formulation have shown to increase the effectiveness of the antifungal activities.

1 Introduction

As with other cultivated plants, banana plants have many obstacles in their cultivation, one of which is the attack of fusarium wilt disease or known as Panama disease which causes the

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destruction of banana plants in the world, especially in tropical and sub-tropical areas. In Indonesia about 8 million traditional banana plantations and more than 5000 ha of commercial plantations were destroyed [1]. Disease incidence can reach 64.45% in Simalungun district [2]. The survey conducted in 16 provinces in Indonesia revealed that Panama disease had spread from NAD to Papua [3]. In Nangro Aceh Darussalam Province (NAD), the percentage of disease attack reached 70% and even some farms experienced a Puso attack above 75% [4]. In West Sumatra, the highest intensity of wilt disease in Indonesia is estimated at more than 60%, with the most severe incidence in Tanah Datar, Solok, Agam and Bukit Tinggi districts [5]. The cause of the disease is *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Of the 50 *Foc* isolates collected in NAD Province, grouped into two VCGs, 37 isolates (74%) were VCG01213/16 Tropical race 4 (TR4), found in Barangan, Kepok and Raja. Nine isolates (26%) grouped into VCG 01218 race 1, were found to attack Siam variety [4].

Fusarium wilt disease causes extensive failure of commercial plantations in all banana-producing areas. This pathogen is effortful to control because it has a survival structure in the form of chlamydospores which can survive saprophytes and last a long time in the soil. This fungus infects through lateral roots or short branches of roots, then penetrates the transport network and expand widely in the xylem [6]. Pathogens can survive in the soil for up to 30-40 years [7].

Several control studies that have been carried out using synthetic chemical fungicides include the use of fungicides. Prochloraz and propiconazole, at concentrations of 1 and 5 µg/ml, were significantly reduced mycelium growth respectively. Furthermore, benomyl and demethylation significantly reduced the severity of *Foc* disease at root dip treatment, which showed a reduction in disease of up to 80.6% [8]. From the results of the control tests put forward by [9], that the phosphitic acid fungicide (Agrifos) was more effective at inhibiting the growth of the *Foc* than the aluminum-fosetyl fungicide (Aliette).

The sustain use of synthetic chemical fungicides will cause damage to plants, ecosystems and humans, therefore an effective and environmentally friendly control effort is needed. Essential oils are one possible alternative at this time, because essential oils have antifungal, antibacterial properties and also act as insecticides and molluscicides and easy to get.

The main components of lemongrass essential oil are citral-a 33.1%, citral-b 30%, granyl acetate 12 %, and linalool 1.2% [10]. Lemongrass and citral oils have potential antifungal activity against *Candida albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis* [11]. At an oil concentration of 500 ppm, it suppressed fungal sporulation and reduced germination of *Colletotrichum coccodes*, *Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger in vitro* [12].

Citronella oil with the main components of citronellal, 41.74%, graniol 14.89%, citronellol 9.86%, naphthalenol 4.83%, citral 1.06% and other components [13], has the potential to be utilized and developed as a botanical pesticide to control plant diseases. Citronella essential oil at a concentration of 2000 ppm for all clones (G1, G2 and G3) was able to suppress the growth of *Sclerotium rofsii* 100% and *Fusarium oxysporum* 87.18% which causes wilting and stem rot of chili plants [14]. While, the main component of cloves leaf oil is eugenol 76.8%, β-caryophyllene 17.4%, alpha-humulene 2.1% and eugenyl acetate 1.2% [15]. Eeugenol is effective for controlling the fungi *Penicillium* sp, *Aspergillus* sp and *F. avenaceum* and *F. oxysporum* [16].

Cinnamon leaf oil with the main component cinnamaldehyde 63.61% [17], is quite effective against the fungus *F. sambucinum* [18], *F. oxysporum* f.sp. *vanillae* and *F. oxysporum* f.sp. *zyngiberi* which cause vanilla stem rot and ginger rhizome rot [19].

Studies on the utilization of essential oils so far are still in the form of a single essential oil yet combined with other essential oil. Thus, few studies used the essential oil in the form of formulations. Based on the description above, the use of environmentally friendly essential oil pesticides is a possible alternative for controlling fusarium wilt in banana plants. The aim

of the study was to obtain an effective botanical pesticide formulation and the right concentration for controlling the *F. oxysporum* f.sp. *cubense* causing Fusarium wilt disease in banana plants.

2 Material and methods

The research was carried out from March to July 2023 at the pest and disease laboratory of KP. Laing Agency for Standardization of Agricultural Instruments Spices, Medicinal and Aromatic Plants, Solok, West Sumatra. Analysis of essential oil components was carried out in the laboratory of the Faculty of Animal Andalas University.

2.1 Essential oil distillation

Essential oils are obtained by distillation of raw materials in the form of cinnamon harvest waste leaves, cloves leaf litter, citronella leaves, lemongrass leaves, using a prototype kettle with steam method in the Centre for Agricultural Instrument Standardization Spices, Medicinal and Aromatic Plants, KP Laing Solok. The essential oils obtained were analysed for chemical components contained with GC-MS at the Faculty of Animal Andalas University.

2.2 Formulation of botanical pesticides

The formulation is 25% of emulsifiable concentrate (EC). The ingredient consists of 25% essential oil as the main ingredient, 63% solvent (ethanol), 10% emulsifier (tween 80), 2% (teepol) moisturizer and levels up to 100%. The formula was stirred with a stirrer for 20 minutes. Then it is stored in colored bottles, and the formulation is ready to be used for testing.

2.3 Isolation of pathogens

The pathogenic *Foc* VCG1213/16 TR4 was obtained from diseased banana plants and identified the VCG according to [4], then the isolates were propagated onto Potato Dextrose Agar (PDA) medium. The isolate cultured for 9 days before used for treatment.

2.4 Antifungal activities assay

2.4.1 Suppression of colony diameter growth by essential oils

The test was carried out by mixing the essential oil until homogeneously into sterile PDA medium, refer to the treatment and concentration tested. After temperature of media reach ±45ºC, it then pouring it into a petri dish and letting it harden, after which the fungus was inoculated. A diameter of 8 mm fungal mat from the *Foc* culture was cultured in the centre of the plate containing PDA, then maintained at 28º C for 7 days.

2.4.2 Colony biomass suppression

The experiment was used liquid media Potato Dextrose Broth (PDB) as a culture medium. 25 ml of media was put into each treatment tube, then the medium was sterilized in an autoclave and cooled. Subsequently, the essential oil with proper concentration according to

the treatment was mixed into the PDB media, then fungal mat from *Foc* cut with a sterile cork hole with a diameter of 8 mm, loaded into the PDB medium, then incubated at 28º C for 7 days. Furthermore, the growing *Foc* mycelium were harvested and dried at 80º C for 48 hours. The dried mycelium was measured for obtaining dried biomass.

The experiment was arranged in a factorial complete randomized design (CRD), each of which was repeated three times. The treatment was a formulation of a botanical pesticide with the first factors: F1 (lemongrass essential oil), F2 (citronella oil), F3 (cloves leaf litter oil), F4 (cinnamon leaf oil), F5 (F1+F2), F6 (F1 +F3), F7 (F1+F4) and F8 (F2+F3), F9 (F2+F4) and F10 (F3+F4). Concentration level (C) as a second factor C1 (1000 ppm) and C2 (2000 ppm).

2.5 Data collection/ Observation

This research was carried out in the laboratory of Postharvest and Parasitology of Agricultural Technology Research and Assessment Installation Laing Solok West Sumatra from April 2022 to December 2022. The activities are: to obtained a data of inhibition of fungal colony growth and fungal biomass. For calculating the inhibition of growth of colony diameter and colony biomass, it is calculated using the formula described by [20].

$$I = \frac{C-T}{T} x\ 100\% \quad (1)$$

I = colony growth inhibition
C = diameter of colony/biomass of control
T = diameter of colony/biomass of treatment

2.6 Data processing/analysis

The data obtained from the calculation of colony diameter and fungal colony biomass were tested statistically, if the F test at the 5% significance level there was a significant difference between the treatments, then it was continued with the DNMRT test.

3 Results and discussion

The results showed that all the essential oil tested and their mixtures showed very good antifungal effectiveness in inhibiting the growth of the diameter of the colony of *Foc* which causes Fusarium wilt disease of banana plants. At a concentration level of 1000 ppm, the average colony diameter of the *Foc* mycelium was ranged from 8.00 – 19.33 mm, while the control without pesticides, the growth of the colony diameter had reached 84.33 mm 7 days after inoculation, thereby inhibiting the growth of the colony diameter. ranged from 77.08 – 90.51%. The higher the level of concentration the inhibition of growth in the diameter of the colony also increased and even in some treatments it could not grow and died (Table 1).

Table 1. Effect of various formulations of essential oil botanical pesticides and their mixtures on the growth of fungal colony diameter growth of *F. oxysporum* f.sp. *cubense* (7DAI).

Treatment	Concentration level	Colony diameter (mm)	Inhibitor potential (%)
F1. Lemongrass EO (*C. flexuosus*)	1000 ppm	11.00 ef	86.95
	2000 ppm	8.66 ij	89.73
F2. Citronella grass EO (*C. nardus*)	1000 ppm	13.00 c	84.58
	2000 ppm	9.33 ghi	88.93
F3. Clove leaf EO (*E. aromatica*)	1000 ppm	8.00 j	90.51
	2000 ppm	0.00 k	**100.00**
F4 Cinnamon leaf EO	1000 ppm	19.33 a	77.08

(*C. burmanii*)	2000 ppm	12.33 c	85.37
F5. (F1+F2) 1:1	1000 ppm	10.33 fg	87.75
	2000 ppm	9.00 hij	89.33
F6. (F1+F3) 1:1	1000 ppm	9.66 ghi	88.54
	2000 ppm	0.00 k	**100.00**
F7. (F1+F4) 1:1	1000 ppm	13.00 c	84.58
	2000 ppm	9.66 ghi	88.54
F8. (F2+F3) 1:1	1000 ppm	10.33 fg	87.75
	2000 ppm	0.00 k	**100.00**
F9. (F2+F4) 1:1	1000 ppm	17.33 b	79.45
	2000 ppm	11.66 de	86.17
F10. (F3+F4) 1:1	1000 ppm	10.00 fgh	88.14
	2000 ppm	8.00 j	90.51
Control (without treatment)		84.33	0.00
CV (%)		4.88	-

Note: numbers followed by the same letters in the same column are not significantly different at 0.05 level at DNMRT method. DAI is days after inoculation and EO is essential oils.

From Table 1 it can be seen at the concentration level of 2000 ppm of cloves leaf essential oil (F3), a mixture of lemon grass essential oil with cloves leaf oil (F6) and a mixture of citronella essential oil and cloves leaf oil (F8) showed the highest inhibition rate of colony diameter reaching 100%. Cinnamon leaf essential oil at the same concentration level showed the lowest inhibition rate of colony diameter growth, namely 85.37%. In general, the combination of essential oil pesticides showed an increase in inhibition of the growth diameter of the fungus colony *F. oxysporum* f.sp *cubense*.

The results of testing the effect of essential oil vegetable pesticides and their mixtures on the biomass of the mushroom colonies of *F. oxysporum f sp cubence*, in liquid media Potato Dextrosa Broth (PDB) showed that the suppression of the growth of the mushroom colony biomass was quite effective. At a concentration level of 1000 ppm the average colony biomass in the cloves leaf oil treatment (F3) was 3.33 mg (96.96% control potential), significantly different from the lemon grass oil treatment (F1) with a biomass of 8.66 mg (control potential 92.10%), citronella (F2) with a biomass of 11.33 mg (control potential 89.67%), and cinnamon leaf oil (F4) with a biomass of 17.00 mg (control potential 84.50%), these results after compared to the colony biomass of the fungus *F. oxysporum f.sp cubence* in the treatment without pesticides with a colony biomass of 109.66 mg (Table 2).

Table 2. Effect of various formulations of essential oil as a botanical pesticide and their mixtures on the growth of *Foc* colony biomass (7 DAI).

Treatment	**Concentration level**	**Colony biomass mg**	**Control potential (%)**
F1. Lemongrass EO	1000 ppm	8.66 de	92.10
(*C. flexuosus*)	2000 ppm	4.66 fg	95.75
F2. Citronella grass EO	1000 ppm	11.33 c	89.67
(*C. nardus*)	2000 ppm	7.00 def	93.62
F3. Clove leaf EO	1000 ppm	3.33 g	96.96
(*E. aromatica*)	2000 ppm	0.00 h	**100.00**
F4 Cinnamon leaf EO	1000 ppm	17.00 a	84.50
(*C. burmanii*)	2000 ppm	11.66 c	89.37
F5. (F1+F2) 1:1	1000 ppm	8.00 de	92.70
	2000 ppm	4.66 fg	95.75
F6. (F1+F3) 1:1	1000 ppm	6.33 ef	94.23
	2000 ppm	0.00 h	**100.00**
F7. (F1+F4) 1:1	1000 ppm	12.66 bc	88.45
	2000 ppm	6.33 ef	94.23

F8. (F2+F3) 1:1	1000 ppm	6.33 ef	94.23
	2000 ppm	0.00 h	**100.00**
F9. (F2+F4) 1:1	1000 ppm	14.00 b	87.23
	2000 ppm	8.33 de	92.40
F10. (F3+F4) 1:1	1000 ppm	9.00 d	91.79
	2000 ppm	5.33 fg	95.14
Control (without treatment)		109.66	0.00
CV (%)		6.95	-

Note: Numbers followed by the same letters in the same column are not significantly different at 0.05 level DNMRT. DAI is days after inoculation dan EO is essential oils.

From Table 2, the higher the concentration level, the higher the inhibition on the growth of the *Foc* colony biomass, so that the colony biomass is getting smaller. At a concentration level of 2000 ppm cloves leaf oil (F3), a mixture of lemongrass oil with cloves leaf oil (F6) and a mixture of citronella oil and cloves leaf oil (F8) showed the highest inhibition rate of colony biomass reaching 100% or not grow. At the same concentration level, the lowest suppression of fungal colony biomass growth was in cinnamon leaf oil treatment (F4) with an inhibition of colony biomass of 89.37%.

Table 1 and 2 shows that the clove leaf oil (F3) with the main component eugenol has the highest antifungal activity compared to lemongrass oil (F1), citronella oil (F2) and cinnamon leaf oil (F4). The mixed treatment generally showed an increase in its antifungal effectiveness and the best were the F6 (F1+F3) and F8 (F2 + F3) treatments, this was due to the synergism of the essential oil mixtures tested. The same thing was stated by Tombe et al [21], that the formulae of clove oil combined with citronella oil was more effective and consistent in inhibiting mycelium growth, conidia production and the disease intensity of *F. fsp.vanillae*. The main components of clove leaf oil according to [15], include eugenol (76.8%), β-caryophyllene (17.4%), alpha-humulene (2.1%), and eugenyl acetate (1.2%). The essential oils of clove leaves, stalks and flowers are also very effective against the pathogen *Phytophthora* sp, *S. rofsii*, *R. lignosus* and also the fungus *Fusarium oxysporum* [22].

The main components in citronella essential oil (*C. nardus*) are 41.74% citronellal, 14.89% graniol, 9.86% citronellol, 4.83% naphthalenol, 2.88% limonene, 1.06% citral, and 45% other components [13]. The active compounds that have great potential as antifungals in citronella oil are citronellal, linalool, α-pinene, β-pinene and methone, while geraniol, citral, and terpenes have moderate antifungal activity [23].

Lemongrass (*C. flexuosus*) essential oil with the main oil content is citral-a 33.1%, citral-b 30.0%, geranyl acetate 12.0% and linalool 2.6% [10], has potential antifungal activity against *C. albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis* [11]. At an oil concentration of 500 ppm it suppressed fungal sporulation and reduced germination of *Colletotrichum coccodes* spores, *B. cinerea*, *C. herbarum*, *R. stolonifer* and *A. niger* in *in vitro* [12].

The constituent components of cinnamon essential oil vary depending on where it grows even though they come from the same species [24]. The main components of *C. burmanii* cinnamon bark are cinnamaldehyde, proanthocyanidines or catechins, and other ingredients that are still suspected, namely procyanidin B1, procyanidin B2, procyanidin trimer, procyanidin dimer, procyanidin tetramer, epicatechin and cinnamic acid [25]. The essential oil from the leaves, twigs and bark of cinnamon has great potential as a fungus control. at a concentration of 500 ppm the skin oil was able to suppress the growth of *F. oxyporum* f.sp. *vanillae* and *F. oxysporum* f.sp. *zingiberi* 100%, whereas for leaf and twig essential oils a higher concentration of essential oil was needed, namely 1250 ppm [19].

4 Conclusion

From the research results it can be concluded that clove leaf oil treatment (*E. aromatica*) has the best antifungal effectiveness against *Foc*. When compared with essential oils of lemongrass (*C. flexuosus*), citronella grass (*C. nardus*) and cinnamon leaf oil. (*C. burmanii*). At a concentration of 2000 ppm clove leaf essential oil was able to suppress the diameter growth and the biomass of the *Foc* colonies reached 100%, and than followed by the treatment of Lemongrass (*C. flexuosus*) essential oil 89.73% and 95.75% and than citronellagrass essential oil (*C. nardus*) 84 .58% and 89.67% and the lowest emphasis on cinnamon leaf essential oil (*C. burmanii*) 77,08% and 84,50%. In general mixing pesticides of essential oils have shown to increase the effectiveness of the antifungal formulations and the best was lemongrass oil + clove leaf oil, and citronella grass oil + clove leaf oil, at the same concentration they were able to suppress the growth of diameter and biomass of *Foc* colonies reaching 100%.

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