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Effectiveness of several dosage formula of oil and nano emulsion of citronella against vascular streak dieback (VSD) disease on cocoa

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Abstract. The disease of *Vascular streak dieback* (VSD) is a deadly disease of cocoa plants, because it attacks the vascular tissue of cocoa at growing point of the plant. In West Sumatra the disease was first reported in 2015 with an incidence of disease range 58.82% - 100% and an intensity of disease range 24.29% - 44.7%. The purpose of this study was to examine the effectiveness of dosage application of oil formula and nano emulsion of citronella formula against *Vascular streak dieback* (VSD) disease on cocoa plants in West Sumatra (in Padang Pariaman District and Limapuluh Kota District). The results showed that the percentage of VSD disease attacks in both testing sites was 100% .The oil and nano emulsion of citronella formulas can reduce the intensity of VSD disease on cocoa plants in West Sumatra, particularly in Padang Pariaman District and Limapuluh Kota District. The reduction of VSD intensity in Padang Pariaman district ranged from 8.32 to 21.13%; while in Limapuluh Kota district ranged from 4.33 to 11.80%. The nano emulsion of citronella formulation is effective to suppress the intensity of VSD disease on cocoa plants at doses 0.1% ($\geq$ 30% of effectiveness level).

1. Introduction

VSD (*Vascular streak dieback*) disease is a deadly disease of cocoa plants, because it attacks the vascular tissue at growing point. The development and spread of the disease in Indonesia is very rapid, because VSD disease was first discovered in 1983 in Sebatik island (East Kalimantan), and in 2013 almost all cocoa plantations are infected with the disease [1]. In West Sumatra the disease was first reported in 2015 with disease incidence are 58.82% - 100% and an intensity of disease are 24.29% - 44.7% [2]. Losses due to VSD disease worldwide can reach 30,000 tons per year equivalent to US $ 28 million [3].

In Indonesia, especially in West Sumatra the potential for loss of results has not been reported. However, on the basis of farmers' surveys and interviews many farmers' gardens have been destroyed and replaced with other crops, because the diseases cause deciduous leaves, barren plants and stop production. On the other hand, poor garden maintenance can accelerate the spread of disease in the field. Cocoa cultivation in Indonesia is generally a smallholder plantation, where in the management and maintenance has not done well. Various efforts to control the disease have been applied, but not

yet effective. Wahap and Sulle [4] reported that VSD disease can be controlled by (1) placing the seedlings in isolated and quarantine locations for 6 months, (2) pruning the sick branch once a month and good drainage, (3) resistant clone cultivation and (4) the use of propiconazole and biloxazole fungicides, [5] reported that the disease is difficult to control because it is in the vessel network. Therefore it is necessary to find a technology that utilize the natural plant potential as pesticide that may be able to control it.

Citronella is known to control several pests. Natural pesticides from citronella plants can be utilized to overcome the spread of mold spores causing plant diseases. The citronella plant is reportedly one of the most potential plant-based pesticides to control plant pathogens [6]. The use of clove oil and potential citronella extracts for control the VSD disease that it can suppress disease progression up to 38.6% and 31.6% [5]. The potential of active ingredients on biopesticides can be improved by using nano technologies such as nano emulsion and nano encapsulation.

Nanoemulsion is a transparent, translucent emulsion system and is a water oil dispersion stabilized by a film coating of a surfactant or surfactant molecule, having a droplet size of 50 - 500 nm [7]. Small size nanoemulsion droplets make nanoemulsically stable kinetically to prevent sedimentation and kriming during storage [8]. In addition, nanoemulsion with an oil-in-water emulsion system (o/w) is an alternative to increase the solubility and stability of the bioactive components present in oil [2].

Nanotechnologies, which can reduce particle size to nano size (10^{-9} m), are expected to increase the efficiency and effectiveness of the active ingredients contained in such materials on a broader scale or wider scale test. An improved perfumed oil formulation using spontaneous emulsification (nanotechnology) and greenhouse test results showed biopesticide nano emulsion at doses of 1-1.5% suppressed viral progression up to 82.5%; While the oil formula of citronella is only 65-70% [9][10].

The purpose of this study was to examine the effectiveness of dosage application of oil formula and nano emulsion of citronella against *Vascular streak dieback* (VSD) disease in cocoa plants in West Sumatra.

2. Materials and method
This research was conducted at Indonesian Spice and Medicinal Crop Research Institute (Plant Protection Laboratory) and Nano Technology of Post Harvest Technology, Bogor (for preparation of citronella formula) and cocoa farmers' land of VSD disease in Padang Pariaman and Limapuluh Kota District of West Sumatra Province from March to December 2016.

2.1. Preparation of Citronella Formula. Citronella plant oils obtained from Indonesian Spice and Medicinal Crop Research Institute of Manoko experimental garden in Lembang, Bandung. The oil is distilled from the leaves of 3 year-old citronella by steaming. Citronella oil formula was prepared according to the method of Wang and Liu [11] and nano-emulsion formula citronella oil is made from the modified method of Bouchemal *et al.* [12].

2.2. Application of biopesticides in the field. The study used a randomized block design with 2 levels of treatment (dosage of biopesticide formula and type of formula (oil biopesticide and nano emulsion of citronella oil biopesticide), each with 6 replicates. The treatment was (1) the formula of citronella oil at a dose 0.1%, 2) the formula of citronella oil at a dose 0.5%, (3) the formula of citronella oil at a dose 1%, (4) the formula of citronella oil at a dose 1.5%, (5) nano emulsion formula of citronella oil at a dose 0.1% 6) nano emulsion formula of citronella oil at a dose 0.5%, (7) nano emulsion formula of citronella oil at a dose 1%, (8) nano emulsion formula of citronella oil at a dose 1.5%, (9) synthetic fungicide with mankozeb active site, and (10) control (without treatment). Fifteen cocoa trees was observed for each treatment as a samples.

2.3. Technique application of biopesticide formula. Applications biopesticide formula is done by spraying using sprayer in all parts of the plant with a total volume of 500-1000 ml per plant (because the plant already aged over 2 years). Spraying is done every single month for 6 months application times. Cultivation of cocoa plants follows the standard operational procedures carryout out by farmers.

2.4. Observation parameters. Observations were made on disease symptoms, disease progression by counting the percentage of diseases, the intensity of diseases each month, and the degree of efficacy of the formula at the end of the observation.

Observations percentage VSD disease using the formula of Strange [13]:

$$\text{Percentage of diseases} = \frac{\text{Number of plants affected by VSD}}{\text{Number of all the plants observed}} \times 100\% \tag{1}$$

Observation of disease intensity was done on each tree by counting the number of leaves and branches affected by the disease based on the attack category in Table 1. The intensity of disease was calculated by the formula of Strange [13]:

$$I = \left(\frac{\Sigma(ni \times vi)}{Z \times N}\right) x \ 100\% \tag{2}$$

I = intensity of disease; ni = number of plants in each attack category; vi = scale values of each attack category; Z = the scale value of the highest attack category; N = number of plants observed.

Table 1. Scoring of VSD symptoms in cocoa plants.

Scoring	Attack category	Symptom
0	Healthy	0% are infected.
1	Light Mild	1-10% leaves are infected.
2	Mild	11-50% leaves are infected, chlorosis, necrosis, deciduous leaves, existing lenticell swelling.
3	Light Heavy	51-75% leaves are infected, chlorosis, necrosis, deciduous leaves, lenticell swelling, there is a fruit body.
4	Heavy	>75% leaves are infected, chlorosis, necrosis, deciduous leaves, lenticell swelling, there is a fruit body and thre are dead branches.

Source: Susilo and Anita-Sari [14] (modified).

The level of efficacy (EI) of biopesticide is calculated by comparing the intensity of the disease in the treated plots with the untreated plots, using the formula:

$$EI = \left(\frac{Ca - Ta}{Ca}\right) X \ 100\% \tag{3}$$

EI = the level of efficacy; Ca = the intensity of the disease in the control (untreated pesticide plant); Ta = Intensity of disease attack on the treatment of natural pesticides. The formula studied can be considered effective if the value of efficacy level (EI) ≥ 30%.

3. Results and discussion

The result of VSD disease survey on cocoa plant in West Sumatera based on symptoms showed that the severity of VSD disease in Limapuluh Kota district was higher than the severity of VSD disease in Padang Pariaman district (figures 1 and 2). The percentage of VSD disease attacks at both sites reached 100%. Both sites are used as research sites for the application of biopesticide citronella oil and nano emulsion of citronella formulation.

Figure 1. VSD disease symptoms on the cocoa crop in the district of Limapuluh Kota (a. Vascular tissue infected with VSD, b and c. Necrosis on leaves).

Figure 2. Symptoms of VSD disease attack on cocoa plants in Padang Pariaman District (a & b, leaf necrosis symptoms, c. VSD infected tissue vessels) and plant conditions after application of nano emulsion of citronella formula (d)

In the observation of disease intensity of VSD that has been done every month in Limapuluh Kota district, some treatments still showed an increasing trend and it was decreasing trend of disease compared to with the control without treatment (figure 3). Similarly, with the cocoa plant in Padang Pariaman district (figure 4), the downward trend is more evident when compared to the disease intensity of VSD before application (0 month observation) with disease intensity of VSD after application (observation at 1 to 5 months). This suggests that both tested formulas were able to decrease the progression of VSD disease.

Citronella oil has been reported to be effective against some pathogenic fungi such as *Aspergillus* sp. and *Penicillium* sp.[15], as well as *Phytophthora palmivora* cause of fruit rot of cocoa [1][16][17][18].
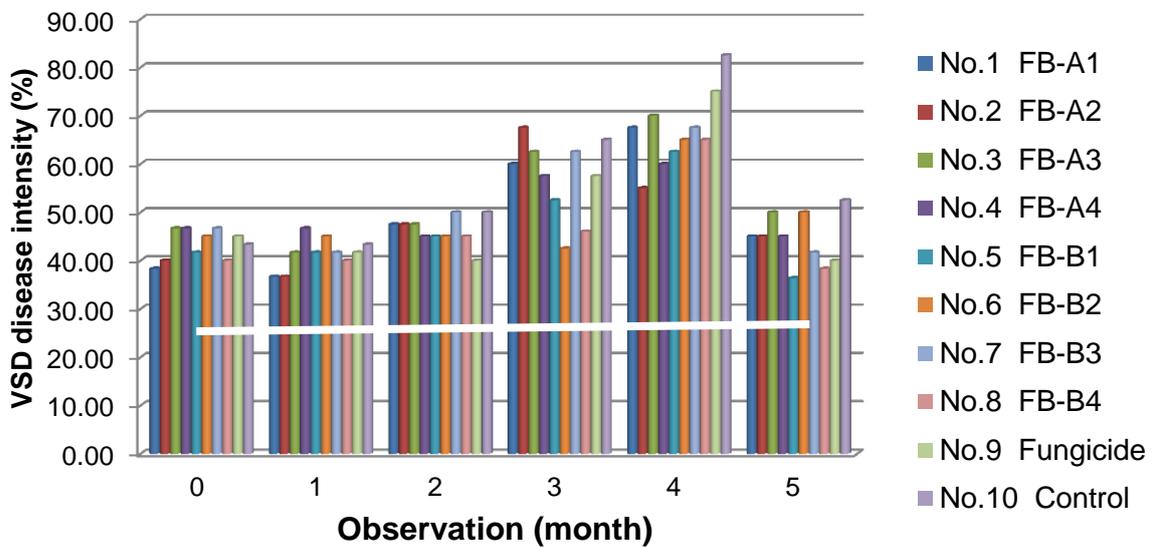
Figure 3. Percentage disease intensity of *Vascular streak dieback* (VSD) on cocoa plants in Limapuluh Kota district before and after application of natural biopesticide. *Description*: No.1 = nano emulsion of citronella oil at a dose 0.1%. No.2 = nano emulsion of citronella oil at a dose 0.5%. No.3 = nano emulsion of citronella oil at a dose 1%. No.4 = nano emulsion of citronella oil at a dose 1.5%. No.5 = citronella oil at a dose 0.1%. No.6 = citronella oil at a dose 0.5%. No.7 = citronella oil at a dose 1%. No.8 = citronella oil at a dose 1.5%. No.9 = no treatment as a comparison for control. No.10 = Fungicide with active ingredient mankozeb.
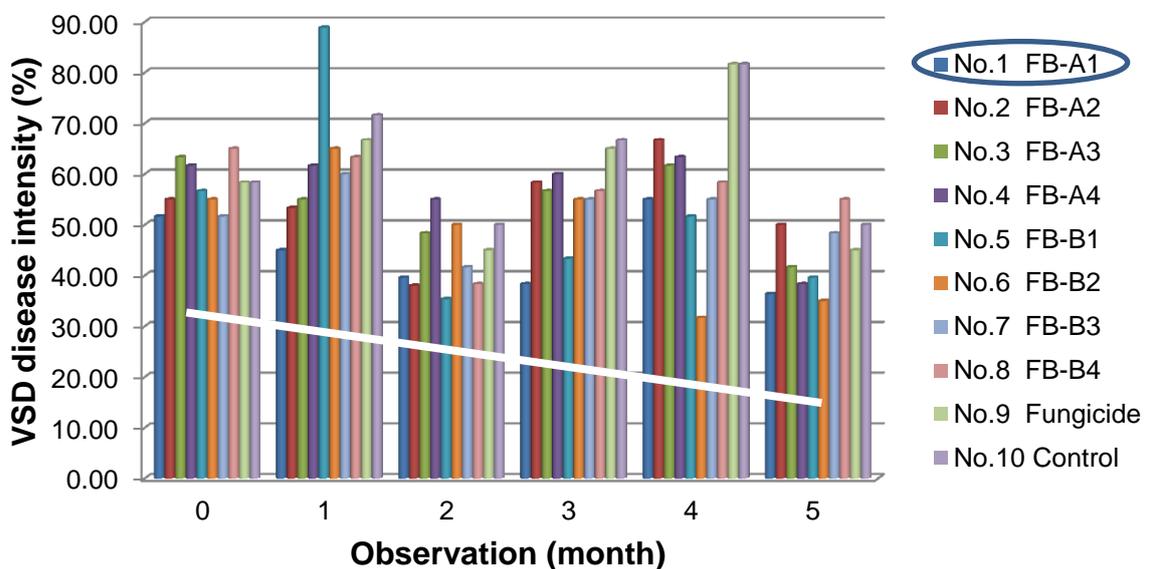
Figure 4. Percentage intensity of *Vascular streak dieback* (VSD) disease in cocoa plants in Padang Pariaman District before and after application of biopesticide. *Description*: No.1 = nano emulsion of citronella oil at a dose 0.1%. No.2 = nano emulsion of citronella oil at a dose 0.5%. No.3 = nano emulsion of citronella oil at a dose 1%. No.4 = nano emulsion of citronella oil at a dose 1.5%. No.5 = citronella oil at a dose 0.1%. No.6 = citronella oil at a dose 0.5%. No.7 = citronella oil at a dose 1%. No.8 = citronella oil at a dose 1.5%. No.9 = no treatment as a comparison for control. No.10 = Fungicide with active ingredient mankozeb.

Table 2. Climate data (average of air temperature, average of relative humidity and rainfall) from June to December 2016 in Padang Pariaman and Limapuluh Kota district.

Month (Bulan)	Average of air temperature (ºC) (Temperatur Udara Rata-rata)	Average of relative humidity (%) (Kelembaban Udara Rata-rata)	Rainfall (mm) (Curah hujan)
Parit Malintang-Padang Pariaman district			
June	25.7	89	157
July	26.0	84	324
August	25.6	84	385
September	25.5	86	317
October	29.2	87	559
November	28.0	86	288
December	25.3	88	341
Limapuluh Kota district			
June	23.2	81	91
July	22.0	83	124
August	21.9	82	60
September	21.7	84	61
October	21.6	86	34
November	26.0	87	308
December	21.5	86	149

On the basis of analysis of the measurement of Area Under Disease Progress Curve (AUDPC) in Padang Pariaman district, the lowest AUDPC was found in the treatment of nano emulsion formula with a dose of 0.1% (FB-A1) compared with control. While in the area of Limapuluh Kota district the lowest AUDPC was found in the treatment of citronella oil formula at a dose 1.5% (FB-B4) (Table 3). This suggests that the two biopesticide formulations can decrease the development of VSD disease at both test sites. The best activity in controlling VSD disease using nano emulsion formula at a dose 0.1% with decrease of disease intensity up to 21.13%, while formula of citronella oil at a dose 1.5% with decrease of disease intensity up to 11,80%. From several studies it is revealed that citronella at doses of 12 ppm could decrease the intensity of *Colletotrichum* sp that infected onion plants to 20% [19], citronella oil with concentration 0.2-1.5 μl/ml could control *Alternaria alternata* in tomato plant [20] and with concentration 0.5% can kill the conidia of *Aspergillus niger* [21].

Table 3. Reduced VSD disease intensity (%) of VSD and efficacy level (%) after treated with oil and nano emulsion biopesticide formulations for 6 months of application time.

No Treatment	Treatment Bio-pesticide	Padang Pariaman district				Limapuluh Kota district			
		AUDPC	Decrease in intensity of disease (%)	Efficacy level (%)	Assessment of efficacy level	AUDPC	Decrease in intensity of disease (%)	Efficacy level (%)	Assessment of efficacy level
1	FB-A1	221.93	21.13	32.08	Effective	253.34	7.33	12.11	Not Effective
2	FB-A2	268.83	10.72	16.08	Not Effective	249.17	8.33	12.83	Not Effective
3	FB-A3	274.17	11.32	16.53	Not Effective	270.01	4.33	6.52	Not Effective
4	FB-A4	289.99	8.32	11.94	Not Effective	255.01	7.83	11.08	Not Effective
5	FB-B1	267.38	12.22	19.54	Not Effective	240.69	11.06	17.	Not

								61	Effective
6	FB-B2	246.67	16.65	23.59	Not Effective	245.00	9.17	13.35	Not Effective
7	FB-B3	261.67	11.99	17.27	Not Effective	265.84	6.00	9.30	Not Effective
8	FB-B4	276.66	9.66	13.7	Not Effective	235.17	11.80	19.02	Not Effective
9	Fungicide	310.01	3.32	5.88	Not Effective	256.67	7.83	13.65	Not Effective
10	Control	324.11	-	-		288.75	-	-	

Description: No.1 = nano emulsion of citronella oil at a dose of 0.1%. No.2 = nano emulsion of citronella at a dose of 0.5%. No.3 = nano emulsion of citronella oil at dose of 1%. No.4 = nano emulsion of citronella oil at a dose of 1.5%. No.5 = citronella oil at a dose of 0.1%. No.6 = citronella oil at a dose of 0.5%. No.7 = citronella oil at a dose of 1%. No.8 = citronella oil at a dose of 1.5%. No.9 = no treatment as a comparison for control. No.10 = fungicide with active ingredient mankozeb.

The observations also show that formula of nano emulsion of citronella oil was more effective and efficient in suppressing the development of VSD disease than the formula of citronella oil. Improving the formula of citronella oil using nanotechnology can improve the effectiveness of the biopesticide, because of its small particle size (nanometer in size) so it can directly reach the target microorganism. Moreover, citronella oil is more stable in the field. According to [22], nanoparticles can improve the stability of neem products (biopesticides from *Azadirachta indica*) against ultraviolet radiation and increase dispersion in the aqueous phase.

4. Conclusion
The percentage of VSD disease attacks in Padang Pariaman and Limapuluh Kota district was 100%. Citronella oil and nano emulsion formulation can reduce the intensity of *Vascular streak dieback* (VSD) disease in cocoa plants in West Sumatra, especially in Padang Pariaman and Lima Puluh Kota districts. The decrease in intensity of VSD disease in Padang Pariaman district ranged from 8.32 to 21.13%; while in the district of Lima Puluh Kota ranged from 4.33 to 11.80%. The nano emulsion formula at a dose of 0.1% is effective and efficient in suppressing the intensity of VSD attacks on cocoa plants compared to the formula of citronella oil itself.

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