# Effectiveness of Clove Nano Biopesticides Against Mosaic Virus in Patchouli

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**ABSTRACT**

*Clove oil has the potential to suppress the development of the mosaic virus in patchouli plants,* but its effectiveness in the field has not been *studied. This study aimed to evaluate the effect of clove nano biopesticide on controlling patchouli mosaic disease. The research was conducted at the Manoko Experimental Garden, Bandung, West Java from March to November 2018. The patchouli used was Patchoulina-2 variety, which originated from the Seed Breeder Garden in Lembang, Bandung. This study was arranged in a Randomized Block Design (RBD), consisting of five treatments and ten replications within each treatment, with one hundred plants in each replication. The results obtained showed that nano biopesticides of citronella, clove, and commercial citronella (Asimbo) were able to reduce the incidence and intensity of mosaic diseases in patchouli plants, showing the efficacy levels of 14.68%, 9.06%, and 5.83%, respectively. The application of citronella and clove biopesticides on Patchoulina-2 every month could increase plant fresh weight, when compared to the plants without treatment. Patchoulina-2 plants treated with nano biopesticides of clove and commercial citronella (Asimbo) showed higher value of fresh weight compared to those treated with citronella nano biopesticide. The clove nano biopesticide can also be developed to control mosaic diseases in patchouli plants.*

Keywords: Mosaic virus, Nano virucide, *Pogostemon cablin*

**ABSTRAK**

Minyak cengkeh berpotensi menekan perkembangan virus mosaik pada tanaman nilam, namun efektivitasnya di lapangan belum didiketahui. Tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh biopestisida cengkeh nano pada pengendalian penyakit mosaik nilam. Penelitian dilaksanakan di Kebun Percobaan Manoko, Bandung, Jawa Barat pada bulan Maret sampai November 2018. Nilam yang digunakan adalah varietas Patchoulina-2 yang berasal dari Kebun Penangkar Benih di Lembang, Bandung. Penelitian ini disusun dengan Rancangan Acak Kelompok (RAK) dengan lima perlakuan sepuluh ulangan dan tiap ulangan seratus tanaman. Hasil yang diperoleh menunjukkan bahwa formula nano biopestisida seraiwangi, cengkeh, dan seraiwangi komersial (Asimbo) telah mampu mengurangi kejadian dan intensitas penyakit mosaik pada tanaman nilam, tingkat efikasinya masing-masing adalah 14,68%, 9,06%, dan 5,83%. Aplikasi biopestisida seraiwangi dan cengkeh pada varietas Patchoulina-2 setiap bulan dapat meningkatkan berat basah tanaman, dibandingkan tanpa perlakuan. Berat basah tanaman Patchoulina-2 lebih tinggi pada nano biopestisida cengkeh dan seraiwangi komersial (Asimbo) dibandingkan dengan nano biopestisida seraiwangi. Formula nano biopestisida cengkeh juga dapat dikembangkan untuk mengendalikan penyakit mozaik pada tanaman nilam

Kata kunci: Mosaik virus, Nano virusida, *Pogostemon cablin*

## INTRODUCTION

The mosaic disease is transmitted through patchouli seeds and insect vectors so that the spread is very fast, and it has been found in many patchouli plantations in Sumatra, Java, and Sulawesi. Patchouli seedlings are susceptible to mosaic diseases caused by viruses because this disease is transmitted by aphids, which attack patchouli plants in nurseries. Mosaic patchouli disease has developed very rapidly, which in 3 years, spreading to patchouli cultivation centers in Sumatra, Java, and Sulawesi. This is mainly due to the vegetative propagation of patchouli plants (Noveriza, 2016).

One of the aphids that have been reported to attack patchouli plants is *A. gossypii* (Mardiningsih and Soetopo, 1999) with the percentage of attacks ranging from 7.77-27.35% (Baringbing et al., 2004). However, the attack has not reduced patchouli production. These insects harm patchouli plants in nurseries. Therefore, the most worrying issue about the existence of this aphid in patchouli is its ability to transmit *Potyvirus* (TeMV). From the results of the study by Noveriza (2013), *A. gossypii* in the acquisition period of 15 minutes and the 4-hour inoculation period was able to transmit TeMV with the percentage of infections reaching 80%. With the high incidence of TeMV virus infection and the quite dense population of vector *A. gossypii* in patchouli production centers in Indonesia, it is necessary to pay attention to the appropriate disease control strategies to prevent mosaic diseases caused by TeMV from widespread (Noveriza, 2013). This aphid was also reported to be able to transmit *Cucumber mosaic virus* (CMV) and *Broad bean wilt virus* 2 (BBWV2) (Santz et al., 2001; Gildow et al., 2008; Belliure et al., 2009; Shi et al., 2016), in which both viruses were also reported to infect patchouli plants (Sukamto et al., 2007; Miftakhurohmah et al., 2015).

Clove oil is an essential oil that has been used as an ingredient in natural pesticides because it contains several volatile compounds, such as eugenol. Eugenol is the main component in clove oil, ranging from 70-95% depending on the part of the clove plant. The eugenol content of clove's leaf oil reaches 70%, while that of clove flowers can reach 90% (Wiratno, 2010).

Clove oil has been reported as a potential fungicide and insecticide. According to Manohara et al. (1993), the use of clove oil at a concentration of 200-300 ppm showed very good effectiveness in inhibiting the growth of several pathogenic fungi, such as *Phytophthora capsici*, *Phytophthora palmivora*, and *Rigidoporus lignosus*. The results of the research by Djiwanti and Supriadi (2012) showed that clove oil at a concentration of 5,000 ppm was very effective in suppressing the symptoms of stem rot in ginger root caused by *Fusarium oxysporum* with 100% inhibition. According to Siswanto et al. (2011), clove oil can function as a contact and stomach poisons against *Longitarsus* sp. (patchouli leaf beetle). Besides, clove oil also has the potential as an antiviral. The results of the research by Noveriza et al. (2016) showed that clove oil has the potential to suppress the development of the mosaic virus in patchouli plants. A concentration of 1% clove oil can reduce the number of lesions by up to 45%.

The clove oil formulation has been refined using the spontaneous and inverse phase of nano emulsification technique so that it is named citronella nano biopesticide. The results of the formula testing in the greenhouse scale showed that the clove nano biopesticide formula at a concentration of 0.5% suppressed viral development by 43.55% (Noveriza et al., 2017), but its effectiveness in the field has not been studied. The use of appropriate and environmentally friendly technology needs to be developed before it is introduced to farmers. Therefore, this study aimed to evaluate the effect of nano biopesticide of clove on controlling patchouli mosaic disease in the field.

## MATERIALS AND METHODS

The research was conducted at the Manoko Experimental Garden, Bandung, West Java from March to November 2018. The patchouli used was Patchoulina-2 variety, which originated from the Seed Breeder Garden in Lembang, Bandung. This study was arranged in a Randomized Block Design (RBD) consisting of 5 treatments and 10 replications within each treatment, with 100 plants in each replication. The treatment was (A) nano biopesticide formula of citronella at a concentration of 1%, (B) nano biopesticide formula of clove at a concentration of 0.5%, (C) a recommended dose of asimbo formula, (D) insecticide, and (E) without treatment (control).

### The technique of applying nano-biopesticide formulas on a field scale

Clove and citronella nano biopesticide formula (50-100 ml) was sprayed to all parts of patchouli plants, and the intensity of spraying was done every month for 7 times spraying. Spraying was done since the plants are in nursery. Land preparation was carried out by following the patchouli cultivation Standard Operational Procedure (SOP), which began with the preparation of patchouli seeds, planting, fertilizing, and harvesting.

## Variables observed

*The incidence of mosaic disease symptoms* was recorded in each row of the experimental plot. The percentage of disease incidence was determined by calculating the total number of infected plants in a row divided by the total number of plants multiplied by 100 (Akram and Naimuddin, 2016). Symptoms of mosaic disease were recorded before treatment and one day after treatment.

The intensity of the disease attack was observed in each plant by calculating the mosaic symptoms that appear, with the attack category according to the scoring (Table 1).

**Table 1**. Scores and descriptions of mosaic symptoms in patchouli plants

| Scoring | Symptom description |
|---|---|
| *0* | Healthy, without any symptoms on the leaf of plants |
| *1* | Mild, striped symptoms in some parts of the leaf and chlorosis |
| *2* | Moderate, all parts of the symptomatic mosaic plant |
| 3 | Heavy, all parts of the plant are mosaic symptomatic with malformations |

Notes: Asare-Bediako et al. (2014) with modification

**Table 2.** Category and criteria of attack

| Category | Level (%) | Criteria |
|---|---|---|
| 0 | X = 0 | No attack |
| 1 | 0 ≤ X ≤ 25 | Mild attack |
| 2 | 25 ≤ X ≤ 50 | Moderate attack |
| 3 | 50 ≤ X ≤ 75 | Heavy attack |
| 4 | 75 ≤ X ≤ 100 | Very heavy attack |

The intensity of disease attacks was calculated using the following formula (Strange, 2008):

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

I = Attack intensity

Ni = the number of plants in each attack category

vi = scale value from each attack category

Z = scale value from the highest attack category

N = number of plants observed

The incidence of the *Aphis gossypii* attack was carried out by scoring the percentage of leaf shoots that rolled up (due to aphid attack) (Asare-Bediako et al., 2014). The development of the population of *A. gossypii* was calculated before and after the application by counting each shoot that was attacked. The level of attack was calculated using the following formulae.

The level of efficacy of the nano pesticide formula for mosaic disease and *A. gossypii* was calculated by:

$$\textit{Aphid attack level} = \frac{\textit{the number of damaged plants}}{\textit{total number of plants}}\ x\ 100\%$$

Meanwhile, the intensity of damage was calculated using the formula (Unterstenhofer 1963) as followed:

$$P = \frac{\sum(n\,v)}{z\,x\,N} x100\%$$

IP = intensity of damage (%)

n = the number of plants attacked according to categories (score 0, 1, 2, 3, 4)

v = the scale value (score) of each category

z = the scale value (score) of the highest attack category

N = the number of all plants observed ($n_0$ + $n_1$ + . . . . + $n_6$)

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

EI = The effectiveness of the nano pesticide formula tested (%)

Ca = Percentage of plant damage in the control plot

Ta = Percentage of plant damage in treatment plots after application of the nano pesticide formula

The tested formula is considered effective if the value of the efficacy level (EI) is 30%.

Loss of yield was determined by calculating fresh (g) and dry biomass (g) of patchouli plants in the first harvest (6 months) and the second harvest (3 months from the first harvest). Crop yield losses will be calculated based on standard formulas and compared with untreated controls. Furthermore, analysis of oil yield and patchouli alcohol content (PA) for each treatment was performed.

### Virus Detection with Serological Methods

Detection of *Potyvirus* in leaf samples from patchouli plants was carried out by the DIBA serological method, the modification of the technique of Chang et al. (2011) using nitrocellulose membranes (Thermo Scientific, USA).

### Data analysis

The collected data were transformed using the square root to ensure the homogeneity of the variance and the normal distribution of the data. The data then were subjected to an analysis of variance (ANOVA) using the SAS Program.

## RESULTS AND DISCUSSION

### The incidence and intensity of mosaics

The percentage of mosaic disease incidence and intensity in Patchoulina 2 variety in West Bandung Regency (West Java) can be seen in Table 3. The lowest average mosaic disease incidence was observed in the plants treated with citronella nano biopesticide (53.86%), which was compared to those without treatment (59.53%). This result shows that the application of nano biopesticide formula of 1% citronella and clove once a month can suppress the development of mosaic disease in Patchoulina 2 plants.

Volatile oil and plant extracts contain active substances that can inhibit viral infections in plant tissues. According to Meneses et al. (2009), essential oil from several plants is antiviral, whose mechanism is to activate the virus directly. Besides, it also induces plant resistance to viruses and induces plant growth (Wang and Fan, 2014; Venkatesan et al., 2012).

**Table 3.** The average percentage of mosaic disease incidence and intensity in West Bandung Regency (West Java) and the efficacy level of the tested formula

| Treatments | Incidence of mosaic disease (%) | The intensity of mosaic disease (%) | Level of efficacy (%) |
|---|---|---|---|
| *Formula of citronella nano biopesticide 1%* | 53.86 b | 19.58 c | 14.68 |
| *Formula of clove nano biopesticide 0.5%* | 56.67 ab | 20.87 bc | 9.06 |
| *Formula of commercial citronella (Asimbo) 0.5%* | 58.01 ab | 21.61 abc | 5.83 |
| Deltamethrin (Chemical insecticide) | 58.04 ab | 22.23 ab | 3.14 |
| Control without treatment | 59.53 a | 22.95 a | |
| CV | 10,44 | 10,64 | |

Notes: Means followed by the same letters in the same column are not significantly different at the level of 5% (LSD). This study was arranged in a Randomized Block Design (RBD) consisting of 5 treatments and 10 replications within each treatment, with 100 plants in each replication.

**Table 4.** The average percentage of incidence and intensity of leaf shoots roller (aphids) in West Bandung Regency and the efficacy of the formulas supported

| Treatments | Incidence of mosaic disease (%) | The intensity of mosaic disease (%) | Level of efficacy (%) |
|---|---|---|---|
| *Formula of citronella nano biopesticide 1%* | 1.57 a | 0.44 a | 38.03 |
| *Formula of clove nano biopesticide 0.5%* | 1.80 a | 0.46 a | 35.21 |
| *Formula of commercial citronella (Asimbo) 0.5%* | 2.62 a | 0.71 a | 0.0 |
| Deltamethrin (Chemical insecticide) | 1.63 a | 0.48 a | 32.39 |
| Control without treatment | 2.60 a | 0.71 a | |
| CV | 37,54 | 29,18 | |

Notes: Means followed by the same letters in the same column are not significantly different at the level of 5% (LSD). This study was arranged in a Randomized Block Design (RBD) consisting of 5 treatments and 10 replications within each treatment, with 100 plants in each replication.

**Table 5.** Potyvirus detection results from patchouli leaf samples before and after application using the ELISA method

| Treatments | Detection of *Potyvirus* | | | |
|---|---|---|---|---|
| | Before application | | After application | |
| | The value of absorbance | Results | The value of absorbance | Results |
| *Buffer* | 0,091 | | 0,087 | |
| *Control (-)* | 0,129 | | 0,104 | |
| *Control (+)* | 0,588 | | 0,934 | |
| *Formula of citronella nano biopesticide 1%* | 0,992 | Positive | 0,859 | Positive |
| Formula of clove nano biopesticide 0.5% | 0,838 | Positive | | Positive |
| Formula of commercial citronella (Asimbo) dose 0.5% | 0,812 | Positive | 0,807 | Positive |
| Deltamethrin (Chemical insecticide) | 0,646 | Positive | 0,820 | Positive |
| Control without treatment | 0,610 | Positive | 0,695 | Positive |

The efficacy rate of the 1% citronella nano biopesticide formula was the highest (14.68%) compared to other treatments. This result was not different compared to the study by Noveriza et al. (2019) in 2017 (20.63%) in the same location using Sidikalang variety. This shows that there are differences in the effectiveness of the application of the citronella nano biopesticide formula on patchouli plants in different varieties. Also, clove nano biopesticides able to reduce the intensity of mosaic diseases, although not as good as citronella nano biopesticides. This result was no different from the testing in the greenhouse. According to Noveriza et al. (2017), nano biopesticides of clove and citronella oil have the potential to be virucides, especially against patchouli mosaic viruses. The highest percentage of mosaic virus inhibition in *Chenopodium amaranticolor* by nano formulation of citronella at a concentration of 1% and clove at a concentration of 0.5% was 74.87% and 43.55%, respectively.

### The incidence and intensity of attack of leaf roller (Aphids)

The lowest number of the rolling shoots due to the attack of aphids in patchouli plants was 1.63% (insecticide) and 1.57% (citronella nano biopesticide) (Table 4). This shows that citronella insecticides and nano biopesticides can reduce the incidence of aphid attack. Meanwhile, the lowest intensity of leaf rollers caused by aphids in patchouli plants for 6 months was 0.13%, resulted by insecticide treatment (deltamethrin), followed by citronella nano biopesticide (0.22%) and with-

**Table 6.** Average fresh biomass weight per plant (g), dry weight per plant (g), fresh and dry weight ratio, and increase in yield (%) of Patchoulina 2 variety after 6 months at an altitude of 1200 m asl

| Treatments | The weight of wet biomass per plant (g) | The weight of dry biomass per plant (g) | Ratio wet: dry biomass | Yield loss of dry biomass weight (%) |
|---|---|---|---|---|
| Formula of citronella nano biopesticide 1% | 268.71 ab | 84.69 a | 3.20 :1 | 5.14 |
| Formula of clove nano biopesticide 0.5% | 282.28 a | 89.50 a | 3.19 : 1 | 11.11 |
| Formula of commercial citronella (Asimbo) 0.5% | 282.43 a | 9.60 a | 3.11 : 1 | 14.96 |
| Deltamethrin (Chemical insecticide) | 281.84 ab | 91.45 a | 3.14 : 1 | 13.53 |
| Control without treatment | 24.61 b | 80.55 a | 3.15 : 1 | |
| CV | 13.92 | 20.16 | | |

Remarks: Means followed by the same letters in the same column are not significantly different at the level of 5% (LSD). This study was arranged in a Randomized Block Design (RBD) consisting of 5 treatments and 10 replications within each treatment, with 100 plants in each replication.

**Table 7.** Average oil production per plant (ml), oil yield (%), and alcohol content (%) of Patchoulina 2 variety after 6 months at 1200 m asl

| Treatments | Production of oil per plant (ml) | The yield of oil (%) | The yield of patchouli alcohol (%) |
|---|---|---|---|
| Formula of citronella nano biopesticide 1% | 0.748 a | 0.86 a | 30.99 |
| Formula of clove nano biopesticide 0.5% | 0.803 a | 0.87 a | 30.37 |
| Formula of commercial citronella (Asimbo) 0.5% | 0.760 a | 0.78 a | 31.40 |
| Deltamethrin (Chemical insecticide) | 0.791 a | 0.84 a | 31.02 |
| Control without treatment | 0,693 a | 0.82 a | 30.56 |
| CV | 24.79 | 13.28 | |

Remarks: Means followed by the same letters in the same column are not significantly different at the level of 5% (LSD). This study was arranged in a Randomized Block Design (RBD) consisting of 5 treatments and 10 replications within each treatment, with 100 plants in each replication.

out treatment (0.72%). The intensity of the leaf roller attack was below 1% because the incidence of attacks was also low in 2018, compared to 2017 in the same location. In 2017, the incidence of leaf roller attacks on patchouli Sidikalang variety ranged 4.76-23.29%, in which the lowest was in insecticide treatment (Noveriza et al., 2019).

The use of essential oil for controlling insects has obtained satisfactory results (Lima et al., 2011). Citronella grass essential oil at 1% (w $v^{-1}$) was more toxic to *Myzus persicae* (aphid) than to *Frankliniella schultzei* (thrips). It is promising for developing pesticides to manage aphid (Pinheiro et al., 2013). According to Noveriza et al. (2019), nano biopesticide of citronella oil was more effective to reduce the intensity of aphid attack on patchouli plant. Besides, clove essential oil possessed anti-inflammatory, cytotoxic, insect repellent, and anesthetic properties (Chaieb et al., 2007).

### Mosaic virus verification

The detection of *Potyvirus* (mosaic virus) in patchouli leaf samples before (1 month of age in the field) and after the application of citronella nano biopesticide (5 months in the field) by ELISA method showed that all positive samples were infected with *Potyvirus* (Table 5).

### Potential Loss of Yields

Patchoulina 2 variety planted in Bandung, West Java (with an altitude of 1,200 m above sea level (asl)) produced dry weight per plant that was not significantly different in all treatments tested (Table 6). According to the results of the study in

2017, the dry weight of patchouli plants infected by the virus would be decreasing. The ratio of the fresh and dry biomass was the highest in the treatment of nano biopesticide formula with a concentration of 1%. The yield loss of patchouli in the first harvest (6 months after planting) due to mosaic disease compared to untreated control ranged from 5.14-14.96%, and the highest loss of yield was in the application of the commercial formula of citronella (Asimbo). This shows that all three biopesticide formulas can increase the potential loss of dry weight of the plants infected by the viral mosaic disease.

In this study, the patchouli oil yield was below 1, while PA level of all treatments was not different (above 30%) (Table 7). According to Sukamto et al. (2007), the mosaic disease was recorded as one of the limiting factors in the production of patchouli (*P. cablin*) in Indonesia. This disease could reduce the fresh and dry weight of patchouli plants, reaching 34.65% and 40.42% (Noveriza et al., 2012). The reduction of fresh and dry weight was due to the plant metabolic disorders. According to Agrios (2005), the decrease in growth hormone produced by plants was accompanied by a decrease in the amount of chlorophyll, this is a common influence that occurs in plants infected by the virus, which consequently decreased plant biomass.

## CONCLUSION

The citronella nano biopesticide, clove nano biopesticide, and commercial citronella (Asimbo) formulas were able to reduce the incidence and intensity of mosaic diseases in patchouli plants, with efficacy levels below 30%. The application of citronella and clove nano biopesticide on Patchoulina 2 variety every month could increase plant fresh weight, compared to the plants without treatment. The fresh weight of Patchoulina 2 plants was higher in the clove nano biopesticide and commercial citronella (Asimbo) compared to that in citronella nano biopesticide. The clove nano biopesticide can also be developed to control mosaic diseases in patchouli plants.

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