EFFECT OF CITRONELLA NANO BIOPESTICIDE AGAINST MOSAIC VIRUS AND ITS VECTOR ON PATCHOULI

Pengaruh Nano Biopestisida Citronella terhadap Virus Mosaik dan Vektornya pada Tanaman Nilam

Rita Noveriza 1*), Maya Mariana 1), Tri Lestari Mardiningsih 1) and Sri Yuliani 2)

1) Indonesian Spices and Medicinal Crops Research Institute
Jalan Tentara Pelajar No. 3, Bogor 16111 Indonesia

2) Indonesian Center for Agricultural Post Harvest Research and Development
Jalan Tentara Pelajar No. 12, Bogor 16124 Indonesia

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ABSTRACT/ABSTRAK

The mosaic disease declines production and oil quality of patchouli. Antiviral-based citronella oil has been formulated using a spontaneous emulsification technique (nanotechnology). The previous result of the greenhouse trial showed the formula at 1-1.5 % concentrations suppressed the development of virus of about 82.5 %. The field-scale tests is necessary to be performed to validate the effectiveness of citronella nano biopesticide (CNB) against the mosaic virus and its vector on patchouli. The study was conducted in patchouli plantation at two locations (Pandeglang, Banten and Manoko, West Java). The research was arranged in a Randomized Completed Block Design (RCBD) with 6 treatments and 10 replications, each replication consisted of 50 plants. The treatments were formula of CNB at the concentration of (1) 0.5 %, (2) 1 %, (3) 1.5 %, (4) 2 %, (5) insecticide (deltamethrin 0.2 %), and (6) control. The results showed that CNB formula at 1 % concentration with a monthly application effectively suppressed the development of mosaic disease in patchouli plants, and at 2 % concentration to control rolled-leaf aphid. The lowest intensity of mosaic disease (at 1 % concentration) was in Banten (23.12 %) and in West Java (18.35 %), while in control ranged from 26.31-44.94 % (Banten) and 19.60-23.12 % (West Java). Efficacy Index (EI) in Banten ranged from 12.12-48.55 % and in West Java was 6.38-20.63 %. The lowest intensity of aphid attack was showed by insecticide and CNB at 2 % concentration. The EI of CNB was 35.33 % (Banten) and 51.71 % (West Java) respectively.

Penyakit mosaik menyebabkan penurunan produksi dan kualitas minyak nilam. Formula anti virus berbasis minyak serai wangi dengan menggunakan teknik pengemulsi spontan (teknologi nano) yang diuji pada skala rumah kaca menunjukkan formula biopestisida nano serai wangi pada konsentrasi 1-1.5 % menekan perkembangan virus 82.5 %. Validasi formula skala lapangan diperlukan untuk mendapatkan konsentrası biopestisida nano sitronela (BNS) yang paling sesuai untuk mengendalikan virus mosaik dan vektornya pada tanaman nilam. Penelitian telah dilakukan di dua lokasi penanaman nilam (Pandeglang, Banten dan Manoko, Jawa Barat). Rancangan yang digunakan adalah Rancangan Acak Kelompok (RAK) dengan 6 perlakuan dan 10 ulangan, 50 tanaman/plot. Perlakuan yang diuji adalah formula BNS pada konsentrası (1) 0.5 %, (2) 1 %, (3) 1.5 %, (4) 2 %, (5) insektisida sintetik (deltamethrin 0.2 %), dan (6) kontrol. Hasil penelitian menunjukkan bahwa formula nano biopestisida dari minyak serai wangi pada konsentrası 1 % yang diaplikasikan setiap bulan efektif menekan perkembangan penyakit mosaik pada tanaman nilam, sedangkan konsentrası yang efektif untuk pucuk daun menggulung akibat serangan kutu adalah 2 %. Intensitas penyakit mosaik terendah (1 %) adalah di Banten (23.12 %) dan di Jawa Barat (18.35 %).

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INTRODUCTION

The mosaic disease of patchouli has been developing very fast, and within three years period, the disease had spread to the central producing patchouli in Sumatera, Java, and Sulawesi due to vegetatively-cutting multiplication system of patchouli. In 2013, the existence of a viral infection of patchouli plantation was reported in Cicurug, Manoko, and Cijeruk, West Java, caused by Potyvirus, Potexvirus, Cucumber Mosaic Virus (CMV) and Fabavirus (Miftakhurohmah et al. 2013). In 2015, there was also a report on Potyvirus and Fabavirus infection in Banten (Mariana dan Noveriza 2015). In addition, the mosaic disease has also been found in patchouli cultivation in Southeast Sulawesi (Taufik et al. 2012; Taufik et al. 2014). Therefore, the use of virus-free seeds and early detection methods for patchouli seeds are the main concerns. Furthermore, the handling of mosaic disease-free patchouli seeds and its vector is essential (Noveriza 2016).

Biological control comprises of various technologies, e.g. the use of botanical pesticide. Many kinds of plant species and techniques have been used in the production of botanical pesticides (Tiilikala et al. 2011) for plant protection. Mariana and Noveriza (2013) reported that citronella oil at 1.2 % concentration was able to suppress the development of Potyvirus (89.78 %) in the patchouli plant. This suggested that citronella oil can reduce the population of Potyvirus above 80 %, whereas clove oil at 1 % concentration could suppress the mosaic virus up to 45 % (Noveriza et al. 2016). Formulation of citronella oil has been conducted by using the method of spontaneous emulsification technique. The result of the greenhouse scale study showed the formula of citronella nano biopesticide at the concentrations of 1-1.5 % repressed the development of virus of about 82.5 %; while citronella oil in the regular form was only 65-70 % (Noveriza et al. 2017). Validation in the field is important to obtain the effective and efficient concentration to control mosaic disease in patchouli. This research was aimed to obtain the effective concentration of citronella nano biopesticide to control the mosaic virus and its vector on patchouli plant in the field.

MATERIALS AND METHODS

The field trials were located at two patchouli plantations at (1) Babakan Kalanganyar Village, Pandeglang, Banten; and (2) Manoko, Lembang, West Java (Figure 1). The research were arranged in a Randomized Completed Block Design (RCBD) with 6 treatments and 10 replications, each replication consisted of 50 plants.

Preparation of patchouli plant material

The patchouli plant used was the Sidikalang variety from Seeds Production Management Unit of Indonesian Spices and Medicinal Crops Research Institute (ISMCRI), Bogor (Figure 2). Nano biopesticide formula with a standard particle size of citronella oil 100-200 nm has been made in the Plant Protection Laboratory of the ISMCRI using spontaneous emulsification techniques. Patchouli seedlings were propagated following the patchouli propagation SOP (GAP on Patchouli, Minister of Agriculture Regulation No 138-2014). One month-old patchouli seedlings were then transplanted to the field.
Nano biopesticide formula application in the field

The formula of citronella nano biopesticide (volume 50-100 ml) was sprayed to the whole patchouli plant every month during 6 months. The treatments were four concentrations of nano biopesticide of citronella, (0.5 %, 1 %, 1.5 %, and 2 %), synthetic insecticide (deltamethrin as active ingredient) at 0.2 % concentration as positive control and without pesticide as negative control.

Data collection

The incidence of mosaic disease was recorded every month. The percentage of disease incidence was calculated by counting the total number of infected plants divided by the total number of plants.
The intensity of the mosaic disease was conducted every month by observing the mosaic symptoms appeared in each plant which were then categorized following the score as presented in Table 1. Disease intensity was calculated using the formula as follows (Strange 2008):

$$I = \left( \frac{\sum (n_i \times v_i)}{Z \times N} \right) \times 100\%$$

$I =$ Disease intensity/Intensitas penyakit,
$n_i =$ the number of plants in each category/jumlah tanaman pada setiap kategori serangan,
$v_i =$ the scale value of each category/nilai skala pada setiap kategori serangan,
$Z =$ the scale value of the highest category/nilai skala pada kategori serangan tertinggi,
$N =$ the number of plants observed/jumlah tanaman yang diamati.

Table 1. Scores and description mosaic symptoms on patchouli.
Tabel 1. Skor dan deskripsi gejala mosaik pada nilam.

| Scores | Description of mosaic symptoms                                      |
|--------|---------------------------------------------------------------------|
| 0      | Plants are healthy, no symptom/ Tanaman sehat, tidak ada gejala.   |
| 1      | Mild, striped symptoms on some parts of the leaves and chlorosis/Gejala ringan, belang pada beberapa bagian daun dan klorosis. |
| 2      | Medium, symptomatic plants 15-25%/ Sedang, tanaman simptomatik 15-25%. |
| 3      | Heavy, symptomatic plants > 50% and plant malformations/Berat, tanaman bergejala > 50% dan malformasi tanaman. |

Incidence of aphids attacks was observed every month by counting the rolled leaf due to aphids attack. The percentage of aphid attack was calculated by counting the total number of damaged plants divided by the total number of plants multiplied by 100 (Asare-Bediako et al. 2014).

Damage intensity (%) was conducted every month by observing each plant which showed roll leaf symptom and then scored as presented in Table 2. The percentage of damage intensity was calculated following (Pustika et al. 2012) formula.

$$IP = \sum \left( \frac{a \times v}{z \times N} \right) \times 100\%$$

$IP =$ damage intensity (%)/intensitas serangan,
$n =$ number of affected plants by category (score 0, 1, 2, 3, 4)/jumlah tanaman yang terserang (skor 0, 1, 2, 3, 4),
$v =$ scale value (score) of each category/skala nilai pada setiap kategori,
$z =$ scale value (score) of the highest attack category/skala nilai pada serangan tertinggi,
$N =$ total number of plants observed (n0 + n1 +... + n6)/jumlah tanaman yang diamati.

Efficacy level of nano biopesticide formula against mosaic disease and aphid A. gossypii was calculated following (Harni dan Baharuddin 2014).

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

$EI =$ The effectiveness of the nano biopesticide formula (%)/Efektivitas formula biopestisida nano (%),
$Ca =$ Percentage of crops damage in control plot after nano biopesticide application/Persentase kerusakan tanaman di petak kontrol setelah aplikasi biopestisida nano,
Ta =$ Percentage of crops damage in treated plots after nano biopesticides application/Persentase kerusakan tanaman di petak yang dirawat setelah aplikasi biopestisida nano.

Formula tested was considered effective if the value of the level of efficacy (EI) was ≥ 30%Formula yang diuji dianggap efektif jika nilai tingkat kemanjuran (EI) ≥ 30%.

Loss of yield was observed by weighing fresh and dry weight of patchouli plants harvested at 6 months after planting.
RESULTS AND DISCUSSION

Mosaic disease and vector incidence

At both study sites, the lowest average percentage of mosaic disease incidence was at 1% concentration (Figure 3). Therefore, 1% concentration was the most effective concentration to suppress the development of mosaic disease in the field. The results of Potyvirus detection with the serology method showed that viral concentration in the patchouli leaf sample from Pandeglang (Banten) was higher than from Manoko (West Java) (data were not shown). The Potyvirus has been detected at high concentration within the plant tissue since June 2017.

The average rolled leaf percentage due to aphid attack on both study sites indicated that the lowest one, other than insecticide, was shown at 2% concentration (Figure 4). The aphid attack was higher in West Java than Banten, the opposite to mosaic virus incidence.

DISEASE INTENSITY AFTER TREATMENT

In Pandeglang (Banten), the lowest intensity of mosaic disease was indicated at 1% concentration with the efficacy level ranged from 12.12 to 48.55% (Figure 5). However in Manoko (West Java), there was no significant difference between the treatment of 1% concentration and control, although it was significantly different from other treatments. The efficacy level ranged from 6.38-20.63% (Figure 6).

Harni and Baharuddin (2014) stated that the efficacy levels of biopesticides above 30% have been affirmed effective. Therefore, the citronella nano biopesticide was effective for controlling mosaic disease in patchouli plants after 5 times applications (at 1% concentration), especially in the areas with high disease incidence. Essential oils of some plants were antiviral, with...
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It also induced plant resistance to viruses as well as enhancing plant growth (Wang and Fan 2014; Venkatesan et al. 2012).

**INTENSITY OF APHID ATTACK AFTER TREATMENT**

In Pandeglang (Banten), the lowest intensity of the leaf roller attack (aphid) was indicated by 2 % concentration of nano biopesticide and insecticide treatments (Figure 7) with efficacy level of 35.33 %. The similar result also occurred in Manoko (West Java) (Figure 8) with the efficacy level 51.71 %. Based on the statistical analysis, Deltametrin treatment was significantly different to control at 1.5 %, and 2 % concentrations. Gibson et al. (1982) revealed deltamethrin could reduce the transmission of persistent, semi-persistent and non-persistent viruses by *Myzus persicae* (aphid) in greenhouses and in the field.

Figure 5. Percentage of mosaic disease intensity in Pandeglang-Banten from May-October 2017. Note : 1 = nano biopesticide at 0.5 % concentration; 2 = 1 %; 3 = 1.5 %; 4 = 2 %; 5 = insecticide (Deltamethrin) 0.2 %; 6 = without treatment (control).

Gambar 5. Intensitas persentase penyakit mosaik di Pandeglang-Banten dari Mei-Oktobr 2017. Keterangan : 1 = konsentrasi nano biopestisida 0.5 %; 2 = 1 %; 3 = 1,5 %; 4 = 2 %; 5 = insektisida (Deltamethrin) 0.2 %; 6 = tanpa perlakuan (kontrol).

Figure 6. Percentage of mosaic disease intensity in Manoko-West Java from May-October 2017. Note : 1 = nano biopesticide at 0.5 % concentration; 2 = 1 %; 3 = 1.5 %; 4 = 2 %; 5 = insecticide (Deltamethrin) 0.2 %; 6 = without treatment (control).

Gambar 6. Intensitas persentase penyakit mosaik di Manoko-Jawa Barat dari Mei-Oktobr 2017. Keterangan : 1 = konsentrasi nano biopestisida 0.5 %; 2 = 1 %; 3 = 1,5 %; 4 = 2 %; 5 = insektisida (Deltamethrin) 0.2 %; 6 = tanpa perlakuan (kontrol).
PLANT PRODUCTION

In Manoko, the highest dry weight of plant was obtained at 1% concentration application of citronella nano biopesticides (294.91 g/plant), and higher than 1.5% concentration (272.62 g/plant), but there were no significant differences. However, the percentage of dead plants was found lower at 1% treatment than at 1.5% (Table 3). The application of 1% citronella nano biopesticide on patchouli plants can avoid yield losses of patchouli 22.73-43.27%, harvested at 6 months after planting.

In Pandeglang, the highest dry weight of plant was found at insecticide treatment (49.33 g/plant). The low yield in Pandeglang was due to the high incidence and intensity of mosaic disease, compounded by the infection of budok disease and bacterial wilt. In addition, the planting season in Pandeglang was also started at the beginning of the dry season, due to the prolonged dry season. Patchouli was very sensitive to drought, as indicated by the high percentage of plant deaths in control treatment (86.8%). Nevertheless, the data of plant production were still able to be collected in the first harvest (6 months after planting).
The 1% concentration of citronella nano biopesticide was the most effective concentration to suppress the intensity of mosaic disease attack on patchouli. The result was similar to the green house study on Chenopodium amaranticolor, where 1-1.5% concentration could inhibit the Potyvirus population in the tested plants (Noveriza et al. 2016; 2017). Meneses et al. (2009) stated that the essential oil of Lippia alba, L. origanoides, Oreganum vulgare and Artemisia vulgaris were also effective as antiviral. However, the mechanism was merely different, e.g. to kill the virus directly and inactivate the virus. Furthermore, fraction and subfraction of essential oils from Cymbopogon nardus were reported to be more effective in protecting cells against the entry of virus particles into inoculated cells than other phases in the viral replication (Aini et al. 2006). Therefore, protecting patchouli plants from viral infections is better than controlling infected plants. Thus, the plant protection using nano biopesticide can be done in the nursery.

The rolled leaf shoot caused by aphid attack could be suppressed with the application of citronella nano biopesticide at 2% concentration. Geraniol and citronella caused a reduction in some biochemical parameters, such as protein and sugars (A Guedes et al. 2018). Moreover, citronellal compounds were known to act as an antifeedant and repellent insecticides, as well as terpene compounds, which was suspected to influence the proliferation of insects (Usmiati et al. 2005; Koul et al. 2008; Zaridah et al. 2003; Hierro et al. 2004).

The results of this study indicated that there was no correlation between the high damage patchouli plants by aphids attack with the high intensity of mosaic disease (Figure 2), because the aphids transmitted the virus in a non-persistent manner. Alegbejo and Abo (2002) stated there was a positive correlation between the number of winged aphids captured in the field with the occurrence of Pepper veinal mottle virus (PVMV). However, in this study, no winged aphid was observed, although it was estimated that the aphids population in one rolled shoot leaf was 176.7. The life cycle of Aphis gossypii ranged from 9.5-21.5 days with the number of offspring borned by one imago ranging from 12-46 aphids (Mardiningsih and Soetopo 1999).

The main concern in the spread and transmission of the mosaic virus was plant material that was not virus free and the source of virus inoculums in the field. Thus, the application of
insecticides and the eradication of infected plants were not enough to reduce the virus spread (Fajinmi 2013). Therefore, it is necessary to provide active ingredients which possess antiviral efficacy, such as citronella nano biopesticides.

The incidence of the mosaic disease was higher in Pandeglang (155 m asl) than in Manoko (1200 m asl). Dahal (1992) reported that the epidemic viral infections usually occurred in the lowlands (around 250 m asl) and decreased at the higher altitudes (> 1500 m asl). Fajinmi (2011) also revealed that ecological characteristics, plant vegetation (the number of secondary host plants) and the warm humid climate influenced the incidence and severity of viral infections (Potyvirus).

CONCLUSION

Nano biopesticide formula from citronella oil at 1% concentration which were applied every month, effectively suppressed the development of mosaic disease in patchouli plants. Further, at 2% concentration, the formula found to be effective to control aphids attack. The efficacy index (EI) of the formula was 12.12-48.55% in Pandeglang and 6.38-20.63% in Manoko.

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