Perspektif Vol. 15 No. 2 /Des 2016. Hlm 87 -95
ISSN: 1412-8004
DOI: http://dx.doi.org/10.21082/psp.v15n2.2016.87-95

CURRENT STATUS OF MOSAIC DISEASE ON PATCHOULI AND ITS CONTROL

Status Terkini Penyakit Mosaik pada Tanaman Nilam dan Pengendaliannya

RITA NOVERIZA
Indonesian Spices and Medicinal Crops Research Institute
Jalan Tentara Pelajar No. 3 Bogor 16111, Indonesia
E-mail: rita_noveriza@litbang-pertanian.go.id

ABSTRACT

The patchouli plant (*Pogostemon cablin* Benth*)* has been developed in twenty one Province in Indonesia and now there are 10 provinces that would be the area of development of patchouli with an area of 150 ha and a nursery garden is focused in North Sulawesi with an area of 6 hectares. Mosaic disease on patchouli has been developing very fast, within a period of 3 years have spread to the central cultivation of patchouli in Sumatra, Java and Sulawesi due to the multiplication of patchouli by vegetative cutting. Therefore, the use of virus-free seed patchouli and early detection methods of patchouli seed is a main concern. Furthermore, the handling of patchouli seeds to be free of mosaic disease and its vector is very important. Sanitation and spraying the plants with formulation of clove and citronella oil needs to be done every one or two weeks to protect the seed patchouli in nursery and every four weeks in the field. Currently, being developed technique for controlling this virus in the nursery and field with the strategy of integrated management techniques.

Keywords: *Pogostemon cablin*, Potyvirus, biopesticide, mosaic disease control.

ABSTRAK

Tanaman nilam (*Pogostemon cablin* Benth*)* telah dikembangkan di duapuluh satu Provinsi di Indonesia dan sekarang ada 10 provinsi yang akan menjadi daerah pengembangan nilam dengan luas areal 150 ha dan kebun benih nilam difokuskan di Sulawesi Utara dengan luas 6 hektar. Benih nilam rentan terhadap penyakit mosaik yang disebabkan oleh virus, karena penyakit ini telah berkembang sangat cepat, dalam jangka waktu 3 tahun telah menyebar ke sentra budidaya nilam di Sumatera, Jawa dan Sulawesi disebabkan oleh perbanyakan tanaman nilam dengan cara vegetatif. Oleh karena itu, penggunaan benih nilam bebas virus dan metode deteksi dini benih nilam merupakan perhatian utama. Selain itu, pemeliharaan benih nilam di persemaian untuk bebas dari penyakit mosaik dan vektor yang sangat penting. Sanitasi dan penyemprotan tanaman dengan formulasi cengkeh dan minyak seraiwangi perlu dilakukan setiap satu atau dua minggu untuk melindungi benih nilam di persemaian dan setiap empat minggu di lapangan. Saat ini, sedang dikembangkan teknik pengendalian virus ini di pembibitan dan lapangan dengan strategi teknik manajemen terpadu.

Kata kunci: *Pogostemon cablin*, *Potyvirus*, biopestisida, pengendalian penyakit mosaik.

INTRODUCTION

The patchouli plant has been developed in the province of Aceh, North Sumatera, West Sumatera, Riau, Jambi, South Sumatera, Bengkulu, Lampung, West Java, Central Java, Yogyakarta, East Java, South Kalimantan, East Kalimantan, Central Kalimantan, South Sulawesi, Southeast Sulawesi, Central Sulawesi, Gorontalo, East Nusa Tenggara and Bali. There are 10 provinces that would be the area of development of patchouli with an area of 150 ha and a nursery garden is focused in North Sulawesi with an area of 6 hectares (Noveriza, 2013b). Part of patchouli is the leaves and stems has been used, which will be extracted and retrieved oil. Patchouli oil is widely used as a fixative in perfume, soaps and cosmetics, shampoos and products for non-cosmetics such as detergents (Bhatia *et al.*, 2008).

There are four types of viruses have been reported to infect patchouli, namely *Potyvirus* (Noveriza *et al*., 2012a), *Broad bean wilt virus* 2, *Cymbidium mosaic virus*, *Cucumber mosaic virus* (Miftakhurohmah *et al.*, 2013; Miftakhurohmah *et al*., 2015). All of these viruses show mosaic symptoms on the leaves of patchouli, and cannot distinguish the symptoms of each mosaic virus.

According to Noveriza *et al*. (2012a), the dominant virus infects patchouli is *Potyvirus*, base on molecular characterization has close homology with *Telosma mosaic virus* (TeMV). All of varieties of patchouli provided are susceptible to all these viruses.

Among different strategies adapted for plant virus disease management, detection and diagnosis at primary level is one of the most primitive and essential strategy to control the overspread of viral diseases for future concerned (Biswas *et al*., 2016). In addition, the important thing to do to get a virus controls strategies that are environmentally friendly in the nursery and in the field. According to Tiilikkala *et al*. (2011), in the future the use of pesticides will be tightly regulated because of well-documented environmental risks in the use of synthetic chemicals. Various extraction technologies will be used for production of plant-based liquids. Many kinds of raw material can be used as the source of the bioactive molecules. In practice, the efficacy of botanicals depends very much on the formulation of the product and on the application technology. So that appropriate biopesticide formulation technology and application techniques need to be developed. A selection of plant material and virus-free is also important to prevent transmission of the virus to another area, especially the new development areas. Now, the development of this disease is very rapid and widespread in Indonesia.

DEVELOPMENT OF MOSAIC DISEASE

Mosaic disease incidence on patchouli in Indonesia has been known since 1991. According Soemardiyono *et al*. (1995), mosaic disease was found in young plant or on crops that have repeatedly harvested with the intensity of the disease between 54-73%. A symptom of viral infection depends on the vulnerability of their appearance for the host, the virulence of the virus strain, and the circumstances of the plants grow. In 2007 was reported that patchouli in Bogor and Cianjur were infected by *Cucumber mosaic virus* (CMV) (Sukamto *et al*., 2007).

Based on observation and survey results of patchouli in Bogor-West Java in October 2008, Garut and Ciamis-West Java in April 2009, shows that the patchouli was infected by mosaic disease caused by *Potyvirus*. In the same year, patchouli in Pasaman-West Sumatera also infected by *Potyvirus*, while in Lampung not find any infection of virus. Then in May-July 2010, the survey results in patchouli cultivation in Brebes-Central Java was found *Fabavirus* infection with similar symptoms (mosaic), whereas in Pakpak Bharat-North Sumatera and Cicurug-West Java not found any infection virus on patchouli

Figure 1. Spreading of mosaic virus on patchouli plant in Indonesia.

(Noveriza, 2013a; Wahyuno *et al*., 2011). In 2013, reported the existence of a viral infection of patchouli plantation in West Java Cicurug caused by *Potyvirus*, *Potexvirus*, CMV and *Fabavirus*. So also in another location found the area of Manoko and Cijeruk-West Java (Miftakhurohmah *et al*., 2013). The development of mosaic disease on patchouli very fast, Cicurug area reported no mosaic disease but after the next 2 years already infected by four types of virus (see above). In addition, the mosaic disease has been found also in patchouli cultivation in Southeast Sulawesi (Taufik *et al*., 2012; Taufik *et al*., 2014).

So all viruses that infect patchouli showing mosaic symptoms and can not distinguish between types of the virus based on symptoms. To distinguish them, can be done by serology or molecular detection.

MECHANISM TRANSMITTED OF MOSAIC DISEASE ON PATCHOULI SEED

Patchouli seeds derived from shoot cuttings or stem cuttings of patchouli. Patchouli infected by *Potyvirus* will be a source of patchouli seeds that carry the virus (Noveriza *et al*., 2012a). This method will rapidly spread the virus from the seed to planting area to another or from one region to another central patchouli, so conserved virus from season to season. It is very worrying in getting virus-free seed patchouli. In 2014 and 2015 no longer exist plants are healthy and free of the virus in Bogor (Mariana *et al*., 2015; Noveriza *et al*., 2015).

The results detection of virus mosaic on the seed patchouli plant tissue culture (developed by Seed Resources Management Unit Balittro, Bogor) using ELISA method (Table 1), in 100 samples of seeds patchouli Patchoulina 1 varieties (100% positive *Potyvirus*, 60% positive BBWV2 and negative CymMV), in 100 samples of patchouli seeds of Patchoulina 2 varieties (60% positive *Potyvirus*, 20% positive and negative BBWV2 CymMV), and in 100 samples of patchouli seeds of Sidikalang varieties (30% positive *Potyvirus*, but negative BBWV2 and CymMV). *Potyvirus* is the dominant virus infects the three varieties namely Pachoulina 1, Pachoulina 2 and Sidikalang (Mariana *et al*., 2015).

Based on a survey conducted on planting patchouli in Banten in early July 2015, then the patchouli plant samples taken from two places visited and performed virus detection by ELISA. Pulosari patchouli cultivation in the village were found infected by *Potyvirus* and BBWV2, while from the village of Pandeglang negative against both types of the virus (Table 2). Patchouli farmer from the village of Pandeglang said that the seeds of patchouli obtained from the Aceh Province.

Furthermore, the results of the sample detection patchouli from patchouli seed multiplication farm in the village Ciburayut, Cigombong District of Bogor in mid-November 2015 that 100 percent from 45 samples were observed are infected with *Potyvirus* and 60 percent infected with CymMV. Patchouli varieties are propagated in the nursery garden are Sidikalang, Patchoulina 1 and 2, because a lot of demand from some areas. Patchouli cultivation of all varieties are used as a source of nursery, and found their symptoms mosaic virus infection (Figure 2). Detection results are not shown here.

Patchouli seed from a nursery will be sent to the area cultivation of patchouli in Java and Sumatra. This is very alarming because mosaic virus would spread quickly. If the seeds are seen in morphology, mosaic virus symptoms are not visible. But the seed had been infected with the virus. So it is necessary to develop early detection techniques of patchouli seed in the nursery and the field, because spreading that disease so fast (see Figure 1). According Noveriza and Mariana (2017), has been developed detection techniques on patchouli seeds in the nursery and in the field with direct methods of **tissue blot immuno assay** (DTBIA). Data shown that DTBIA more effective compare with ELISA method, especially for *Broad bean wilt virus* 2 (BBWV2-*Fabavirus*) and all of virus that are infected on patchouli can detect by DTBIA technique (Figure 3).

Patchouli that will serve as seed sources should be tested first by using the above techniques. This technique is very cheap and easy to do, in a short time has been known to result if the seed is infected or not. If patchouli not infected by the virus, it could be cutting with

Table 1. Detection of mosaic virus (*Potyvirus, BBWV2* and *CymMV*) on patchouli leaf samples varieties of Patchoulina 1, Patchoulina 2 and Sidikalang from plant culture using ELISA method (Mariana *et al*., 2015).

No	Samples	Absorbance value			ELISA results		
		Potyvirus	BBWV2	CymMV	Potyvirus	BBWV2	CymMV
1	Buffer	0.299	0.187	0.322	Negative	Negative	Negative
2	Negative control	0.240	0.233	0.345	Negative	Negative	Negative
3	Positive control	0.408	1.274	3.156	Positive	Positive	Positive
4	Patchoulina 1.1	0.451	0.382	0.325	Positive	Positive	Negative
5	Patchoulina 1.2	0.663	0.312	0.351	Positive	Negative	Negative
6	Patchoulina 1.3	0.382	0.354	0.299	Positive	Positive	Negative
7	Patchoulina 1.4	0.542	0.475	0.354	Positive	Positive	Negative
8	Patchoulina 1.5	0.532	0.317	0.376	Positive	Negative	Negative
9	Patchoulina 1.6	0.640	0.376	0.297	Positive	Positive	Negative
10	Patchoulina 1.7	0.458	0.411	0.305	Positive	Positive	Negative
11	Patchoulina 1.8	0.701	0.412	0.347	Positive	Positive	Negative
12	Patchoulina 1.9	0.575	0.296	0.367	Positive	Negative	Negative
13	Patchoulina 1.10	0.540	0.250	0.325	Positive	Negative	Negative
14	Patchoulina 2.1	0.306	0.269	0.367	Negative	Negative	Negative
15	Patchoulina 2.2	0.394	0.247	0.308	Positive	Negative	Negative
16	Patchoulina 2.3	0.299	0.284	0.351	Negative	Negative	Negative
17	Patchoulina 2.4	0.377	0.365	0.284	Positive	Positive	Negative
18	Patchoulina 2.5	0.420	0.291	0.298	Positive	Negative	Negative
19	Patchoulina 2.6	0.522	0.265	0.357	Positive	Negative	Negative
20	Patchoulina 2.7	0.321	0.293	0.333	Negative	Negative	Negative
21	Patchoulina 2.8	0.369	0.342	0.319	Positive	Positive	Negative
22	Patchoulina 2.9	0.349	0.265	0.324	Negative	Negative	Negative
23	Patchoulina 2.10	0.483	0.242	0.325	Positive	Negative	Negative
24	Sidikalang CPR 1	0.291	0.220	0.323	Negative	Negative	Negative
25	Sidikalang CPR 2	0.299	0.301	0.338	Negative	Negative	Negative
26	Sidikalang CPR 3	0.426	0.247	0.287	Positive	Negative	Negative
27	Sidikalang CPR 4	0.331	0.230	0.327	Negative	Negative	Negative
28	Sidikalang CPR 5	0.465	0.207	0.358	Positive	Negative	Negative
29	Sidikalang CPR 6	0.317	0.270	0.365	Negative	Negative	Negative
30	Sidikalang CPR 7	0.606	0.243	0.330	Positive	Negative	Negative
31	Sidikalang CPR 8	0.309	0.237	0.456	Negative	Negative	Negative
32	Sidikalang CPR 9	0.344	0.248	0.344	Negative	Negative	Negative
33	Sidikalang CPR 10	0.347	0.297	0.323	Negative	Negative	Negative

Note: Positive are detected, negative are not detected, BBWV2 = *Broad bean wilt virus* 2, CymMV = *Cymbidium mosaic virus* and CPR = culture plant result.

Table 2. Detection of mosaic virus (*Potyvirus*, BBWV2) on patchouli leaf samples from Banten Province (Mariana and Noveriza, 2015).

No	Samples	Absorbance value		ELISA results	
		Potyvirus	BBWV2	*Potyvirus*	BBWV2
1	Buffer	0.104	0.090	Negative	Negative
2	Negative control	0.105	0.089	Negative	Negative
3	Positive control	1.839	0.332	Positive	Positive
4	From Pandeglang 1	0.098	0.104	Negative	Negative
5	From Pandeglang 2	0.094	0.104	Negative	Negative
6	From Pulosari 1	0.203	0.142	Positive	Positive
7	From Pulosari 2	0.197	0.096	Positive	Negative
8	From Pulosari 3	0.097	0.097	Negative	Negative

Note: Positive are detected, negative are not detected, BBWV2 = *Broad bean wilt virus* 2.

scissors virus-free as a seed or seedling. During patchouli seeds in the nursery, need care and protection from virus and vector virus.

MANAGEMENT AND DISEASE CONTROL TECHNIQUE

Biological control comprises various technologies of which one option is the use of botanical products. Many kinds of plant species and technologies have been used in the production of botanical pesticides (Tiilikkala *et al*., 2011) for plant protection.

Figure 2. Mosaic virus symptoms on varieties Sidikalang (left) and Patchoulina 1 (right)

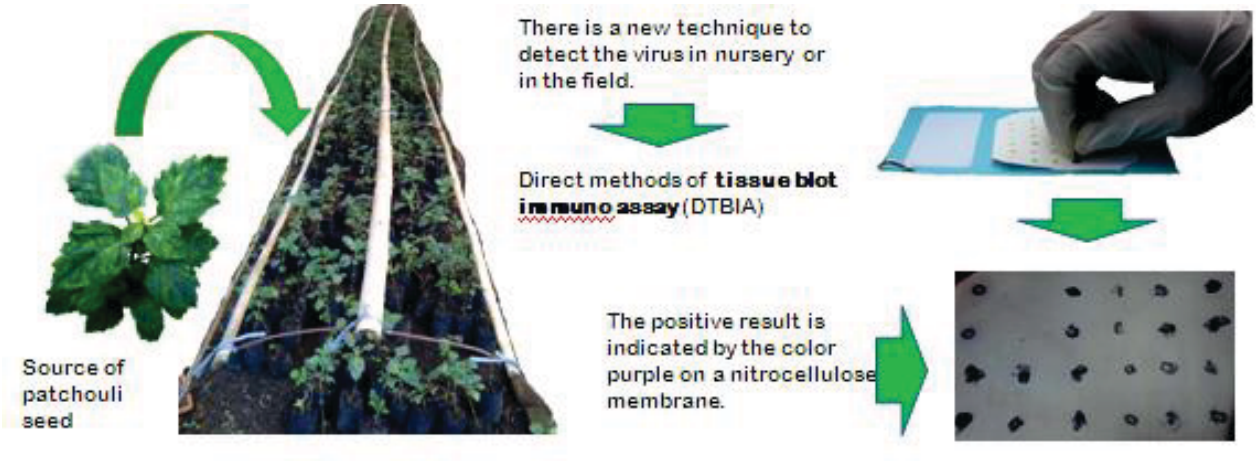

Figure 3. Technique detection mosaic virus of patchouli seed in the field (Noveriza and Mariana, 2017).

Avoid sources of infection is one way to reduce the occurrence of epidemic diseases. Some actions that can be taken to avoid sources of infection are as follows: a) crop rotation, in a manner similar plants do not grow continuously; it aims to break the life cycle to prevent disease epidemics, b) planted in an isolated area, it is more geared to produce seeds or seedlings are free of viruses, c) ensure that the plant remains healthy (hygienic) to deal with virus attacks mainly stable disease, d) the use of seeds and seedlings free of viruses which negates the source of infection so that it can delay the occurrence of epidemics of viral diseases in the field, e) to avoid vectors, specifically for the nursery, selecting a location free of viral vectors is an effective way to get virus-free seed.

In addition, to find a technique to eliminate the virus in infected patchouli, in order to obtain seed virus-free patchouli and prevent the virus from spreading to the entire planting area of patchouli. In addition, patchouli seedlings infected with the virus will affect plant vigor and decrease biomass and oil content (Noveriza *et al*, 2012b). According Pearson and Cole (1991), the plants are propagated vegetative if infected with the virus will cause the seed be no vigor.

According Noveriza *et al*. (2012c), has successfully carried out the elimination *Potyvirus* on three varieties of patchouli (Sidikalang, Lhokseumawe, Tapak Tuan) using apical meristem culture techniques. In the varieties of Lhokseumawe, successfully obtained virus-free plants reached 90.9% with a size of 0.5-1.0 mm

apical meristem, and then followed by Sidikalang varieties and Tapak Tuan successive amounted to 66.7% and 33.3%. Virus-free plants produced in plants varieties Tapak Tuan patchouli not many (33.3%), this might be due to the size of the meristem that is used is still too big. So they need to reduce the size of the apical meristem were used and it is not easy to do. According to Brown *et al*. (1988), meristem isolation techniques are extremely sensitive area and the very small size also reported relatively difficult in some types of plants.

According to Amal *et al*. (2012) that control of the disease may be achieved by introducing a plant free period to break the cycle of virus transmission between successive crops. The development of new strategies of integrated pest management to improve processing crop yield and sustainable quality, are requested by the market (Pane *et al*., 2013).

Many plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities (Koul *et al*., 2008), antivirus (Meneses *et al*., 2009). Most essential oil chemical are relatively non-toxic to mammals and fish in toxicological test, and meet the criteria for "reduced risk" pesticides (Koul *et al*., 2008).

Volatile oils and plant extracts contain active substances that can inhibit viral infection. Essential oil of *Melaleuca alternifolia* at concentrations of 100, 250 and 500 ppm reported to be effective to reduce the number of local lesions of TMV in *Nicotiana glutinosa* (Bishop, 1995). Likewise, the oil of *Ageratum conyzoides*, *Callistemon lanceolatus*, *Carum copticum*, *Ocimum sanctum* and *Peperomia pellucid* effectively inhibit the activity of *Cowpea mosaic virus* (CPMV), *Mungbean mosaic virus* (MBMV), *Bean common mosaic virus* (BCMV) and *Southern bean mosaic virus* (SBMV). *Ocimum sanctum* at 3000 ppm gave the highest inhibition against CMV, MBMV, BCMV, and SBMV respectively, are 89.6, 90, 92.7, 88.2%. (Rao *et al*., 1986) and the *Tobacco mosaic virus* (TMV) reached 62%. Other ways that is easier to do in order to control the mosaic disease and its vector is to protect plants by spraying while in nursery with mixed of citronella and clove oil at a concentration of 1%. According to Noveriza *et al.* (2015), clove oil and mixture of clove oil and citronella oil at a concentration of 1 % can suppress the growth of viruses respectively 45% and 32%.

Another thing to be considered during the patchouli in the nursery is an attack by aphids *Aphis gossypii*, because aphids are vector *Potyvirus* and BBWV2. According Mardiningsih *et al.* (2014), a mixture of neem oil, clove and citronella reduce the population of *A. gossypii*. Neem has a systemic nature, can be translocated into the plant tissue slowly. Therefore, it is effective to control various insect pests (Kardinan and Atmadja, 2004). We should take measures to prevent aphids in the early stage of their occurrence in the filed to prevent virus outbreak (Shi *et al*., 2016).

Citronella (*Cymbopogon nardus*) essential oil has been used for over fifty years both as insect repellent and an animal repellent (Zaridah *et al*., 2003). The action of different monoterpenic compounds against *Anisakis simplex* larvae and found that geraniol, citronellol, citral, carvacrol and cuminaldehyde were active at 12.5 µg/ml concentration (Hierro *et al*., 2004).

Several studies have been done to control the Tomato spotted wilt virus (TSWV) and trips (vector of TSWV) in the field using essential oils such as geraniol, citronella (*Cymbopogon flexuosus*), and tea tree (*Melaleuca alternifolii*) combined with kaolin; can reduce the incidence of disease by 32 to 51% on tomatoes. The results are roughly the same as using a standard insecticide (Reitz *et al*., 2008). A biopesticide like neem oil and a mixture of neem with clove oil, and citronella oil itself was able to inhibit the development of *Potyvirus* that are transmitted by *Aphis gossypii* (Noveriza *et al*., 2015).

According to Sohail *et al.* (2012), extracts of tobacco (2%) showed its superiority in terms of efficacy against aphid on tea and resulted in the death of insects highest 89%. Then proceed with neem extract (2%) with a mortality rate reached 61%. And the garlic extract (2%) with the lowest mortality rate of 52%. The results showed that all botanical pesticides lose their efficacy after two weeks of spray and aphids population began to

evolve after 72 hours, one week and two weeks of treatment. According to Oparaeke (2007), increasing the concentration of black pepper extract to 20% w/v with four weekly applications is effective, safe and cheap for the control of post flowering insect pests of cowpea without degrading the environment.

CONCLUSION

At present, the technique to get healthy and free patchouli seed virus to be very important as well as techniques for early detection of mosaic virus in nursery and in the field to prevent the spread of the virus mosaic at patchouli to the new development areas. Besides that, the technique in the prevention and control of viral or mosaic disease in patchouli during the growth process in the nursery becomes very important, because the seed becomes the main base in the spread of mosaic disease on patchouli. In addition, integrated control techniques have been developed to mosaic disease using formulations of nano biopesticides and biofertilizer.

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