Study on *Ferrisia virgata* and *Planococcus minor* as vectors of mottle disease in black pepper

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**Abstract.** Two species of mealybug, *Ferrisia virgata* and *Planococcus minor* were previously reported as vectors of *Piper yellow mottle virus* (PYMoV), the causal agent of mottle disease in black pepper (*Piper nigrum*). Several activities were performed to study the PYMoV transmission through *F. virgata* and *Pl. minor*, involving transmission experiment in ISMCRI greenhouse (Bogor, West Java), field observation of mealybugs, and mottle disease incidence at ISMCRI field station (Sukabumi, West Java). The result showed that the transmission rate of PYMoV through *F. virgata* and *Pl. minor* in black pepper reached 40.00 and 46.67%, respectively. Field temperature >33 °C, light intensity >700.00 lux, wind speed <1.10 m/s, and rainfall <80 mm/month were recorded during the occurrence of a peak population of mealybugs. Mealybugs population in the field was affected by insecticide application. Its population was lower in a block with insecticide spraying than in a block without insecticide spraying. Furthermore, mottle disease incidence was correlated with the population level of mealybugs. Based on PCR detection, the frequency of viruliferous mealybugs did not differ in a block with or without insecticide spraying, i.e., 6/39 and 6/37, respectively. This indicated that other factors than insect vectors might play an important role in disease spread.

**Keywords:** disease incidence, insecticide application, mealybug, *Piper nigrum*, virus transmission

1. Introduction

Black pepper (*Piper nigrum* L.) is one of the most important export commodities of plantation crops for Indonesia. The average black pepper production in Indonesia is 85.92 tons per year; it contributes 31.84% to the total black pepper production of ASEAN in 2009 to 2013 [1]. In the world trade, the famous Indonesian black pepper, i.e., “Lampung black pepper”, comes from Lampung, whereas Indonesian white pepper, called is “Muntok white pepper”, comes from Bangka Belitung. There is another white pepper from East Kalimantan, known as “Samarinda white pepper” [2]. Green pepper occupies a smaller portion of Indonesia’s total export than black pepper and white pepper [3].

Mottle disease is caused by *Piper yellow mottle virus* (PYMoV) and *Cucumber mosaic virus* (CMV), is one of the main diseases in black pepper plants. The disease was first reported in Vietnam (1952), then it spreads to Malaysia (1959), Brazil (1970), Sri Lanka (1979), Thailand, and India (1990).
Recently, this disease has also been reported in China [4]. In Indonesia, this disease has been observed in black pepper plants since 1976. A more recent report indicated that PYMoV is the main pathogen causing mottle disease in black pepper plantations in Sukabumi District of West Java, Indonesia [5] and Hainan, China [4].

Several insect species have been reported as the vector of PYMoV, i.e., *Planococcus minor*, *Pl. citri*, and *Ferrisia virgata* (Pseudococcidae: Hemiptera), and *Diconocoris distanti* (Tingidae: Hemiptera) [6, 7, 8, 9]. Two species of mealybugs, i.e. *Pl. minor* and *F. virgata* was previously found in black pepper plants in Sukabumi, Bangka and Lampung [10]. It is becoming interesting in confirm the role of these two species of mealybugs in the spread of mottle disease in black pepper plants. Many factors, among others are weather conditions and pesticide application, influence the potential of insect vectors as an important component to disease development. Therefore, research on insect population dynamics in the plantation is needed to study the effectiveness of insecticides to control the insect population and to determine how weather conditions such as temperature and rainfall will influence insect population [11, 12, 13]. Furthermore, the correlation between insect population with disease incidence can be studied by monitoring viruliferous insects in the field [14].

This study was conducted to obtain information on the transmission rate of PYMoV through *Pl. minor* and *F. virgata* in the greenhouse. The effect of the occurrence of these insect species in the field on the incidence of mottle disease also confirmed in this study.

2. Materials and methods

2.1. Transmission study of PYMoV using *Pl. minor* and *F. virgata* in greenhouse

The experiment was conducted in the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) greenhouse in Bogor (West Java). Black pepper plants were used as test plants by first sowing the seeds on cocopeat media. Seedlings having a pair of leaves above the cotyledons on cocopeat media were transferred to growing media composed of soil and fertilizer (2:1; v/v). Ten months-old plants are ready for transmission study. Polymerase chain reaction (PCR)-based detection method was carried out to confirm all test plants were virus-free [5].

Mealybugs were collected from the field and reared in the laboratory to obtain nonviruliferous insects. Potato tubers and pumpkin fruit was used as feeding medium for rearing *Pl. minor* [8] and *F. virgata*, respectively. This study successfully reared *F. virgata* in pumpkin fruit, updating the previous rearing technique on black pepper plants [8]. After three generations on feeding medium, mealybugs can be used for transmission experiments.

Black pepper plants infected by PYMoV were used as a source of virus inoculum, and maintained in the experimental station of ISMCRI in Bogor. The diseased plants showed mottle and stunted symptom, and, has been detected by RT-PCR to confirm the infection of PYMoV [15]. Transmission study was carried out by the following procedure [8]: 10 mealybug nymphs or adults were given acquisition feeding period for 24 hours in an infected plant, then the mealybugs were transferred to healthy plant for 48 hours inoculation feeding period, and finally, the plants were sprayed with insecticides (Deltamethrin) to kill mealybugs. Non-inoculated healthy plants were used as check controls. The transmission experiment was replicated three times; each, consisted of 5 black pepper seedlings. Observation on test plants was carried out every day, for about three weeks, involving symptom development, disease incubation period, and number of symptomatic plants (disease incidence). In the previous study, disease symptom was first observed at three weeks after transmission [8].

The presence of PYMoV on test plants were confirmed by PCR. Optimization of primers for amplification of PYMoV was carried out using three pairs of primers, i.e., AIB 104/105, AIB 225/226, and AIB 35/36, following previous protocols [5, 16, 17]. Primers of AIB 35/36 were selected for detection of PYMoV from leaves of test plants due to its best amplification results (data not shown). For negative control of PCR, nuclease-free water was used as a template.
2.2. Field survey and PYMoV detection on mealybugs and black pepper plants
The population of *Pl. minor* and *F. virgata* were observed once every two months, from June 2019 to October 2020 at ISMCR1 field station in Sukabumi (West Java). Weather parameters including rainfall, temperature, light intensity and wind speed were taken from weather data sources in this location, and used for the analysis of its effect on the population of *Pl. minor* and *F. virgata*.

The observation was conducted in two blocks: block I, Natar-1, and Petaling-1 varieties, without pesticide application; block II, Natar-1, Petaling-1, and Ciinten varieties, with application of contact insecticide (Deltamethrin) once a month. Thirty plants at each block were randomly selected as sample plants. All plants were in reproductive stages about 3 to 4 m high. Mealybug is an insect with low mobility, infesting plant parts permanently. Therefore population of *Pl. minor* and *F. virgata* were counted directly from the stem base to a plant height of about 150 cm [12,18].

A total of 20 plants from each block were randomly selected for leaf sampling for further identification of PYMoV. Samples were stored in banana stem sheets, kept in plastic bags, and transferred to the laboratory using an icebox. PYMoV presence in leaf samples was detected by PCR method using AIB 104/105 primers [5].

The frequency of viruliferous mealybugs in the field was determined by collecting the insects, both nymphs and adults, at the time of a peak insect population (in April - August) [8]. Insects were collected directly using a wet brush, stored in an appendorf tube containing absolute alcohol [19], then brought to the laboratory. Specific fragment of PYMoV was amplified from single insect following total DNA isolation of nymph or imago using CTAB method [20]. Amplification was carried out using AIB 225/226 primers according to the PCR program in the previous protocol [16], with a modification of the annealing temperature to 57 °C.

3. Result and discussion
3.1. Transmission rate of PYMoV through *Pl. minor* and *F. virgata*
Disease incubation period is one week, indicated by first chlorotic symptoms appeared after transmission. In this study, disease incubation period was two weeks faster than the previous transmission test [8]. Two weeks later, some of chlorotic symptoms developed into severe mottling. Other symptoms observed were weak mottle and vein banding. Similar symptoms have been reported in black pepper plants in the field [5]. PYMoV amplification using AIB 35/36 primers succeeded in obtaining the target DNA measuring 450 bp (Figure1). Further sequence analysis of the DNA fragment confirmed the presence of PYMoV [17].

![Figure 1](image.png)

*Figure 1.* Visualization of PYMoV specific DNA fragments amplified by PCR using AIB 35/36 primers. M. DNA marker 100 pb; 1. negative control of PCR; 2. positive control of PCR; 3 and 4. control plants; 5. non inoculated plant; 6 - 9. PYMoV-inoculated plants.

The number of plants showing symptoms after transmission using *Pl. minor* dan *F. virgata* were 73.33 and 60.00%, respectively; but confirmation by PCR revealed that infection of PYMoV were only 40.00 and 46.67%, respectively (Table 1). This fact suggests that symptoms may be caused by factors other than infection of PYMoV. A previous study reported that the transmission efficiency of
PYMoV through *Pl. minor* dan *F. virgata* were 100% based on disease incidence and PYMoV detection by ELISA using BSV antiserum [8]. Detection by ELISA is suspected not only detects PYMoV in black pepper samples, but also other *Badnavirus* that are serologically related to BSV.

| Treatments              | No. plants showing symptom (%) | No. plants infected (%)** |
|-------------------------|--------------------------------|---------------------------|
|                         | 1          | 2          | 3    | average |                        |
| *Pl. minor*             | 60.00      | 80.00      | 80.00| 73.33    | 40.00                    |
| Control of *Pl. minor*  | 0.00       | x          | x    | 0.00     | 0.00                      |
| *F. virgata*            | 80.00      | 40.00      | 60.00| 60.00    | 46.67                    |
| Control of *F. virgata* | 0.00       | x          | x    | 0.00     | 0.00                      |

* x: not tested, control was not repeated  
** confirmed by PCR test

3.2. Effect of weather and black pepper varieties on population of mealybugs in the field

The population of *Pl. minor* and *F. virgata*, was found infesting axils, leaf sheaths, the base of stem segments, and spike of black pepper plants. In the dry season, both insects were observed inside the leaf edge roll, previously infested by thrips (Figure 2). It has been reported that *Pl. minor* colonizes flowers, spike, leaves, and stem segments of black pepper plants, while *F. virgata* presents on leaves and stem internodes [21, 22].

![Figure 2. Mealybugs infestation on black pepper plants. a and b. *Pl. minor* at the base of stem and leaf sheaths; c and d. *F. virgata* on spike and leaf surfaces; e and f. *Pl. minor* dan *F. virgata* inside the leaf edge roll.](image-url)
The population of *Pl. minor* was higher than *F. virgata* during 1.5 years of observation at the block I. The high population of *Pl. minor* was observed in June - November 2019, and June - October 2020 with the peak reached in August 2019 and 2020, then the population declining in December 2019 to Mei 2020. Meanwhile, *F. virgata* was only observed in October 2019 (Figure 3a). A different situation was noticed at block II in which the population of *F. virgata* was higher than *Pl. minor* within one year of observation, with the peak reached in August 2020 (Figure 3b).

![Graph of population dynamics](image)

**Figure 3.** Population dynamics of *Pl minor* and *F. virgata* on black pepper plantations at the block I (a) and block II (b) from June 2019 to early November 2020.

The weather parameters including rainfall, temperature, light intensity, and wind speed were obtained from weather stations at ISMCRI in Sukabumi. These data were used to determine weather factors that affect the dynamics of the mealybug population in the field. The average minimum and
maximum temperature ranged from 18.74 to 21.40 °C and 31.38 to 38.33 °C, respectively. The minimum and maximum light intensity ranged from 95.37 to 181.94 lux and 550.65 to 789.77 lux, respectively. The minimum and maximum wind speeds ranged from 0.10 to 0.47 m/s and 1.04 to 1.84 m/s, respectively. The monthly rainfall ranged from 6 to 752 mm/month, but during the long dry season of 2019, from June to August, there were no rainy days.

The mealybug population usually increases when maximum temperature, light intensity, wind speed reached >33.00 °C, >700.00 lux, and <1.10 m/s, respectively, with low rainfall. However, the population of mealybug was quite high in October 2019 and 2020 with moderate rainfall (100 and 272 mm/month) and temperature (<33.00 °C), high light intensity (>700.00 lux), and low wind speed (<1.10 m/s). On the other hand, the population of mealybug was low when maximum temperature, light intensity, wind speed reached <33.00 °C, <700.00 lux, and >1.14 m/s, respectively with rainfall >250 mm/month. The population of mealybug was not found in June 2020 when the temperature was <33.00 °C and maximum light intensity <700.00 lux, although the rainfall and maximum wind speed were low (81 mm/month and 0.96 m/s) (Figure 3). Population dynamics of Pl. minor and F. virgata was influenced by temperature, light intensity, wind speed and rainfall. Wind speed and rainfall tend to be negatively correlated with mealybug populations, while temperature and light intensity tend to be positively correlated. It was reported previously that population of the mealybugs Phenacoccus solenopsis was positively correlated with temperature and negatively correlated with rainfall [13]. Our study suggests that the population of Pl. minor and F. virgata is high in the dry season (May-October), and low in the rainy season (November - April). This result is following a previous report that mealybugs are common on black pepper plants after the rainy season [21].

As of the effect of the varieties, there was no preference of mealybugs for black pepper varieties. In August 2020, when the overall population reached its peak, F. virgata at block I was not found, while population of Pl. minor was high, both in Natar-1 and Petaling-1 varieties. In contrast, population of F. virgata at block II was higher than Pl. minor in all observed varieties (Figure 4). No host preference behavior of F. virgata was also reported previously when tested on five cotton cultivars [23].

![Figure 4](image.png)

**Figure 4.** The population of Pl. minor dan F. virgata in 3 varieties of black pepper at the block I and II, in August 2020. The data was the average of insect numbers on 8-10 plants.
3.3. Incidence of PYMoV and association of PYMoV with mealybugs in the fields

The abundance of mealybugs and incidence of PYMoV in the block I was >50% higher than those in block II (Table 2). Insecticide application seems to reduce the population of Pl. minor and F. virgata, which in turn lower disease incidence and frequency of PYMoV. Similar results were reported in a previous study, in which the application of insecticide on cucumber plants succeeded in reducing aphid population and incidence or severity of disease due to CMV infection [24]. The high population of insect vectors can be used as an indicator to predict the high incidence of PYMoV. This phenomenon was found in vineyards in which the spread of Grapevine leafroll-associated virus 3 (GLRaV-3) between plantation blocks occurred when more than five mealybugs per leaf were found [25]. Although the abundance of mealybugs and incidence of PYMoV in block I were higher than those in block II, the frequency of viruliferous mealybugs collected from blocks I and II did not differ, and were relatively low (Table 2). In this study, the spread of PYMoV through insect vectors is predicted to still occur, but there are other factors plays a more important role in the spread of mottle disease in the field.

| Observation sites | Incidence of PYMoV (%) | Number of mealybugs* | Frequency of viruliferous mealybug |
|-------------------|------------------------|-----------------------|-----------------------------------|
| Block I           | 90                     | 480                   | 6/39                              |
| Block II          | 35                     | 108                   | 6/37                              |

* Data was collected in August 2020

Mealybugs are generally hidden in plant parts which make them difficult to be controlled. Systemic insecticide application is recommended to control mealybugs [21]. The results of previous studies also showed that systemic insecticide was more effective than the contact one for controlling mealybugs in a vineyard in Tunisia [26]. In this study, monthly spraying of contact insecticide was successfully reduced the mealybugs population and frequency of PYMoV in black pepper plants. However, a more suitable control measure is needed in the future in order to minimize insecticide negative effects. Further research is required to evaluate the application of systematic pesticides in decreasing the mealybugs population. Data on the population dynamics of mealybugs is important to determine the application time for pesticides spraying [11]. The effect of the insect vector population and of disease severity on black pepper production should be further studied to determine its role in disease development.

4. Conclusions

Mealybug species, Pl. minor and F. virgata are confirmed as the vector of PYMoV, with transmission rate of 40.00 and 46.67%, respectively. The occurrence of these two mealybug species in the field contributes to the spread of mottle disease. Application of insecticide to control the mealybugs population might be recommended as part of the black pepper mottle disease control strategy.

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