Recent condition of clove death syndrome disease in Indonesia: epidemiology and causal agents

Widodo1*, Supriyanto1, G D Prasetyo1, S Wiyono1 and H Triwidodo1

1 Department of Plant Protection, Faculty of Agriculture, IPB University, Jl. Kamper, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia.

*Email: widodo@apps.ipb.ac.id

Abstract. The aim of this study was to determine symptoms and causal agents associated with clove death syndrome, and factors related to disease severity. This research was carried out through field observations on the severity of the disease, associated symptoms, and interviews with farmers regarding the cultivation techniques carried out. The results of the study were presented quantitatively with cross tabulations for the three research location districts, and one location, including Trenggalek District, complemented with correspondence analysis using the Chi square test. From surveys in three districts, Trenggalek, Semarang and Tegal, the highest probability of symptoms associated with clove death syndrome was stem borer + canker, stem canker, and stem borer + canker, respectively. Based on the results of the correspondence analysis in Trenggalek District using the chi square test ($\chi^2$: $p <0.1$), some factors, i.e. plant age, cropping patterns, doses of animal manure, Phosphate (P) fertilizer dosage, weed conditions in the field, and frequency of weed control showed an association with the severity of the clove death syndrome. Further experimental research is needed to ensure that these factors are related to the severity of the disease syndrome.

Keywords: cultural techniques, epidemic, stem canker, stem borer

1. Introduction

Clove (Syzygium aromaticum L. Merr. Perry) is one of among spice plants originating from Indonesia, namely the Maluku Islands. With these tropical climatic conditions, Indonesia has great potential to cultivate clove plants as evidenced by the existence of the small scale plantation in all areas. This commodity has also been an important part of the spice business since the 1600s during the Dutch colonization. Plant disease has been a major factor to suffice for the national manufacture of kretek cigarettes. Bennett et al. [1] reported the clove death in Sumatra, the most important area of national production, called as Sumatra disease associated with xylem-limited bacteria. The causal agent, then, was identified as Pseudomonas syzygii by Lomer et al. [2], and again updated as Ralstonia syzygii subsp.indonesiensis [3].

Based on our field observation in some areas in Java, many individuals dead clove trees were also found other symptom, e.g. stem borer and canker on stem, branch, or twig. Based on the theory of plant disease triangle, the occurrence of disease epidemics is determined by three main factors, including plants, pathogens, and planting environment conditions, both macro and micro environments. These three components in the field are also influenced by humans as the fourth factor, mainly related to the cultural techniques applied by farmers [4]. A good understanding of these factors that cause clove death...
syndrome is needed to devise more strategic management to this problem [5]. This study was performed to determine some symptoms associated with clove death syndrome and factors triggering the disease development.

2. Methodology

2.1. Survey sites
Survey in this study was conducted in 3 districts, namely District of Semarang and Tegal (Province of Central Java), and District of Trenggalek (Province of East Java). Then in each of the district, some subdistricts and villages which are known to have a large number of clove trees were selected as survey sites (Table 1).

| District | Sub District | Village                      |
|----------|--------------|------------------------------|
| Semarang | Jambu        | Bedono, Kelurahan, Gertas   |
|          | Banyubiru    | Wirogomo, Kemambang, Banyubiru |
| Tegal    | Bojong       | Lengkong, Danasari, Sangkan Ayu |
|          | Bumijawa     | Begawat, Muncang Larang, Cintamanik |
| Trenggalek | Trenggalek | Dawuhan                      |
|          | Bendungan    | Depok, Boto Putih, Dompyong |
|          | Panggul      | Gayam, Ngrencak, Depok, Besuki |

2.2. Number of plants observed and farmers interviewed
The number of plants displayed in this paper is the total of each district to determine the probability of the symptom type occurred in each individual plant. In the District of Semarang and Tegal, interview to farmers observed their plantation did not conduct, while in the District of Trenggalek interview to all sampling farmers was performed (table 2).

| District | Total plant sample | Plantations/plots | Interviewed farmers |
|----------|--------------------|-------------------|--------------------|
| Semarang | 480 (24)           | 24                | 0                  |
| Tegal    | 480 (24)           | 24                | 0                  |
| Trenggalek | 1200 (60) | 60               | 60                 |

2.3. Death syndrome disease incidence and severity
The observation of disease incidence and disease severity was carried out on total plant sample (Table 2) and half of the total plant sample, respectively. Sample plants were determined using a systematic sampling technique following a diagonal or "Z" pattern depending on the land topography and plant distribution in the individual plot. Plant samples selected for observation of disease incidence and severity were determined with an interval of every 3 and 5 plants, respectively.

Disease incidence and severity were calculated using the formula as follows:

\[ DI = \frac{n}{N} \times 100\% \]  

(1)
DI: disease incidence, n: individual plant sample showed symptom, N: total number of plant sample observed.

\[
DS = \frac{\sum n_i x v_i}{N \times V} \times 100\%
\] (2)

DS: disease severity, \( n_i \): number plant samples with \( i \)\(^{th} \) disease score, \( v_i \): \( i \)\(^{th} \) disease score, \( N \): total of plant sample observed, \( V \): highest score set. Disease score was described in table 3 as follow:

### Table 3. Disease score based on canopy condition

| Score | Percentage | Description |
|-------|------------|-------------|
| 0     | \( x = 0 \) | Healthy, No dieback symptom |
| 1     | \( 0 < x \leq 25 \) | Mild symptom up to 25%, about 25% of branches infected, or < 25% of canopy show leaf falling or yellowing |
| 2     | \( 25 < x \leq 50 \) | Medium symptom, between 25 to 50% of branches infected, or \( \geq 25\% \) of canopy show leaf falling or yellowing, twig drying |
| 3     | \( 50 < x \leq 75 \) | Severe symptom, plant dying, 50-75% branches infected followed by branches death |
| 4     | \( x > 75 \) | Very severe, 75 – 100% of canopy dying or permanently die |

2.4. Associated symptom observation

Each sampled plant showing death syndrome symptom were observed regarding the presence or absence of canker and/or borer symptoms. If on the symptomatic plant observed did not show both of canker and borer symptom, some branches and twigs were cut transversally and moistened to see the presence of bacterial ooze comes out of its surface. When oozes detected on the samples, It was assumed that the symptom associated is caused by bacteria.

2.5. Farmers interview

Interviews of farmers were carried out in order to see the relationship between the cultural practices and the condition of the death syndrome disease in their plantations. Clove cultural practices information of each plantation observed was collected by interviewing the owner using structured questionnaire. To determine the association between cultural practices and disease severity was performed by using Chi square (\( \chi^2 \)) and correspondence analysis.

3. Results

3.1 Clove death syndrome incidence, severity and other associated causal agents or symptoms

The most common symptom found in the field during the survey was canopy death that started from the shoots due to falling of younger leaves. This symptom is very easy to see from a distance which is marked by white-crowned moltiths in the clove plant population (figure 1). Of all the individual sample plants observed in the three districts, more than half of them showed the symptom. Even in one district, namely Tegal, the disease incidence was almost 100 percent. (table 4).
Table 4. Clove death syndrome disease incidence.

| District   | Total sampled plants | Disease incidence (%) |
|------------|----------------------|------------------------|
| Semarang   | 240                  | 78.0                   |
| Tegal      | 240                  | 98.5                   |
| Trenggalek | 1200                 | 57.3                   |

Figure 1. Clove death syndrome started with canopy die back symptom.

Figure 2. Other associated symptom with clove death syndrome disease on stem. (a) Brown frass of stem borer and (b) stem cracking or canker.
During field observation, many sample plants which showed canopy death symptom also associated with stem borer (figure 2a), canker (figure 2b) or combination of both, and only a few without one or both of them. From the observation in Semarang and Tegal, out of 120 sample plants from each district, the incidence without showing any association with stem borer, canker or both was only 16.7% in Semarang, and even 0.0% in Tegal. The associated symptom in these two districts mostly was dominated with canker or stem borer + canker (table 5). After moistening of transversally cut twig or branch samples from 120 plants of each district, bacterial ooze showed with frequency of 20.8 and 28.3% from Semarang and Tegal, respectively. We assumed that the individual diseased sample plants without any association with borer, canker, or both symptom were caused by bacterial as reported by Bennett et al. [1] and Lommer et al. [2]. However, we did not perform further identification.

| District | Stem borer (%) | Canker (%) | Stem borer + canker (%) | Without both stem borer and canker (%) |
|----------|----------------|------------|-------------------------|---------------------------------------|
| Semarang | 4.2            | 61.7       | 17.5                    | 16.7                                  |
| Tegal    | 0.0            | 39.2       | 60.8                    | 0.0                                   |

To elaborate the possibility of various causal agents associated with this disease, then in a survey in Trenggalek District all sample plants were checked for bacterial infections. Out of 600 sample plants, the symptom(s) or causal agent(s) associated with clove canopy death syndrome was dominated by combination of stem borer+canker and followed by stem borer, while the others were less then 10% (table 6). Interestingly, we did not obtain any sample plants associated with bacteria as single causal agent.

| Symptom or causal agent | Incidence (%) |
|-------------------------|---------------|
| N = 600                 |               |
| Stem borer              | 31.8          |
| Canker                  | 8.7           |
| Bacteria                | 0.0           |
| Stem borer + canker     | 55.0          |
| Stem borer + bacteria   | 3.5           |
| Stem borer + canker + bacteria | 1.0 |

The severity of the disease in three surveys location was dominated with mild to medium and followed with the categories of severe to very severe or death. We still obtained the healthy sample clove trees in Trenggalek, but did not in Semarang and Tegal (table 7). Because of our findings that indicate variations in disease severity and the presence of healthy plants in Trenggalek, the data was further followed up with correspondence analysis using chi square.
Table 7. Probability of sample plants with various clove canopy death syndrome severity.

| District          | Healthy (%) | Mild (%) | Medium (%) | Severe (%) | Very severe or death (%) |
|-------------------|-------------|----------|------------|------------|--------------------------|
| Semarang (N=120)  | 0.0         | 57.5     | 29.2       | 10.0       | 3.3                      |
| Tegal (N=120)     | 0.0         | 55.8     | 32.5       | 9.2        | 2.5                      |
| Trenggalek (N=600)| 21.3        | 32.7     | 23.8       | 16.7       | 5.5                      |

3.2. **Plantation and cultural practices actors related with the disease severity**

Analysis of cultural practices and its relationship with disease severity using Chi square test showed that the cropping pattern, the use of animal manure, dose of P element per plant/year, weed condition, and weeding frequency were significantly influence disease severity. In addition to the cultural practices, the plant age also significantly affected the disease severity (Table 8).

Table 8. Summary of Chi square analysis of epidemic factors related with the disease severity

| Epidemic factors                          | $\chi^2$ | p-value |
|-------------------------------------------|----------|---------|
| Plantation location                       | 1.143    | 0.285   |
| Land condition                            | 0.188    | 0.665   |
| Cultivar                                  | 1.143    | 0.285   |
| Seedling/propagation source               | 0.554    | 0.457   |
| Plant population per hectar               | 0.286    | 0.593   |
| Plant age                                 | 3.135    | 0.077*  |
| Plant spacing                             | 2.169    | 0.141   |
| Cropping pattern                          | 8.247    | 0.004*  |
| Dose of animal manure                     | 3.502    | 0.061*  |
| Frequency of animal manure application    | 1.422    | 0.233   |
| Dose of N fertilizer/plant/year           | 0.180    | 0.672   |
| Dose of P fertilizer/plant/year           | 3.502    | 0.061   |
| Dose of K fertilizer/plant/year           | 0.180    | 0.672   |
| Weed condition                            | 6.465    | 0.011*  |
| Altitude                                  | 0.046    | 0.830   |
| Rainfall                                  | 0.219    | 0.640   |

Note: *significantly affected ($\alpha = 0.1$)

Older plantations, which are more than 20 years old have a tendency for higher severity. Clove plant populations with lower severity tended to occur in intercropping pattern rather than monoculture. Intercropping plants commonly used by farmers include vegetables, medicinal plants, and tuber crops. Application of animal (goat) manure with a dose of more than 60 kg/plant/year also tended to increase the number of individual clove plants with a low severity category. Farmers who apply P fertilizer with a dose of more than 500 grams/plant/year, their plants tended to show a low severity level. Weed conditions in plantations that are not too dense, the clove death syndrome mostly occurred with low
category. The level of closeness of each component of the plantation condition and the cultivation techniques used were presented in figure 3.

Figure 3. Correspondence analysis plot between plantation condition and cultural practices with clove death syndrome disease severity. (1a) N fertilizer dose < 500 g/plant/year, (1b) N fertilizer dose ≥ 500 g/plant/year, (2a) P fertilizer dose < 500 g/plant/year, (2b) P fertilizer dose ≥ 500 g/plant/year, (3a) K fertilizer dose < 250 g/plant/year, (3b) K fertilizer ≥ 250 g/plant/year, (4a) sparse weed, (4b) dense weed, (5a) weeds are just cut, (5b) weeds are cut and removed, (6a) weeding frequency 3 times/year, (6b) weeding frequency ≥ 3 times/year, (7a) goat manure ≤ 60 kg/plant/year, (7b) goat manure > 60 kg/plant/year, (8a) without animal manure application, (8b) animal manure application, (9a) monoculture, (9b) intercropping, (10a) rainfall < 2000 mm/year, (10b) rainfall ≥ 2000 mm/year, (11a) plantation distance from residence < 2 km, (11b) plantation distance from residence ≥ 2 km, (12a) less rocky land, (12b) more rocky land, (13a) clove plantation ownership < 0.5 ha, (13b) clove plantation ownership ≥ 0.5 ha, (14a) plantation altitude ≤ 350 m from sea level, (14b) plantation altitude > 350 m from sea level, (15a) proper plant spacing, (15b) improper plant spacing, (16a) mixed cultivar Sikotok+Zanzibar, (16b) mixed cultivar Sikotok+Zanzibar+Siputih, (17a) seedling from free market, (17b) own seedling+free market, (18a) population < 80/ha, (18b) population ≥ 80/ha, (19a) plantation age < 20 years, (19b) plantation age ≥ 20 years, (20a) disease severity < 30 %, (20b) disease severity ≥ 30 %, (21a) disease incidence < 50 %, (21b) disease incidence ≥ 50 %.

4. Discussion
Clove trees death, which has the specific symptom started from canopy die back, followed by branch and twig drying and later the death of the whole individual trees, currently has been as one of limiting factors in the production and sufficing for the industrial needs in Indonesia. Historically, in Indonesia the disease with same and similar symptom has been recognised in 1950s by Hadiwidjaja [6]. He called the disease as “mati budjang” (death during youth) and described as slow decline of the clove tree before
the complete death in 2-3 years after the onset of the symptom. In 1970s, the devastated disease of clove plantation in West Sumatra that show the same symptom was recognised by Waller and Sitepu [7] and called as Sumatra disease. They isolated some fungal species from clove specimens and indicated that this transmissible pathogenic agent is responsible for the disease. They also indicated that climatic factors, soil condition and cultural practices predispose clove trees to severe attack. Further taxonomically studies reported that xylem-limited pathogenic bacterial [1], P. syzigii [2], R. syzigii subsp. indonesiensis [3] as the causal agent of the disease. The results from our study showed that the clove death syndrome disease with same symptom as described above was associated with other symptom and/or causal agent, mostly canker, stem borer insect and combination of both. Meanwhile only a few probability was singly associated with bacteria or in combination with the others. One of the most significant finding in our study were canker and in a combination with stem borer symptom associated with the disease. Stem canker caused by picnidial or perithecial forming fungi is very common and currently was indicated as one of the threat disease to plantation forests in Indonesia [8]. One of the canker causal agent fungus, Cryphonectria cubensis, has been recognised on clove trees in North Sulawesi and Samosir Island, North Sumatra [9]. In the same year, van Wyk et al. [10] reported the close morphologically fungus, Ceratocystis polychroma, and wood borer (Hexamitodora semivelutulina) occur and associate with decline clove trees in Sulawesi. We also constantly isolated the picnidial forming fungi from colour streak discoloration in the clove wood showing death syndrome as previously described by other researchers (data not shown).

Based on correspondence analysis from the depth observation in Trenggalek District showed that the disease severity has a relationship with some factors of plantation condition and cultural practices. These factors mostly have a contribution in determining of plant fitness, e.g. plant age, fertilizer input, cropping pattern, weed condition, and animal manure application. Plantation with age older than 20 years tended to obtain individual sample plants with higher severity. This is very reasonable, because most farmers interviewed apply inadequate amounts of fertilizer, while the frequency of harvest increases with age. This inadequate of nutrients will make plants weak and more susceptible to invading organisms. Lower disease severity was showed in intercropping patterns system. In intercropping system farmers always apply sufficient fertilizer for their intercropping plants, and it might be also have an impact on the surrounding clove plants through water flow and/or residual effect. Application of animal manure into the soil will improve the physical, chemical and biological properties of the soil. One important impact of the physical improvement of the soil is the increased ability of the soil to store water, and eventually will avoid the possibility of water stress. From several studies reported that drought stress is responsible as predisposition factor to development of several diseases in woody plants, especially caused by weak pathogen, including canker, and borer insects [11-14]. In this study, we observed that clove plantations amended with adequate animal manure have tendency to lower disease severity. In other word, our finding showed that improper cultural practices and clove plantation care is responsible for speeding up of the clove death syndrome outbreak. In line with actual current issue of global climate change, based our study results we propose that better understanding of holistic causal agents of clove tree death syndrome and its predisposition factors, especially factors inducing plant stress, should be paid attention.

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