Morphological Confirmation of the Fungi that Causes Strawberry Wilt Disease in Bali Indonesia

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ABSTRAK

Konfirmasi secara Morfologi Jenis Jamur Penyebab Penyakit Layu Tanaman Stroberi di Bali Indonesia

Stroberi adalah salah satu buah yang sangat digemari oleh konsumen karena mengandung banyak phytochemical yang merupakan senyawa fenolik yang bermanfaat bagi kesehatan. Perkembangan stroberi di Bali mengalami beberapa kendala yang menyebabkan penurunan produksi, salah satunya adalah penyakit layu. Namun, hingga saat ini belum banyak informasi spesifik tentang agen penyebab penyakit layu tersebut khususnya di Bali. Identifikasi penyebab penyakit layu tersebut sangat perlu diakukan, oleh karena itu pada penelitian ini dilakukan identifikasi secara makroskopis dan mikroskopis terhadap jamur penyebab penyakit tersebut. Selain itu penelitian ini juga bertujuan untuk mengetahui patogenisitas jamur yang diisolasi dari tanaman stroberi bergejala layu tersebut. Hasil uji patogenisitas menunjukkan bahwa isolat jamur patogen tersebut memiliki daya patogenesitas terhadap tanaman setroberi yang diuji. Persentase indeks penyakit adalah 80% dari isolat Gobleg, 90% dari isolat Pancasari dan Candi Kuning dibandingkan dengan kontrol. Berdasarkan identifikasi morfologi secara makroskopis dan mikroskopis jamur penyebab layu pada tanaman stroberi adalah dari genus *Fusarium*.

Keywords: phytochemical, strawberry, morphological, fusarium

1. Introduction

The development of strawberry in Bali experiencing some obstacles that cause a decrease of production, such as wilting disease. Strawberry wilt disease reported as a soil-borne disease (Hannum, 2004). The disease that can cause the outer leaves first become yellow and eventually take on a scorched appearance, so that cause the physiological processes of strawberry plants to be disrupted and strawberry plants can suddenly wilt and die. Strawberry wilting with red spots on the leaves may rapidly become so numerous that the entire leaf dries up, wilted and looks "scorched" as though by fire (Industry & Investment NSW, 2009).

Strawberry wilt disease that caused by *Fusarium oxysporum* has been reported in many countries such as Spain (Arroyo *et al*., 2009), Western Australia (Golzar *et*

al., 2007), South Carolina (Williamson *et al.*, 2012), Serbia (Stankovic *et al.*, 2014), and Korea (Nagarajan *et al.*, 2006). According to research conducted Iran (Ayoubi *et al*., 2016) also reported specifically that strawberry wilt disease is caused by the *Fusarium oxysporum* f. sp. *fragariae*. In addition, from the results of the research in Iraq (Kamil *et al*., 2014) and Turkey (Dinler *et al*., 2016) that the fungus identified was *Fusarium oxysporum* f. sp. *fragariae*. In contras Sari *et al*., (2018) reported in Bali the fungi associated with rooting of strawberry plants were pathogens of the genus *Verticillium* sp. The symptom is very similar with the strawberry wild disease caused by *Fusarium*. Based on the phenomena the confirmation the species of fungi caused the strawberry wilt disease is needed.

2. Material and method

2.1 Isolation and morphology identification of fungi on strawberry

The samples were collected from the strawberry plants with wilt disease at the center of strawberry plantations (Pancasari Village, Gobleg Village, and Candi Kuning Village). The isolations were started by cutting the infected parts (leaf, trunk or root) with the size about (1x1) cm^2, then dipped it in alcohol for 2 minutes to remove the outside contaminations. The pieces of plant then washed off by dipped into the sterile water for 3 times and put it on PDA medium which already contains chloramphenicol antibiotics (100mg/L) (Samson *et al.* 1995, Ando *et al.*, 2003 & Ilyas *et al.*, 2006), and incubated for 3-5 days. The fungi then purified by re-emerging fungal hyphae in PDA medium (Vawdrey, 2001). The fungi then propagated and stored for subsequent test. Finally, the pure cultures were identified macroscopically and microscopically to determine morphological characteristics.

2.2 Pathogenicity test of isolated fungi on healthy strawberry

The fungi were isolated from strawberry plants with wilt disease. 1 x 10^5/ ml conidial suspensions were used as inoculum. The fungi were inoculated by sprinkling of conidial suspensions on the plants at the volume of 2 ml per plant. Strawberries that have been inoculated with pathogenic candidate were observed the development of the disease progress by noting the symptoms. The observations were performed on day 3 after inoculation until symptoms appeared (Golzar *et al.*, 2007 & Dinler *et al.*, 2016). Disease were assessed by counting the disease incidence by following equation:

$$\%\text{Disease incidence} = \frac{No.of\ infected\ plants}{Total\ no.of\ plants} \times 100 \ldots\ldots\ldots(1)$$

3. Result and Discussion

3.1 Isolation and Morphology Identification of Fungi on Strawberry

The results of the isolation showed that one type of pathogen was obtained when it was observed macroscopically. Pathogens that have been isolated and purified are pathogens from the fungal group.

The macroscopic observation showed that the overall shape of the fungus colony was white which in the middle was purplish and if seen at the bottom of the purple color it was clearly visible dark purple (Fig. 1).

Fusarium fungi grown on PDA medium are initially white mycelium, getting older of culture age, it will be pale yellow, in certain circumstances pinkish purple with insulated mycelium and form branching.

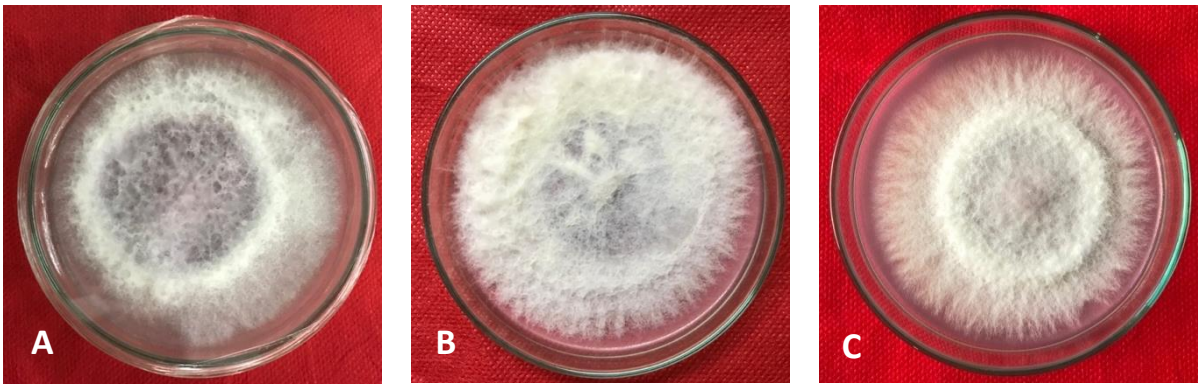

Figure 1. Pure culture at 7 days on PDA medium of fungal that isolated from strawberry plant with wilt disease in A) Pancasari Village, B) Gobleg Village, and C) Candi Kuning Village.

Fusarium fungi grown on PDA medium had characteristics such as white mycelium and will be yellow even to form a cream color. Some Fusarium isolates can also form pink pigments rather purple, blue, red or purple in certain circumstances. It supported by Susetyo (2010), that the mycelium produced by pathogenic fungi causing wilting is initially cloudy white, then becomes pale yellow, and pale pink to purplish.

In general, *Fusarium* that observed microscopically usually had microconidia and macroconidia. Microconidia is ovoid which generally has 0-1 septum, whereas the macroconidia form is generally tapered and has 2-6 septums.

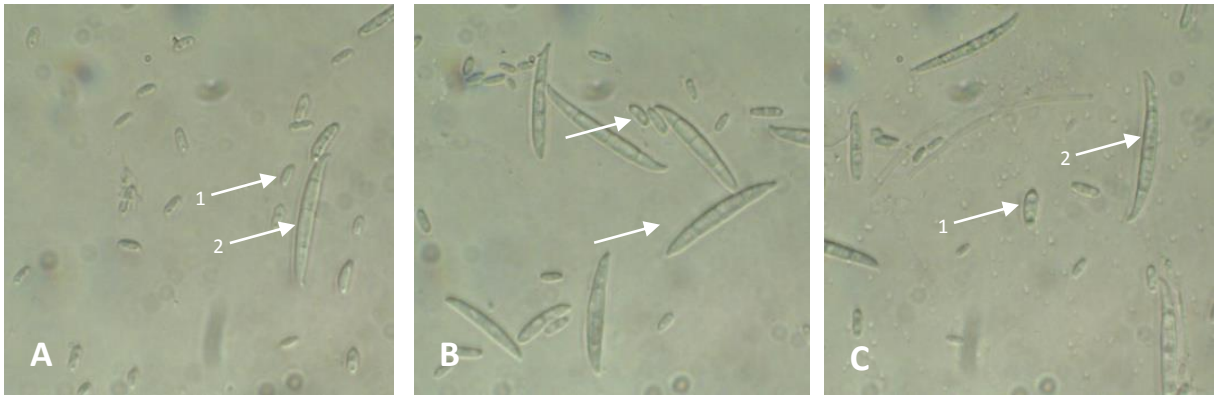

Figure 2. Microscopic observation of conidia from the sample at A) Gobleg, B) Candi Kuning, and C) Pancasari. Each image was designated by number 1) microconidia and number (2) macroconidia from pathogenic fungi

Microscopic observation of the samples showed that those were had the same characteristics, namely microconidia in the form of ovoid which has 1-2 septums, while macroconidia is spindle, oval with pointed tip and has 3-5 septums (Figure 2). Bridge & Spooner (1993) reported that the macroconidia of *Fusarium oxysporum* were spindle, oval, sharp edge, has 3-5 septums and Agrios (1996) stated that microconidia has one or two septums, it was present in large quantities.

The fungi that caused strawberry wilt in Bali that observed macroscopically and microscopically was the fungi from the genus *Fusarium*. However, it is still unclear, therefore molecular identification is needed.

3.2 Pathogenicity Test of Isolated Fungi on Healthy Strawberry

The pathogenicity test was carried out to ensure the fungus that was isolated and identified morphologically, was a fungus that caused strawberry wilt in Bali. On 8 days after inoculation showed that a mycelium was appeared in the all inoculated plants (Figure 3). It possibly that, the conidia contained in suspension that inoculated on strawberry plants was able to germinate, the hyphae grow (Lagopodi *et al*., 2002; Xiao-min *et al*., 2011).

Figure 3. Observation on 8 days after inoculation on strawberry plants that inoculated with pathogenic fungi from fungi isolate at A) Pancasari, B) Gobleg, C) Candi Kuning. Mycelium that appears on the strawberry plant (arrow)

The color changes in the former inoculation on 17 days after inoculation. The color changes in the former inoculation plant area were clearly seen in the Pancasari and Candi Kuning isolate treatments, namely the red color that had previously appeared had turned into black. Whereas the Gobleg isolate treatment still shows a red color that is still clear in the inoculated former plant. The mycelium possibly causing the process of plant physiology to be disturbed. Disruption of the physiological process of strawberry plants due to hypha colonizes epidermal and cortex cells, then enters the xylem vessels, eventually blocking the vascular system of strawberry plants (Lagopodi *et al*., 2002; Xiao-min *et al*., 2011). Plant stems will remain hard and green on the outside, but in the vascular tissue of the plant, discoloration occurs in the form of narrow brown wounds. The symptoms such as the outer leaves first become yellow and eventually take on a scorched appearance seen almost the same symptoms, namely scorched appearance on the leaves, so that causes physiological processes of strawberry

plants to be crushed and strawberry plants can suddenly wilt and die. Results of pathogenicity test showed that pathogenic fungi isolates were highly pathogenic to strawberry plants. The percentage of disease index in the amount of 80% in the treatment of Gobleg isolate, 90% in treatment of Pancasari and Candi Kuning isolates compared with zero in control. In accordance with the results of a research from Essa (2016), that fungi that were isolated from strawberry plants that infected with wilt in Egypt and used in the pathogenicity test showed a high disease incidence reach to 86.7% (range of 80%-90%). These results were in agreement with Fang *et al*. (2011). That *F. oxysporum*, was reported as the most virulent pathogen responsible for causing strawberry wilt in Western Australia. Koike *et al*. (2009) and Williamson *et al*. (2012) first time reported of the occurrence of strawberry wilt disease caused by *F. oxysporum* on strawberry plants in California and South Carolina. Previous research work showed that *Fusarium oxysporum* was most frequently isolated from diseased crown and root tissues of strawberry (Nagarajan *et al*., 2006; Suga *et al*., 2013, Ebihara & Uematsu, 2014).

After successfully observing the symptoms that appeared in each treatment and the percentage of disease index was calculated. Furthermore, the fungus was successfully re-isolated from the part of strawberry plant with wilting symptoms, which was subsequently identified.

The macroscopic observations on the three treatments showed the same form of fungal colonies, which are overall white and in the center of dark purple. Microscopic observations in all three treatments also showed the same characteristics, namely microconidia in the form of an ovoid which has 1-2 septums, while the form of macroconidia is spindle, oval with a pointed tip and has 3-5 septums. Based on these results, the fungus that was successfully isolated was the same fungus as the pathogenic fungus that caused wilt on strawberry plants in the field.

4. Conclusion

The fungi that caused strawberry wilt in Bali that observed macroscopically and microscopically was the fungi from the genus *Fusarium*. The fungi that isolated from strawberry plant with wilting disease in Bali (Pancasari Village, Gobleg Village and Candi Kuning Village) having pathogenicity and it were highly pathogenic to strawberry plants. The percentage of disease index in the amount of 80% in the treatment of Gobleg isolate, 90% in treatment of Pancasari and Candi Kuning isolates compared with zero in control.

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