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Distribution of banana *Fusarium* wilt in Banyumas, Indonesia, and characterization of *F. oxysporum* isolates from infected bananas and taro growing on the same farm

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Abstract. *Rahayuniati RF, Kurniawan REK, Mugiastuti E. 2024. Distribution of banana* Fusarium *wilt in Banyumas, Indonesia, and characterization of* F. oxysporum *isolates from infected bananas and taro growing on the same farm. Biodiversitas 25: 1344-1351.* *Fusarium* wilt disease is a major constraint to banana production in Indonesia. The disease has also affected banana production in Banyumas, one banana-producing region. However, information on the distribution and characteristics of *Fusarium* wilt in Banyumas is limited. Growing mixed bananas and taro on the same farm in Banyumas is common. During the banana wilt survey, we found that taro also showed a symptom of *Fusarium* wilt**.** Therefore, this study aims to determine the distribution of *Fusarium* wilt disease on bananas in Banyumas, determine the characteristics of *F. oxysporum* on bananas and taro that grow on the same farm, and conduct pathogenicity tests of taro *Fusarium* isolates on bananas. The study consists of two steps, i.e., survey and sampling using purposive sampling technique conducted at the banana-growing area in Baturraden, Rawalo, Wangon, Purwojati, Gumelar, Ajibarang, Kemranjen, and Somagede districts. The second step involved the identification, characterization, and pathogenicity test of *Fusarium* isolate banana compared to isolate taro. A complete randomized design was used in an experimental study. All samples with *Fusarium* wilt symptoms were identified morphologically as comprised of macroscopic, microscopic characters and volatile odor of isolates. The results indicated that *Fusarium* wilt was equally distributed in Banyumas District, with the disease incidence reaching 50%. *F. oxysporum* isolates, which cause *Fusarium* wilt of banana and taro in Banyumas District, have indicated the same characteristics as *Foc*, i.e., diverse colony morphology, dominated by white to purple colonies with a cottony, smooth, and dry surface. Colonies are circular, irregular, dispersed, and undispersed on the surface of the medium with a filamentous margin; the single-septate oval-shaped microconidium and typical straight to sickle-shaped macroconidium. There were no significant differences between *F. oxysporum* isolate banana and taro in terms of disease incubation period, disease intensity, and Area Under Disease Progress Curve (AUDPC). Generally, this study found that *Fusarium* wilt was equally distributed in the lowlands. The *Fusarium* in bananas was the same as the *Fusarium* on taro in the same area, and the *Fusarium* isolated from taro effectively infected the banana.

Keywords: Banana, disease, *Fusarium*, infection, taro

INTRODUCTION

Banana is a horticultural fruit product developed in many developing countries, including Indonesia, that rely on fruit as a staple food (Warman and Aitken 2018). Banyumas District is one of the banana central regions. According to the BPS-Statistics of Banyumas District (2022), banana production in 2020 reached 31,375.6 tonnes and increased by 5.31% to 33,042.2 tonnes in 2021. Despite the potential and economic importance, the overall yield and quality of the banana sector have declined, mainly due to increasing pest and disease pressure.

Wilt disease, caused by the fungus *Fusarium oxysporum* fsp. *cubense* (Foc) is one of the factors limiting banana production (Ploetz 2015). After invading the roots of banana plants, they colonize and obstruct the vascular system, causing the plant to wilt severely before dying (De Ascensao and Dubery 2020; Ahmad et al. 2020). Worldwide, including in Indonesia, *Fusarium* wilt disease causes major crop losses and seriously threatens banana production (Mostert et al. 2017; Mengesha et al. 2018; Olivares et al. 2021).

Symptomatic plants are essential in understanding epidemic development and the movement of new inoculum into the environment (Pegg et al. 2019). The typical external signs of banana *Fusarium* wilt included plant wilting, yellowing of older leaves, collapse of leaves at the base of the petiole, fallen and dried leaves surrounding the pseudostem, wrinkling, and deformation of leaf blades, and splitting at the base of the pseudostem. The internal symptoms were yellow, reddish-brown, or black discolorations in vascular tissues (Heck et al. 2021). *Fusarium* wilt is distributed throughout Indonesia, and geographic characterization has been performed on isolates from West Sumatra, Central Java, North Sumatra, East Java, East Kalimantan, South Sulawesi, and Lampung (Molina et al. 2010). Up-to-date information on the distribution of *Fusarium* wilt of banana is still needed. Based on the literature review, there is only one piece of information on the distribution of *Fusarium* wilt disease on banana plants in Banyumas District, namely Sumbang Sub-district (Kusuma et al. 2020). Therefore, this study surveyed the distribution of *Fusarium* wilt disease of bananas in Banyumas District.

Various hosts can harbor and transmit Foc. Other families of Zingiberales and Alismatales may serve as alternative hosts for spreading disease to new areas, including decorative plant species from other families involved in Musa (Waman et al. 2013), but information on Araceae families is lacking. In rural areas, bananas are frequently grown alongside other food crops. In general, banana plants are planted mixed with cassava, sweet potato, and taro plants (Michael 2020). Taro (*Colocasia esculenta* L) is one of the crops farmed in the same area in Banyumas. Like all crops, Taro is susceptible to pathogen infections, severely influencing food sources and commercial profits. Some pathogens that have been reported to infect taro include *Phytophthora colocasiae*, which causes taro leaf blight (Oladimeji et al. 2022), Dasheen mosaic virus, Taro bacilliform virus, Colocasia bobone disease virus, Taro vein chlorosis virus, Taro bacilliform CH virus which caused viral symptoms on leaf and plant (Yusop et al. 2019), *Dickeya fangzhongdai* a causal agent of soft rot (Huang et al. 2021), and *Fusarium oxysporum* and *F. proliferatum* causing corm rot (Ye et al. 2023).

Studying the distribution of banana *Fusarium* wilt disease and its alternative host is still important to gain information about the pathogen evolution. Therefore, we research to collect information regarding banana *Fusarium* wilt disease in Banyumas District. In addition to observing *Fusarium* wilt disease on bananas, symptoms of *Fusarium* wilt disease were also observed on other plants in the same field. During this survey and observation, it was found that some taro plants growing in the same area also showed symptoms of *Fusarium* wilt disease. However, there is no information on *Fusarium* wilt disease in taro.

According to these problems, the objectives of this study are to determine the distribution of *Fusarium* wilt disease on bananas in Banyumas, determine the characteristics of *F. oxysporum* on bananas and taro that grow on the same farm, and conduct pathogenicity tests of taro *Fusarium* isolates on banana.

MATERIALS AND METHODS

Survey

A survey was conducted in banana growing areas, including mixed cropping farms of banana and taro at Baturraden, Rawalo, Wangon, Purwojati, Gumelar, Ajibarang, Kemranjen, and Somagede Sub-districts, Banyumas, Indonesia. Banana production in these eight districts is relatively high, around 631.0-7,272.9 tons in 2021 BPS-Statistics of Banyumas District 2022). We selected 2 to 4 banana-producing villages from subdistricts situated at 15 to 25 m asl for sampling, where we observed banana and taro with symptoms of *Fusarium* wilt. Then, we counted the disease incidence rates by comparing the percentage of bananas with *Fusarium* wilt symptoms (x_i) with the total number of banana plants observed in a land unit (N_i) (Heck et al. 2021; Teixeira et al. 2021).

$$Disease\ incidence\ (y_i) = \frac{x_i}{N_i} \times 100\%$$

Disease incidence rates were classified according to Zuo et al. (2018), with y_i = 0 (very low/no incidence of disease), $0 \leq y_i < 25$ (low), $25 \leq y_i < 50$ (moderate), $50 \leq y_i < 75$ (high), $75 \leq y_i \leq 100$ (very high).

Collection of isolates

*Fusarium*s were isolated from diseased bananas and also taro plants around banana plantations. We collected leaves, stems, and soil around the root of banana and taro samples that showed symptoms of *Fusarium* wilt for further identification. Samples were washed with running water and air-dried before processing. Small sections (2×3 mm) from advancing regions of discolored pseudostem beaches were attained. These samples were sterilized using 10% sodium hypochlorite for 5 min, washed in three changes of sterile distilled water, and grown in Potato Dextrose Agar (PDA). After 5-7 days of incubation at room temperature, mycelial growth from the seeded isolate typical of a *Fusarium*, generally white, violet, and magenta, was transferred to PDA slants for sanctification. The isolation was carried out by modifying the method according to Pattikawa et al. (2020) on Potato Dextrose Agar (PDA) medium by taking 4 pieces of stem tissue with a size of ± 1 cm^2. The surface of the stem tissue was sterilized by soaking them in 70% alcohol for 60 seconds and continued with rinse in sterile water 3 times. The cutting tissue was drained in a petri dish containing sterile filter paper. Each dried sample was placed on a PDA medium containing 0.1 g/L streptomycin (Li et al. 2013) and then incubated at 25°C for 3-5 days. After the microscopy identified process, each fungus was grown separately on other PDA medium and incubated for 5-7 days.

Morphological identification

Macroscopic observations were carried out by observing the shape of the colony (also its edge and surface) and the color of the upper and lower surface colonies. We also observed the microscopic characteristics, namely the septum on the hyphae and the shape of macroconidium and microconidium.

Volatile odor were tested by culturing the isolates on steamed rice. Thirty mL of rice grains and 90 mL of water were added to 250 mL Erlenmeyer flasks, plugged with cotton wool, and steamed at 103°C for 1 h on each of 2 consecutive days. After 10-14 days, cultures were classified by the presence or absence of a strong volatile odor. According to Thangavelu et al. (2019), the Foc race 4 isolate exhibits aldehyde aromas (odoratum) on steamed rice. In contrast, Foc race 1 and 2 cultures on steamed rice lack the odoratum characteristic (inodoratum).

Pathogenicity test

The pathogenicity test was conducted to obtain information on the ability of the Foc from banana and taro to infect some banana cultivars. Four banana seed cultivars: cv. Mas, cv Cavendish, cv. Raja, and cv. Kepok were treated with Foc isolate banana and Foc isolate taro. The inoculation was carried out according to Gabrekiristos et al. (2018) with slight modifications. Some roots were cut to prevent the pathogen penetration before being immersed in the isolate solution with a spore concentration of 10^7 spores/mL for 10 min. The seedlings were then dried for 15 min before being planted on the sterile soil and placed in a screen house and the incubation period started from the day of inoculation to the first symptom appearing.

Disease intensity

The intensity of *Fusarium* wilt disease was measured based on the severity of leaf wilt symptoms and calculated using the equation according to Widyantoro et al. (2020).

$$IP = \frac{\sum(n \times v)}{N \times Z} \times 100\%$$

Where: IP: Disease intensity (%); n: Number of leaves on each corresponding score; v: Score of disease on corresponding leaf; N: Highest score; Z: Number of observed leaves

Infection score values were determined based on Widinugraheni et al. (2018), namely: 0 = healthy leaves; 1 = Mostly green, one leaf starting to turn yellow; 2 = Some leaves have yellowing streaks; 3 = Yellowing on all leaf surfaces or some leaves with necrosis with necrosis; 4 = wilting and/or necrosis

Area under Disease Progress Curve (AUDPC)

The AUDPC was calculated to determine the relationship between disease intensity and time using the formula by Rahayuniati et al. (2021).

$$AUDPC = \sum\left(\frac{Y_{i+1} + Y_i}{2}\right).(t_{i+1} - t_i)$$

Where: Y_i : Disease intensity at I; t_i : Time at i

RESULTS AND DISCUSSION

Disease distribution

Fusarium wilt disease is distributed equally in the eight sub-districts of Banyumas District, Central Java, Indonesia, including Baturraden, Rawalo, Wangon, Purwojati, Gumelar, Ajibarang, Kemranjen, and Somagede (Figure 1). The incidence of *Fusarium* wilt in Banyumas District was accounted for 0.20-50.00% and thus classified as moderate. The incidence rates from highest to lowest based on areas were Baturraden 1.33-14.29%, Rawalo 1.33-15.12%, Wangon 0.33-15.00%, Purwojati 0.20-22.22%, Gumelar 1.47-30.56%, Ajibarang 0.92-50.00%, Kemranjen 0.70-16.67%, and Somagede 0.49-20.00%.

Differences in topography and slope characteristics of the survey area presumably affect the spread of *Fusarium* wilt. The most symptoms of *Fusarium* were found in 16 locations at areas with an altitude of 0-200 m asl, and the least in 2 locations at 500-1,500 m asl. While in areas with flat slopes, the most symptoms of *Fusarium* wilt were found in 69 locations, compared to 6 at the very steep slopes. Bosman (2016) stated that steep slopes have more disintegration and complementation failures than gentle slopes, where debris regularly accumulates. Disintegration also contributes to the spread of Foc inoculum, which is transported with silt and runoff. In the expansion, soak and slope area affect the water accessibility within the soil, the sum of runoff water, and the development of Foc inoculum near the root framework.

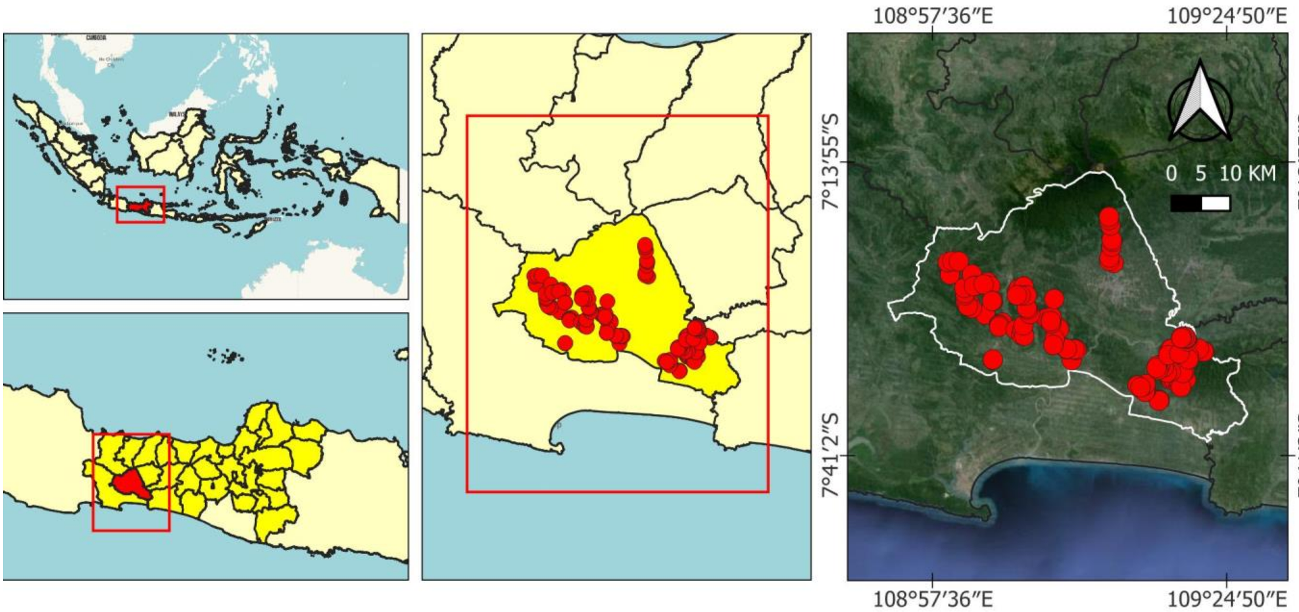

Figure 1. Map of distribution and incidence of *Fusarium* wilt (*red dot*) in Banyumas District, Central Java, Indonesia

Altitude relates to temperature and humidity that support the development of the disease. Pathogenic fungi require high relative humidity (>80%) for abundant sporulation and to infect host plants. Germination and infection rates during wet or humid periods are affected by temperatures ranging from 25 to 30°C (Bebber 2019; Devika et al. 2017; Gheorghe et al. 2015). The slope level affects the gravitational force generated for the soil mass movement. This shows the potential of an area to concentrate or spread pathogenic propagules through water flow and soil movement due to differences in topographical slope (Cardillo et al. 2018). Banyumas District's low-to-medium height resulted in no major difference in temperature or humidity between locations (Kusuma et al. 2023).

Different disease incidence rates are probably attributed to different agro-climatic conditions and the environment that support the development of pathogens, such as temperature, humidity, rainfall, and sunlight intensity. The survey and sampling were carried out from August through December 2021 in which the consecutive rainfalls were 71 mm^3, 227 mm^3, 238 mm^3, 452 mm^3, 407 mm^3, and rainy days ranged from 7-21 days (BPS-Statistics of Banyumas District 2022). Based on the Schmidt-Ferguson (1952) climate classification, the rainfall of a wet month is >100 mm rainfall, a dry month is <60 mm, and a humid month is 60-100 mm. Mengesha et al. (2018) state that *Fusarium* wilt shows a more severe disease incidence under warm and wet conditions throughout the year.

Disease symptoms

Typical symptoms of *Fusarium* wilt include yellowing leaves, chlorosis, and progressive wilting starting from older leaves (Figure 2A). Pathogens infect roots through the xylem vessels colonized in the xylem at the top of the plant, obstructing water flow and causing wilting. Symptoms were also seen on the pseudostem, showing reddish-brown discoloration (Figures 2B and 2C). This follows the observation of symptoms by O'Neill et al. (2016) and Pegg et al. (2019). In intercropping areas in Baturraden District, taro was found near banana plantations with symptoms of *Fusarium* wilt and yellowing of the leaves (Figure 3). The cropping pattern used is irregular with other plants such as cassava, papaya, and sengon. The land area is very humid with dense weed vegetation, and there is shade from annual plants around the land. This indicates that land sanitation is not carried out regularly.

Some factors encourage the development of plant diseases, including virulent pathogens, large amounts of inoculum, abiotic environment suitable for pathogens but suppresses plant development. Pathogens can easily infect a host under a suitable environment, such as weeds around the plants and lack of fertilization, which may cause the main crop to compete for nutrients. This can make the main plants weak and susceptible to *Fusarium* wilt (Orr et al. 2022). The spread of pathogens is also possible from seeds, former planting soil, vector transfer, water flow around the land/irrigation, and even human activity.

Figure 2. *Fusarium* wilt symptom on taro (yellowing and wilting)

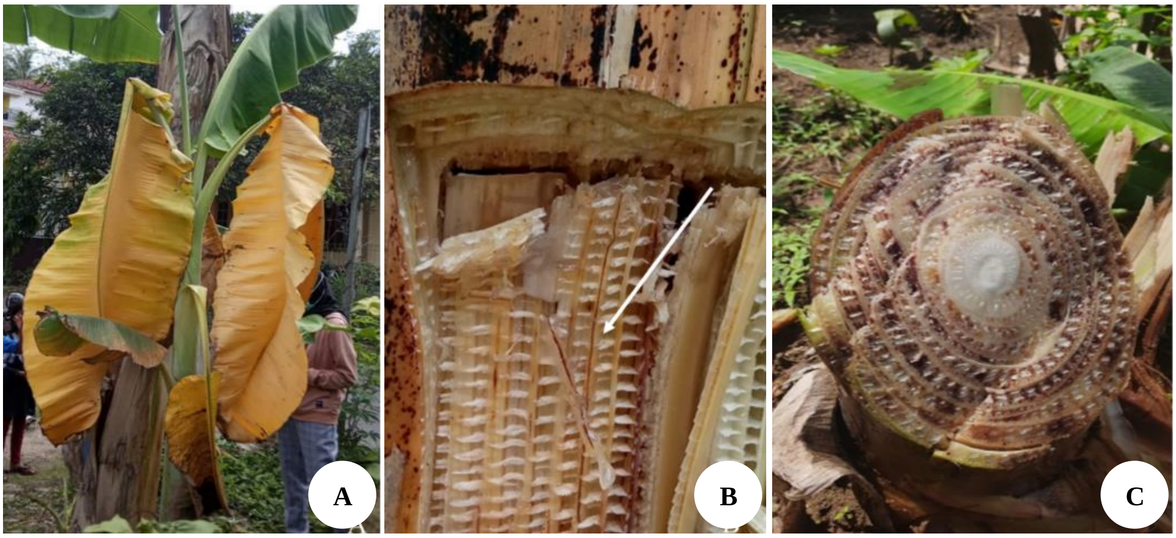

Figure 3. Symptoms of banana *Fusarium* wilt in Banyumas District: A. Yellowing of old leaves and broken leaf sheaths, B. Longitudinal discoloration of stems, C. Transversal discoloration of stems

Morphological identification

Based on the morphological identification, the isolates from banana and taro are *Fusarium oxysporum* (Figures 4-5). The morphological variations shown by the isolate samples follow the identification results of Aguilar-Hawod et al. (2020) on *Foc* from the Philippines. Colors range from pale purple-pale orange on *Fusarium* isolate banana to dark magenta or dark purple on isolate taro; some are not pigmented on isolate banana. The mycelium varies from flat to aerial, woolly to cotton-like, and circular to irregular shape both on isolate banana and isolate taro. Conidiophores are varied, slender, simple, stout, short, irregular branches, single or grouped into sporodochia. Macroconidium consists of several cells, slightly curved or bent at the pointed end, typically canoe-shaped. Microconidia are single-celled, ovoid, or oblong (Burgess et al. 1994) (Figure 5).

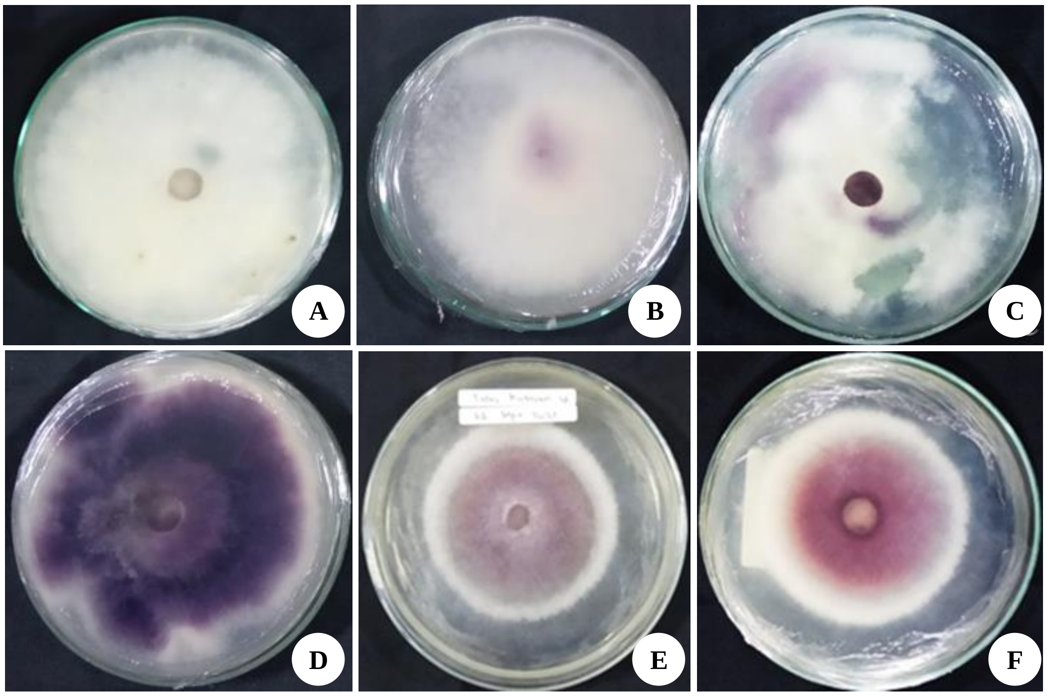

Figure 4. Colony color of *Fusarium oxysporum* isolate banana (A-D) and isolate taro (E-F)

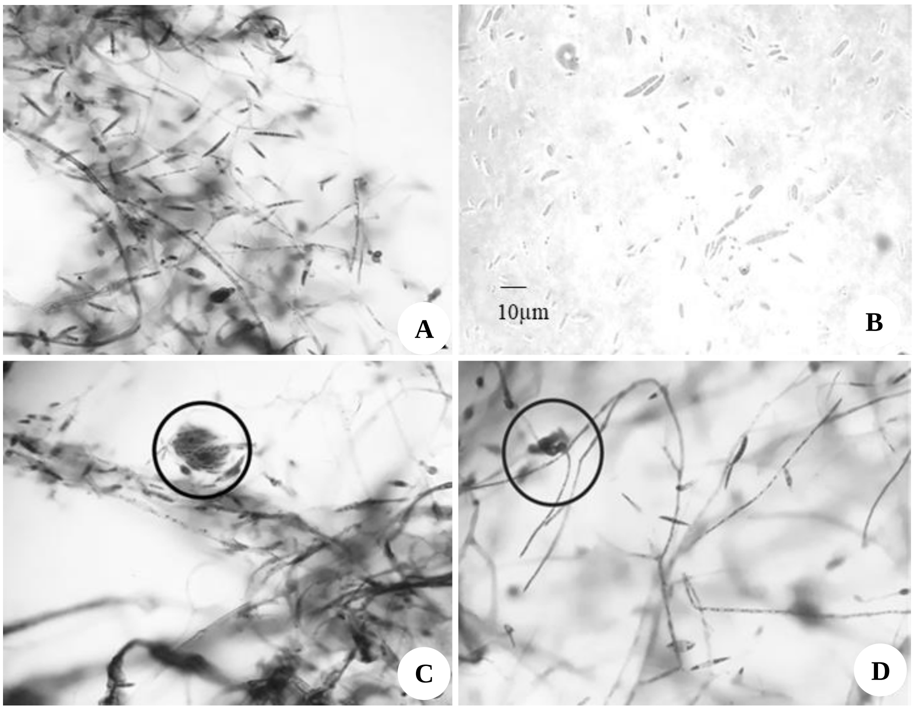

Figure 5. Conidium characteristic of *Fusarium oxysporum.* A. Macroconidium, B. Microconidium, C. False head, D. Clamidospora

Table 1. Incubation period, disease intensity, and AUDPC of *Fusarium* wilt disease on four banana cultivars

Treat-ments	Incubation Period (DAI)	Disease Intensity (%)	AUDPC (%* week)
B_1I_1	6.17 ± 0.289 a	90.14 ± 0.138 a	2627.78 ± 2.961 ab
B_1I_2	6.50 ± 0.500 a	90.73 ± 0.018 a	2588.76 ± 3.156 ab
B_2I_1	7.67 ± 1.155 a	86.00 ± 0.216 a	2057.32 ± 0.671 b
B_2I_2	8.33 ± 4.041 a	93.24 ± 0.094 a	2363.01 ± 2.844 b
B_3I_1	7.67 ± 1.155 a	83.16 ± 0.110 a	2400.88 ± 4.052 b
B_3I_2	6.33 ± 0.289 a	89.69 ± 0.107 a	2677.31 ± 3.637 ab
B_4I_1	6.17 ± 0.289 a	92.34 ± 0.032 a	3413.62 ± 6.646 a
B_4I_2	6.33 ± 0.577 a	85.28 ± 0.181 a	2704.82 ± 8.327 ab

Note: B_1: Cavendish, B_2: Mas, B_3: Raja, B_4: Kepok, I_1: *F. oxysporum* isolate banana, I_2: *F. oxysporum* isolate taro. Numbers with different letters indicate DMRT significant differences at a 5% level

The volatile odor test of the isolate showed various volatile aromas, from strong sour to weak sour, accompanied by sweet aroma, sour to putrid. Similarly, the *Foc* volatile production test conducted by Thangavelu et al. (2019) showed the isolate from India produced a strong, sweet aroma similar to that of *Foc* race 4. However, the isolate *Foc* race 1, which served as a control, did not produce any aroma. In this study, the volatile odor test results were not used to differentiate isolates based on the aroma of the aldehyde fermentation produced because the parameters for strong volatile aromas or odoratum and inodoratum were inconclusive.

Pathogenicity test

Four banana cultivars, namely Cavendish (AAA), Mas (AA), Raja (AAB), and Kepok (ABB) that were inoculated with *F. oxysporum* isolates banana and isolate taro showed *Fusarium* wilt symptoms during greenhouse pathogen testing, such as yellow turning leaves, gradual decomposition, necrosis, and eventual death. The analysis of variance is presented in Table 1.

On average, symptoms appeared on day 6 after inoculation. The earliest symptoms appeared in the treatment of Cavendish cultivar inoculated with *F. oxysporum* from banana (B1I1) and Kepok cultivar inoculated with *F. oxysporum* from banana (B4I1). The disease intensity and AUDPC of both cultivars were not different. Meanwhile, the Mas cultivar inoculated with *F. oxysporum* from taro (B2I2) showed the longest symptoms, but the incidence was high (93.24%) related to its AUDPC (2,363 %*week). This phenomenon contradicts Aprilia et al. (2020), who state that the correlation between incubation period and disease intensity shows that the longer the incubation period, the lower the disease intensity. Disease intensity (IP) showed different results in each isolate up to the 12^{th} week of observation after inoculation (Table 1). The lowest and highest IP in the last week ranged from 83.16 to 93.24%. Cultivars Cavendish, Mas, and Raja inoculated with *F. oxysporum* from taro showed higher IP than bananas. The opposite was true for cultivar Kepok. Based on the analysis of variance, the four banana cultivars inoculated with *F. oxysporum* suspension from banana and taro showed non-significantly different effects. It is suggested that the two isolates have the same ability to infect the four banana cultivars. Another possible explanation is that the four cultivars have similar resistance to *F. oxysporum* infection from bananas and taro.

The analysis of variance showed that the treatments resulted in different effects when Kepok cultivar was inoculated with *F. oxysporum* from banana (B4I1), producing the highest AUDPC value of 3,413.62%. The other treatments showed that the results did not significantly affect the lowest AUDPC value (2,057.32%) in the treatment of Mas inoculated with *F. oxysporum* of banana origin (B2I1). We also found that Kepok (ABB) and Cavendish were the cultivar with the shortest incubation period. The incubation period positively correlates with disease intensity and infection rate; the higher the disease intensity, the higher the infection rate (Aprilia et al. 2020). However, in this study, the incubation period does not seem to be related to disease intensity. This may be related to differences in resistance response of each cultivar. Some triploid genotypes with double Balbisiana (B) genomes, such as 'Pisang Awak' (ABB) and 'Bluggoe' (ABB), are known to be susceptible to Foc races 1 and 2, respectively. This suggests a significant variation in the level of resistance found in B genome types (Li et al. 2015).

Based on the pathogenicity test results, the four cultivars had short incubation periods, disease intensity, and progressively increasing symptom development (AUDPC), indicating that *F. oxysporum* of banana and taro origin was pathogenic to the four banana cultivars. Kai-li et al. (2019) stated that no resistant and cultivated varieties have good agronomic characteristics. For the prevention and control of *Fusarium* wilt disease, further research can probe into the development of resistant varieties and monitor the dynamic physiological changes of Foc in the region. Therefore, Table 1 shows that banana and taro isolates have the same effect as banana cultivars. The data also indicate that *Fusarium* isolates taro should be noted as the causal agent of wilt in banana plants, so it is recommended that it should not be grown intercropped with taro.

In conclusion, *Fusarium* wilt disease spread equally in the lowlands of Banyumas District with a moderate incidence rate. *Fusarium oxysporum* that caused banana *Fusarium* wilt in Banyumas District were found in banana and taro plants and indicated the same characteristics as *Fusarium oxysporum* f. sp. *cubense* (*Foc*). It is important to study the influence of the banana ecosystem on the development and spread of banana *Fusarium* wilt. Further research can determine the appropriate disease management technique for increased banana production.

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