Microscopic Characterization of *Fusarium* sp. Associated with Yellow Disease of Pepper (*Piper nigrum* L.) in South Bangka Regency

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Ayu Lestari[1], Henri[1]*, Eka Sari[1], Tri Wahyuni[2]

[1]*Department of Biology, Faculty of Agriculture, Fisheries, and Biology, Universitas Bangka Belitung, Balunijuk, Merawang, Bangka, Bangka Belitung 33172, Indonesia*

[2]*Regional Development Planning and Research Agency, Central Bangka Regency Jl. Titian Puspa 2, Koba, Central Bangka Regency, Bangka Belitung 33181, Indonesia*

**Corresponding author, email: biology.henry@gmail.com*

ABSTRACT

Pepper production has decreased recently, especially due to yellow diseases of Fusarium sp. Thus, this research aimed to isolate and characterize Fusarium sp. from soil and root of healthy and diseased pepper plants. The sampling technique used was purposive sampling. Soil and root pepper samples were taken from lands in Payung and Ranggung Village, Payung District, South Bangka Regency. There were 3 varieties of pepper plant used, including Petaling 1, Nyelungkup, and Merapin Daun Kecil. The characterization of Fusarium sp. isolate included macroscopic and microscopic observation. Macroscopic observation included colony color, colony base color, and growth rate/colony diameter size (cm), while microscopic observation included hyphae structure, and the shape and size of microconidia, macroconidia, chlamydospore, and conidiophore. The research found 66 isolates of Fusarium genus based on the colony color. Most of the isolates were white or purple and red. Colony color of Fusarium sp. showed white color, which then turned to orange color. All isolates showed septate hyphae. Isolates with macroconidia 3-4 septate and micronidia 0-1 septate showed the character of Fusarium oxysporum, while isolates with macroconidia 3-5 septate and microconidia 0-2 septate showed the character of Fusarium solani.

Keywords: Fusarium sp.*, yellow disease, Piper nigrum* Linn.

ABSTRAK

Beberapa tahun terakhir, produksi lada mengalami penurunan, terutama disebabkan oleh serangan penyakit kuning oleh jamur *Fusarium* sp. Penelitian ini bertujuan untuk mengisolasi dan mengkarakterisasi *Fusarium* sp. dari tanah dan akar pada tanaman lada sehat dan terserang penyakit kuning. Metode penelitian yang digunakan adalah metode *purposive sampling*. Sampel tanah dan akar tanaman lada sehat dan sakit diperoleh dari kebun petani lada Desa Payung dan Desa Ranggung, Kecamatan Payung, Kabupaten Bangka Selatan dengan tiga jenis lada, yaitu Varietas Petaling 1, varietas Nyelungkup dan aksesi Merapin Daun Kecil. Karakterisasi isolat *Fusarium* spp. meliputi pengamatan secara makroskopis yang dilihat dari warna koloni, warna dasar koloni dan kecepatan tumbuh/diameter (cm) koloni, sedangkan pengamatan mikroskopis dilihat dari struktur hifa, bentuk dan ukuran mikrokonidia, makrokonidia, klamidospora dan konidiofor. Penelitian ini memperoleh 66 isolat genus *Fusarium*. Berdasarkan warna koloni, mayoritas isolat berwarna putih atau disertai warna ungu atau merah. Warna koloni *Fusarium* spp. pada awalnya berwarna putih kemudian berwarna oranye. Semua isolat menunjukkan ciri hifa berseptat. Isolat yang memiliki makrokonidia 3-4 septat dan mikrokonidia 0-1 septat, menunjukkan karakter *Fusarium oxysporum*, sedangkan isolat yang memiliki makrokonidia 3-5 septat dan mikrokonidia 0-2 septat, menunjukkan karakter *Fusarium solani*.

Kata Kunci: *Fusarium* sp., penyakit kuning, *Piper nigrum* Linn.

INTRODUCTION

Pepper (*Piper nigrum* Linn.) is a spice plant with high economic value (Kardinan et al., 2018). Pepper is important plant type of the Piperacea Family and native to Southeast Asia. Peper plants are divided into two groups, namely black pepper and white pepper, in which the difference lies in the time of harvest and processing methods (Prabhu et al., 2015). *P. nigrum* has significant economic importance due to its valuable edible spicy taste worldwide. Moreover, *P. nigrum* has anti-platelet aggregation, anti-atherogenic, antioxidant, and anti-inflammatory activities (Kim et al., 2012; Son et al., 2012).

The Province of Bangka Belitung Islands is one of the most famous and biggest pepper producing regions in Indonesia through the product of white pepper called Bangka White Pepper (Muntok White Pepper). Around 80-97% of Indonesia's white peppers are from the Bangka Belitung Islands Province (Daras & Pranowo, 2009). According

Ditjenbun (2018), South Bangka Regency contributed the highest pepper production in 2016 in the Bangka Belitung Islands Province, with a production of 16,269 thousand tons. However, in the last few years, the production of pepper in the South Bangka Regency decreased from 17,009 thousand tons in 2017 to 14,859 thousand tons in 2018 (BPS Babel, 2018).

One of the factors causing the decrease in pepper production is the attack of yellow disease by *Fusarium* fungus. Yellow disease is a complex disease caused by *Fusarium* sp., *Phytophthora* sp., and nematodes. Infected plants quickly died and, it is difficult for replanting, thereby causing significant losses for the farmers (Taufik et al., 2011). The yellow disease damages pepper plants in Bangka Island because it causes the plants to become stunted and stop growing. As a result, the yields decrease, and losses reach 41% (Harni & Munif, 2012; Munif & Sulistiawati, 2014). Fusarium is a soil-borne pathogenic fungus that has a surviving structure in the form of chlamydospores (Ropalia, 2017).

This research focused on the observation of the *Fusarium* sp. fungus. Studies by Ropalia (2017) and Suryanti et al. (2015) stated that yellow disease in pepper plants in the Bangka region was caused by Fusarium pathogenic fungi. The disease caused by soil-borne pathogens is a major obstacle to pepper cultivation in the pepper production centers in Indonesia, such as Bangka Islands.

The yellow disease is one of the important diseases in pepper cultivation in the Bangka Islands. Damage and yield loss due to yellow disease in this region have not been a major concern so that information about yellow disease is still very limited. Therefore, research on the characterization of *Fusarium* sp. as a cause of pepper yellow disease in the Bangka Islands is important to do to increase pepper production.

MATERIALS AND METHODS

Study Area

This research was conducted in Payung District, South Bangka Regency. Geographically, Payung District is located at 2°29'16" - 2°43'59" South Latitude and 106°2'48"- 106°15'36" East Longitude. The study was conducted from June 2018 to March 2019. Sampling locations were in the pepper farmer villages of Payung Village and Ranggung Village. Isolation and microscopic characterization of *Fusarium* sp. were conducted at the Laboratory of the Regional Development Planning, Development and Research Agency in Central Bangka Regency; Laboratory of Biology and Laboratory of Agrotechnology of Bangka Belitung University.

Sampling on Pepper Plants

Pepper fields used as sampling locations was determined using purposive sampling, in which the inclusion criteria were that field was planted with *Petaling 1*, *Nyelungkup*, and *Merapin Daun Kecil* varieties with plant ages between 2.5-3 years, and there were pepper plants with yellow diseases in the field. The pepper fields cover an area of 0.3 ha, 0.1 ha, and 0.4 ha. The determination of was based on a higher percentage of yellow disease in plants aged 2.5 years compared to the younger plants. The evaluation of yellow disease symptoms in pepper referred to general symptoms in the field. Variables observed included leaf color and root condition (Munif & Kristiana, 2012).

Soil samples were taken from the rhizosphere of healthy and diseased pepper plants. In each field, 1 healthy plant and 3 yellow pepper diseased plants were randomly selected. Soil sampling was carried out referring to the method used by Suwandi (2001), in which soil samples from the rhizosphere of healthy pepper plants were taken using a ground drill (diameter 8 cm) at a depth of

0-10 cm. A total of four sampling points were made in each location. The samples were then compiled and put into plastic bags of ± 300 gr for analysis in the laboratory and labeled according to the sample. This procedure was repeated for soil sampling from the rhizosphere of the diseased pepper plants.

Isolation of *Fusarium* sp.

Isolation of Fusarium sp. from the soil was done using serial dilution techniques with 3 replications of 12 rhizosphere soil samples from healthy pepper plants and diseased pepper plants. Soil samples from each field were taken as much as 10 gr and put into Erlenmeyer tubes that had been filled with 90 mL of sterile distilled water. The soil was then homogenized using an orbital shaker at a speed of 120 rpm for 15 minutes. The suspension resulted was then made into a dilution series up to level 10^{-5} by taking 1 mL (1000 µL) suspension and putting it in a test tube containing 9 mL of sterile distilled water. The dilution series from levels 10^{-3} to 10^{-5} were then taken as much as 1 mL (1000 µL) and cultured on PDA media with 3 replications of the petri dish by pour plate method and were incubated for 3 days at room temperature (Suryanti et al., 2015). Fungal colonies that grew were purified by transferring the fungus colonies to a new sterile PDA media.

Isolation of Fusarium sp. from the roots was done by direct osolation from the roots of healthy pepper plants and the roots of the diseased pepper plants. Isolation of *Fusarium* sp. from the roots of healthy pepper plants was carried out referring to the method of Shahnazi et al. (2012), which was modified in pepper plants samples. The roots were washed clean with running water to remove excess soil. The roots were then cut ±2 cm, and the surface was sterilized by sinking them in 1% NaOCl solution for 5 minutes, then rinsed with sterile distilled water twice for 1 minute each, and drained on sterile tissue until completely dry using sterile tweezers. A 2 cm root section was cut from both ends of the root under aseptic conditions, and a piece of 1 cm middle part of the root was grown on PDA media in a petri dish with three replications and incubated at room temperature for 3 days (Suryanti et al., 2013). Fungal colonies that grew from the roots of healthy pepper plants were purified by transferring the fungus colonies to a new sterile PDA media until the *Fusarium* sp. isolates were obtained and suspected as a cause of yellow disease in pepper plants.

Microscopic Observation

Microscopic observations were made using block agar on WA media (Fadilah, 2016). The characters observed from Fusarium isolates included hyphae structure and the shape and size of macroconidia, microconidia, chlamydospore, and conidiophores using OPTILAB tools that were directly connected to computers. The characterization of *Fusarium* spp. referred to the identification book of The Fusarium Laboratory Manual (Leslie & Summerell, 2006), Illustrated Genera of Imperfect Fungi (Barnett & Hunter, 2006), and Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Keys to Species (Watanabe, 2010).

RESULTS AND DISCUSSION

Symptoms of Yellow Disease in Pepper

The symptoms of yellow disease on the leaves and roots of the *Petaling 1, Nyelungkup and Merapin Daun Kecil* varieties/accessions are illustrated in Figure 1 and 2. Based on the results of the assessment of the symptoms of yellow disease in the field, the leaf color changed slowly in each variety/accession. Pepper plants that had yellow disease changed the color of the leaves, which were initially green (healthy), into half yellow and eventually turned yellow or pale as a whole (Figure 1). Besides being

Figure 1. Symptoms of yellow disease on the leaf. A. Healthy leaves, B. Symptoms of half yellowing of the leaves, C. Symptoms of yellow disease on leaves

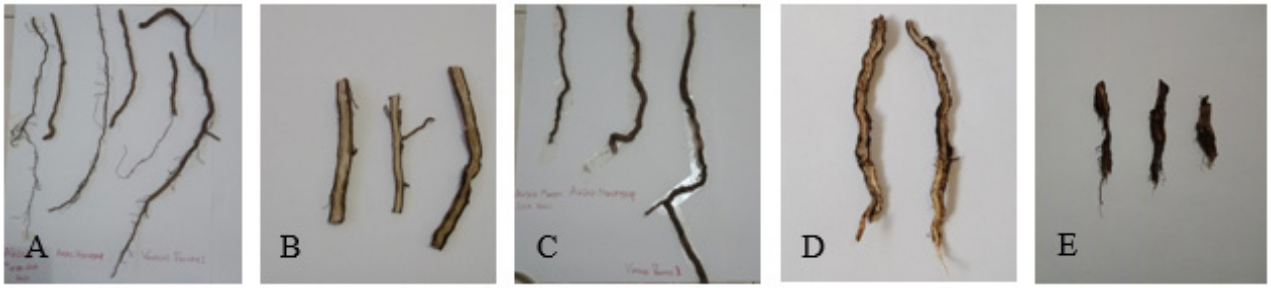

Figure 2. Symptoms of yellow disease in the Root. A. Healthy roots, B. Cut longitudinal roots healthy, C. The root hurts, D. Longitudinal pieces of diseased root, E. Root rot

seen in the color of the leaves, the symptoms can also be seen in several other parts of the plant, especially the roots. The symptoms of yellow disease were clearly visible on the root pieces (Figure 2d). *Fusarium*-infected roots showed dark patches of wound on the root fragments and experienced a brown discoloration in the medium vessel bundle on healthy root pieces (Figure 2b), which were pure white. The pepper plant roots infected by *Fusarium* eventually rot (Figure 2e). According to Suryanti et al. (2017), the common symptoms in the field, shown by pepper plants with yellow disease include stunted growth with stiff yellowing leaves, hanging upright, and finally leading to the stem. The yellowing leaves do not wither but are so fragile that they will gradually fall off. Yellowing leaves indicate that there has been a decrease in the chlorophyll content in the leaves due to the infection from *M.incognita* and *F. solani*.

Fusarium infected plant roots become rotten (Shahnazi et al., 2012), and if the roots of the diseased plant are cut, there will be visible patches on the attacked parenchymal cells, experiencing brown discoloration in the vascular bundles (Semangun, 2008). Severe attacks by yellow disease will cause death in plants (Shahnazi et al., 2012). According to Mukarlina et al. (2010), the brown discoloration of Fusarium infected plant cells is due to the melanin polymerization, which is brown, of phenol compounds and phenol oxidase enzymes produced by host plants. The roots of pepper plants infected with yellow disease showed swelling, which is a symptom of the nematode (*Meloidogyne* sp.) attack, and partly root rot, which is a symptom of an advanced attack of *R. similis* nematodes (Munif & Sulistiawati, 2014).

The high percentage of yellow disease incident in *Nyelungkup* variety was related to the age of the pepper plants used, which were still relatively young, ranging from 2-3 years. This is in line with the results of Munif & Sulistiawati (2014), stating that the yellow disease is relatively high in Bangka Regency, Central Bangka Regency and South Bangka Regency. This is related to the age of pepper plants observed, which was relatively young (2 years old). The high percentage of yellow disease

can also occur due to unfavorable planting environment conditions, one of which is the lack of hygiene of the field and insufficient ground water content. Cultivation techniques (maintaining hygiene) are very necessary, because it is one of the components of proper control of yellow disease (Sudarma et al., 2015)

The increase in the percentage of disease incident in *Nyelungkup* variety was presumably because farmers did not take proper control, and the infected plants were left in the field, assuming the plants could be still productive. According to Suryanti et al. (2015), infected pepper plants that remain on the ground can cause parasitic nematodes and multiply the yellow disease so that the population increases and has the potential to be a source of inoculum in the land.

Yellow disease is a major problem for farmers in pepper cultivation in Bangka region. The limited information about yellow disease causes the population of this disease to remain high. An effective strategy or method for controlling yellow disease has not been found. Evaluation and development of effective yellow disease control technology are needed in order to increase the productivity of pepper, especially in Bangka region.

Isolation of *Fusarium* spp. from the Soil and Roots of Healthy and Diseased Pepper Plants

Isolation from 12 samples of soil and roots of healthy and diseased pepper plants of *Petaling 1* variety, *Nyelungkup* variety, and *Merapin Daun Kecil* accessions resulted in 66 isolates of *Fusarium* sp., consisting of 27 isolates from soil and 39 isolates from the roots of pepper plants (Table 1). The 66 isolates of *Fusarium* sp. obtained from the soil and roots of healthy and diseased pepper plants were grouped into 13 groups of *Fusarium* sp. isolates colonies based on the color of mycelium colonies formed on PDA media.

Based on Table 1, the number of *Fusarium* sp. at each location was higher in the diseased plants than in the healthy plants, both from soil samples and from root samples. Meanwhile, the number of *Fusarium* sp. from *Payung* Village and *Nyelungkup* variety was higher compared to other location and varieties. In addition, seen from the total number of *Fusarium* sp., there were more diseased plants of *Fusarium* sp. than healthy plants, namely 39 isolates of diseased plants and 27 isolates of healthy plants. The isolation results of *Fusarium* sp. indicated that the number of individuals and total *Fusarium* sp. in each location were higher in diseased plants than

Table 1. Isolates of Fusarium sp. obtained from healthy and diseased pepper plants in different locations

Sampling Locations	Varieties/Accessions	Number individuals of *Fusarium* sp.				∑ *Fusarium* sp.	
		Healthy Plants		Infected Plants		Healthy Plants	Diseased Plants
		Soil	Root	Soil	Root		
Payung Village	*Petaling 1*	1	4	4	9	5	13
Payung Village	*Nyelungkup*	2	3	13	15	15	18
Ranggung Village	*Merapin Daun Kecil*	3	2	4	6	7	8
Total						27	39

Table 2. Abiotic data in the pepper fields

Abiotic	Location 1	Location 2	Location 3
Soil temperature (^{0}C)	24.5	26.75	27.25
Soil pH	6.5	6.68	6.02
Soil moisture (%)	65.58	23.33	66.67

Note: Location 1: Payung Village (Petaling 1); Location 2: Payung Village (Nyelungkup); and Location 3: Ranggung Village (Merapin Daun Kecil)

in healthy plants, both from the soil and from the roots. This is in line with the result of Munif & Kristiana (2012), showing that the abundance of endophytic bacterial populations in healthy pepper field was higher than in diseased pepper field.

Unhealthy plants due to the malfunction of plant hormones will facilitate infection of parasitic fungi in the plant's body. In addition, the formation of secondary metabolites in disturbed plants will reduce the ability of plants to interact and survive the environmental stress. This is in accordance with the principle of the disease triangle , stating that favorable plant conditions will facilitate pathogens to cause disease symptoms (Sari et al., 2017). The number of individuals and total *Fusarium* sp. in each location and the number of all *Fusarium* sp. based on the sampling locations are presented in Table 1. The number of all *Fusarium* sp. was higher in the location 2 for *Nyelungkup* variety compared to other locations, which were 33 isolates, consisting of 15 isolates from healthy pepper plants and 18 isolates from diseased pepper plants. This was because the abiotic environment in the second location strongly supports the growth of *Fusarium* sp.

According to Table 2, shows that both soil temperature and pH in the location 2 are suitable for the development of *Fusarium* sp., namely 26.75°C and 6.68, respectively. This shows that the pH of the soil in the research location is in the acidic category. *Fusarium* can grow at an optimum temperature of 31°C and a maximum of 37°C in acidic soils that have a pH range of 4.5-6.0 and can survive in dry soils compared to damp soils (Shahnazi et al., 2012).

The soil sample taken from in the location 2 was dry. This is because the location 2 had a low level of soil moisture compared to other locations, which was 23.33%. The low soil moisture in the field supports the development of *Fusarium* sp. According to Suryanti et al. (2017), yellow disease in pepper plants, especially in Bangka, is caused by very complex conditions, including the presence of nematode attacks (*R.similis* and *M. incognita*), the presence of parasitic fungi (*F. solani* and *F. oxysporum*), and low soil fertility, and low humidity or soil moisture content. The density of soil vegetation also greatly influences soil moisture levels. In the location 2, the vegetation density in the field is very low. Thus, it is suspected that this causes the level of humidity in the location to be lower than in other locations, which have high vegetation density.

The Microscopic Characterization of *Fusarium* sp.

Microscopic observations at each location showed that some of the isolates had characteristics of slender sickle macroconidia with sharp and curved apical cells 3-4 and oval or elliptical microconidia with 0-1 septate. Chlamydospores were formed separately or in pairs, and monophialid conidiophores were not branched, which had short stems (false heads) and hypnotic structures with peptides. Isolates that have such microscopic characteristics are *F. oxysporum* types (Figure 3). This is in line with Sutejo & Priyatmojo (2008), stating that *F. oxysporum* has a very abundant macroconidium, slick sickle-shaped, thick and smooth walls, with tapered and foot-shaped apical cells at the bottom cells, namely branched or unbranched monophialid. Monophialid, which binds to microconidium, is very short when compared with that of *F. solani* or *F. moniliforme*. Chlamydospore is formed separately or in pairs.

According to Leslie & Summerell (2006), macroconidium in *F. oxysporum* has a pectate 3, is relatively slim, thin-walled with apical cell morphology, tapered and curved, and sometimes with few hooks. Meanwhile, the basal cells morphological characteristics include pointed legs and oval or elliptical microconidia with 0 septate microconidia. *F. oxysporum* has a 4-stage Macroconidia and 1-stratum

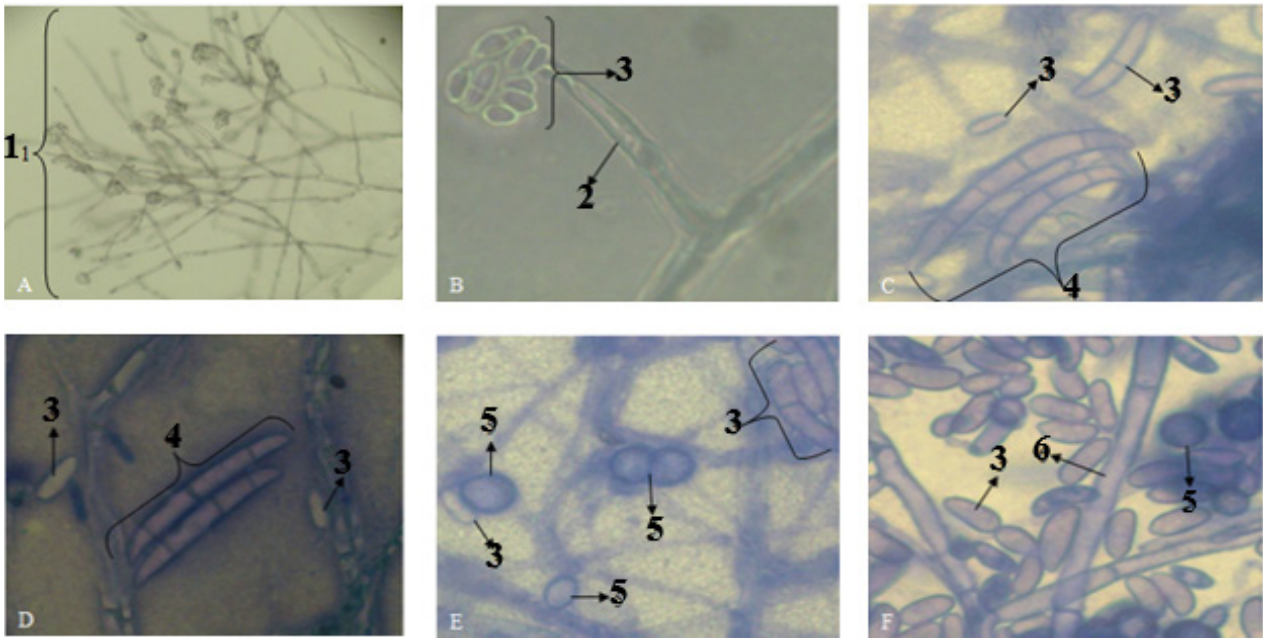

Figure 3. Microscopic morphology of *Fusarium oxysporum* isolates (400x magnification). 1. *Fusarium* sp. on Water Agar (WA) media, 2. Conidiophore, 3. Microconidia, 4. Macroconidia, 5. Chlamydospore, and 6. bulkheaded hyphae

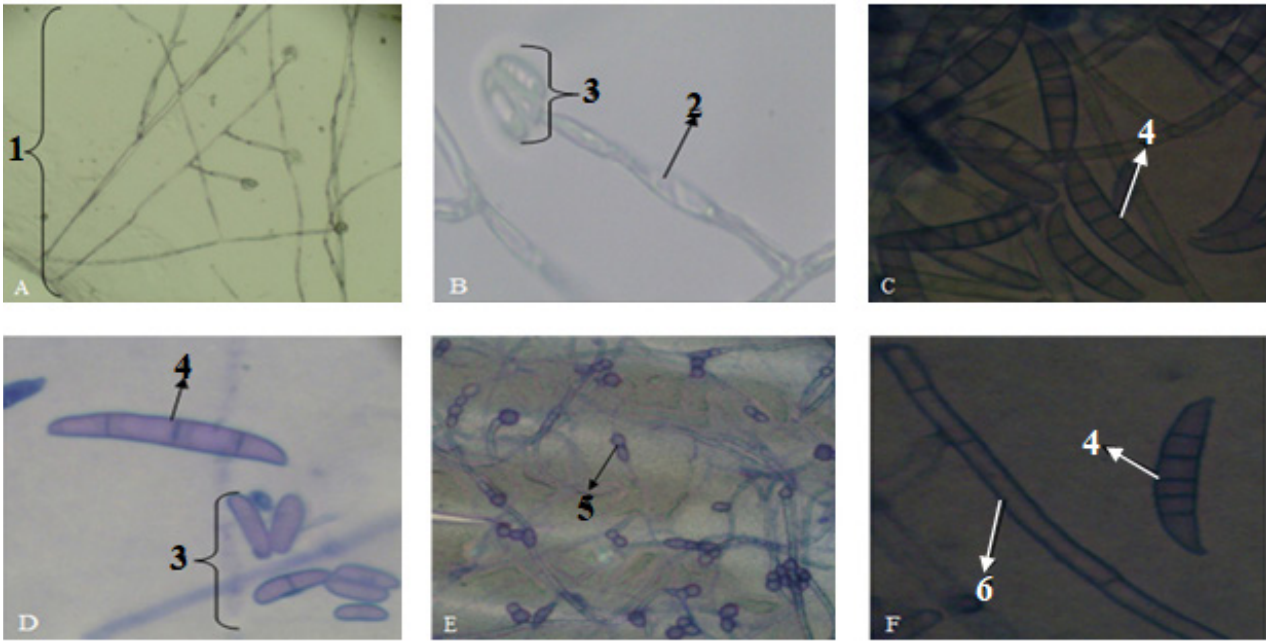

Figure 4. Microscopic morphology of Fusarium solani isolates (400x magnification). 1. Fusarium spp. on Water Agar (WA) media, 2. Conidiophore, 3. Microconidia, 4. Macroconidia, 5. Chlamydiospore, and 6. Insulated hyphae

microconidia. On the other hand, some isolates showed different microscopic characteristics. The crescent-shaped macroconidia were rather flat/wide and fat in blunt and round apical cells with 3-5 septate, the microconidia were oval or elliptical with 0-2 septate, chlamydospores were formed separately or in pairs, and monophialid conidiophores were long and unbranched. Hyphae of each isolate had a pectate structure (Watanabe, 2010).

The isolates showing characteristics as men-

tioned above are included in *F. solani* (Figure 4). The identification book of The Fusarium Laboratory Manual by Leslie & Summerell (2006), states that macroconidia in *F. solani* has 3-5 peptides and has blunt and round apical cells. Microconidia are oval, ellipsoid and 0-2. Chlamydospore is usually formed singly or in pairs. Conidium is formed in monophialid conidiophores, long and unbranched as in the fungus *F. solani*, *F. sacchari*, *F. verticilliodes*, and *Fusarium* sp. (Sutejo & Priyatmojo, 2008).

In the *Petaling* 1 variety, the longest size of microconidia and macroconidia were found in TLKP3 2.1 and TLHP54.3 isolates, which were 12.5 x 5 µm and 59.4 x 6.2 µm, respectively. The *Nyungkungkup* variety had the longest microconidia size in ALKN isolate 3.1, which was 12.0 x 3.3 µm with macroconidia size of 59.7 x 6.4 µm in ALKN 3.1 isolate. Meanwhile, in the *Merapin Daun Kecil* accession, the longest microconidia and macroconidia were found in TLKM3 3.1 isolate, which was 12.0 x 3.3 µm and 52.7 x 6.3 µm, consecutively. Based on the observation of the *Fusarium* spp. isolate colonies, the *Petaling* 1 variety, *Nyelungkup* variety and *Merapin Daun Kecil* accession are included in the type of *F. oxysporum*. According to Leslie & Summerell (2006), *F. oxysporum* has macroconidia and microcutaneous measuring 29.1-45 x 2.9-4.7 µm and 6-15.8 x 1.9-3.7 µm, respectively. Meanwhile, according to Suryanti et al. (2015), *F. oxysporum* has macroconidia measuring 36.42 × 4.37 µm (31.1-42.2 × 3.6-5.0 µm)

CONCLUSION

Of the 12 samples of soil and roots of three pepper varieties, there were 66 isolates of *Fusarium* sp. The number of individual *Fusarium* sp. in each location was higher in the diseased plants compared to in the healthy plants, both from soil samples and from root samples. Likewise, the total *Fusarium* sp. Was higher in the diseased plants (39 isolates) compared to in the healthy plants (27 isolates). The macroscopic and microscopic characteristics found represent the characteristics of *Fusarium oxysporum* and *Fusarium solani* species.

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