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# Biological characterization of *Fusarium* sp. isolates on red chili plants (*Capsicum annuum*) from Boyolali, Central Java, Indonesia

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**Abstract.** *Nugraheni ES, Widadi S, Supyani. 2018. Biological characterization of* Fusarium *sp. isolates on red chili plants (*Capsicum annuum*) from Boyolali, Central Java, Indonesia. Bioteknologi 15: 92-98.* This study examines the biological character, and virulence diversity of *Fusarium* sp. isolates in red chili plants (*Capsicum annuum* L.) from Boyolali, Central Java, Indonesia. This study was conducted from October 2009 to May 2010 at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. This study was designed as exploratory research in the field and an experiment in the laboratory. Observation variables included colony growth rate, colony phenotype, sporulation, and virulence test. Data from growth rate observations, colony phenotype, and sporulation were presented in descriptive form. Meanwhile, data from the virulence test were analyzed for variance with the F test at a 5% level. If the treatment has a significant effect, it is continued with Duncan's Multiple Distance Test (DMRT) at a 5% level. The results showed that the fungus of *Fusarium* sp. on red chili plants from Boyolali had distinguished macroscopic and microscopic diversity, with different levels of virulence. The colony group of *Fusarium* sp. isolates that had a low virulence level was the isolate of group D. In contrast, the biological characters of isolate D showed white mycelium like cotton, long macroconidia shape with a tapered tip, macroconidia with 3-6 septa, microconidia with 0-1 septa, size [(±5.35x3.15)-(±4.75x2.5)] µm x [(±27.36x4.10)] µm, spore density between $25.64x10^6$-$27.76x10^6$ spores/ml. This isolate has the potential as a hypovirulent isolate that can be used as an environmentally friendly biological control agent, particularly by applying the hypovirulent isolate in a biological control system with induced resistance and virocontrol with mycovirus methods.

**Keywords:** Biological characterization, *Fusarium* sp., red chili

## INTRODUCTION

Horticultural products such as fruits and vegetables are always in demand by the community every day to meet the needs of vitamins for the body. One of the horticultural products people need daily is red chili, both used for vegetables and as a mixture in cooking spices. Chili plants are one of the horticultural crop commodities whose fruit has a fairly high nutritional value, especially the content of vitamins A and C. The nutritional content of chili peppers in 100 grams of the edible part contains 71.00 mg of vitamin A and 18.00 mg of vitamin C (Wirakusumah 1995 cit. Harpenas and Dermawan 2010). The demand for red chili has been increasing over the years in line with the increase in population. Yet, chili production is still insufficient. In 2006, the harvested area of red chili in Central Java, Indonesia, reached about 20,415 hectares with a production level of 1,248,392 quintals and productivity of 61.15% per year. However, in the 2003-2006 period, the harvested area of red chili in Central Java seems to have a downward trend of 6.10% per year. Those are due to the emergence of pests and diseases (Agriculture Office of Central Java 2006 cit. Supriyati and Roosganda 2009).

Efforts to increase the production of chili plants still experience obstacles. Insects, mites, nematodes, and pathogens are threats that are always present in every planting. Plant pathogens can be caused by fungi, bacteria, mycoplasma-like organisms, and viruses (Gunawan et al. 2004 cit. Samoosir 2007). One of the pathogens caused by fungi is fusarium wilt caused by *Fusarium* sp. This fungus attack is one of the limiting factors that cause a decrease in red chili production. The spread of *Fusarium* is highly fast and can spread to other plants by infecting plant roots using sprout tubes or mycelium. Plant roots can be infected directly through the root tissue or lateral roots and wounds, which settle and develop in the vascular bundles. Once entering the plant roots, the mycelium will develop until it reaches the root cortex tissue. When the fungal mycelium reaches the xylem, this mycelium will develop to infect the xylem vessels. Mycelium that has infected the xylem vessels will be carried to other parts of the plant so that it interferes with the circulation of nutrients and water in plants which causes plants to wither (Semangun 2005). *Fusarium* forms a polypeptide called lycomarasmin, which can interfere with the permeability of the plasma membrane of plants (Walker 1952 cit. Susetyo 2010).

When the vascular tissue dies, and the air is moist, the fungus forms purplish-white spores on the infected roots. Their spread can occur through wind, irrigation water, and agricultural tools. Complete wilting may occur between 2-3 weeks after infection. Generally, when plants wilt, it is started from the lower leaves, and the leaves turn yellow. When an infection develops, plants wilt within 2-3 days after infection. Diseased plants cut near the understock showed symptoms of a brown ring from the vascular

bundle. The color of the root and stem tissue becomes brown. The infected site is covered with white hyphae like cotton (Directorate of Horticultural Protection 2003 cit. Huda 2010).

The decline in red chili production in the Boyolali area resulted from *Fusarium* sp. attacks which caused huge losses to farmers. Efforts to control fusarium wilt have been carried out, but farmers have not found an effective and environmentally friendly control method. The rapid spread of fusarium wilt has exacerbated the situation, so the use of synthetic pesticides (fungicides) is the last alternative to control *Fusarium* sp. pathogens in red chili plants in the Boyolali area. Various things have been considered before using these fungicides because, in addition to their high price, they also cause pathogens to become resistant to fungicides, create new *Fusarium* familia, and may cause environmental pollution. However, the control of *Fusarium* sp. pathogens can be done naturally without having to cause negative residues for the environment, particularly by applying the induced resistance method and virocontrol with mycoviruses. According to Istikorini (2002), biological control mechanisms can occur through some mechanisms, two of which are virocontrol with mycoviruses and induced resistance. Virocontrol with mycoviruses is controlled by viruses that can infect fungi. Meanwhile, induced resistance develops after plants are inoculated early with biotic elicitors (avirulent, non-pathogenic, saprophytic microorganisms) and abiotic elicitors (salicylic acid, 2-chloroethyl phosphonic acid).

The objective of this study is to examine the biological character, and virulence diversity of *Fusarium* sp. isolates on red chili (*Capsicum annuum* L.) from Boyolali, Central Java, Indonesia.

## MATERIALS AND METHODS

### Materials

The main materials used in this study were chili plant parts infected with *Fusarium* sp. (understock, interstock, scion, root) and PDA (Potato Dextrose Agar) media.

### Research design

This study was designed as exploratory research in the field and an experiment in the laboratory.

### Research implementation

*Collection of isolates of* Fusarium *sp.*

*Fusarium* sp. isolates were collected from the Boyolali area. The collection was done by visiting the area. Red chilies that showed a general symptom of wilting were dug up with a shovel. The entire plant (including all the roots) was removed, then the attached soil grains were released by immersing them in water. Parts of the plant, especially the understock and roots, were examined for additional symptoms of pathogens in the form of spots, wounds, rot, or signs of pathogens in the form of sclerotia.

The plant parts that show symptoms or signs of the pathogen were then put in a plastic bag and labeled. They were then put in a cooler right away. Once arriving at the laboratory, the plant samples were immediately transferred to a refrigerator at 4°C to be further cultured in PDA media.

*Cultures of* Fusarium *sp. isolates in PDA media*

Cultures of *Fusarium* sp. isolates were carried out in LAF (Laminar Air Flow). Tissue surfaces containing spots, wounds, or rot were sterilized with 90% alcohol. A small section of the border area between diseased and healthy plant tissue was cut, removed, and placed in the center of an 8 cm diameter sterile Petri dish containing 20 mL of PDA (Potato Dextrose Agar). The preparations were incubated under standard conditions at 22-26°C for 7-10 days. All isolates were labeled with an identification number corresponding to the label's identity when they were isolated from the field. When the culture was 1 week old, photo shoots were taken for documentation.

While taking the photo shoot, each isolate was stocked, particularly by culturing it on a regeneration medium in a 4 cm diameter Petri dish. The making of stock began with inoculating 3x3x3 mm cubic inoculum taken from the edge of the culture, then placed in the middle of the medium that had been provided. The preparations were incubated under standard conditions at 22-26°C for 1 week. After that, the preparation was stored in a refrigerator at 4°C as stock for the next tests. Each of these stocks was labeled with an identification number corresponding to the identification number of the isolate on the PDA media.

*Characterization of* Fusarium *sp. isolates*

Morphological characterization was carried out by inoculating 3x3x3 $mm^3$ inoculum, taken from the edge of 1-week-old stock culture, in the center of an 8 cm diameter Petri dish containing 20 mL of PDA. The Petri dish was then incubated under standard conditions of 22-26°C. At the same time, virus-free *Fusarium* sp. preparations were also cultured for comparison. The culture was observed on days 3, 5, and 7. The characteristics observed and recorded were colony growth rate, colony phenotype, and sporulation. If the observed isolates showed different characteristics, such as smaller colony diameter of the colony growth rate, darker/lighter color of the colony phenotype, and rough colony surface, the isolates were marked/selected and documented. These isolates had a high chance of low virulence (hypovirulence). The selected isolates were then tested by virulence testing using apples and chili plants.

*Virulence test*

The virulence test was carried out on apples using selected hypovirulent isolates based on the characterization of the isolates. Selected isolates were tested for virulence. First, ripe apples were disinfected with 90% alcohol. Four points around the fruit with a balanced spread position were determined. Each point was then inoculated with *Fusarium* sp. isolates on the part that had been wounded. The inoculum was inserted into each wound in an inward-facing position. It was pressed with a sterile spatula so that there was complete contact with the apple tissue. The

inoculated parts were then wrapped with parafilm to prevent drying. Next, the apples were incubated in a plastic tray measuring 35x25x7 cm at room temperature. The diameter of the lesions was measured on days 5, 7, 9, and 12. Based on the results of the virulence test, it was possible to determine which *Fusarium* sp. isolates had lower virulence (hypovirulent) than the control (virulent).

**Data analysis**

Data on the growth rate, colony phenotype, and sporulation were presented in descriptive form. The virulence test data were then analyzed for variance with the F test at a 5% level. If the treatment has a significant effect, it is continued with Duncan's Multiple Distance Test (DMRT) at a level of 5%.

## RESULTS AND DISCUSSION

**The general condition of the location**

The sampling location is in Boyolali. Boyolali, particularly the sampling location, has an altitude of ±400 m above sea level with an average rainfall of 3000-4000 mm/year, 70% air humidity, and an average temperature of 21-30°C/month (www.scbdp.net). The red chili plantation area at the sampling site looks quite dry (especially the soil). It lacks water because the sampling was carried out during the dry season, and it was difficult to find water for irrigation. Around the location, cassava and peanuts are planted, cultivated food crops. During the sampling, the condition of red chili plantations showed withered leaves and brown stems, and the plants seemed to no longer have the potential to produce red chilies (Appendix 8). Those are presumably caused by the attack of the pathogenic fungus, *Fusarium* sp., which is one of the limiting factors for red chili production in the Boyolali area. Various efforts have been carried out to control the pathogen that causes this wilt, such as using healthy seeds or fungicides. Yet, they have not yielded satisfactory results.

The symptoms caused by the *Fusarium* sp. pathogen to red chili plants are that the affected plants wilt and die. Those are because the fungus attacks the vascular tissue and causes wilting of the host plant by inhibiting the flow of water in the xylem tissue (De Cal et al. 2000). The most typical symptoms are found on the inside of the plant stem. If the understock is split longitudinally, dark brown streaks are seen running in all directions, from the stem upwards through the vascular tissue to the base of the leaves and stems. The root vascular bundles are usually not discolored, but the roots of diseased plants are often black and rot.

**Isolation and pure culture**

Pathogen isolates were taken from the scion, interstock, understock, and roots of red chili plants affected by fusarium wilt. The parts were then aseptically sliced, grown on PDA media, and isolated (Appendix 9). The results of the isolation of *Fusarium* sp. obtained from red chili plantations from Boyolali were 100 isolates with morphological diversity. The 100 isolates obtained were divided into five groups based on the color of the mycelium colonies formed on PDA media, including cream, purple, pink, white, and creamy white colonies. The grouping of the isolate colonies was also based on the macroscopical variation of other morphological characteristics. According to Machmud et al. (2003), one way to determine the type of pathogen is to know its biological character. Therefore, studying various biological and ecological characteristics of pathogenic microbes is also related to the epiphytology and control of agricultural plant diseases.

**Macroscopic observation of *Fusarium* sp. isolates**

Macroscopic observations of *Fusarium* sp. isolates were carried out from the 3$^{rd}$ to the 7$^{th}$ day after isolation. Macroscopic observations were carried out directly by evaluating the development of each colony, starting from the diameter of the colony, colony color, air mycelium, and colony profile. Based on these morphological differences, *Fusarium* sp. isolates were grouped into five colony groups: A, B, C, D, and E (Appendix 10). In each group of isolates that had been isolated, there were 5 selected isolates consisting of 1 isolate with the fastest colony growth (control isolate: K) and 4 isolates with relatively slow colony growth (treatment isolate). The control isolates were used as a comparison with treatment isolates. The results of macroscopic observations of *Fusarium* sp. isolates are presented in Table 1.

**Table 1.** Macroscopic observation of control and non-control of *Fusarium* sp. isolates

| Isolate | Control isolate diameter (cm) | The mean diameter of the treatment isolates (cm) | Colony color | Air mycelium | Colony profile |
|---|---|---|---|---|---|
| A | 7.8 | 5.6 | The base of the colony is cream; the top is white with a smooth surface | little | soft |
| B | 6.5 | 4.2 | The base of the colony is purple; the top is white | little | soft |
| C | 6.8 | 4.5 | The base of the colony is pink; the top is white | little | wavy |
| D | 7.3 | 4.8 | White like cotton. | little | like cotton, uneven edges |
| E | 7.5 | 5.3 | The base of the colony is creamy white | little | rough, forming a ring |

Notes: A: Group, AB: Group, BC: Group, CD: Group, DE: Group E

The observation results showed that the isolated isolates could be identified based on the macroscopic characteristics that appeared, including colony diameter, colony color, air mycelium, and colony profile (Table 1). Each of the selected isolates shows different morphological characters. The red chili plant parts taken as isolation material for the fusarium wilt pathogen may affect the characteristics of the *Fusarium* sp. isolates. The isolate colony of group A is from the scion of red chili plants, the isolate colony of group B is from the interstock of red chili plants, and the isolate colony of group C is from the understock of red chili plants. Meanwhile, the isolate colony of groups D and E are from the root of the red chili plants.

Observation of the growth rate of the isolate colony was carried out by evaluating the size of the colony diameter in each group, which showed different sizes of the isolate diameter (Figure 1). The control isolates in each colony group had a higher growth rate than the treatment isolates. The control isolate and the treatment isolate colonies showed that the colony groups with the highest and lowest diameter values were in the colony of group A and colony of group B. In the control isolate colonies, the highest colony diameter values were found in isolates group A of 7.8 cm. In comparison, the lowest diameter value found in isolates group B was 6.5 cm. In the treatment isolate colonies, the highest colony diameter value was found in the isolate of group A at 5.6 cm.

In comparison, the lowest average diameter value was found in the isolate of group B at 4.2 cm. Gandjar (1999) stated that *Fusarium* sp. on PDA media had colonies reaching a diameter of 3.5-5.0 cm. Those indicate that the isolates obtained from the isolation of red chili plants have a high growth rate. The isolate colony's diameter affects the conidia formation process, which in turn will also affect the level of development of *Fusarium* sp. The growth of the next fungal isolate colony will still occur even though it is slow. That is because the conidia are developmental tools in the Deuteromycetes class, which are produced asexually so that their number determines the development of the next generation. Under favorable conditions, the number of conidia tends to be directly proportional to the rate of development of the *Fusarium* (Burnett and Hunter 1988). In addition to observing the colony growth rate, observations were also made on the color of the colonies in each group. The appearance of the color of the colonies of *Fusarium* sp. isolates in each group of isolate colonies was different. The color difference of this isolate colony was based on the color that appeared at the bottom of the colony and the top surface.

The color of the colonies appeared to be smooth cream, purple, pink, white like cotton, and creamy white (Figure 2). The appearance of the isolate colony of group A had a cream color, smooth colony surface, little air mycelium formation, and hyaline mycelium appearance. The appearance of the isolate colony of group B had purple color, smooth colony surface, and medium air mycelium. The appearance of the isolate colony of group C had pink color, wavy edges, and medium air mycelium. The appearance of the isolate colony of group D had white colors like cotton, uneven edges, and little air mycelium. Finally, the appearance of the isolate colony of group E had a creamy white color, a rough and ring-forming colony profile, and a little air mycelium formation.

Based on these macroscopic observations, it can be considered that, in fungal cultures in solid media such as Potato Dextrose Agar (PDA), *Fusarium* sp. can have different appearances even though they come from the same host plant. And in general, the air mycelium first appears white and then can change to various colors according to the strain (or special shape) of *Fusarium* sp. Some *Fusarium* sp. isolates will also form red pigment in the medium (Agrios 1996; Susetyo 2010). According to Semangun (1996), the hyphae pigments of *Fusarium* sp. are generally varied, hyaline pigmented (colorless), if colored means the fungus is pigmented, generally it is melanin pigment bound to hyphae cell walls.

In Sastrahidayat (1986), the fungus growing on a PDA medium was initially white, then the color became cream or pale yellow. Under certain circumstances, it was pinkish-purple with the mycelium insulated and forming branches. The effect of light on the growth of fungal vegetative hyphae is usually in the form of inhibiting or triggering their growth so that light may affect the concentration of pigment production and hyphae growth. Generally, fungi grown in bright conditions will have more air mycelium than in other conditions. That is due to the nature of fungi that grow in the direction of light (phototropy).

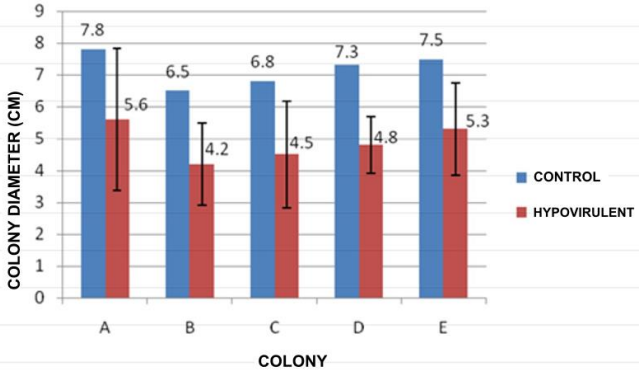


**Figure 1.** Colony diameter of *Fusarium* sp. isolates at the age of 7 days. Notes: A: Isolate colony, AB: Isolate colony, BC: Isolate colony, CD: Isolate colony, DE: Isolate colony E

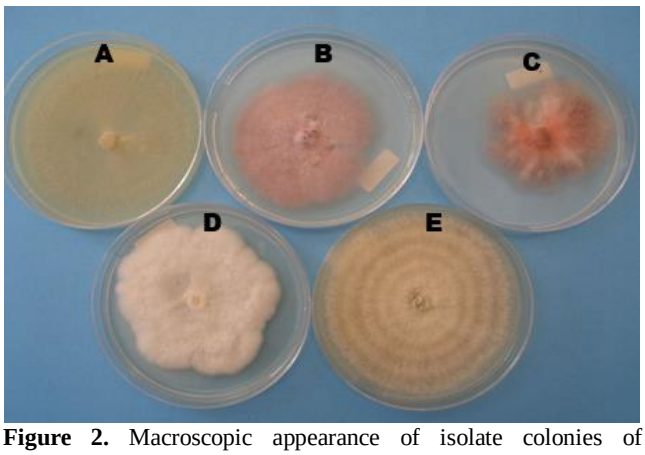


**Figure 2.** Macroscopic appearance of isolate colonies of *Fusarium* sp. Notes: A: Isolate colony, AB: Isolate colony, BC: Isolate colony, CD: Isolate colony, DE: Isolate colony E

**Microscopic observation of *Fusarium* sp.**

Observations of *Fusarium* sp. microscopically were done by observing the size and shape of the parts of *Fusarium* sp. and measuring the spore density of each isolate from each colony group (Figures 3 and 4).

Tables 2 and 3 show the differences in shape, size, conidia septa, and spore density in *Fusarium* sp. control and treatment isolates. In general, *Fusarium* sp. isolates microscopically have an ovoid microspore shape which generally has 0-1 septa, while macrospores generally have a long tapered tip and 2-6 septa.

Observation of the fungal isolates of *Fusarium* sp. is done by observing the size and shape of the conidia/spores of the isolates and the density of the spores of *Fusarium* sp. The colony measurements on control isolates showed that the longest macroconidia were found in the isolate colony of group C of 31.56x4.10 µm; 3-5 septa with microconidia size of 4.38x1.5 µm; 0-1 septa. Meanwhile, the smallest macroconidia size was found in the isolate of group B of 27.36x2.20 µm; 2-5 septa with microconidia size of 3.25x2.15 µm; 0-1 septa. On the other side, in the treatment isolates, the longest macroconidia size was found in the isolate of group D at 31.16x3.2 µm; 3-6 septa with microconidia size of 4.75x2.5 µm. Meanwhile, the smallest macroconidia were found in the colony of group B of 24.16x1.6 µm; 2-5 septa with microconidia size of 3.25x2.15 µm; 0-1 septa (Appendix 12). The size of microconidia and macroconidia in each isolate differed in control and treatment isolate colonies. Still, the general shape of macroconidia and microconidia was the same, particularly ovoid (microconidia) and elongated with tapered tips (macroconidia). In Domsch et al. (1993), macroconidium is spindle-shaped, oval, the sharp tip has 3-5 septa, and the size of the spores is [(20-27) - (46-60) x (3.5-4.5)] µm. Microconidia are arranged in 1 cell, transparent, in long basipetal chains. According to Agrios (1996), microconidium has one or two cells, is present in large numbers, and is often produced in all conditions. This type of spore is commonly found in infected plant tissues. Meanwhile, macroconidium has two to five cells and is curved. This type of spore is commonly found on the surface of plants that die due to this fungal infection.

Observation of spore density of *Fusarium* sp. isolates in the five groups of colonies was seen from the number of spores of each isolate. The spore density was observed by making a suspension for each isolate, particularly by diluting the mushroom culture with 10 mL of distilled water into a Petri dish with a diameter of 8 cm. First, the surface of the mushroom culture was scratched with an L glass for 5 minutes.

**Table 2.** Microscopic observation of *Fusarium* sp. control isolates

| Isolate | Conidia/spora | | Conidia septa | Sporulation |
|---|---|---|---|---|
| | Shape | Size | | |
| A0 | Ovoid (microconidia), macroconidia with a tapered tip | Microconidia of 3.75x2.5µm, Macroconidia of 29.76x3.12 µm | Macroconidia consists of 2-6 cells | $17.86x10^6$ |
| B0 | Ovoid (microconidia), macroconidia with a tapered tip | Microconidia of 5.25x1.87µm, Macroconidia of 27.36x2.20 µm | Macroconidia consists of 2-5 cells | $44.20x10^6$ |
| C0 | Ovoid (microconidia), macroconidia with a tapered tip | Microconidia of 4.38x1.5µm, Macroconidia of 31.56x4.10 µm | Macroconidia consists of 3-5 cells | $15.34x10^6$ |
| D0 | Ovoid (microconidia), macroconidia with a tapered tip | Microconidia of 5.35x3.15µm, Macroconidia of 27.36x4.10 µm | Macroconidia consists of 5-6 cells | $27.76x10^6$ |
| E0 | Ovoid (microconidia), macroconidia with a tapered tip | Microconidia of 3.05x1.75µm, Macroconidia of 30.16x3.26 µm | Macroconidia consists of 2-4 cells | $37.18x10^6$ |

Notes: A0: Control isolate colony A, B0: Control isolate colony B, C0: Control isolate colony C, D0: Control isolate colony D, E0: Control isolate colony E

**Table 3.** Microscopic observation of *Fusarium* sp. treatment isolates

| Isolate | Conidia/spora | | Conidia septa | Average sporulation |
|---|---|---|---|---|
| | Shape | Average size | | |
| A | Ovoid (mikrokonidia), makrokonidia with a tapered tip | Microconidia of 3.15x1.5µm, Macroconidia of 27.26x2.72 µm | Macroconidia consists of 2-5 cells | $15.94x10^6$ |
| B | Ovoid (mikrokonidia), makrokonidia with a tapered tip | Microconidia of 3.25x2.15µm, Macroconidia of 24.16x1.6 µm | Macroconidia consists of 2-5 cells | $42.76x10^6$ |
| C | Ovoid (mikrokonidia), makrokonidia with a tapered tip | Microconidia of 2.52x2.5µm, Macroconidia of 29.36x3.2 µm | Macroconidia consists of 2-4 cells | $13.82x10^6$ |
| D | Ovoid (mikrokonidia), makrokonidia with a tapered tip | Microconidia of 4.75x2.5µm, Macroconidia of 31.16x3.2 µm | Macroconidia consists of 3-6 cells | $25.64x10^6$ |
| E | Ovoid (mikrokonidia), makrokonidia with a tapered tip | Microconidia of 2.64x1.45µm, Macroconidia of 28.76x2.16 µm | Macroconidia consists of 2-6 cells | $36.25x10^6$ |

Notes: A: Isolate colony A, B: Isolate colony B, C: Isolate colony C, D: Isolate colony D, E: Isolate colony E

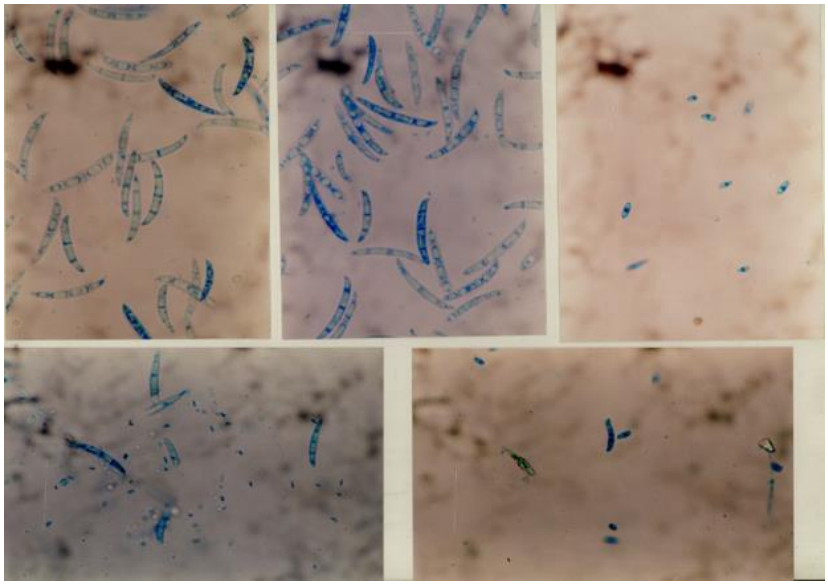

**Figure 3.** Microscopic appearance of *Fusarium* sp. isolates

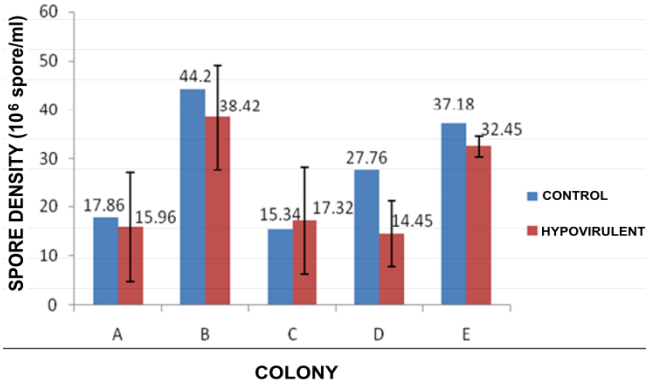


**Figure 4.** Spore density of *Fusarium* sp. isolates. Notes: A: Isolate colony A, B: Isolate colony B, C: Isolate colony C, D: Isolate colony D, E: Isolate colony E

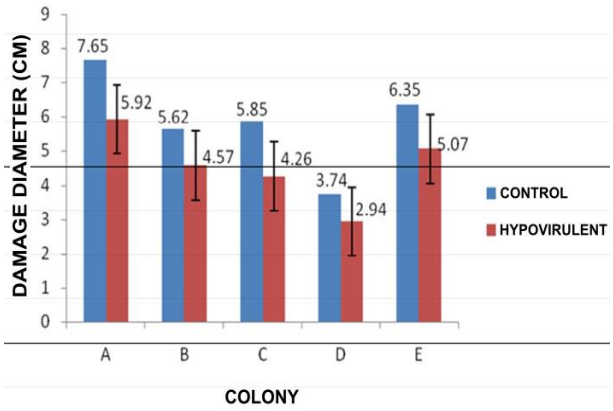


**Figure 5.** Diameter of apple damage on virulence test of *Fusarium* sp. Notes: A: Isolate colony A, B: Isolate colony B, C: Isolate colony C, D: Isolate colony D, E: Isolate colony E

After that, it was filtered and put into a prepared falcon. The suspension dilution was carried out three times. The results of this dilution were used to calculate the spore density of *Fusarium* sp. isolates. The highest spore density of control isolates was found in the isolate colony of group B at 72.38x$10^6$ spores/ml, while the lowest spore density was found in the isolate colony of group C, which was 15.34x$10^6$ spores/mL. On the other hand, in the treatment isolates, the highest spore density was found in the isolate colony of group B at 38.42x$10^6$ spores/mL. In contrast, the lowest density was found in the isolate colony of group D of 14.45x$10^6$ spores/mL (Tables 2 and 3).

Based on the results of observations of the spore density of *Fusarium* sp. isolates that have been obtained and identified, it can be seen that these isolates have spore density values that are almost the same. Specifically, the spore density in both control and treatment isolates has a spore density value ranging from $10^6$ spores/mL.

**Virulence test of *Fusarium* sp. isolates**

The isolates that had been identified macroscopically and microscopically were then tested for virulence to determine the ability of *Fusarium* sp. isolates to cause both symptoms and damage to apples. The parameter observed was the diameter of apple damage caused by *Fusarium* sp. isolates which had been successfully characterized macroscopically and microscopically (Appendix 13). The highest damage diameter in the control isolate's virulence test was found in the isolate colony of group A at 7.65 cm. The lowest was found in the isolate colony of group D at 3.74 cm. Likewise, for the diameter of damage to the treatment isolates, the highest diameter of damage in the isolate colony of group A was 5.92 cm. The lowest damage diameter was found in isolate D at 2.94 cm (Figure 5).

The results of measuring the diameter of the damage caused by the *Fusarium* sp. isolate on the virulence test showed that the isolate colony of group A had the

appearance of cream colony color, smooth colony surface, a little air mycelium formation, and hyaline mycelium appearance as well as fast colony growth rate with high colony diameter values, gave the most serious damage when tested for virulence on apples. In contrast, the isolate colony of group D had a slow colony growth rate with low diameter values. It appeared with pigmented colony colors (purple, pink), smooth colony surface, and medium air mycelium, which gave low damage during virulence tests on apples.

The virulence test of *Fusarium* sp. isolates was carried out to determine the ability of *Fusarium* sp. isolates isolated in artificial culture (PDA) to infect apples. Based on the observations, it was known that *Fusarium* sp. isolates in each colony had different levels of infecting ability. Boisson and Lahlou (1984) and Hadisutrisno (1987) stated that fungi treated with culture will lose their pathogenicity after being transferred several times in the medium or after the isolates have been stored for a long time. The exception is the hyaline variant, from which variant fungi can be obtained stable variants with weak or strong pathogenicity.

Based on the macroscopic, microscopic, and virulence testing of each colony group, it can be seen which isolates are virulent and which are hypovirulent. Isolates considered virulent were control isolates with high and fast growth rates and gave serious damage when virulence tests were carried out on apples. Meanwhile, isolates considered hypovirulent had a slow colony growth rate and gave low damage during the virulence test on apples. Hypovirulent isolates inoculated on apples showed minor disease symptoms; in contrast, control apples that had been inoculated with virulent isolates showed serious damage. According to Latterot (1982) cit. Hadisutrisno (2004), variants characterized by clear or hyaline mycelium with air mycelium, such as cotton, are called *une souche faible* or weak isolates, which are identified with avirulent isolates, while variants that have thin air mycelium are identified with strong isolates or virulent isolate. Avirulent isolates differed from virulent isolates in terms of morphology, the growth rate of macro-microconidium production, and protein content (Susanto, 2000). In addition, the mechanism that causes pathogens to turn non-pathogenic is caused by biochemical changes in these non-pathogenic strains, particularly reduced production of extracellular pectic lyase enzymes, decreased polygalacturonase activity, and deficient secretion of extracellular enzymes (Yamaguchi et al. 1992).

Related to biological control mechanisms based on antagonistic microbes, it can occur directly (competition and antibiosis) or indirectly with host-induced resistance. Induced resistance is resistance that develops after plants are inoculated early with biotic elicitors (avirulent, non-pathogenic, saprophytic microorganisms) and abiotic elicitors (salicylic acid, 2-chloroethyl phosphonic acid) (Agrios 1996). For example, hypovirulent fungi can be used in the biological control of virocontrol mechanisms with mycoviruses, which is by utilizing the viruses present in fungi that play a role in weakening the growth of the fungus so that the growth of the fungus is inhibited. Meanwhile, the control mechanism of induced resistance is resistance that develops after plants are inoculated early with biotic elicitors (avirulent, non-pathogenic, saprophytic microorganisms) and abiotic elicitors (salicylic acid, 2-chloroethyl phosphonic acid) (Agrios 1996). For example, suppose a certain part of the plant or fruit is inoculated with a pathogen or a weak pathogen. In that case, a reaction will occur in the plant body that produces a defense system so that the plant will be resistant to attacks by the same pathogen that is more virulent or even resistant to other pathogens. The occurrence of resistance induction due to local infection is considered to be caused by the presence of infected plant cell fluids that do not have sufficient or suitable food supplies or because the infected plants form toxic compounds that inhibit the development of pathogens (Caruso and Kuc 1979 cit. Hadisutrisno 2004).

Based on the research that has been done, it can be seen: (i) *Fusarium* sp. isolates on red chili plants from Boyolali that have been successfully isolated, obtained five groups of isolate colonies. (ii) The colony group of *Fusarium* sp. isolates that had a high virulence level was the isolate colony of group A, while the biological character of isolate A was cream-colored mycelium, long macroconidia shape; tapered tip, macroconidia with 2-6 septa, microconidia with 0-1 septa, conidia size of [(3.15 x 1.5) - (3.75 x 2.5)] µm x [(27.26 x 2.72) - (29.76 x 3.12)] µm, spore density between ($15.94x10^6$-$17.86x10^6$ ) spores/ml. (iii) The colony group of *Fusarium* sp. isolates which had low virulence was the isolate colony of Group D, while the biological character of isolate D was white mycelium like cotton, long macroconidia shape; tapered tip, macroconidia with 3-6 septa, microconidia with 0-1 septa, conidia size of [(4.75 x 2.5) - (5.35 x 3.15)] µm x [(31.16 x 3.2) - (27.36 x 4.10)] µm, the spore density was between ($25.64x10^6$-$27.76x10^6$ ) spores/ml. (iv) The isolate colony of group D had the potential as hypovirulent isolates that can be developed as environmentally friendly biological control agents, particularly by the induced resistance method.

## REFERENCES

Agrios GN. 1996. Ilmu Penyakit Tumbuhan. Penerjemah: Busnia M. Universitas Gadjah Mada Press, Yogyakarta. [Indonesian]

Barnet, Hunter. 1988. Illustrated Genera of Imperfect Fungi. Burgress Publishing Company, West Virginia.

Boisson C, Lahlou H. 1982. Etude du polymorphisme intraclonal chez le Verticillium alboatrum, forme a microsclerotes. II. Morphologie et morphogenese comparees de deux isolats de phenotype sauvage et de leurs principaux variants. Can J Bot 61: 188196. DOI: 10.1139/b83-020.

De Cal A, Garcia-Lepe R, Melgarejo P. 2000. Induced resistance by Penicillium oxalicum against *F. oxysporum* f.sp. *lycopersici*: Histological studies of infected and induced tomato stem. Phytopathology 90: 260-268. DOI: 10.1094/PHYTO.2000.90.3.260.

Domsch KH, Anderson TH, Gams W. 1993. Compendium of Soil Fungi. Vol I. IHW-Velag.

Gandjar I. 1999. Pengenalan Kapang Tropik. Yayasan Obor Indonesia, Depok. [Indonesian]

Gunawan AW, Dharmaputra OS, Rahayu G. 2004. Cendawan dalam Praktik Laboratorium. IPB Press, Bogor. [Indonesian]

Hadisutrisno B. 1987. Etude de la variabilite intraclonale du pouvoir pathogene du Verticillium dahlia Klebahn vis-à-vis de la tomate et du cotonnier. These de Docteur Ingenieur de l'Ensam. Unpublished.

Hadisutrisno B. 2004. Pemanfaatan Isolat Avirulen Dalam Pengendalian Penyakit Tanaman. Disampaikan pada pertemuan teknis Pengembangan Teknologi Perkebunan Regional Kalimantan, 25-28 Agustus di Pontianak. Fakultas Pertanian, Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Harpenas A, Dermawan R. 2010. Budidaya Cabai Unggul. Penebar Swadaya, Jakarta. [Indonesian]

Huda M. 2010. Pengendalian Layu *Fusarium* pada Tanaman Pisang (*Musa paradisiaca* L.) secara Kultur Teknis dan Hayati. [Skripsi]. Fakultas Pertanian, Institut Pertanian Bogor, Bogor. [Indonesian]

IstikoriniY. 2002. Pengendalian Penyakit Tumbuhan Secara Hayati Yang Ekologis Dan Berkelanjutan. Makalah Falsafah Sains (PPs 702) Program Pasca Sarjana / S3 Institut Pertanian Bogor, Bogor. [Indonesian]

Machmud M, Suhendar MA, Suryadi Y, Jumanto, Sudjadi M. 2003. Seleksi dan Karakterisasi Patogen Tanaman. http://biogen.litbang.deptan.go.id/terbitan/prosiding/fulltext_pdf. Diakses pada tanggal 10 Mei 2009. [Indonesian]

Samoosir J. 2007. Inventarisasi Penyebab Penyakit Pada Tanaman Stroberi (*Vragaria vesca* L.) di Kecamatan Bertasgi. [Skripsi]. Fakultas Pertanian, Universitas Sumatra Utara, Medan. [Indonesian]

Sastrahidayat IR. 1986. Ilmu Penyakit Tumbuhan. Usaha Nasional, Surabaya. [Indonesian]

Semangun H. 1996. Pengantar Ilmu Penyakit Tumbuhan. Universitas Gadjah Mada Press, Yogyakarta. [Indonesian]

Semangun H. 2005. Penyakit-penyakit tanaman pangan di indonesia. Universitas Gadjah Mada Press, Yogyakarta. [Indonesia]

Supriyati, Roosganda E. 2009. Pensejahteraan Petani dan Pengembangan Agribisnismelalui Pengembangan Kelembagaan Kemitraan dalam Pemasaran Cabai Merah. Seminar Nasional Peningkatan Daya Saing Agribisnis Berorientasi Kesejahteraan Petani. Bogor. [Indonesian]

Susetyo, AP. 2010. Hubungan Keanekaragaman Cendawan Rizosfer Tanaman Pisang (*Musa* spp.) dan Penyakit Layu Fusarium. [Skripsi]. Fakultas Pertanian, Institut Pertanian Bogor, Bogor. [Indonesian]

Yamaguchi K, Kida, Arita M, Takahashi M. 1992. Induction of systemic resistance by *Fusarium oxysporum* MT0062 in solanaceous crops. Ann Phytopath Soc Japan 58: 16-22. DOI: 10.3186/jjphytopath.58.16