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Antagonistic Test of *Bacillus* spp. against *Fusarium* sp., the Causal Agent of Wilt Disease of Red Chili Plants

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ABSTRACT

Wilt disease caused by *Fusarium oxysporum* is one of the diseases affecting chili plants. The utilization of *Bacillus* spp. can be a solution for plant disease control because it can colonize plants and produce useful microbe compounds to inhibit the development of plant pathogen. This study aimed to determine the ability of *Bacillus* spp. to suppress the growth of *Fusarium* sp. isolates from chili plants in vitro. *Bacillus* sp. isolates Ba-6, Ba-9, Ba-12, Ba-15, Ba-17, and a control (aquadest) were used as treatments, each replicated four times. The observation parameters were the inhibition zone test and microscopic observations of the morphology of *Fusarium* sp. after the inhibition zone test. The highest inhibition was shown in the treatment of *Bacillus* sp. isolate Ba-15, which was 20.02%. The observation of *Fusarium* sp. hyphae after the inhibition zone test showed abnormal growth, which was different for each isolate. Some hyphae were bent, coiled, shrunken, swollen, curled, or lysed.

Keywords: *Bacillus* spp., *Fusarium* sp., antagonist bacteria, inhibition

Uji kemampuan antagonistik *Bacillus* spp. terhadap patogen *Fusarium* sp. penyebab penyakit layu pada tanaman cabai merah

ABSTRAK

Penyakit layu fusarium merupakan salah satu penyakit pada tanaman cabai yang disebabkan oleh jamur *Fusarium oxysporum*. Pemanfaatan *Bacillus* spp. dapat menjadi solusi pengendalian penyakit tanaman karena dapat mengolonisasi tanaman dan menghasilkan senyawa antimikroba yang berguna untuk menghambat perkembangan patogen penyebab penyakit tanaman. Penelitian ini bertujuan untuk mengetahui kemampuan *Bacillus* spp. dalam menekan pertumbuhan *Fusarium* sp. isolat cabai secara *in vitro*. Penelitian ini menggunakan bakteri *Bacillus* sp. isolat Ba-6, Ba-9, Ba-12, Ba-15, Ba-17, dan kontrol (akuades) sebagai perlakuan serta di ulang sebanyak empat kali. Parameter pengamatan yaitu uji daya hambat dan pengamatan morfologi *Fusarium* sp. dari uji daya hambat secara mikroskopis. Hasil uji daya hambat tertinggi ditunjukkan pada perlakuan *Bacillus* sp.isolat Ba-15 yaitu sebesar 20,02 %. Pengamatan hifa *Fusarium* sp. pasca uji daya hambat menunjukkan pertumbuhan abnormal, yang masing-masing isolat berbeda, ada yang membengkok, melilit, mengecil, membengkak, mengeriting atau lisis.

Kata Kunci: *Bacillus* spp., *Fusarium* sp., bakteri antagonis, daya hambat

INTRODUCTION

Fusarium spp. is a soil-borne pathogen that causes wilt disease in many plants including chili. The species of *Fusarium oxysporum* is known to attacks the roots particularly through wounds and causing infected plant stems turning brown and losing a lot of fluids (Raharini, *et al*., 2012). This fungus infect all plant stages from the germination phase to maturity and causes crop losses of up to 50%.

Currently, synthetic fungicides are still being utilized to control fungal diseases. However, their prolonged usage can lead to pathogen resistance, destruction of useful microorganisms, and leave behind chemical residues in the environment and foods (Palmieri *et al*., 2022). To overcome this issue, the utilization of *Bacillus* spp. bacteria to control *Fusarium* sp. fungus is a promising alternative that can potentially reduce the dependence on synthetic fungicides

The use of *Bacillus* spp. as an option for antagonist testing because it is easy to formulate and can relatively colonize plants. According to Djaenuddin, (2018) colonialization that occurs in roots can also trigger plants to produce jasmoic acid and plant ethylene to induce plant resistance to pathogens. In previous study bacterial isolates of *Bacillus* sp. isolated from chili plant from Kandat, Kediri East Java were shown to be able to suppress the pathogen

Ralstonia solanacearum by forming inhibition zones of varying sizes (Prasetyawati & Wiyatiningsih, 2020). Those isolates were also capable of causing abnormal *Phytophthora palmivora* hyphae (Anjarsari *et al*., 2022). This study was conducted to determine the ability of *Bacillus* spp. in controling the fungal pathogen pathogen *Fusarium* sp. of chili isolate *in vitro*.

MATERIALS AND METHODS

Experimental Design

The research was conducted in October 2022 at the Laboratory of Plant Protection, Faculty of Agriculture, UPN "Veteran" East Java. The research design was Completely Randomized Design (CRD) with six treatments, namely control and five isolates of *Bacillus* spp. with isolate codes of Ba-6, Ba-9, Ba-12, Ba-15, and Ba-17. All the treatments were replicated 4 times so that gave a total trial of 24 units.

Isolation of Fusarium sp.

Fusarium sp. was isolated from pieces of chili plants with wilt symptom. The base of the infected chili stem was cut into a size of 0.5 x 0.5 cm^2, dipped in 70% alcohol for 1 minute and then rinsed with sterile distilled water 3 times. Stem pieces were grown on PDA medium in petri dishes and incubated at room temperature for 7 days (Sudantha & Abadi, 2007).

Fusarium sp. macroscopic observations based on the morphologycal characteristic mycelium or hyphae. While microscopic observations were done by observing the shape of the conidia, hyphae, and the characteristics of the conidiophores using an Olympus microscope.

Recultured of Bacteria Bacillus spp.

Bacterial isolates was recultured by scraping the ose needle of bacterial isolates *Bacillus* spp. on the nutrient agar medium in Petri dish. Bacterial isolates to be used for testing incubated at room temperature for 24 hours.

Antagonistic Test of Bacillus spp. against Fusarium sp. in Vitro

The antagonistic test used by dual culture method by growing *Fusarium* sp. together with *Bacillus* sp. on PDA medium. Isolate *Fusarium* sp. (7 days old culture) was perforated using a cork borer (diameter of 0.5 cm) and it was placed 3 cm from the edge of the petri dish. For bacterial isolates, filter paper with a diameter of 0.5 cm was soaked in the antagonist suspension (10^8 cells/ml) for 30 minutes and it was placed at a distance of 3 cm from the pathogenic fungus (Flori *et al*., 2020)

Observations of the antagonist test were carried out every day until forth days by measuring the radius of the pathogenic fungus colony. Calculation of the percentage of inhibition can be calculated by the formula according to Oktania & Asniwita, (2018) :

$$DH = \frac{R1-R2}{R1} \times 100\ \% \quad \ldots (1)$$

Description :
DH = Percentage of inhibition (%)
R1 = Radius of pathogenic fungal colonies away from antagonistic bacteria
R2 = Radius of pathogenic fungal colonies approaching antagonist bacteria

Microscopic Observation of Fusarium sp. in Antagonist Test

This observation was aimed to determine the effect of giving antagonistic bacteria that cause pathogenic malformations after the antagonist test. Hyphae of *Fusarium* sp. in the inhibition zone observed on an Olympus microscope.

Data Analysis

Data were analyzed using *Analysis of Variance* (ANOVA) to determine whether there was an effect of each treatment. If there was a significant difference, a Tukey test or *Honestly Significant Difference* (HSD) 5% follow-up test was carried out.

RESULT AND DISCUSSIONS

Isolation of *Fusarium* sp.

Fusarium sp. isolated from chili plants showing symptoms of fusarium wilt had white colonies with a yellowish lower surface and, the mycelia was textured like cotton (Figure 1.a). *Fusarium* sp. colonies. has an uneven rim shape, yellowish white bottom color of the colony, the direction of mycelium growth is sideways, the mycelium structure is smooth (Sholihah *et al*., 2019).

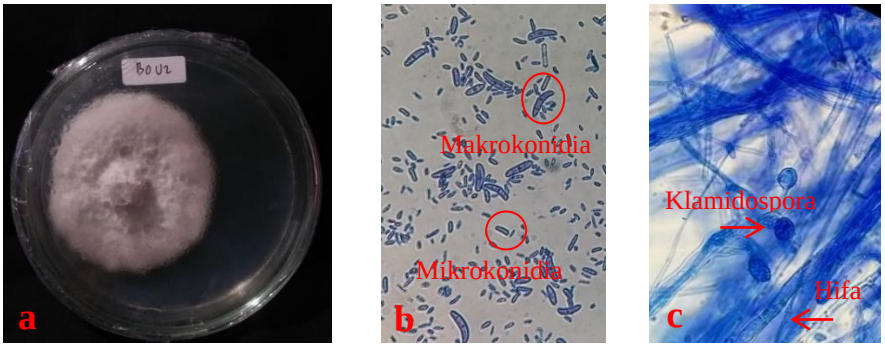

Figure 1. Morphology of *Fusarium* sp. (a) Colony of *Fusarium* sp.,(b) Conidia, (c) Hyphae

The results of microscopic observations of *Fusarium* sp. showed that the macroconidia are crescent-shaped and septum, the ends of the microconidia were rounded (Figure 1.b). *Fusarium* sp. had straight and septum hyphae, while chlamydospores were also found at the tips of the hyphae which were round in shape (Figure 1.c). According to Sari, *et al*., (2017) microconidium *F. oxysporum* is shaped like an oval or kidney-shaped, and there are single or branched fialids, while chlamidospora is round, thick-walled, formed at the end of the hyphae. *Fusarium* sp. which had been observed was then subjected to a Koch's postulate test on chili plants. The results showed the symptoms of fusarium wilt in chili plants then it confirmed that the isolated *Fusarium* sp. was pathogenic.

Antagonism of *Bacillus* spp. Against *Fusarium* sp. in *Vitro*

The results of the analysis of variance showed that there was a significant effect on some of *Bacillus* spp. as seen from the percentage of inhibition at the age observation time of 4 days after inoculation (DAI) which is presented in (Table 1). Mean while, the treatment that had the same notation showed no significant effect. The lowest percentage of inhibition is 0,00 %, namely the control treatment without *Bacillus* sp. so that there was no inhibition. Treatment of *Bacillus* sp. Ba-15 had the highest percentage of inhibition of 20,02 %. *Bacillus* sp. isolate Ba-15 was not significantly different from other *Bacillus* sp. isolates because it had the same notation. Putro, *et al*., (2014) stated that the differences in inhibition of each biological agent isolate are suspected to have different inhibition mechanisms from each other.

Table 1. Mean Percentage of Inhibition of *Bacillus* spp. against *Fusarium* sp. *in Vitro* 4 DAI

Treatment	Percentage of inhibition (%)
Control	0,00 **a**
Bacillus sp. Ba-6	5,48 **ab**
Bacillus sp. Ba-9	11,55 **ab**
Bacillus sp. Ba-12	5,57 **ab**
Bacillus sp. Ba-15	20,02 **b**
Bacillus sp. Ba-17	7,59 a**b**
HSD 5%	15,84

Note: Numbers with the same letter notation in the same column show no significant difference in the HSD 5% test

Nevertheless, antagonistic test of *Bacillus* spp.on *Fusarium* sp. in this study showed low age inhibition percentage (Table 1). Previous study conducted by Anjarsari *et al*. (2022) using same *Bacillus* spp. isolates were able to inhibit *Phytophthora palmivora* with higher inhibition percentage of more than 30%. The results of this low inhibition percentage could be affected by several factors such as differences in environmental pH, incubation period, size of inoculum, metabolic activity of bacteria, and stability of active substances (Dutta *et al*., 2013). Other factors can also occur because not all fungi can be inhibited by the secondary metabolites produced by *Bacillus* spp. (Afifah, 2017).

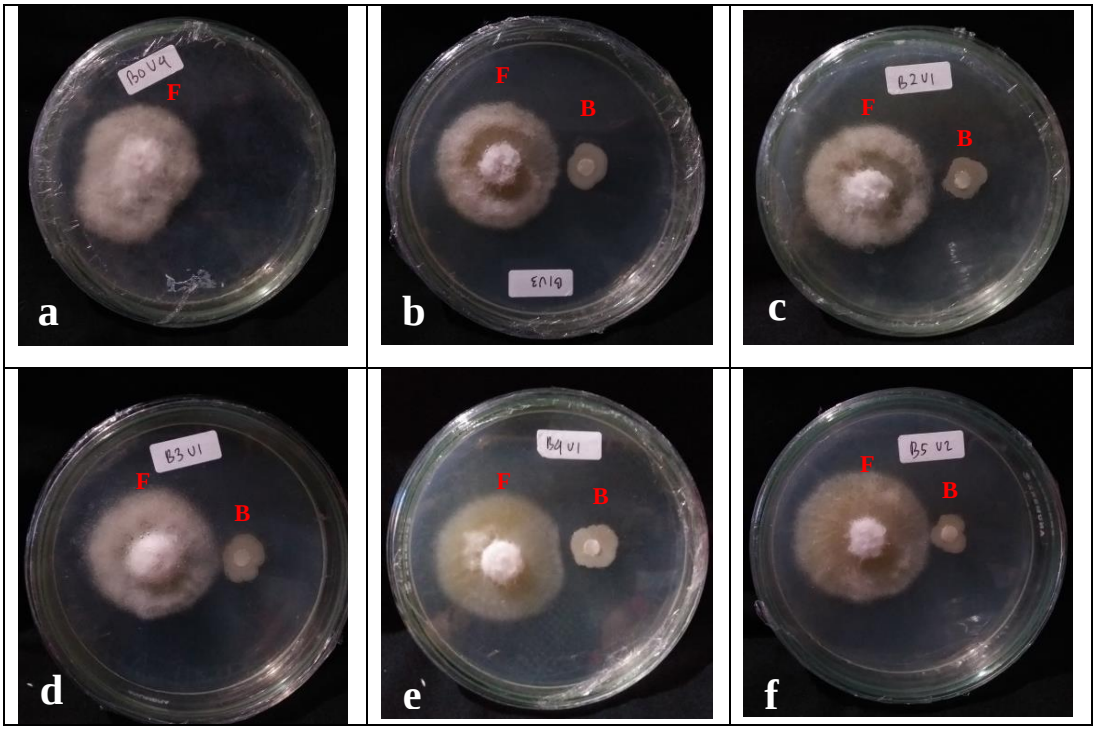

Figure 2. Antagonistic Test of *Bacillus* spp. against *Fusarium* sp. on PDA media at 4 DAI (a) Control, (b) *Bacillus* sp. Isolate Ba-6, (c) *Bacillus* sp. Isolate Ba-9, (d) *Bacillus* sp. Isolate Ba-12, (e) *Bacillus* sp. Isolate Ba-15, (f) *Bacillus* sp. Isolate Ba-17. Note : B = *Bacillus* spp., F = *Fusarium* sp.

Observations of antagonist tests was carried out up to 4 DAI to avoid overlapping between *Bacillus* sp. and *Fusarium* sp. if observed for a prolonged duration. Observations showed the formation of inhibition zone between bacteria and pathogens. Each treatment of *Bacillus* sp. Ba-6, Ba-9, Ba-12, Ba-15, Ba-17 had different inhibition level, indicated by the size of the formed inhibition zone (Figure 2). The clear zone is considered to occur due to the production of antifungal compounds produced by bacteria that can reduce the population of plant pathogens through competition and production of antifungal compounds (Bawantari *et al*., 2020).

The Morphology of *Fusarium* sp. in the Antagonistic Test

Microscopic observation of *Fusarium* sp. hyphae in the in *vitro* test showed that the hyphae of *Fusarium* sp. experiencing abnormal growth so that it did not develop properly. Hyphae *Fusarium* sp. in the treatment of *Bacillus* spp. isolate Ba-6 was swelled and some hyphae appeared to shrink (Figure 3.b), treatment with isolate Ba-9 caused hyphae of *Fusarium* sp. were shrinking and bent (Figures 3.c and 3.d), while the Ba-12 isolate caused *Fusarium* sp. hyphae was convolved each other (Figure 3.e). The Ba-15 isolate treatment caused *Fusarium* sp. hyphae bent, curled and lysed (Figures 3.f and 3.g), while Ba-17 isolate treatment caused *Fusarium* sp. hyphae was lysis (Figure 3.h). Research by (Anjarsari *et al*., 2022) with the same isolates of *Bacillus* spp. was also resulted in the abnormality of the *Phythopthora palmivora* hyphae due to the content of amylase, protease, and cellulase enzymes in *Bacillus* spp. which has been tested for extracellular enzyme activity. It is known that amylase enzyme has the ability to break down pathogenic cell walls (Muis, 2016), while protease enzyme is able to break down protein compounds that are in the cell walls of pathogenic fungi (Singh & Chhatpar, 2011), Cellulase enzyme can lyse cellulose compound, in which cellulose compound is a compound that makes up fungal cell walls (Semêdo *et al*., 2004).

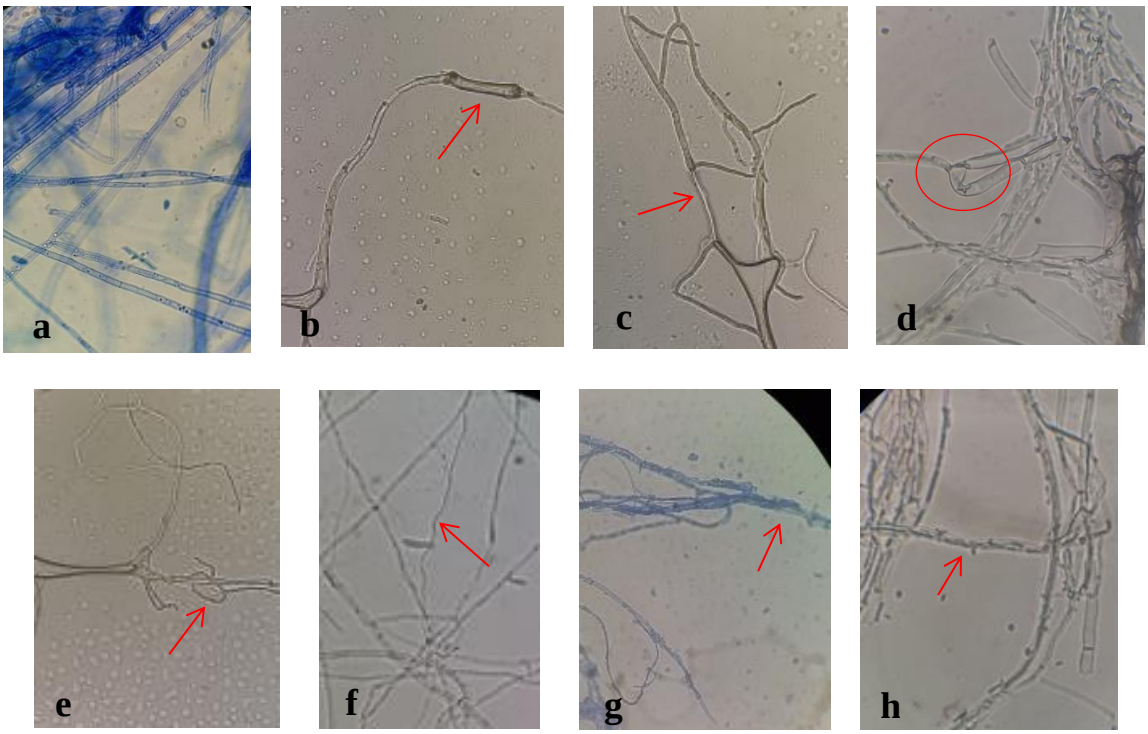

Figure 3 Hyphae *Fusarium* sp. Post In vitro Antagonist Test Abnormal (a) Control, (b) Swelling, (c) Shrinking, (d) Bending, (e) Twisting, (f) Curling, (g,h) Lysis.

Control mechanism of *Bacillus* spp. against *Fusarium* sp. by competition and antibiosis. The results of the antagonist test showed the appearance of an inhibition zone although it was classified as low, but on microscopic observation there was abnormal hyphae. Abidin *et al*., (2015) stated that *Bacillus* spp. also produces chitinase enzyme compounds that cause lysis of pathogenic cell walls. The chitinase enzyme works by degrading the chitin component which is a constituent of the cell walls of the fungi *Fusarium oxysporum* and *Scelrotium rolfsii* (Raaijmakers *et al*., 2010). *Bacillus* sp. had antifungal compounds, but not all of them can inhibiting fungi, because each isolate produces different types and amounts of secondary metabolites (Afifah, 2017).

CONCLUSIONS

Treatment of *Bacillus* spp. against the pathogen *Fusarium* sp. produced different inhibition with the highest percentage by the treatment of *Bacillus* sp. isolate Ba-15 of 20,02 %. The hyphae of *Fusarium* sp. in the inhibition test showed abnormal growth, namely bending, twisting, shrinking, swelling, curling and lysis.

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