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Effect of *Gliocladium* sp. and mung bean sprouts extract (*Vigna radiata* (L.) R. Wilczek) induction on Fusarium wilt in tomato plants (*Solanum lycopersicum* L.)

Nashaza Aishwardani[1]*, Bambang Irawan[1], Rochmah Agustrina[1], Yulianty[1]
Department of Biology, Faculty Mathematics and Natural Science, University of Lampung, Jl. Prof. Dr. Ir. Sumantri Brojonegoro No.1, Kota Bandar Lampung, Lampung, 35141.
Corresponding Author: aishwardani1709@gmail.com

Abstract: *Tomatoes (Solanum lycopersicum L.) are widely cultivated due to high demand. However, tomato cultivation still encounters many obstacles, one of which is Fusarium oxysporum infection, which causes wilt in plants. Controlling wilt disease using chemical fungicides harms the environment, so other alternatives are needed, such as utilizing biological agents. Gliocladium sp. is known to inhibit the growth of F. oxysporum through several mechanisms. Efforts to accelerate plant recovery can be made by applying growth regulators. Natural growth regulators in mung bean sprouts extract (Vigna radiata (L.) R. Wilczek) are known to contain the hormones auxin, gibberellin, and cytokinin. This study aims to determine the effect of the combination of Gliocladium sp. and mung bean sprouts extract in inhibiting F. oxysporum and increasing plant growth and find the right dose combination of Gliocladium sp. and mung bean sprouts extract in inhibiting F. oxysporum and increasing tomato plant growth. This study used a randomized design with seven treatments and three replications. ANOVA results at the α = 5% level proved that the induction of Gliocladium sp. and 60% mung bean sprouts extract in vivo had a significant effect on disease incidence, disease severity, plant height, leaf area, dry weight, and chlorophyll content with the best dose at plant height, leaf area, dry weight, and chlorophyll content with the best dose in the P_{G30T} treatment.*

Keywords: *Fusarium oxysporum, Gliocladium* sp., *mung bean sprouts extract, tomatoes*

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I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a horticultural commodity that is widely consumed because it contains vitamins and minerals that are good for health, so it is widely cultivated in Indonesia (Marina & Sukmawati, 2017; Suleman *et al*., 2022). Tomato cultivation still encounters several challenges, including a pathogenic fungal infection. One of the pathogenic fungi that inhibit the growth of tomato plants is *Fusarium oxysporum*. Fungal pathogen infection in plants can occur through wounds on the roots and then spread to the stem, leaves, flowers, and fruit. Tomato plants infected with *F. oxysporum* show symptoms of discoloration of roots and stems to brown, yellowing leaves, wilting plants, decreased fruit production, and finally plant death (Renu, 2018). Disease control in plants is generally carried out using chemical fungicides containing active ingredients to control the growth and spread of fungi (Huang *et al*., 2020). Chemical fungicides are considered effective in controlling fungal infections of plant pathogens, but their long-term use harms the environment and creates resistance to pathogenic strains (Jaihan *et al.*, 2018). Another effort to control plant pathogens is to use biological agents in the form of endophytic fungi that are more environmentally friendly (Gu *et al.*, 2020), one of which is *Gliocladium* sp.

Gliocladium sp. has antagonistic characteristics against pathogenic fungi. *Gliocladium* sp. antagonism inhibits pathogenic fungi growth through competition, parasitism, and antibiosis mechanisms (Fadiji & Olubukola, 2020). *Gliocladium* sp. can synthesize antibiotic compounds including gliotoxin, gliovirin, and viridin. Gliotoxin can inhibit the mycelial growth of pathogenic fungi (Kalimutu *et al.,* 2020), gliovirin can agglutinate the cytoplasm of pathogenic fungi and cause fungal cell wall damage, while viridin can inhibit spore germination of pathogenic fungi such as *Fusarium* sp. (Vinale *et al.,* 2014). Previous research proved that *Gliocladium* sp. can inhibit the growth of pathogenic fungi that cause infections in plants Ramadhina *et al.* (2013).

Efforts to increase the growth of plants infected with pathogens can be made by giving natural or synthetic growth regulators. Natural growth regulators can come from mung bean sprouts extract, which is known to contain the hormones auxin 1.68 ppm, gibberellin 29.94 ppm, and cytokinin 96.26 ppm (Ulfa, 2014). In addition

to growth hormones, mung bean sprouts extract contains secondary metabolite compounds flavonoids, saponins, and triterpenoids (Moniharapan *et al.*, 2018). The research results by Aunila (2022) prove that giving 60% mung bean sprouts extract as much as 15 ml to chili plants can increase chlorophyll content.

Based on the description above, this study used a combination of *Gliocladium* sp. and mung bean sprouts extract to inhibit the growth of *F. oxysporum* and spur the growth of tomato plants. Double application is expected to increase the efficiency of plant protection and plant growth.

II. EXPERIMENTAL PROCEDURE

This research was conducted in December 2023 - March 2024 at the Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung.

Propagation of *Gliocladium* sp.

Isolates were propagated using rice media. 500 g rice was soaked in water for 2 hours and then drained. The rice was then steamed for 15 minutes. The steamed rice was placed on a tray to cool. After cooling, the rice was put into heat-resistant plastic, each containing 150 g, and then sterilized using an autoclave for 15 minutes at 1 atm pressure with a temperature of 121 ^{0}C. *Gliocladium* sp. isolates rejuvenated for seven days were inoculated using an ose needle on rice media. Then, the media was incubated for 14 days at room temperature, and then the spore density was calculated. If the spore density has reached 10^6, then the media can be applied (Afriani *et al.*, 2019).

Preparation of *Fusarium oxysporum* suspension

F. oxysporum culture was added to 10 ml of sterile distilled water and then scraped until the top was detached. The suspension was put into a test tube and then vortexed to separate the spores from the mycelium. Then 1 ml of suspension was taken using a micropipette and dripped on the surface of the haemocytometer. The number of spores was counted under a microscope at 400 magnification times. The density used for application is 10^6 spores/ml (Sujadmiko, 2012).

Preparation of mung bean sprouts extract

Mung bean seeds were soaked in water for 24 hours, then drained and placed on a tray covered with a damp towel, then the mung bean seeds were placed in a dark place and given water using a sprayer for 2-3 days to keep them moist. Mung bean sprouts as much as 500 g were mashed in 500 ml of distilled water using a blender and then filtered with a sterile cloth to get 100% mung bean sprouts extract (Latunra *et al*., 2020). Mung bean sprouts extract with a concentration of 60% was obtained by dissolving 60 ml of 100% mung bean sprouts extract into 40 ml of distilled water (Jariah, 2022).

Induction test of the combination of *Gliocladium* sp. and mung bean sprouts extract *in vivo*

Fusarium oxysporum infection was carried out through the planting media by sprinkling 10 ml of *F. oxysporum* suspension. Watering the *F. oxysporum* suspension was done 14 days before planting (Rahayu, 2020). *Gliocladium* sp. was applied with treatment doses (12 g, 18 g, 24 g, and 30 g). *Gliocladium* sp. propagation media was spread on the planting media seven days before planting the seedlings (Ramadhani *et al*., 2013). Mung bean sprouts extract was sprayed using a sprayer on the lower part of the leaves and watered near the roots as much as 15 ml/plant. The application of mung bean sprouts extract was carried out when the tomato plants were seven hst, then repeated every seven days (Berlintina *et al*., 2020; Pamungkas & Rudin, 2020).

***Gliocladium* sp. antagonist test in vitro**

The antagonist test was conducted using the dual culture method. *Gliocladium* sp. and *Fusarium oxysporum* isolates were placed on petri dishes containing PDA media. Each isolate was placed on the edge of the petri dish at a distance of 3 cm. One Petri dish was inoculated using *F. oxysporum* isolate to serve as a control. The culture was incubated at room temperature, and the growth of the two fungi was observed by measuring the diameter of their growth. The results of the antagonist test were described descriptively (Kalimutu *et al.*, 2020).

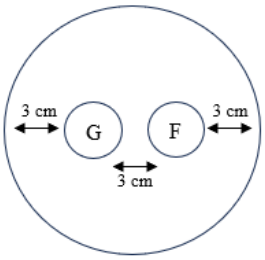


Figure 1. Illustration of antagonistic test of dual culture method *in vitro* (Kalimutu *et al.*, 2020).

Observation

Observations of *F. oxysporum* growth inhibition include the incubation period. The incubation period was calculated from the time after inoculation of *F. oxysporum* suspension on tomato plants until the first symptoms of Fusarium wilt appeared (Purwantisari *et al.*, 2016). The growth variables observed were leaf area and chlorophyll content. Leaf area was assessed 28 days after planting by weighing all leaves, taking a 2x2 cm sample, and weighing it. Dry weight was determined by oven-drying the plants at 80°C for 48 hours and then weighing them. Chlorophyll content was measured by extracting 0.1 g of fresh leaves with 10 ml of 95% ethanol, filtering, and analyzing the extract with a spectrophotometer at 648 nm and 664 nm (Miazek, 2002).

Data analysis

Data on the inhibition of *Gliocladium* sp. against the growth of *F. oxysporum in vitro* were presented in descriptive form. The test of *Gliocladium* sp. and mung bean sprouts extract *in vivo* were analyzed using analysis of variance (ANOVA). Then to determine the differences between treatments Tukey test was conducted with α = 5%

III. RESULTS AND DISCUSSIONS

3.1. *Gliocladium* sp. antagonist test *in vitro*

The results of the *in vitro* study showed that *Gliocladium* sp. was able to inhibit the growth of *F. oxysporum*, as seen from the clear zone between the two fungi. *Gliocladium* sp. grew more rapidly than *F. oxysporum*. The inhibition percentage of *Gliocladium* sp., which was included in the high inhibition percentage of 87.5%, is shown in Fig. 2.

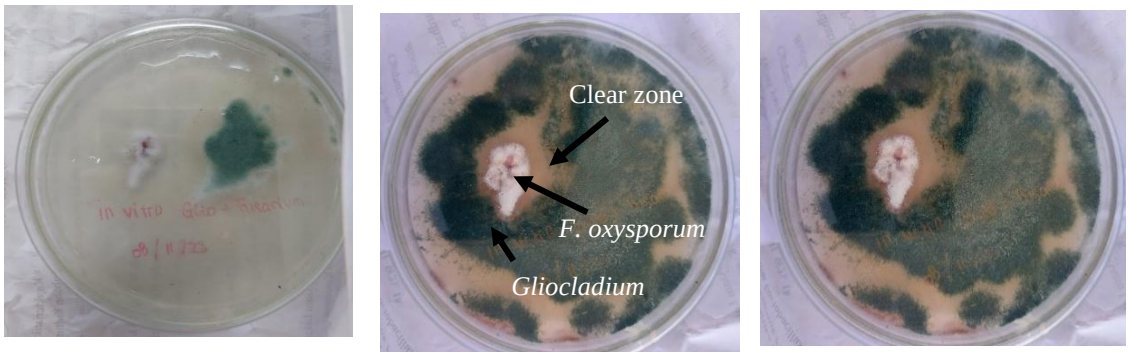


Figure 2. Inhibition test results of *Gliocladium* sp. against *F. oxysporum in vitro*. a) 3 days post-incoulation; b) 5 days post-incoulation; c) 7 days post-incoulation

Based on observations (Figure 2) After three days of inoculation, *Gliocladium* sp. outpaced *F. oxysporum* in terms of growth. By day five, *F. oxysporum* growth was suppressed by *Gliocladium* sp., creating a clear zone separating the isolates. *Gliocladium* sp. had a diameter of 9 cm at seven days post-inoculation, whereas *Fusarium oxysporum* diameter was only 1 cm. This aligns with Kalimutu *et al.* (2020), who state that *Gliocladium* sp. secretes gliotoxins, forming clear zones by inhibiting pathogenic fungi. *Gliocladium* sp. surpassed *F. oxysporum* in the

competition for nutrients and space because of its quicker growth in the PDA medium. As supported by Ropalia (2017) and Octriana (2011), the rapid growth of *Gliocladium* sp. on PDA media made it dominant, outcompeting *F. oxysporum* for space and nutrients. *Gliocladium* sp. might inhibit *F. oxysporum* growth by competing with it for resources and space. Pathogenic fungi are prevented from growing because of the antagonistic fungal-pathogenic fungi competition mechanism, which denies them space and resources.

3.2. Observation

The incubation period of *F. oxysporum* on tomato plants was obtained by observing plants that showed symptoms of *F. oxysporum* infection after planting. The incubation period was observed for 28 days during the vegetative growth of tomato plants.

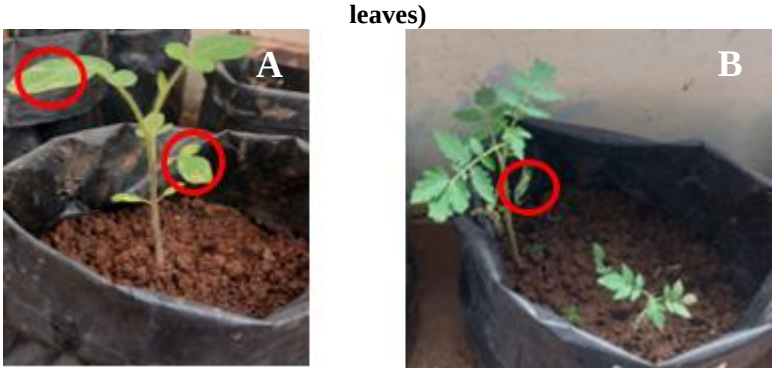
Table 1. The incubation period of *F. oxysporum* on tomato plants after induction of *Gliocladium* sp. and mung bean sprouts extracts

Treatment	Day after inoculation
P0	28
P_G	-
P_T	28
P_{G12T}	-
P_{G18T}	-
P_{G24T}	-
P_{G30T}	-

Notes: The sign (-) indicates no symptoms of *F. oxysporum* infection on tomato plants until the end of the study.

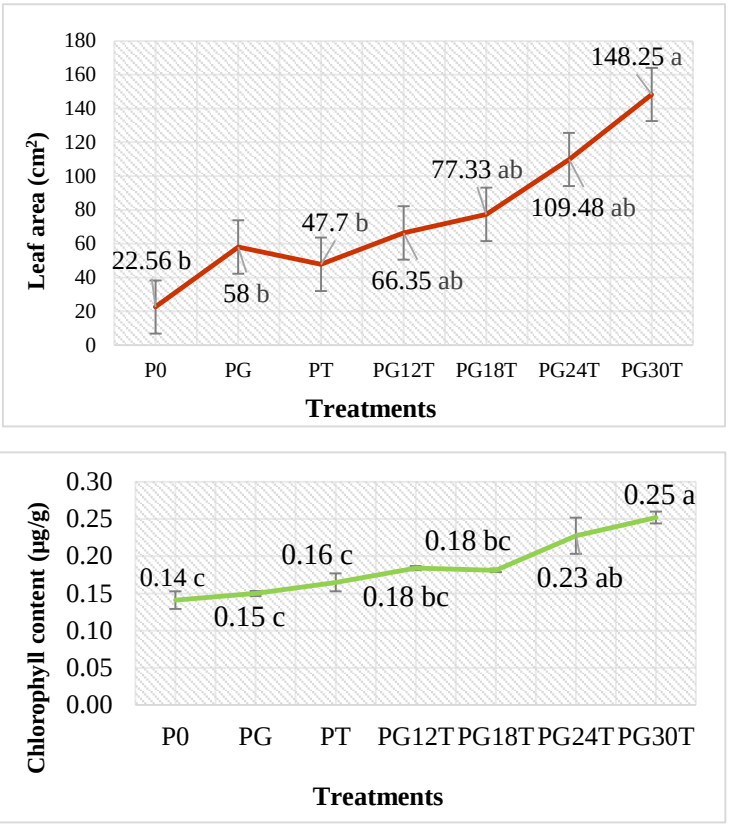
Observations of the incubation period in (Table 1) show that the incubation period occurred when tomato plants were 28 hst old in the P0 treatment (only given *F. oxysporum*) and P_T (15 ml of mung bean sprouts extract 60% + *F. oxysporum*). This is in line with the study results by Mahmoud (2016), which show that the first signs of F. oxysporum infection in plants include wilting and subsequently turning yellow leaves, which disrupts photosynthesis and prevents plants from growing.

Figure 3. Tomato plants showing symptoms of Fusarium wilt. a) P0 (yellow spot leaves); b)P_T (wilted leaves)



P_G and P_{G12T} to P_{G30T} treatments did not cause symptoms of Fusarium infection. This shows that combining *Gliocladium* sp. and 60% mung bean sprouts extract in plants can extend the incubation period of *F. oxysporum* in tomato plants. *Gliocladium* sp. as a biological agent can colonize the roots first compared to *F. oxysporum* to inhibit Fusarium infection through the roots. In line with the opinion of Agustina *et al.* (2019), *Gliocladium* sp. grows faster on plant roots so that the roots of the plant will be dominated by *Gliocladium* sp. which causes *F. oxysporum* to have difficulty getting infection sites and nutrients. In addition, *Gliocladium* sp. secretes antibiotic compounds such as gliovirin, gliotoxin, and viridin, which are antibiotics *to F. oxysporum* so that they can lyse the cell wall of *F. oxysporum* and cause death to *F. oxysporum* (Agustina *et al*., 2013).

Figure 5. leaf area, and chlorophyll content 28 days after being induced by *Gliocladium* sp. and mung bean sprouts extract at various treatment doses.



Data are presented based on Tukey further test at α = 5%. Values followed by the same letter are not significantly different at α = 5%.

The growth of leaf area and chlorophyll content can be seen in (Figure 5), which shows that the growth of tomato plants increased as the dose of *Gliocladium* sp. and 60% mung bean sprouts extract increased. The highest leaf area and chlorophyll content were produced in the PG30T treatment. This is because applying *Gliocladium* sp. can inhibit the growth of *F. oxysporum* so that tomato plants can absorb nutrients and water through the roots optimally. *F. oxysporum* infects plants from the roots and causes clogs in the xylem vessels, disrupting the transportation of nutrients and water to all plant parts (Susanna, 2023).

Mung bean sprouts extract contains cytokinins that can increase cell division and help the formation of chloroplasts in leaves (Mutryarny & Septrida, 2018). Cytokinin can stimulate cell division, thus increasing the leaf area of tomato plants. This is supported by Pratama (2019), who states that the increase in the number of leaves and the size of the leaf area is caused by cell enlargement and division. The leaf area is related to chlorophyll content. A large leaf area contains more chlorophyll. This statement is supported by Misbahulzanah et al. (2014), who states that the higher the leaf area, the more chlorophyll content in the leaves. In addition, Sakya et al. (2015) also argue that the wider the leaf area, the more light is absorbed. The intensity of the absorbed light affects the chlorophyll content in the leaves. High chlorophyll content causes the photosynthesis process in plants to increase so that more photosynthate is produced. Photosynthate is channeled to all parts of the plant to support plant growth.

IV. CONCLUSION

The induction of *Gliocladium* sp. and 60% mung bean sprouts extract significantly affected the incubation period, leaf area, and chlorophyll content of tomato plants. The best tomato plant growth results were obtained from the P_{G30T} treatment with a dose of 30 g *Gliocladium* sp. and 15 ml of 60% mung bean sprouts extract.

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