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Antifungal activity of papaya leaf extract against *Fusarium oxysporum* in red chili (*Capsicum annuum* L.)

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ABSTRACT

Chili pepper (*Capsicum annuum* L.) is a high-value horticultural commodity in Indonesia; however, its production is frequently constrained by Fusarium wilt caused by *Fusarium oxysporum*, a destructive soil-borne pathogen capable of inducing substantial yield losses. The intensive use of synthetic fungicides for disease management poses environmental risks and may disrupt soil microbial balance, thereby necessitating the development of sustainable and eco-friendly control strategies. This study aimed to assess the antifungal efficacy of papaya (*Carica papaya* L.) leaf extract against *F. oxysporum* under in vitro conditions. The experiment was conducted using a Completely Randomized Design (CRD) with five extract concentration treatments and three replications. Antifungal activity was evaluated based on the inhibition of fungal mycelial growth on Potato Dextrose Agar (PDA) medium. The results indicated that papaya leaf extract significantly suppressed the radial growth of *F. oxysporum*, with the inhibitory effect increasing proportionally with extract concentration. Statistical analysis revealed a highly significant effect of treatment concentration on fungal growth inhibition. The highest antifungal activity was observed at a concentration of 8.5 ml, suggesting optimal suppression of mycelial development. These findings demonstrate that papaya leaf extract possesses strong antifungal properties and considerable potential as a plant-based biocontrol agent for managing Fusarium wilt in chili pepper cultivation.

Keywords: *Fusarium oxysporum*, infection, inhibition, papaya leaf extract, red chili (*Capsicum annuum*)

1. Introduction

Horticultural crop cultivation in Indonesia is highly popular, and one of the prioritized commodities is red chili (Capsicum annuum L.) [1]. One of the diseases that commonly attacks chili plants is wilt, caused by the fungus *Fusarium oxysporum*. *F. oxysporum* is a pathogenic fungus that generally infects chili plants and belongs to the *Fusarium* genus [2], [3]. Symptoms such as wilting or other abnormal plant conditions characterize Fusarium wilt. Fungi and bacteria are two main parasitic organisms causing wilt in chili plants [4], [5]. *F. oxysporum* is particularly feared because it is challenging to control or cure once the plant is infected. It is a soil-borne pathogenic fungus and a significant cause of seedling damping-off disease [6]. Moreover, this fungus is considered a highly destructive pathogen that can cause significant agricultural losses.

Fusarium wilt can lead to crop failure. It can occur at any time, during both dry and rainy seasons. The symptoms include yellowing of the leaf veins, especially on the upper leaves, followed by curling of the older

leaves as the petioles droop, eventually causing the entire plant to wilt [7], [8]. According to Muhammad [9], the quality of seeds plays a crucial role in the success of crop cultivation. High-quality seeds also contribute positively to economic outcomes. However, when chili plants are attacked by Fusarium wilt, it can result in losses and crop failures of up to 50% or more if not promptly and effectively controlled [10], [8].

The most common method farmers use to control Fusarium wilt is the application of fungicides or other chemical substances [7]. However, fungicides are often ineffective because they do not eliminate the fungus. Furthermore, long-term use of fungicides can lead to soil ecosystem degradation [11]. Red pepper (*Capsicum annuum* L.) is highly susceptible to *Fusarium oxysporum f. sp. capsici*, which can cause substantial wilt and yield losses under conducive conditions because the pathogen colonizes its vascular tissue and persists in soil. Studies have documented that Fusarium wilt is a significant constraint for chilli production in multiple regions and emphasise susceptibility across chilli varieties in comparison to other crops that also suffer from *F. oxysporum* infections, such as banana (*F. oxysporum f. sp. cubense*) and tomato (*F. oxysporum f. sp. lycopersici*), which similarly exhibit severe disease but have benefited from resistance breeding efforts in some cases. *Fusarium oxysporum* affects a wide range of hosts beyond chilli—including legumes, tobacco, and sweet potatoes—illustrating that many economically important plants are vulnerable to vascular wilt, though host-specific formae speciales determine the exact host range and disease expression.

2. Materials and Methods

2.1. Time and Place

This research was conducted at the Agrotechnology Laboratory, Pancabudi University, from October to November 2024.

2.2. Materials and Equipment

The materials used in this study included: Potato Dextrose Agar (PDA), 96% ethanol, distilled water, Fusarium fungal inoculum, and papaya leaves. The equipment used consisted of: petri dishes, tissue paper, masks, an analytical balance, aluminum foil, a steamer, a stirrer/spatula, a pan, gloves, glass beakers, Erlenmeyer flasks, a refrigerator, a hot plate, cotton wool, a knife, a camera, a calculator, a blender, and stationery.

2.3. Research Procedure

2.3.1. Material Collection, Papaya Leaves and Powder

Papaya leaves were dried without direct exposure to sunlight until crisp. The dried leaves were then ground into a fine powder using a blender. The process was repeated until a homogenous powder was obtained, which was subsequently sieved.

2.3.2. Sterilization of Supporting Equipment

All equipment was sterilized prior to use to eliminate unwanted contaminants. The items were first cleaned and then wiped with 96% ethanol. They were then sterilized in an autoclave at 120°C and 1 atm pressure for 20 minutes.

2.3.3. Preparation of Potato Dextrose Agar (PDA)

Forty grams of PDA granules were weighed and transferred into a 2-liter Erlenmeyer flask. One liter of distilled water was added, and the mixture was heated on a hot plate while stirring continuously. It was then boiled for 30 minutes at 40°C. The prepared PDA was poured into ten 100 ml Erlenmeyer flasks. For fungal culturing, 20 ml of PDA was poured into each of 5 petri dishes and allowed to solidify. The remaining PDA in the Erlenmeyer flasks was stored in a refrigerator.

2.3.4. Cultivation of Fusarium oxysporum Isolate

The Fusarium oxysporum isolate was obtained from infected chili fruits. Chili fruits showing Fusarium wilt symptoms were placed on 3 petri dishes containing PDA medium. The dishes were incubated in a closed room at 16°C, covered with cloth, for 5 days until white fungal growth appeared. The fungal isolate was then transferred to 2 new PDA-containing petri dishes for sub-culturing. Macroscopic identification was conducted to confirm the isolate as Fusarium oxysporum.

2.3.5. Preparation of Papaya Leaf Solution and Extract

Papaya leaf powder was placed into three 3-liter Erlenmeyer flasks. A 96% ethanol solution was added to each until the total volume reached 2000 ml. The mixture was soaked and stirred, then left to stand for 24 hours. This soaking process was repeated over 3 days. After 24 hours, the solution was transferred to a 3000 ml Erlenmeyer flask and filtered using filter paper. The resulting 8 liters of filtrate were then concentrated to yield 400 ml of papaya leaf extract.

2.3.6. Preparation of PDA Supplemented with Papaya Leaf Extract

PDA stored in 100 mL Erlenmeyer flasks in the refrigerator was liquefied on a hot plate at 30 °C. Once liquid, PDA was measured with a syringe at the specified volumes: 0 ml, 2.5 ml, 4.5 ml, 6.5 ml, and 8.5 ml. An equivalent volume of papaya leaf extract (0 ml, 2.5 ml, 4.5 ml, 6.5 ml, and 8.5 ml) was added to each PDA dose. The mixtures were stirred until homogeneous and then poured into petri dishes at 20 ml per dish.

2.3.7. Fungal Inoculation into PDA

A pure isolate of Fusarium oxysporum was sampled using a 0.5 cm diameter cork borer. The resulting mycelial agar plug was placed at the center of each petri dish containing the PDA mixed with papaya leaf extract.

2.4. Observation Parameters

2.4.1. Isolation and Identification of Fusarium oxysporum

Isolation and identification involved observing infected red chili plants exhibiting characteristic Fusarium wilt symptoms, such as plant and fruit wilting.

2.4.2. Characterization of Fusarium oxysporum

The fungus was characterized through macroscopic observation. The macro-morphological traits observed included colony shape, color, and growth pattern.

2.4.3. Measurement of Fungal Mycelium Growth Diameter

Measurement of the Fusarium oxysporum colony diameter commenced at 3 days after inoculation (3 DAI). Measurements were taken daily using a ruler to record the colony's developmental diameter.

2.4.4. Fungal Colony Diameter

The fungal colony diameter was measured every 24 hours during the incubation period. A cross-diameter measurement method (two axes) was employed. Using a ruler, the colony diameter was measured along two perpendicular lines intersecting at the center of the inoculation point. The recorded diameter value was the average of these two measurements, expressed in millimeters (mm).

2.5. Research Method

The research was conducted using a Completely Randomized Design (CRD) with a non-factorial design, namely the concentration of papaya leaf extract, consisting of 5 levels:

K0 = 100 ml = 20 ml/PDA
K1 = 97.5 ml = 20ml/PDA
K2 = 4.5 ml + 95.5 ml = 20ml/PDA
K3 = 6.5 % = 6.5 ml + 93.5 ml = 20ml/PDA
K4 = 8.5 ml + 91.5 ml = 20ml/PDA

The experiment consisted of five treatments, each replicated three times, resulting in a total of fifteen experimental units.

2.6. Observation Parameters

2.6.1. Percentage Inhibition of Fusarium oxysporum Mycelium Growth

The percentage inhibition of mycelial growth for Fusarium oxysporum was calculated using a modified formula from Pandey et al. as follows:

$$P = \frac{(R-r)}{(R)} \times 100\% \quad (1)$$

where: P = Percentage inhibition (%); R = Colony diameter in the control treatment (0 ml extract) (mm); r = Colony diameter in treatments with various extract concentrations (mm)

2.6.2. Macroscopic Colony Characteristics

During the incubation period, macroscopic characteristics were observed, including: colony shape (circular, irregular), mycelium color (white, cream, purple), and surface texture (cottony, filamentous, wool-like).

2.7. Data Analysis

Data obtained from colony diameter measurements and inhibition percentages were analyzed statistically. One-way Analysis of Variance (ANOVA) was used to test the significant effect of various concentrations of papaya leaf extract on fungal growth. If the ANOVA results indicated significant differences ($P < 0.05$), a Tukey's Honest Significant Difference (HSD) post-hoc test was conducted to determine specific differences between treatments. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 25.

3. Results and Discussion

3.1. Fungal Characterization

Fungal characterization confirmed that the isolate used in this study corresponded to the pathogenic fungus *Fusarium oxysporum*, based on macroscopic features such as cotton-like hyphal growth and white to yellowish cream mycelium. These characteristics are consistent with previous descriptions, which report rapid colony expansion and the formation of white aerial hyphae that gradually darken as the culture matures [12]. Beyond morphological confirmation, the research results demonstrated a clear concentration-dependent relationship between papaya leaf extract and fungal growth inhibition. Increasing extract concentrations resulted in progressively smaller colony diameters, indicating stronger suppression of mycelial development. This trend suggests that higher concentrations contain greater levels of bioactive secondary metabolites—such as alkaloids, flavonoids, tannins, and papain enzymes—that interfere with fungal cell wall integrity, membrane permeability, and metabolic activity. The strongest inhibition observed at the highest concentration aligns with earlier antifungal studies using papaya leaf extracts, which similarly reported enhanced inhibitory effects with increasing extract dosage against soil-borne fungi. Comparable findings have also been documented for other plant-based extracts, where antifungal efficacy increased proportionally with concentration due to higher availability of active compounds. Therefore, the results of this study reinforce existing evidence that plant-derived extracts, particularly papaya leaves, exhibit significant antifungal potential and that their effectiveness is closely linked to extract concentration.

The macroscopic observations showed that *F. oxysporum* has branching hyphal structures and produces two types of conidia: macroconidia and microconidia. According to [13], [14]. The sizes of reproductive spores vary: microconidia measure 5–12 × 2.3–3.5 µm, macroconidia measure 23–54 × 3–4.5 µm, and chlamydospores range from 5–13 µm. Macroconidia are larger, cylindrical, thick-walled, and have blunt ends. Microconidia are elliptical, thick-walled, and smaller [15]. The *Fusarium* genus is characterized by having shorter conidia and conidiophores compared to *Fusarium solani*. [16] stated that *F. oxysporum* forms conidia on long, unbranched conidiophores. Microconidia are generally single-celled, elliptical, or oval. Macroconidia are cylindrical, with dorsal and ventral ends aligned, and have blunt, rounded apical cells. Overall, the macroconidia and microconidia of *F. oxysporum* are smaller and have thicker walls than those of *Fusarium solani* [17].

3.2. Mycelial Growth Diameter of F. oxysporum

The analysis of variance on the mycelial diameter of *F. oxysporum* at 3 to 12 Days After Inoculation (DAI) showed that the treatment with papaya leaf extract had a highly significant effect in inhibiting the growth of *F. oxysporum* in red chili plants. The average diameter of *F. oxysporum* mycelial growth is shown in Table 1 below.

Based on the observations in Table 1 at 3 days after inoculation (DAI), the highest mycelial growth of *Fusarium oxysporum* was observed in the K0 treatment (16.20 mm), while the lowest was in the K4 treatment (8.10 mm), which showed a highly significant difference compared to treatments K3, K2, and K1. At 12 DAI, the highest mycelial growth was again recorded in the K0 treatment (90.20 mm), and the lowest in the K4 treatment (41.60 mm), which was also significantly different from the K3, K2, and K1 treatments. These results

indicate that *F. oxysporum* mycelial growth, measured by colony diameter, was affected by the application of different concentrations of plant-based fungicides at 12 DAI.

Table 1. Average Mycelial diameter of *F. oxysporum*

Treatments	Diameter of *F. oxysporum* fungus (mm)									
	3 DAI	**4 DAI**	**5 DAI**	**6 DAI**	**7 DAI**	**8 DAI**	**9 DAI**	**10 DAI**	**11 DAI**	**12 DAI**
K0 = 0	$16,20^{aA}$	$27,85^{aA}$	$34,50^{Aa}$	$39,60^{Aa}$	$45,45^{aA}$	$52,70^{aA}$	$63,40^{aA}$	$68,70^{aA}$	$78,10^{aA}$	$90,20^{aA}$
K1 = 2,5	$13,20^{aAbB}$	$22,12^{bA}$	$24,00^{bB}$	$38,50^{aA}$	$43,83^{aA}$	$50,17^{aA}$	$55,33^{aA}$	$63,83^{aA}$	$67,67^{bA}$	$72,32^{aAB}$
K2 = 4,5	$9,83^{AbBc}$	$13,50^{cB}$	$18,00^{BcC}$	$25,33^{bB}$	$31,50^{bB}$	$35,67^{bB}$	$34,83^{bB}$	$44,67^{bB}$	$50,00^{cB}$	$55,70^{bBC}$
K3 = 6,5	$8,50^{bBc}$	$12,67^{cB}$	$13,00^{cC}$	$18,67^{bBc}$	$24,67^{bB}_{C}$	$28,33^{Bc}_{C}$	$34,00^{bB}$	$39,17^{bB}$	$45,00^{cB}$	$50,30^{bBC}$
K4 = 8,5	$8,10^{cB}$	$11,35^{cB}$	$13,10^{cC}$	$14,78^{cB}$	$16,70^{cC}$	$19,50^{dC}$	$22,30^{cC}$	$24,25^{cC}$	$26,70^{dC}$	$41,60^{bC}$

Note: Values within the same column followed by different letters indicate significant differences at the 5% level (lowercase) and 1% level (uppercase)

The research findings indicate that the phytochemical profile test of papaya leaf extract demonstrates its ability to inhibit fungal hyphal growth, as reflected in the reduced colony diameter of *F. oxysporum*. This effect is attributed to secondary metabolites in papaya leaf extract, such as saponins, flavonoids, and tannins [18]. These three secondary metabolites are suspected of interfering with fungal metabolic processes, inhibiting growth, and even triggering cell death. In several studies, papaya leaf extract has been tested *in vitro* against *F. oxysporum*. The results demonstrated that papaya leaf extract significantly suppressed the fungus growth with a notable inhibition percentage.

3.3. Percentage of Mycelial Growth Diameter of F. Oxysporum

Table 2 shows the percentage of mycelial inhibition of *F. oxysporum*, which was calculated based on the colony diameter approaching the edge of the Petri dish at 3 to 12 DAI. These results show that the percentage of fungal inhibition tends to decrease over time.

Table 2. Percentage of inhibition of *Fusarium oxysporum*

Treatments	Percentage of inhibition of *F. oxysporum* (mm)									
	3 DAI	**4 DAI**	**5 DAI**	**6 DAI**	**7 DAI**	**8 DAI**	**9 DAI**	**10 DAI**	**11 DAI**	**12 DAI**
K0 = 0	80%	69%	63%	55%	48%	42%	32%	25%	14%	0%
K1 = 2,5	85%	77%	73%	56%	52%	45%	38%	29%	24%	19%
K2 = 4,5	89%	84%	80%	72%	64%	60%	61%	50%	43%	38%
K3 = 6,5	92%	85%	85%	79%	72%	68%	63%	55%	50%	44%
K4 = 8,5	93%	87%	86%	84%	81%	78%	75%	73%	70%	55%

Based on the observations in Table 2, 3 days after inoculation (DAI), the highest percentage of mycelial inhibition of *Fusarium oxysporum* was found in the K4 treatment at 93%, while the lowest was in the K0 treatment at 80%. These results showed a highly significant difference compared to treatments K1, K2, and K3. At 12 DAI, the highest percentage of inhibition was again observed in the K4 treatment at 55%, while the lowest was in the K0 treatment at 0%, with a highly significant difference compared to treatments K1, K2, and K3.

From the observations at 12 DAI in Table 2, it can be seen that the lowest inhibition was in the K_0 treatment at 0%, while the highest was in the K4 treatment at 55%. Each treatment and extract concentration significantly affected the treatment (control). This is due to the presence of antifungal compounds produced by plant-based

fungicides [19]. The damage caused by these compounds interferes with the fungal cell growth and development, resulting in delayed growth.

From the observation of the papaya leaf extract treatment at 12 DAI, the inhibition value was 54% with a concentration of 8.5 ml, and even at the lowest concentration of 2.5 ml, it still inhibited *F. oxysporum* growth by 19%. These results indicate that the papaya leaf extract is already inhibiting the growth of *F. oxysporum* [20], [21].

These findings align with research conducted by [22] on the phytochemical activity and antibacterial effects of papaya leaf extract [23], which states that higher concentrations of papaya leaf extract result in more excellent antibacterial activity. The increased inhibition may be due to higher concentrations of the extract, which contain higher amounts of antibacterial compounds, thus more effectively inhibiting bacterial growth.

The findings of this study are consistent with research by [7], [24], which show that plant-based fungicides, such as soursop leaf extract [25], [26], have the most effective inhibition percentages due to their high toxicity, which can suppress the growth of *F. oxysporum* colonies more effectively than papaya and kaffir lime leaves.

This opinion is also supported by research by [27], who stated that papaya leaf extract contains active compounds such as flavonoids, alkaloids, and terpenoids that act as contact poisons on vectors. Active compounds like flavonoids can cause nerve paralysis and damage to the respiratory system [28]. Alkaloids act as contact poisons and disrupt the growth system when entering the body, preventing fungal development and leading to cell death. Terpenoids, when inhaled, can suppress appetite, eventually leading to death.

This is in line with the research conducted by [29], which found that 70% ethanol extract from papaya leaves has antifungal activity against *Candida albicans* [30]. The mechanisms of flavonoids and alkaloids involve affecting fungal cell components by damaging cell membranes and denaturing proteins. Flavonoids can act as antifungal agents because they have phenolic groups that can irreversibly denature proteins [24] and disrupt cell membranes. The mechanism of tannins involves damaging the fungal cell wall, which consists of lipids and amino acids [18], [31]. Membrane damage increases cell permeability, which can cause fungal cell destruction [32]. The antifungal and antibacterial effects of saponins are disrupted by the presence of monosaccharide groups and their derivatives [33], [34].

4. Conclusion

Papaya leaf extract has shown toxic effects at a concentration of 2.5 ml, inhibiting the growth of *Fusarium oxysporum*. The increase in the concentration of papaya leaf extract is directly proportional to the percentage of inhibition of *F. oxysporum* growth. The papaya leaf extract can optimally inhibit the growth of *F. oxysporum* mycelial diameter at a concentration of 8.5 ml. Based on the conclusions drawn, it is recommended that future research should focus on conducting in vivo studies to determine the effective dose, toxic dose, and side effects on the growth and production of red chili plants.

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