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Article

Effect of *Trichoderma* spp. Application Timing on the Control of Fusarium Wilt and Vegetative Growth of Cayenne Pepper

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Abstract: Cayenne pepper (*Capsicum frutescens* L.) is a valuable horticultural crop, yet its productivity is frequently reduced by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici*. Although *Trichoderma* spp. is widely known as a biological control agent, the optimal timing for its application to achieve maximum disease suppression and plant growth enhancement remains insufficiently studied. This research aimed to evaluate the effect of *Trichoderma* spp. application timing on the incidence of Fusarium wilt and the vegetative growth of cayenne pepper. A completely randomised design with five treatments—application at 21, 14, and 7 days before planting, at planting, and a no-treatment control—was used with four replications. Observed parameters included disease incubation period, incidence rate, plant height, number of leaves, and root length. The results showed that *Trichoderma* spp. application timing significantly influenced all variables ($p < 0.05$). Application 21 days prior to transplanting resulted in the lowest disease incidence (8%), longest incubation period (28 days), and improved vegetative growth (plant height 66 cm, 33 leaves, root length 17 cm), whereas untreated plants exhibited the highest disease incidence (75%) and the poorest growth. These results suggest that early application of *Trichoderma* spp. enables effective colonisation of the rhizosphere and may activate induced systemic resistance, thereby enhancing plant defence against Fusarium infection. In conclusion, applying *Trichoderma* spp. 21 days before planting offers an effective strategy for controlling Fusarium wilt and promoting cayenne pepper growth in a sustainable manner.

Keywords: Biological Control, *Capsicum frutescens,* Disease Control, Fusarium, ISR

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Introduction

Cayenne pepper (*Capsicum frutescens* L.) is a high-value horticultural commodity cultivated widely in tropical and subtropical regions [1]. It plays a critical role in food, industry, and traditional medicine due to its nutritional content and pungent properties, which are primarily attributed to capsaicinoids [2]. In Indonesia, cayenne pepper holds substantial economic importance, with increasing consumer demand observed annually. However, despite its commercial potential, productivity remains suboptimal. One of the principal limiting factors is the prevalence of soil-borne diseases, particularly Fusarium wilt caused by *F. oxysporum* f. sp. *capsici* [3,4]. This pathogen invades the plant's vascular

system, leading to wilting, chlorosis, and eventual plant death, thereby causing substantial yield losses [5].

Fusarium wilt is notoriously difficult to manage due to its persistence in the soil and its ability to infect through wounds or root tips [6]. Although chemical fungicides have traditionally been employed for disease control, their indiscriminate and prolonged use has raised concerns regarding environmental safety, pathogen resistance, and the disruption of beneficial soil microbiota. Consequently, biological control strategies have garnered increasing interest, especially those involving antagonistic fungi such as *Trichoderma* spp.. This genus is well recognised for its multiple mechanisms of action, including competition, mycoparasitism, antibiosis, and the induction of plant systemic resistance [7–12].

Numerous studies have confirmed the efficacy of *Trichoderma* spp. in suppressing soil-borne pathogens [13]. However, most research has focused on dosage and formulation, while the influence of application timing—particularly in relation to planting—remains inadequately addressed. Since the interaction between *Trichoderma* spp. and plant roots is time-sensitive, establishing the optimal application window could significantly enhance its biocontrol performance and contribute to sustainable crop protection strategies. This study aims to evaluate the effect of *Trichoderma* spp. application timing on the incidence and progression of Fusarium wilt in cayenne pepper and determine the most effective timing for application to improve plant health and vegetative development. The findings are expected to support the practical integration of biological agents into field-based management programmes for cayenne pepper cultivation.

Materials and Methods

1. Material and Equipment

The materials included *Trichoderma* sp. and *F. oxysporum* isolates (sourced from Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan Surabaya), Mediterranean red topsoil, aquadest, PDA medium, 70% alcohol, aluminium foil, labelling paper, local cayenne pepper seeds, seedling trays, organic manure, and maize-rice for fungal culture. The equipment used included a Bunsen burner, autoclave, beaker glass, Petri dishes, Erlenmeyer flasks, hot plate, inoculating needle, lighter, oven, ruler, volumetric pipette, pH meter, laminar air flow cabinet, spatula, and digital balance.

2. Experimental Design

The study used a Completely Randomised Design (CRD) with five treatments and four replications, totalling 20 experimental units. Each unit consisted of four plants, with three used for sampling, resulting in 60 plants overall. *F. oxysporum* inoculum was applied to all treatments at 7 days after transplanting (DAT). The treatments were as follows:

P0 = Control (no *Trichoderma* spp.)
P1 = *Trichoderma* spp. application 21 days before planting
P2 = *Trichoderma* spp. application 14 days before planting
P3 = *Trichoderma* spp. application 7 days before planting
P4 = *Trichoderma* spp. application at planting.

3. Soil Preparation, Seedling Transplantation, and Fungal Inoculation

The topsoil used in this experiment was air-dried and sieved using a 2 mm mesh to ensure uniform texture, then filled into 30 × 30 cm polybags at a rate of 2 kg per bag. Cayenne pepper (*C. frutescens* L.) seedlings, 20 days old, were transplanted individually into each polybag. The pathogenic fungus (*F. oxysporum* f. sp. *capsici*) and the antagonistic fungus (*Trichoderma* spp.) were initially cultured on potato dextrose agar (PDA) and subsequently multiplied using a solid maize-rice medium (150 g per Erlenmeyer flask), which had been soaked for 16 hours and sterilised at 121–126°C for 20 minutes. Fungal

discs (3–5 discs per flask) were inoculated into the medium, and cultures were shaken daily to promote uniform colonisation.

For *F. oxysporum* inoculation, 10 grams of colonised maize-rice medium were buried 3 cm deep in the rhizosphere zone at 7 days after transplanting. The soil was kept moist to support pathogen activity. Meanwhile, *Trichoderma* spp. was applied at 20 grams per polybag according to the designated treatment schedule: 21, 14, or 7 days before transplanting, or on the day of transplanting. The inoculum was incorporated 3 cm deep into the soil during the evening to minimise evaporation, followed by light watering to support establishment.

4. Observation Parameters

The incubation period was recorded as the number of days from *F. oxysporum* inoculation to the first visible symptoms of wilt, including leaf yellowing, wilting, and necrosis. Daily observations were conducted until the onset of symptoms was confirmed. Disease incidence was calculated as the percentage of infected plants relative to the total number of plants observed, using the formula 1 [14].

Vegetative growth was evaluated through several parameters. Plant height (cm) was measured weekly from the base of the stem to the apical tip at 7, 14, 21, and 28 days after transplanting (DAT). The number of leaves per plant was also recorded weekly by counting all fully expanded leaves at the same time points. Root length (cm) was measured at 28 DAT by carefully removing the plant from the polybag and measuring the distance from the root base to the tip.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants evaluated}} 100\% \quad (1)$$

5. Data Analysis

All data were analysed using Analysis of Variance (ANOVA) at a 5% significance level. Where significant differences occurred, Duncan's Multiple Range Test (DMRT) was applied for mean separation at $p < 0.05$.

Results and Discussion

1. Identification of *F. oxysporum* and *Trichoderma* spp.

The pathogenic fungus was obtained from BBPPT Surabaya, isolated from cayenne pepper plants showing symptoms of Fusarium wilt. The biocontrol agent, *Trichoderma* spp., was collected from the rhizosphere of cayenne pepper plants. The purpose of identification was to confirm the fungal species used in this study.

F. oxysporum colonies exhibited colours ranging from white to pink. The hyphae were septate and branched, with a velvety texture and characteristic purple-yellow hue at the colony margins. The fungus produced two spore types: macroconidia (crescent-shaped, 3–5 septa, 40–70 μm × 15–20 μm) and microconidia (oval, 1–2 septa, 20–25 μm × 15–20 μm) [6,15]. *Trichoderma* spp. colonies ranged in colour from white to various shades of green over 7 days. The mycelium displayed two radial growth patterns with septate hyphae. The conidiophores were branched in a pyramidal arrangement. Phialides were short-stalked, measuring approximately 11.1 μm in length, and grouped in clusters of 2–3. Conidia were green, round, and located at the tips of the conidiophores [16–18] (Figure 1).

2. Effect of *Trichoderma* sp. on Suppression of *F. oxysporum*

Results showed that *Trichoderma* sp. application timing significantly affected disease incubation period and severity. The earliest application (21 days before transplanting) delayed symptom appearance until 28 days after transplanting, whereas untreated plants showed symptoms at 14 days. This delay was likely due to root colonisation by *Trichoderma* spp., which reduced pathogen access and competition for space and nutrients.

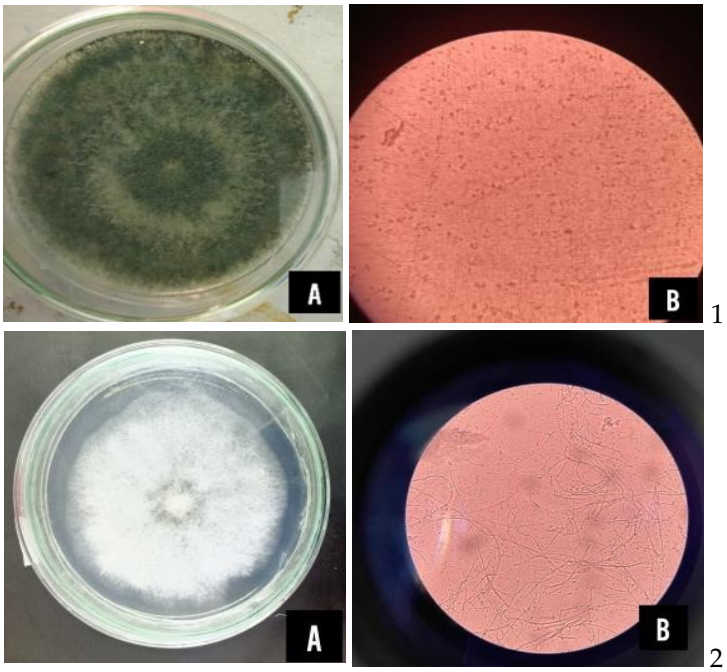

Figure 1. Morphological characteristic of *Trichoderma* spp. (1) and *F. oxysporum* (2) (A=colony; b=microscopic miselia/conidia/spore).

Table 1. Effect of pre-planting application timing of *Trichoderma* spp. on disease incubation period.

Treatment	Incubation periods (day after inoculation (DAI))
Control (no *Trichoderma*)	14
P1	28
P2	21
P3	14
P4	14

Based on the research findings, it was observed that treatments involving different application intervals of *Trichoderma* spp. were effective in delaying the onset of disease symptoms, with an incubation period of 21 days after inoculation (DAI). In contrast, plants grown without *Trichoderma* spp. were more rapidly infected, with an incubation period of only 14 DAI (Table 1). The longer incubation period is attributed to spatial competition between the pathogen and the antagonistic fungus, resulting in a delay in the pathogen's ability to infect the plant. When the root system is predominantly colonised by the antagonistic fungus, it becomes more difficult for the pathogen to establish infection [5,19,20].

This suggests that plants possess an innate ability to defend themselves from pathogens, particularly after non-lethal necrosis occurs, a defence mechanism referred to as Systemic Acquired Resistance (SAR). In addition to SAR, increased resistance in cayenne pepper plants may also be induced through Induced Systemic Resistance (ISR) [21].

Previous study reported that the contact and penetration of pathogens in cayenne pepper plants can be delayed by the presence of *Trichoderma* spp. in the growing medium. This occurs because *Trichoderma* spp. colonises the root zone earlier, forcing the pathogenic fungi to compete for space and nutrients. As a result, *F. oxysporum* faces difficulty infecting the plant. A high population of *Trichoderma* spp. in the soil can significantly delay the appearance of wilt symptoms and even suppress the occurrence of pathogenic fungal attacks [3,5].

Disease incidence was also lowest in the 21-day treatment (8%) and highest in the control (75%). The progression of disease symptoms influences the overall incidence of the disease. Plants that did not receive *Trichoderma* spp. treatment (P0) exhibited a high disease incidence, reaching 42% at 14 days after transplanting (DAT), whereas no disease symptoms were observed in treatments P1 and P2 (0%). At 28 DAT, the highest disease incidence was still observed in the control (P0), reaching 75%. In contrast, the lowest incidence was recorded in treatment P1 (8%), followed by P2 (33%), P3 (50%), and P4 (58%) (Table 2). The higher incidence in the untreated control (P0) compared to P1 is likely due to the absence of competition for nutrients and space between *Trichoderma* spp. and *F. oxysporum* in the soil [5,22]

The antagonistic mechanism of *Trichoderma* spp. against soil-borne pathogens may occur through three primary strategies: (i) production of extracellular enzymes, including β-1,3-glucanase and chitinase, which degrade the pathogen's cell wall; (ii) secretion of trichodermin, a toxin that inhibits pathogen propagules in the rhizosphere; and (iii) activation of host defence responses [23]. β-1,3-glucanase is considered one of the pathogenesis-related proteins due to its capacity to break down fungal cell walls.

Based on these mechanisms, the low disease incidence in treatment P1 suggests that *Trichoderma* spp. was able to establish effectively in the rhizosphere of cayenne pepper plants, owing to its earlier application prior to *F. oxysporum* inoculation. *Trichoderma* spp. rapidly colonised the root system, thereby suppressing the growth of *F. oxysporum* through spatial and nutritional competition. In addition, the plant likely developed resistant mechanism more rapidly in P1 compared to P2, P3, and P4. Conversely, the high disease incidence in treatment P0 may be attributed not only to the absence of biocontrol competition but also to the plant's delayed activation of SAR and ISR mechanisms, which require time to develop in the absence of early microbial stimulation, as plant growth fungi [22].

Trichoderma spp. treatment also improved plant height, leaf number, and root length. Plants treated 21 days before transplanting reached 66 cm in height, had 33 leaves, and developed 17 cm long roots. In contrast, untreated plants showed poor growth (19 cm height, 12 leaves, 6 cm roots) (Table 3, Table 4, Table 5).

Table 2. Effect of pre-planting application timing of *Trichoderma* spp. on disease incidence in cayenne pepper.

Treatment	Disease incident (%)		
	14 DAT	21 DAT	28 DAT
Control (no *Trichoderma*)	42 c	67 c	75 c
P1	0 a	0 a	8 a
P2	0 a	8 a	33 b
P3	17 b	50 b	50 b
P4	33 c	58 b	58 b
ANOVA 5%	*	*	*

Table 3. Effect of pre-planting application timing of *Trichoderma* spp. on plant height.

Treatment	Plant height (cm)			
	7 DAT	14 DAT	21 DAT	28 DAT
Control (no *Trichoderma*)	9 a	14 a	16 a	19 a
P1	15 d	29 d	45 d	66 e
P2	14 c	29 d	43 d	61 d
P3	11 b	17 c	27 c	42 c
P4	11 b	15 b	20 b	29 b

ANOVA 5%	*	*	*	*

Table 4. Effect of pre-planting application timing of *Trichoderma* spp. on leaf number.

Treatment	Leaf number (			
	7 DAT	14 DAT	21 DAT	28 DAT
P0	7 a	10 a	10 a	12 a
P1	13 d	19 d	27 d	33 d
P2	12 d	18 d	24 d	30 d
P3	10 c	15 c	20 c	25 c
P4	8 b	12 b	15 b	19 b
ANOVA 5%	*	*	*	*

Table 5. Effect of pre-planting application timing of *Trichoderma* spp. on root length.

Treatment	Disease incident (%)			
	7 DAT	14 DAT	21 DAT	28 DAT
P0	7 a	10 a	10 a	12 a
P1	13 d	19 d	27 d	33 d
P2	12 d	18 d	24 d	30 d
P3	10 c	15 c	20 c	25 c
P4	8 b	12 b	15 b	19 b
ANOVA 5%	*	*	*	*

The application of *Trichoderma* spp. to suppress *F. oxysporum* had a significant impact on the early growth of cayenne pepper plants. This was evidenced by increases in early vegetative growth, number of leaves, and root length. The best plant performance was observed in treatment P1 (*Trichoderma* spp. applied 21 days before transplanting), with an average plant height of 15 cm and 13 leaves at 7 days after transplanting (DAT), increasing to 66 cm in height, 33 leaves, and 17 cm root length by 28 DAT. Application of *Trichoderma* spp. 21 days prior to planting likely improved soil nutrient availability, contributing to better plant growth compared to other treatments.

The increased vegetative growth observed in the treated plants can be attributed not only to *Trichoderma* spp. acting as a biocontrol agent against *F. oxysporum*, but also to its function as a biofertiliser, known as a Plant Growth Promoting Fungus (PGPF) [22]. *Trichoderma* spp. can also function as a decomposer that accelerates the composting process. Previous research reported that *Trichoderma sp.* is capable of producing plant growth hormones such as auxins and cytokinins [24]. When *Trichoderma* spp. is applied to the soil, it not only protects plants from soil-borne fungal attacks but also produces plant growth regulators [25]. An increase in plant height was also associated with a greater number of leaves. *Trichoderma* spp. plays a role in decomposing organic matter in the soil, which contains essential nutrients such as N, P, S, Mg, and others required for plant growth [26]. *Trichoderma* spp. breaks down organic compounds such as nitrogen, which is then used by plants to stimulate growth and enhance leaf greenness. Nitrogen contributes to chlorophyll formation and gives leaves their green colour. During vegetative growth stages, especially for stems and leaves, plants require high levels of nitrogen, phosphorus, and potassium. Its found that *T. harzianum* increased root and leaf growth, indicating that it effectively promotes overall plant development [27].

Root length varied significantly among treatments, likely due to improved nutrient availability. *Trichoderma* spp. positively influences root growth, plant development, and reproductive yield [10,26]. *Trichoderma sp.* enhances the speed of plant growth and root system development, particularly by promoting the formation of healthy and deeper roots. *Trichoderma* spp. supported this by showing that improved water and nutrient

uptake by rice roots was due to the compatibility between indigenous *Trichoderma* spp. and its host plant [26,28]. Indigenous *Trichoderma* spp. in soil can enhance nutrient availability. The rhizosphere is a specialised soil zone rich in nutrients, and plant roots exude organic acids that support microbial communities. This microbial interaction within the rhizosphere benefits plant growth, nutrient assimilation, and development [26,29]. Plant–*Trichoderma* interactions regulate root architecture by increasing lateral and primary root length, thereby improving nutrient absorption efficiency [25].

The effectiveness of *Trichoderma* spp. in suppressing *F. oxysporum* wilt was also reflected in the percentage of plant wilting. *Trichoderma* spp. has the ability to degrade cellulose, starch, lignin, and soluble organic compounds such as proteins and sugars, enabling it to utilise diverse nutrient sources for growth. Further reported that *Trichoderma* spp. could inhibit the growth of several pathogenic fungi including *C. capsici*, *Fusarium sp.*, and *Sclerotium rolfsii* in vitro, with the highest inhibition observed against *C. capsici*, followed by *Fusarium sp.* and *S. rolfsii* [30–34].

Conclusions

This study demonstrated that the timing of *Trichoderma* spp. application plays a critical role in the suppression of *F. oxysporum* and the enhancement of early vegetative growth in cayenne pepper (*Capsicum frutescens* L.). Among the treatments tested, the application of *Trichoderma* spp. 21 days prior to transplanting (P1) proved to be the most effective, significantly delaying disease onset with an incubation period of 28 days after inoculation and reducing disease incidence to just 8%. In addition, this treatment consistently improved plant height, leaf number, and root development throughout the observation period.

These findings highlight the importance of optimising the application timing of biocontrol agents to maximise their effectiveness in disease management and plant growth promotion. Integrating early *Trichoderma* spp. application into cayenne pepper cultivation practices could serve as a sustainable alternative to chemical controls..

Additional Section

Author Contributions: Conceptualization, A.D. and S.K.; methodology, A.G.S.; validation, A.D. and S.K.,; formal analysis, A.G.S.; investigation, A.G.S.; resources, A.G.S., A.D., and S.K..; data curation, A.D.; writing—original draft preparation, A.G.S; writing—review and editing, S.K.; visualization, A.G.S; supervision, A.D.; proofreading, M.A.; funding acquisition, A.G.S., A.D., and S.K.. All authors have read and agreed to the published version of the manuscript.

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