

ANALYSIS OF THE RATE OF SYMPTOM DEVELOPMENT OF *FUSARIUM* SP. CAUSING VANILLA STEM ROT ON ARTIFICIAL MEDIUM AND LIVING PLANTS

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ABSTRACT

Vanilla stem rot disease (Vanilla planifolia Andrews) is one of the most destructive fungal diseases affecting vanilla cultivation worldwide. It is caused by the soil-borne fungus Fusarium sp., which significantly reduces crop productivity and quality through stem necrosis, tissue softening, and plant death. This study aimed to analyze the rate and pattern of symptom development of Fusarium sp. infection under two different conditions: artificial medium (Potato Dextrose Agar/PDA) and living vanilla plants. The research was conducted at the Biotechnology Laboratory and the Laboratory of Plant Pests and Diseases using a descriptive observation method without statistical analysis. Inoculation was performed by culturing fungal isolates on vanilla stem cuttings placed on PDA medium and on healthy vanilla plants under controlled humidity and temperature. Daily visual observations were made to record the onset and progression of disease symptoms. The results showed that fungal mycelium began to develop on PDA on the second day after inoculation and fully covered the stem surface by the sixth day. In contrast, disease symptoms on living plants appeared on the sixth day, characterized by brownish lesions, tissue discoloration, and mild stem wilting. These findings confirm that Fusarium sp. has a high infection potential under humid environments and fulfills the initial criteria of Koch's Postulates as the causal pathogen of vanilla stem rot disease.

Keywords: *Fusarium sp., vanilla stem rot, PDA medium, infection symptoms, Koch's Postulates*

1. INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) is one of the most valuable horticultural crops and a major export commodity with high economic importance. However, vanilla production is highly susceptible to attack by plant pathogens, particularly vanilla stem rot disease (VSRD), which causes severe losses in the field (Directorate General of Estate Crops, 2022). The main pathogen responsible for VSRD is *Fusarium oxysporum* f. sp. *vanillae*, a fungal species highly specific to vanilla. This pathogen possesses strong infection capabilities and can survive under diverse environmental conditions. It spreads through contaminated soil, water, cultivation tools, and infected plant materials such as cuttings, roots, and dust particles. Moreover, *Fusarium oxysporum* f. sp. *vanillae* produces chlamydospores that allow it to survive for years in the absence of a host plant, making long-term control extremely difficult (7–10 years or more) in infested soils.

Typical symptoms of the disease include stem discoloration to brown, tissue softening, necrosis, and eventual plant death under severe infection (Directorate General of Estate Crops, 2022). In plant pathology studies, *in vitro* (artificial medium) models are frequently used to assess fungal virulence, screen biological control agents, and perform biochemical tests because they allow controlled conditions and faster observation. However, although *in vitro* systems accelerate observations, they do not always represent the infection dynamics occurring in *in vivo* (living plant) systems, which are influenced by plant defense mechanisms, local microbiota interactions, environmental variability, and other complex factors (Shi et al., 2018).

Fusarium sp. can persist in soil and plant debris for extended periods. Infection typically occurs through wounds on roots or stems and spreads into soft plant tissues. Environmental conditions such as temperatures between 28–30°C and high humidity strongly favor disease development (Windels, 1993). To confirm the causal relationship between the pathogen and the disease, Koch's Postulates are applied, involving the processes of pathogen isolation, inoculation, and observation of symptoms in healthy plants (Agrios, 2005).

In this study, a descriptive observation was conducted to analyze the rate of symptom development of *Fusarium* sp. under two conditions: artificial medium (Potato Dextrose Agar/PDA) and living vanilla plants. The findings are expected to provide a preliminary understanding of the infection dynamics of *Fusarium* sp. and serve as a scientific basis for developing effective control strategies against vanilla stem rot disease.

2. RESEARCH METODOLOGY

2.1. Research Time and Location

The research was conducted every Friday at the Biotechnology Laboratory and the Laboratory of Plant Pests and Diseases, Faculty of Agriculture.

2.2. Tools and Materials

The tools used in this study included a weighing balance, stove, stirrer, measuring cylinder, Erlenmeyer flask, cotton, bottles, aluminum foil, autoclave or pressure cooker, Petri dishes, laminar flow cabinet, tweezers, inoculating needle, syringe, cotton buds, microscope, and digital camera.

The materials used were 250 g of potatoes, 17 g of agar, 25 g of dextrose, 1 L of distilled water, soil for planting medium, healthy and diseased vanilla plants, and *Fusarium* sp. fungal isolates.

2.3. Preparation of PDA Medium

Potatoes were peeled, washed, and diced, then boiled in 750 mL of water for 20 minutes until soft. The potato extract was filtered and mixed with dextrose, agar, and distilled water to a final volume of 1 liter. The mixture was dispensed into test tubes (approximately 10 mL each), plugged with cotton and covered with aluminum foil, then sterilized using an autoclave for 10 minutes at a pressure of 1 bar. After sterilization, the tubes were slanted until the medium solidified and became ready for use.

2.4. Isolation of Microorganisms

Petri dishes were sterilized using an autoclave and exposed to UV light under a laminar flow cabinet. PDA medium was poured into sterile Petri dishes and allowed to solidify.

Diseased vanilla stem tissues (approximately 1 × 2 cm) were cut, surface-sterilized with 70% ethanol for 3 minutes, rinsed three times with sterile distilled water, and air-dried on sterile filter paper. The sterilized tissue pieces were then placed onto PDA medium using a sterile inoculating needle under a Bunsen flame. The Petri dishes were sealed and incubated at room temperature until fungal growth appeared.

The fungal colonies were observed visually and under a microscope. Isolates producing spores were purified using the single-spore isolation or hyphal tip cutting technique. Germinated spores were transferred to fresh PDA medium until pure cultures were obtained. Morphological identification was carried out by comparing the shape and structure of spores and conidia with standard taxonomic references (Barnett, 1983; Drenth & Sendall, 2001).

2.5. Koch's Postulates Test

Purified *Fusarium* sp. isolates were inoculated onto healthy vanilla plants. The inoculating needle was sterilized over a Bunsen flame and used to take the fungal inoculum from pure culture, then applied or inserted into the stems and leaves of healthy vanilla plants.

Inoculated plants were covered with transparent plastic to maintain humidity. For inoculations on cut vanilla stems, the samples were placed on PDA medium in sealed Petri dishes.

Daily observations were carried out to record symptom development, including discoloration, tissue decay, and mycelial growth. The infection process and resulting symptoms were documented visually using a digital camera.

3. RELATED RESEARCH/LITERATUR REVIEW

3.1. Infection of *Fusarium* sp. in Vanilla

Vanilla stem rot disease is caused by the fungus *Fusarium oxysporum*, which infects plant tissues through mechanical or natural wounds. The infection process begins with the discoloration of tissues to brown, followed by soft rot and wilting symptoms (Singh et al., 2022). This fungus produces toxins and cell wall-degrading enzymes such as pectinase and cellulase, which accelerate tissue maceration and decay (Kumar et al., 2021).

A study by Pavitra et al. (2023) reported that *Fusarium oxysporum* exhibits different levels of virulence among isolates, and disease symptoms develop more rapidly under high humidity conditions. These findings suggest that environmental factors play a critical role in determining the severity and progression rate of *Fusarium* infection in vanilla.

3.2. Koch's Postulates in Pathogenicity Verification

Koch's Postulates are used to establish a causal relationship between a microorganism and the disease it induces. The four main steps include isolation, cultivation in pure culture, inoculation into a healthy host, and re-isolation of the same pathogen (Agrios, 2005). This process confirms that the obtained isolate is indeed the causal agent of the disease.

Similar studies by Sutanto et al. (2020) and Pavitra et al. (2023) successfully demonstrated that *Fusarium* isolates derived from diseased vanilla plants were able to reproduce identical symptoms when inoculated onto healthy plants, thereby fulfilling Koch's criteria for pathogenicity confirmation.

3.3. Vanilla Stem Rot Disease (VSRD)

Vanilla Stem Rot Disease (VSRD) is one of the major diseases affecting *Vanilla planifolia*, primarily caused by fungi belonging to the genus *Fusarium*, particularly *F. oxysporum* and *F. solani* (Leslie & Summerell, 2006). Early symptoms include brown discoloration of the stem tissues, followed by softening, rotting, and eventually plant death. According to Pavitra et al. (2023), *F. oxysporum* f. sp. *vanillae* can survive for long periods in soil and infect plants through wounds in roots or stems. The disease develops rapidly under conditions of high humidity and optimal temperatures of 25–30°C. Typical symptoms include internal tissue browning and the exudation of yellowish mucilage from infected stems.

In addition to stem infection, the pathogen can spread through contaminated farming tools and unsterilized planting media (Sutanto et al., 2020). Control efforts for VSRD include the use of disease-free planting materials, sterilization of tools,

and the application of biological control agents such as *Trichoderma harzianum*, which has been proven effective in suppressing *Fusarium* growth in soil (Sharma et al., 2022).

Recent research by Mahendra et al. (2024) indicated that *Fusarium* isolates from different regions exhibited varying levels of virulence, emphasizing the importance of understanding the rate of symptom development to determine effective management strategies against vanilla stem rot.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1 Symptoms of *Fusarium* sp. Infection on Vanilla Stems in Petri Dishes

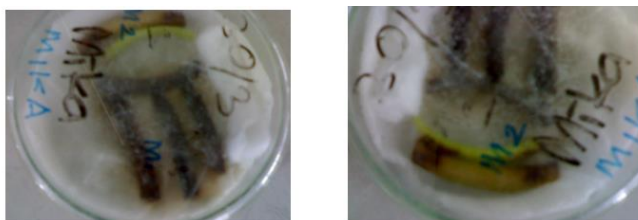


Figure 1. Symptoms observed on day 5
Source: Mika (2025)



Figure 2. Symptoms observed on day 5
Source: Mika (2025)

4.1.2. Symptoms of Infection on Living Vanilla Plants



Figure 3. Symptoms observed on day 6
Source: Mika (2025)

4.2. DISCUSSION

On artificial medium (Petri dishes), vanilla stems inoculated with *Fusarium oxysporum* began to show early signs of infection on the second day after inoculation. By the fifth day, fungal mycelia partially covered the stem surface, and by the sixth day, dense whitish-gray mycelial growth had enveloped the entire stem, indicating complete fungal colonization. In living vanilla plants, symptoms appeared later—on the sixth day after inoculation. Early symptoms included light brown lesions on the stem, slight leaf wilting, and changes in tissue texture around the inoculation site.

These findings indicate a positive result for the first stage of Koch's Postulates, in which inoculation of the suspected pathogenic isolate caused similar disease symptoms on healthy plants. However, the confirmation test (re-isolation) was not conducted in this practical study, so it cannot be concluded whether all four of Koch's criteria were fully satisfied.

According to Matthews (1970) as cited in Agrios (2005), Koch's Postulates consist of four essential criteria to establish a causal relationship between a microorganism and a disease:

1. The microorganism must always be associated with the same disease.
2. It must be isolated and grown in pure culture under laboratory conditions.
3. The pure culture must cause the same disease when inoculated into a healthy host.
4. The same microorganism must be re-isolated from the newly infected host.

Thus, the current observation fulfills only the first three criteria of Koch's Postulates. The disease symptoms observed on the vanilla stems were characterized by soft rot, followed by tissue drying along the stem edges. This suggests that *Fusarium oxysporum* has the ability to infect soft plant tissues and persist in dried tissues. The fungus grows optimally at 28–30°C (Windels, 1993), consistent with the temperature of the laboratory during observation.

According to Pavitra et al. (2023), the rapid formation of *Fusarium oxysporum* mycelia is strongly influenced by tissue moisture content and environmental humidity. In living plant tissues, physiological defense responses such as phenolic compound synthesis and lignification may slow down fungal penetration (Singh et al., 2022). These results are consistent with the findings of Sutanto et al. (2020), who reported that *Fusarium* infection in vanilla typically produces soft rot symptoms between the fifth and seventh day after inoculation. Therefore, the present observations reinforce the conclusion that *Fusarium oxysporum* is the primary causal agent of vanilla stem rot disease (*Vanilla planifolia*).

5. CONCLUSION

Based on the observations, it can be concluded that *Fusarium* sp. is the pathogenic fungus responsible for stem rot disease in vanilla (*Vanilla planifolia*). The development of infection symptoms occurred more rapidly on artificial medium (PDA) than on living plants. On PDA medium, fungal mycelium appeared on the second day and completely covered the stem tissues by the sixth day, while on living plants, symptoms appeared on the sixth day, characterized by soft rot and tissue drying along the stem. The results confirm that *Fusarium* sp. is capable of infecting and developing in accordance with Koch's Postulates, thereby establishing a clear causal relationship between the fungus and vanilla stem rot disease.

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