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Effectiveness of Fobio Biopesticide Concentration and Soil Sterilization Interval for Controlling Stem Rot Disease by *Fusarium oxysporum* in *Vanilla planifolia*

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Abstract

Background: *Stem rot disease caused by Fusarium oxysporum is one of the major diseases affecting vanilla plants (Vanilla planifolia A.), which can cause losses of up to 100%. Current management practices rely heavily on synthetic pesticides and often lack precision, raising environmental concerns and need for sustainable disease management strategies. This study aimed to determine the effectiveness of Fobio biopesticide concentration and soil sterilization intervals as control methods in suppressing the development of vanilla stem rot disease.* **Methodology:** *The study was conducted in vivo in Wonosalam Village, Jombang Regency using a factorial completely randomized design (CRD) with factors of Fobio biopesticide concentration (5 mL/L, 10 mL/L, and 15 mL/L) and application interval (1-week and 2-week intervals). The data were analyzed statistically using the Analysis of Covariance (ANCOVA). The parameters observed included disease intensity, infection rate, and biopesticide effectiveness.* **Findings:** *The results showed that the initial disease intensity had a very significant effect on the final intensity. The treatment with an application interval of once a week reduced disease intensity to 23.89%, lower than the interval of 2-weeks (26.67%) and the control (46.66%). A concentration of 15 ml/L reduced disease intensity to 24.94%, with the highest effectiveness also obtained in the 15 ml/L treatment at 1-week intervals (50.29%). The interaction between interval and concentration was also not significant, indicating that the effect of concentration did not change depending on treatment interval. The biopesticide Fobio was found to be sufficiently effective in suppressing the development of vanilla stem rot, and applications with more frequent intervals showed the highest efficacy.* **Contribution:** *This study highlights the importance of integrating biopesticide concentration and application interval while adjusting for initial disease intensity in vanilla stem rot management.*

Keywords: *Fusarium oxysporum; Fobio Biopesticide; Vanilla planifolia*

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INTRODUCTION

The vanilla plant (*Vanilla planifolia* A.) is a high-value spice commodity widely cultivated in Indonesia. Vanilla is nicknamed "Green Gold" because of its high selling value and increasing market demand (Prasaja et al., 2024). In 2019, Indonesia was recorded as the third largest vanilla exporter in the world after Madagascar and France, with national production reaching 400 metric tons in 2021 and increasing to 500 metric tons in 2022 (Munandar et al., 2023). However, vanilla production in Indonesia has experienced significant fluctuations, as stem rot disease outbreaks were reported to severely affect major production centers in Java, Bali, Nusa Tenggara Timur, and Sulawesi. A drastic decline in production occured in Bali, decreasing from 323.31 tons in 1988 to 91.40 tons in 1994, with disease severity causing plant mortality rates of 70-100% at the farm level (Tombe et al., 2012).

Vanilla plants produce green pod-shaped fruits that have a distinctive aroma and are widely used in various industries such as food, cosmetics, pharmaceuticals, and health (Ramadhani & Fadillah, 2024). High demand for vanilla has encouraged many farmers to develop their cultivation businesses. However, vanilla cultivation in Indonesia faces various obstacles, especially pest and disease attacks that can reduce plant productivity. One of the main diseases that frequently attacks is vanilla stem rot caused by fungus *Fusarium oxysporum*, a soil-borne pathogen that can cause severity levels of up to 100% (Pardede et al., 2022). This disease has caused significant losses in various production centers such as Central Java, Bali, NTT, and Sulawesi, with plant mortality rates reaching 70–100% (Tombe et al., 2012). *Fusarium oxysporum* can attack plants by causing wilting or rot. When attacking during the vegetative phase, it causes abnormal plant growth, while attacking during the generative phase affects fruit production and can reduce yields by up to 50% (Khoirunisa et al., 2024).

Agustina et al., (2022) argued that chemical control of soil-borne diseases is generally ineffective because the active ingredients in fungicides degrade quickly in the soil. Control of vanilla stem rot disease has generally relied on synthetic chemical pesticides. Conventional pesticide use without regard to dosage and application accuracy can have a negative impact on the environment and reduce the population of beneficial microorganisms in the soil. Therefore, environmentally friendly control alternatives such as the use of biopesticides are needed. Natural-based biopesticides have been proven effective in suppressing pathogen populations. For example, clove oil and lemongrass oil can suppress *Fusarium oxysporum* attacks by up to 90% (Tombe et al., 2012), although they are relatively expensive and difficult for farmers to obtain. One potential biopesticide alternative is Fobio.

Fobio is an organic pesticide from microorganisms that live in the rhizosphere of roots siwalan, mangrove, sugar cane, coconut, and tunjang plants. The Fobio biopesticide contains various microorganisms that can act as biological agents, decomposers, and PGPR (*Plant Growth Promoting Rhizobacteria*), as well as containing nutrients that can increase production or crop yields (Wiyatiningsih et al., 2021). The composition of these microorganisms includes yeast, phosphate-solubilizing bacteria, *Lactobacillus* sp., *Rhizobium* sp., amylolytic bacteria, proteolytic bacteria, photosynthetic bacteria, ammonification bacteria, and nitrifying bacteria

(Wiyatiningsih et al., 2020). In addition, Fobio biopesticide also aims to increase plant resistance to pathogen attacks and reduce chemical compound residues in soil and plants (Fitriana et al., 2020).

Control using the biopesticide Fobio against the pathogen *Fusarium oxysporum* has been proven effective, as reported by Rahayu et al., (2021) which shows that attacks on shallot plants can be suppressed to an intensity of less than 20%. Fobio also functions as a soil bio-conditioning that is often used to reduce soil-borne pathogens (Rahayu, 2023). Various sterilization methods such as heat, synthetic chemicals, and irradiation are available, but they are rarely used because they can kill all soil life, so a safer alternative in the form of bio-sterilization is needed. Li et al., (2019) state that there are many soil sterilization methods available, including heat (e.g., dry heat and autoclaving), synthetic chemicals (e.g., sodium azide, formaldehyde, propylene oxide, chloroform, and methyl bromide), and irradiation.

However, these sterilization methods are rarely used in China because they can kill all life in the soil, so a safe and effective alternative soil sterilization method is needed to control soil-borne diseases, one of which is the application of bio-sterilization. The research by Li et al., (2023) shows that bio-sterilization using *allyl isothiocyanate* compounds from Brassica plants can reduce the population of soil pathogens such as nematodes and increase beneficial bacteria. Research by Hasyidan et al., (2021) also shows that soil sterilization treatment at 10 ml/L combined with the biopesticide Fobio at 3 ml/L can suppress *Fusarium oxysporum* attacks by up to 83% in shallots. This indicates that soil sterilization using the biopesticide Fobio has the potential to reduce the population of soil-borne pathogens and increase beneficial microorganisms, prompting research on the effect of concentration and interval of Fobio soil sterilization as a method of controlling stem rot disease in vanilla plants caused by *Fusarium oxysporum*.

METHOD

The research was conducted in Tukum Hamlet, Wonosalam, Jombang from March 2025. Pathogen identification was carried out at the Plant Health Laboratory, Faculty of Agriculture, UPN "Veteran" East Java. The study used a 2-factor factorial RAL: application interval once a week (I1) and once Every 2 weeks interval(I2), and Fobio concentrations of 5 ml/L (F1), 10 ml/L (F2), and 15 ml/L (F3). There were a total of 7 treatments including controls, with 3 replicates and 105 plants. Observation parameters included disease intensity, infection rate, and biopesticide efficacy. Data analysis was performed using IBM SPSS *Statistics* 24 software with *Analysis of Covariance (ANCOVA)* to evaluate the effects of biopesticide concentration and application interval by adjusting for initial disease intensity as a covariate, as this variable was not included as an experimental factor but affected the research outcomes.

Isolation and Identification of *Fusarium oxysporum* Stem Rot Pathogen

The stem rot pathogen *Fusarium oxysporum* was isolated from vanilla stems infected with *Fusarium oxysporum* stem rot. The isolation process was carried out by applying cotton swabs moistened with 70% alcohol to the surface or inside of split vanilla stems. This step was intended to remove unwanted microorganisms. Next, the vanilla stem was taken from the border between the diseased and healthy areas using a scalpel, then placed on PDA medium in a petri dish using aseptic (Kartubi et al., 2018). The stem pieces were grown on PDA medium in petri dishes and incubated at room temperature for 7 days. The *Fusarium oxysporum* fungus was observed macroscopically and microscopically. Macroscopic observation involved direct observation of colony color compared to the Munsell soil color chart, as well as the morphology of the mycelium or hyphae. Microscopic observation was performed by taking a small amount of *Fusarium oxysporum* mycelium and placing it on a slide stained with methylene blue.

Land Preparation

Land preparation was carried out by cleaning the vanilla plantation area. The land was cleared of debris and weeds in the vanilla planting area. Each bed was divided into plots and marked with research name boards. Plants per plot were observed and the initial disease intensity was calculated.

Treatment Application

Control application using the Fobio biopesticide soil sterilization method was carried out in accordance with the predetermined treatment on vanilla plants in the plots according to the concentration tested, except for the control treatment. The control formula using the Fobio biopesticide soil sterilization method was applied in WS (*Water Soluble*) form. The application of the Fobio biopesticide formula to the soil in the vanilla plant area at concentrations of 5 ml/L water, 10 ml/L water, or 15 ml/L water according to the treatment was carried out by turning the soil over using a hoe, then spraying it with Fobio liquid. Spraying with Fobio biopesticide was carried out at intervals of once a week or once every two weeks.

Research Parameters

The parameters observed included disease intensity, infection rate, and biopesticide effectiveness,

Disease Intensity

Disease intensity according to Tombe et al., (2012) can be calculated using a scoring system with the formula 1:

$$P = \frac{\sum(ni \times vi)}{Z \times N} \times 100\ \% \quad (1)$$

Notes:
P = Disease Intensity
ni = Number of infected plants
vi = Attack score value
Z = Highest attack score value
N = Number of plants observed

The attack value is determined based on the symptoms that appear in the research field from the lowest to the highest category, resulting in the following categories on table 1.

Table 1. Attack Categories Based on Field Conditions by Tombe et al., (2012)

Determination of the value (score) for disease attack symptoms	
Symptoms of stem rot	**Score**
Healthy, with no signs of necrosis on the stem	0
Length of necrosis on the stem > 5 cm, but < 20 cm	1
Length of necrosis > 21 cm, but < 40 cm	2
Length of necrosis > 41 cm, but < 60 cm	3
Length of necrosis on the trunk > 61 cm	4

Disease Infection Rate

Vanilla stem rot is caused by the *Fusarium* pathogen, which is a monocyclic disease. The infection rate of monocyclic diseases can be calculated using the van der Plank monomolecular model formula by Warman et al., (2021).

$$r = \frac{1}{t} \times \left[In\ \frac{1}{1-Xt} - In\ \frac{1}{1-X0} \right] \text{ per unit of time} \qquad (2)$$

Notes:
Xt = Disease proportion at time t
X0 = Proportion of disease at the start of observation (t = 0),
t = Time
r = Disease infection rate

Effectiveness of Fobio Biopesticide

The effectiveness of biopesticides is used to determine the effectiveness of Fobio biopesticide application as a soil sterilization solution on vanilla plants, calculated using a formula modified by Elfina et al., (2017).

$$EF = \frac{IPk - IPp}{IPk} \times 100\% \qquad (3)$$

Notes:
EF = Effectiveness of Biopesticide
IPk = Disease intensity in the control
IPp = Disease intensity in the treatment

Based on the results of biopesticide effectiveness calculations, the ability of Fobio biopesticide as a soil sterilization solution can be assessed based on the biopesticide effectiveness categories (Elfina et al., 2017) (found on table 2).

Table 2. Biopesticide Effectiveness Categories

Biopesticide Effectiveness (%)	Effectiveness Category
0	Incapable
> 20–40%	Very ineffective
> 40–60%	Moderately capable

Biopesticide Effectiveness (%)	Effectiveness Category
> 60–80%	Capable
> 80	Very capable

RESULT AND DISCUSSION

Identification of *Fusarium oxysporum*, the pathogen causing vanilla stem rot

The identification of the vanilla stem rot pathogen was obtained from the isolation of vanilla plants showing symptoms of stem rot originating from a vanilla plantation located in Tukum Subvillage, Wonosalam Village, Jombang Regency. The identification process began with isolating stem samples suspected of being infected with vanilla stem rot. Stems infected with rot caused by *Fusarium oxysporum* are characterized by wilting and yellowing, followed by brownish rot and eventually necrosis that can spread throughout the plant (Figure 1). Kadir et al., (2019), stated that infected plants have wilted and dry stems and leaves, while at the same time, the roots turn brown, dry out, or die. This is followed by browning and death of the roots and stems.

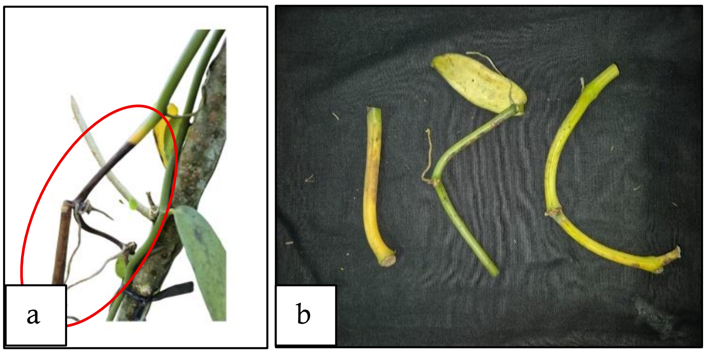

Figure 1. Symptoms of Vanilla Stem Rot. Figure description: a.) Symptoms of attacks on vanilla plants; b.) Samples of vanilla plants showing symptoms of stem rot

The isolation results obtained pure fungal colonies suspected to be *Fusarium oxysporum* with a colony diameter of 4.5 cm at 12 days of age. The macroscopic characteristics were purplish white with a purple center and a cottony *aerial* texture with fibrous colony edges (Figure 2). Macroscopic color identification of the colony was compared using *the Munsell soil color chart*, yielding a result of 5 RP 6/8, indicating that the isolate was reddish purple (*red purple*) with medium brightness (*value* 6) and high color intensity (*chroma* 8).

Microscopic identification shows the presence of microconidia, macroconidia, and chlamydospores at 1000× microscope magnification (Figure 3). Microconidia are oval to oblong in shape, measuring 5.7–8.1 µm (Figure 3.a). Macroconidia are oblong in shape with slightly pointed ends, like a canoe, with four septa, measuring 35.1 µm. Chlamydospores are characterized by a single spherical shape found in Fusarium isolates aged approximately 1 month, with a size of 9.6 µm.

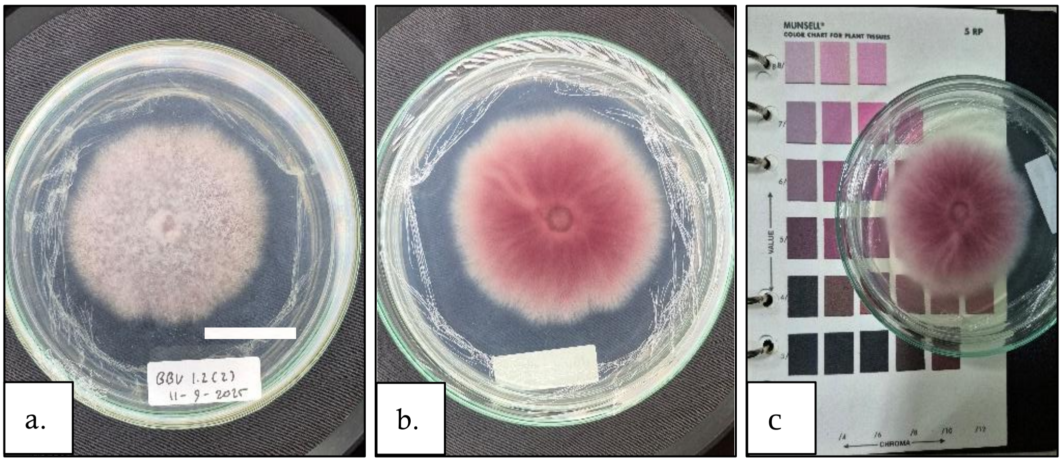

Figure 2. Macroscopic observation of Fusarium oxysporum isolates. Figure description: a.) Front view of Fusarium oxysporum isolates; b) Rear view of Fusarium oxysporum isolates; c.) Comparison of Fusarium oxysporum isolates with the Munsell Soil Color Chart.

This identification is consistent with the identification conducted by Adame-García et al., (2015), which states that *Fusarium oxysporum* isolates produce white or purple colonies with *aerial* mycelium and a texture resembling cotton or fibers. The surface color of the colony varies from pink to light purple or dark purple. Microconidia are oval or elliptical in shape, while macroconidia with 3-5 septa are elongated with pointed ends like a canoe. Chlamydospores appear as single round structures and are rarely found in pairs.

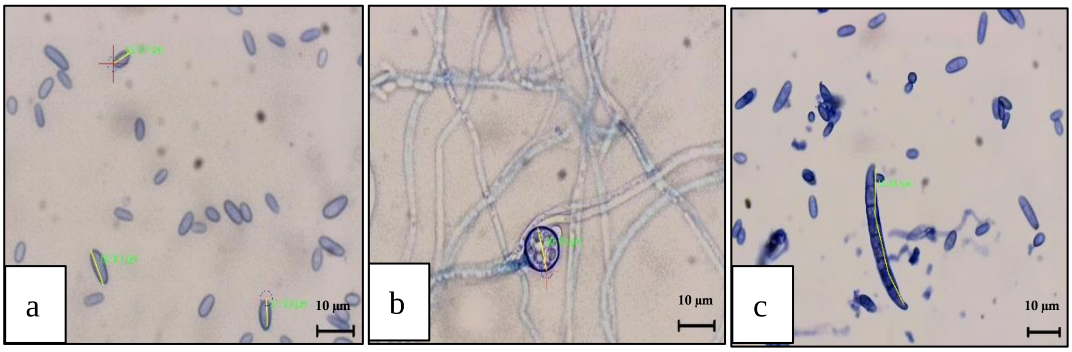

Figure 3. Microscopic Observation of *Fusarium oxysporum* Isolates. Figure description: a.) Microconidia; b.) Chlamydospores; c.) Macroconidia

Disease Intensity

The disease intensity calculated was the initial and final intensity of the study based on predetermined scoring. The *Ancova* analysis results showed that the initial intensity was significant to the final intensity, so that the initial condition of the disease was proven to be the main determining factor in influencing the level of attack at the final observation. The concentration treatment did not have a significant effect on the final intensity (*Sig.* 0.930), and similarly, the application interval did not show a significant effect at the 5% level (*Sig.* 0.061). In addition to the initial condition of the disease that can affect the final disease intensity, environmental factors can also affect the intensity of plant diseases. Jeni et al., (2025) in their research stated that based on soil chemical analysis, Wonosalam village has a pH ranging from 5.16 to 5.52, which is classified as acidic with an average temperature of 28 to 29°C. These environmental conditions support the development of diseases, particularly the pathogenic fungus *Fusarium oxysporum*, which can increase the intensity of attacks. This is consistent with the statement by Yuliana et al., (2022) that high humidity and acidic soil pH can support the growth of *Fusarium* fungi.

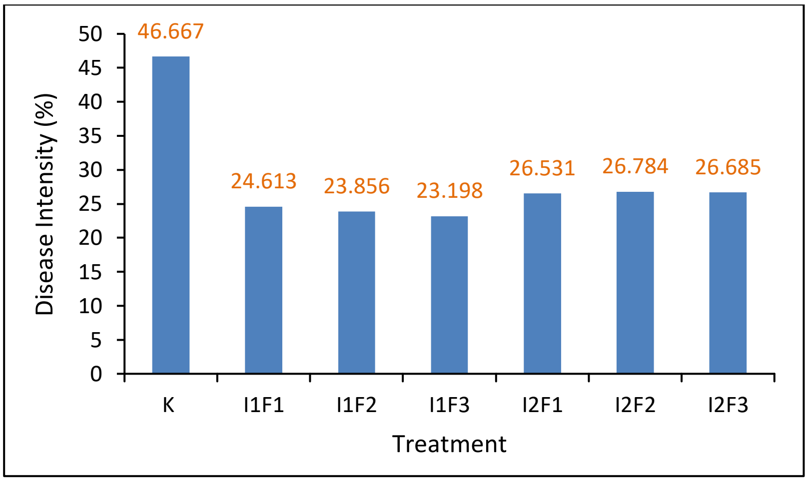

Figure 4. Graph of Average Vanilla Stem Rot Attack Intensity. Notes: K = Control (No Treatment); I1F1 = Once a week interval+ Fobio 5 ml/L; I1F2 = Once a week interval+ Fobio 10 ml/L; I1F3 = Once a week interval+ Fobio 15 ml/L; I2F1 = Every 2 weeks interval+ Fobio 5 ml/L; I2F2 = Every 2 weeks interval+ Fobio 10 ml/L; I2F3 = Every 2 weeks interval+ Fobio 15 ml/L.

The average intensity of vanilla plant disease, the lowest intensity was found in treatment I1F3 (once a week, 15 ml/L) with an intensity of 23.29%, while the highest intensity was found in the control (without treatment) with an intensity of 46.66%. Overall, when comparing the control with all treatments, there is a significant difference. This shows that the application of Fobio biopesticide is able to suppress stem rot disease in vanilla plants caused by the *Fusarium oxysporum* pathogen. In applications with a 1-week interval, the intensity of attacks decreased sequentially with increasing concentration, while at a 2-week interval, the intensity between concentrations showed almost no difference. This indicates that more frequent application intervals of biopesticides can further suppress disease attacks. The best treatment was obtained at a 1-week application interval because it could suppress vanilla stem rot disease by up to 77%. Jauhari & Majid, (2019), in their study on the application of various types of fungicides (*Triadimefon, Trichoderma harzianum*, and betel leaf) on the development of anthracnose disease, stated that the application interval plays a role in maintaining the availability of fungicide active ingredients in soybean plants.

Treatment at intervals of once a week, with the application of Fobio biopesticide concentration, showed that higher concentrations could reduce the intensity of the disease. This indicates that the higher the concentration of Fobio biopesticide sprayed on the soil, the more abundant population of antagonistic microorganisms that can suppress the development of the *Fusarium oxysporum* pathogen. Spraying Fobio biopesticide triggers competition between pathogens and antagonistic microorganisms for limited nutrients and space (Rosyiidah et al., 2025). Putra et al., (2019) argued that spraying antagonistic microorganisms into the soil can increase their population, thereby suppressing and reducing the population of pathogens and reducing their ability to infect plants.

The biopesticide Fobio contains various types of microorganisms, one of which is *Lactobacillus* sp. *Lactobacillus* sp. bacteria can produce an active ingredient in the form of lactic acid, which can act as an antagonist to the plant pathogen (Farisa et al., 2023). Plant resistance compounds can also be formed with the help of other organisms present in biopesticides, which are capable of producing compounds resistant to pathogen attacks. In addition, Fobio also contains yeast, which can also play a role in inhibiting the development of pathogens. Based on research conducted by Puspitasari et al., (2014), yeast can inhibit the growth of the pathogenic fungus *Collectotrichum* sp. with very good potential *in vitro*. Yeast has great potential in suppressing pathogens from different habitats because it acts as a new competitor in terms of space and nutrients, thereby exhibiting its antagonistic properties.

The Fobio biopesticide also contains proteolytic bacteria that can play a role in suppressing plant diseases. Proteolytic bacteria produce protease enzymes that can degrade the cell walls of pathogens and play a role in protecting against infection. Chanifah et al., (2025) explained that proteolytic bacteria can produce protease enzymes that break down proteins into simpler amino acids. These microorganisms are also capable of breaking down extracellular proteins, such as casein and gelatin, through the release of proteolytic enzymes, and the activity of these enzymes results in protein degradation.

Infection Rate of the Disease

The development of vanilla stem rot disease, which is suspected to be caused by *Fusarium oxysporum,* can be seen in the disease infection rate. Based on the results of Ancova analysis of the infection rate variable not yet statistically significant (*Sig.* 0.069). The 2-week interval tended to produce a higher infection rate compared to the 1-week interval. The solution concentration factor (5 15 ml/L, 1015 ml/L, and 15 ml/L) did not have a significant effect (*Sig.* 0.241), although the 15 ml/L concentration showed a lower average infection rate compared to the other two concentrations.

The table 3 shows a significant difference in infection rates between the control and the treatment combinations (Table 3). The infection rate in the control (0.069) tended to be higher than all treatment combinations, indicating that the application of the biopesticide Fobio can suppress the development of vanilla stem rot disease, which is thought to be caused by *Fusarium oxysporum*. The graph shows that the lowest infection rate was found in the treatment combination with a 1-week interval and a concentration of 15 ml/L. The infection rate is influenced by the amount of inoculum; the faster infection rate of a disease, the higher amount of inoculum. Additionally, a high infection rate can shorten the disease development period, thereby accelerating the occurrence of an epidemic of vanilla stem rot disease (Warman et al., 2021). Yuliana et al., (2022), argue that the occurrence of a plant disease is always related to the disease triangle, where a plant can be infected when it is susceptible, the attacking pathogen has high virulence, and the environmental conditions support pathogen growth.

Table 3. Average Infection Rate (r) of Disease per Day

Average Infection Rate (r) of Disease per Day	
Treatment	**Average**
K	0.069[a]
I1F1	0.003[b]
I1F2	0.001[b]
I1F3	0.0003[b]
I2F1	0.006[b]
I2F2	0.005[b]
I2F3	0.002[b]

Notes: K = Control (No Treatment); I1F1 = Once a week interval+ Fobio 5 ml/L; I1F2 = Once a week interval+ Fobio 10 ml/L; I1F3 = Once a week interval+ Fobio 15 ml/L; I2F1 = Every 2 weeks interval+ Fobio 5 ml/L; I2F2 = Every 2 weeks interval+ Fobio 10 ml/L; I2F3 = Every 2 weeks interval+ Fobio 15 ml/L

In this case, the environmental conditions during the study indeed had relatively high humidity, reaching up to 80%, with a temperature of around 26°C. These environmental conditions supported the growth of *Fusarium oxysporum* fungus, enabling it to develop and infect vanilla plants rapidly. Similar conditions occurred in the research conducted by González-Reyes et al., (2020), where the average temperature of 27.1°C and relative humidity of 84.3% in the vanilla field supported the development of the disease caused by *Fusarium*, which was characterized by a high disease incidence of 82.2%.

Effectiveness of Fobio Biopesticide

The effectiveness of Fobio biopesticide is consistent with the intensity of disease attacking vanilla plants. The graph on figure 6 shows the effectiveness of Fobio biopesticide in controlling stem rot disease in vanilla plants. The highest value was obtained in treatment I1F3 (once a week, 15 ml/L) with an effectiveness of ±50%. This shows that more frequent spraying with high concentrations provides the most optimal disease control. The lowest effectiveness occurred in I2F2 and I2F3 (once every two weeks, 10 ml/L and 15 ml/L), which was ±42% (Figure 6). At each of these efficacy levels, the lowest value of 42% falls into the "very ineffective" category for disease suppression, while the highest value of 50% falls into the "sufficiently effective" category for suppressing vanilla stem rot disease, which is suspected to be caused by *Fusarium oxysporum*.

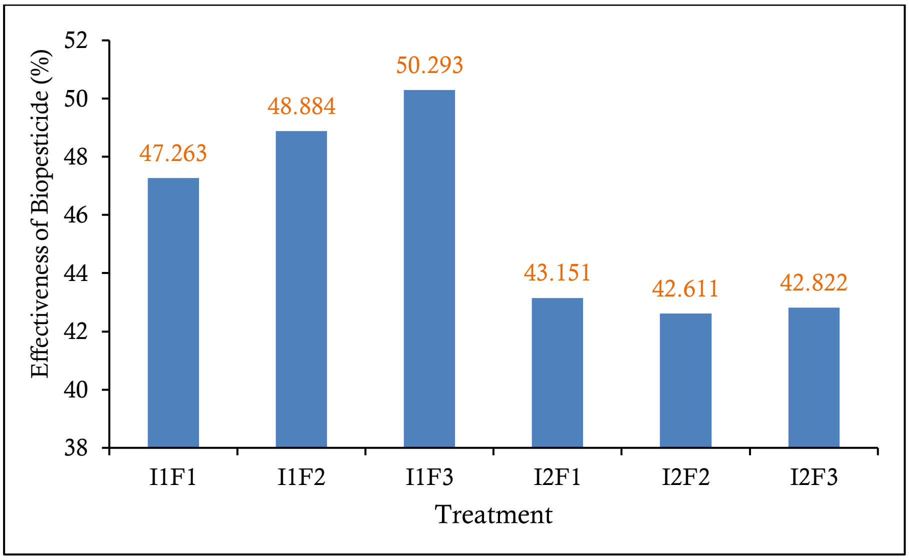

Figure 5. Average Effectiveness of Fobio Biopesticide. Notes: K = Control (No Treatment); I1F1 = Once a week interval+ Fobio 5 ml/L; I1F2 = Once a week interval+ Fobio 10 ml/L; I1F3 = Once a week interval+ Fobio 15 ml/L; I2F1 = Every 2 weeks interval+ Fobio 5 ml/L; I2F2 = Every 2 weeks interval+ Fobio 10 ml/L; I2F3 = Every 2 weeks interval+ Fobio 15 ml/L.

These categories indicate that the application of Fobio biopesticide soil sterilization as a control method has not been able to work optimally. This is due to the relatively short duration of the study. Vanilla is an annual plant with a slow growth period, and a study period of only two months resulted in the suboptimal application of Fobio biopesticide soil sterilization as a control method. This is because the control mechanism of Fobio biopesticide occurs indirectly. With periodic application over a long period of time, Fobio biopesticide can induce plant resistance so that vanilla plants will be more resistant to disease attacks and can increase their growth.

CONCLUSION

The concentration of Fobio biopesticide and the soil sterilization interval significantly contributed to suppresing stem rot disease in vanilla (*Vanilla planifolia* A.) caused by *Fusarium oxysporum*. Te combination of a 15ml/L fobio concentration apllied at a one-week interval was identified as the most effective treatment for disesase control. These results indicate that optimization of biopesticide application strategies can enhance disease suppression while supporting sustainable vanilla production.

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