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The Effect of Shelf Life and the Effectiveness of Biopesticide Fobio in Controlling Stem Rot Disease in Vanilla Plants (*Vanilla planifolia* Andrews)

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Abstract

Stem rot disease caused by *Fusarium oxysporum* poses a significant challenge to vanilla (*Vanilla planifolia* Andrews) cultivation. The use of microbial-based biopesticides, such as FOBIO, provides an environmentally friendly alternative to reduce reliance on chemical fungicides. This study aimed to evaluate the effects of FOBIO biopesticide storage age and concentration on disease intensity and vanilla plant growth (*Vanilla planifolia* Andrews). The research was conducted from March to June 2025 in Dusun Tukum using a factorial completely randomized design with two factors: FOBIO storage age (6 months and 1 year) and concentrations (5, 7.5, and 10 mL/L of water), with three replications totaling 105 plants. Data were analyzed using Analysis of Covariance (ANCOVA), followed by the Least Significant Difference (LSD) test at a 5% significance level. The results demonstrated that the FOBIO application significantly reduced disease intensity compared to the control ($p < 0.05$). The most effective treatment was FOBIO stored for 1 year at a concentration of 7.5 mL/L of water (FP23B), resulting in a final disease intensity of 6.365 compared to 38.255 in the control. FOBIO also increased leaf number and stimulated plant growth during the vegetative phase, including accelerating flower development. The FOBIO biopesticide is an environmentally friendly alternative for controlling stem rot in vanilla and serves as a biological control agent with potential to support sustainable agriculture.

Keywords: ANCOVA, Disease Intensity, *Fusarium oxysporum*, Plant Growth, Vanilla

1. Introduction

Vanilla (*Vanilla planifolia* Andrews) is a high-value plantation commodity widely cultivated in Indonesia. Global demand for vanilla continues to increase in line with the growth of the food and cosmetics industries (Kumolontong & Wongkar, 2024). However, vanilla productivity in the field remains low due to stem rot caused by the fungus *Fusarium oxysporum* (Efi Taufiq et al., 2017). This disease can cause plant death and reduce yields by up to 80% (Pardede *et al*, 2022).

Stem rot disease control is generally carried out using synthetic chemical pesticides, which have negative environmental impacts. A more environmentally friendly alternative is the use of biopesticides. One potential product is the biopesticide FOBIO, which contains various microorganisms, including Rhizobium sp., Lactobacillus sp., and phosphate-solubilizing bacteria, that act as biological agents and plant growth promoters (Hasyidan et al., 2021; Rahayu et al., 2021).

The effectiveness of biopesticides depends heavily on the viability of the microorganisms within them, which can decrease over time during storage (Mulyani et al., 2022; Alfiansyah et al., 2024). The microorganism content in Fobio biopesticide can also help improve plant growth. The microorganisms in it are mostly bacteria, including Rhizobium sp. and *Lactobacillus* sp. It is also known that bacteria generally have a fairly short incubation time. However, research on the shelf life of Fobio biopesticide has never been conducted. Therefore, based on the background, it is necessary to research the application of Fobio biopesticide with different shelf lives to control stem rot in vanilla plants, and the effectiveness of Fobio

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biopesticide in suppressing stem rot caused by the fungus *Fusarium oxysporum*. Therefore, this study was conducted to determine the effect of shelf life and concentration of FOBIO biopesticide on its effectiveness in controlling stem rot disease in vanilla plants. In addition, the FOBIO biopesticide is believed to reduce the incidence of stem rot and stimulate vanilla plant growth.

This study aims to determine the effect of FOBIO biopesticide shelf life on the effectiveness of controlling F. oxysporum, determine the optimum biopesticide concentration, and evaluate the growth response of vanilla plants to FOBIO biopesticide treatment.

2. Material and Methods

The research was conducted in March–June 2025 in Wonosalam Village, Jombang Regency, East Java. The village is located at the foot and on the slopes of Mount Anjasmoro, with coordinates -7.7060, 112.3647, and an average altitude of 500-600 meters above sea level. Wonosalam Village is located 35 km southeast of Jombang Regency with an area of 121.63 km². Climatologically, Wonosalam District has a range of temperatures, namely 17-30˚C. Wonosalam District falls under the E climate classification. Climate E indicates a rather dry rainfall type.

The research used a completely randomized design (CRD) with two factors: shelf life (6 months and 12 months) and concentration (5 ml/L, 7.5 ml/L, 10 ml/L), and a control. The materials used for the implementation of the Fobio application research as a control for the development of vanilla stem rot disease in vanilla plants included vainili plants, samples of vanilla plants with stem rot, and the Fobio trademark pesticide containing microorganisms derived from the rhizosphere of coconut, sugarcane, tunjang, siwalan and mangrove roots with a carrier medium in the form of potato extract, sugar, black sticky rice and meat, PDA media, water, distilled water, 70% alcohol and spirits. Meanwhile, the tools used for this research were an autoclave, laminar air flow (LAF), Erlenmeyer flask, measuring cylinder, petri dish, glass slide, glass object, loop needle, Bunsen burner, stove, microscope, cardboard, raffia rope, marker, scissors, hoe, sickle, hand sprayer, camera and writing instruments. The parameters observed included disease intensity, biopesticide effectiveness, and plant growth response.

Observation data from the two factors, namely the 12-month and 6-month Fobio shelf lives, will be analyzed statistically using IBM SPSS Statistics 24. The data were analyzed statistically using analysis of covariance (ANCOVA). If the F test shows a significant effect, a further test, namely the Least Significant Difference (LSD) at the 5% level, is performed. The parameters observed in this study are disease intensity, infection rate, biopesticide effectiveness and plant growth.

Observations were made on vanilla plant stems by counting the number of damaged plants caused by *Fusarium oxysporum.* Disease intensity was recorded every seven days using the scoring system in Table 3.1 for the control and Fobio treatments for 6 weeks. Disease intensity was calculated using the following systemic damage formula:

$$\boldsymbol{I = \frac{\sum(ni \times vi)}{N \times V} \times 100\%}$$

Information:
I = disease intensity (%)
n_i= number of plants in the i-th attack category
v_i= score value of the i-th attack category
N= total number of plants observed

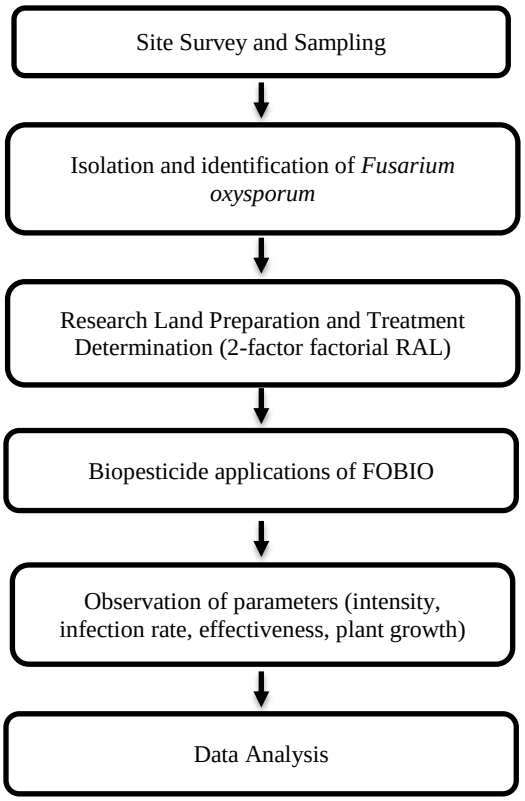

Figure 1 Research Implementation Flowchart

Vanilla stem rot is a monocyclic disease caused by the *Fusarium pathogen.* To determine the growth and development of this pathogen, the monomolecular formula model proposed by Van der Plank (1963) can be used, and the resulting equation can then be calculated (Toni et al., 2023). The monomolecular formula is shown below :

$$\boldsymbol{r = \frac{1}{t}\left(In\frac{1}{1-Xt} - In\frac{1}{1-X0}\right) \text{ per time unit}}$$

Information:
Xt : Proportion of disease at time
t : Proportion of disease at the start of observation (t = 0)
t : Time
r : Rate of disease infection

The effectiveness of biopesticides is used to determine the application of Fobio biopesticide as a soil sterilization solution on vanilla plants, calculated using a formula

modified from that of Elfina et al. (2017).

$$EF = \frac{IPk - IPp}{IPk} \times 100\%$$

Information:
EF = Biopesticide Effectiveness
IPk = Disease intensity in control
IPp = Disease intensity in treatment

Inhibition of stem rot disease development is characterized by the ability of vanilla plants to form new shoots and leaves. This finding is because Fobio treatment alters secondary metabolites and can suppress the growth of the *Fusarium fungus.* The number of new shoots and leaves was monitored every five days after Fobio treatment. Observations were made on all parts of the plants in one experimental plot of vanilla plants for six weeks after Fobio application.

3. Results and Discussion

The study was conducted on 3-year-old plants infected with stem rot disease. Disease intensity was assessed using a scoring method to determine the level of disease infestation. The scoring method used was determined based on field observations during the study, resulting in the scores in Table 1.

Table 1. Attack index for observing the intensity of Vanilla Stem Rot disease attacks

Symptoms of stem rot	Score
Healthy, without any signs of necrosis on the stem	0
Length of necrosis > 5cm, but < 20cm	1
Length of necrosis > 21cm, but < 40cm	2
Length of necrosis > 41cm, but < 60	3
Length of necrosis on the stem > 61cm	4

The ANOVA results showed that the FOBIO biopesticide, with different shelf lives and concentrations, significantly affected disease intensity (Sig = 0.001 < 0.05). Table 2. shows that the BNT test results for the control treatment (K) had a much higher disease intensity than those for the biopesticide treatments (FP23A, FP23B, FP23C, FP24A, FP24B, FP24C).

Table 2. Average Intensity of Stem Rot Disease in Vanilla Plants in Various FOBIO Biopesticide Treatments

Treatment	Average intensity (%)
K	38.25 ± 3.94a
FP23A	13.03 ± 3.71ab
FP23B	6.36 ± 3.71ab
FP23C	7.78 ± 3.67ab
FP24A	7.78 ± 3.67ab
FP24B	8.98 ± 3.80ab
FP24C	12.78 ± 3.67ab
BNT 5%	7.34

Description: The average value followed by the same letter indicates no significant difference according to the ANCOVA further test at a 95% confidence level (Sig. > 0.05), different letters indicate a significant difference between treatments (Sig. < 0.05).

The control treatment showed an average of 38.25%, while all biopesticide treatments showed much lower averages (6.00-13.03%). Since all biopesticide treatments were not significantly different from each other (letter notation "ab") and significantly different from the control (notation "a"), it can be concluded that the application of biopesticide has succeeded in suppressing the disease intensity significantly. The results of the comparison between the concentrations of 5 ml/L, 7.5 ml/L and 10 ml/L of FOBIO biopesticide with a shelf life of 12 months (FP23) and a shelf life of 6 months (FP24) showed no significant difference (notation "ab"), which indicates that the difference in shelf life and concentration is still effective in suppressing the intensity of stem rot disease in vanilla plants.

Based on the analysis results, the best treatment is the one with the largest difference in the average final disease intensity compared to the control. Control (K) has the highest average disease intensity, at 38.3%, indicating that the affected stem portion has dried and shriveled. In accordance with the statement by Subrata and Rai (2019), the symptoms that will appear are spots with less distinct boundaries, black in color, which quickly spread around the stem segments. After that, the affected part wrinkles (shrivels), turns brown and finally dries. In the treatment with FOBIO biopesticide (FP23) with a shelf life of 12 months, at concentrations of 5 ml/L, 7.5 ml/L, and 10 ml/L, the final disease intensity decreased significantly compared to the control (K). The lowest value was obtained at a concentration of 7.5 ml/L, with an average of 6.36%; followed by 10 ml/L, 7.79%; and 5 ml/L, 13.03%. Meanwhile, in the FOBIO biopesticide treatment, the 6-month shelf-life showed that the final disease intensity tended to increase again, although the average value remained lower than in the control (K).

In terms of infection rate, the results of the analysis of variance showed that the FOBIO biopesticide treatment with a storage period of 6 months (FP24) and a storage period of 12 months (FP23) had no significant effect on the rate of stem rot disease infection in vanilla plants (Sig. > 0.05).

Table 3. Average Infection Rate of Stem Rot Disease in Vanilla Plants

Treatment	Average (%)
Control	1.750 ± 0.012a
FP23A	1.660 ± 0.012b
FP23B	1.644 ± 0.012b
FP23C	1.644 ± 0.012b
FP24A	1.644 ± 0.012b
FP24B	1.651 ± 0.012b
FP24C	1.659 ± 0.012b
BNT 5%	0.0287

Description: The average value followed by the same letter indicates no significant difference at the 95% confidence level (Sig. > 0.05), different letters indicate a significant difference between treatments (Sig. < 0.05).

The average infection rate in the control (K) was 1.750%, while in each FOBIO biopesticide treatment, it ranged from 1.644% to 1.659%, indicating a consistent decrease in stem rot infection rate. The FOBIO biopesticide treatment with a 6-month shelf life (FP24) tended to produce slightly lower infection rates compared to the FOBIO biopesticide with a 12-month shelf life (FP23) at each concentration level given. Table 3 shows that none of the treatments showed significant differences in suppressing the disease infection rate. The lack of difference in infection rates between treatments is thought to be due to decreased viability of antagonistic microorganisms in the FOBIO biopesticide during storage or field application. The same was also conveyed by Setiawan et al. (2020): a biopesticide shelf life that is too long decreases the stability of secondary active compounds that play a role in the antibiosis mechanism.

The test results showed that the control (K) was significantly different from the FOBIO biopesticide treatment with a shelf life of 6 months (FP24) and a shelf life of 12 months (FP23), while between the biopesticide treatments with a shelf life of 6 months (FP24) and a shelf life of 12 months (FP23) with concentrations of 5 ml/L, 7.5 ml/L and 10 ml/L there was no significant difference. Although the difference was not statistically significant in each FOBIO biopesticide treatment, the results showed that the 6-month shelf-life FOBIO biopesticide had a better ability to suppress stem rot disease infection in vanilla plants than the 12-month shelf-life FOBIO biopesticide. In the biopesticide treatment with a shelf life of 6 months (FP24) at a concentration of 5 ml/L, the average infection rate was 1.644%, while in the biopesticide treatment with a shelf life of 12 months (FP23) at the same concentration, the average infection rate was 1.660%. A similar pattern was also observed at concentrations of 7.5 ml/L and 10 ml/L, where the 6-month shelf-life biopesticide treatment (FP24) consistently showed a slightly lower average infection rate.

The effectiveness of the FOBIO biopesticide was analyzed using analysis of variance (ANOVA) and followed by a Tukey HSD post hoc test at the 5% level. The results of the follow-up test at the 5% level in Table 4 show that the effectiveness of the FOBIO biopesticide in controlling stem rot disease in vanilla plants differed across treatments. The highest average effectiveness was observed in the FP23B treatment (86.66%), while the lowest was in the control (K; 0.00%).

Table 4. Average effectiveness of FOBIO biopesticide in suppressing stem rot disease in vanilla plants.

Type of Treatment	Average (%)
K	0.00 ± 7.91c
FP23A	50.00 ± 7.91b
FP23B	86.66 ± 7.91a
FP23C	66.66 ± 7.91b
FP24A	54.43 ± 791b
FP24B	66.66 ± 7.91b
FP24C	53.33 ± 7.91b
BNT 5%	18.98

Description: The same letters in the same column indicate no significant difference at the 5% confidence level ($\alpha = 0.05$) based on further testing.

The test results showed that the FP23B treatment was in a different subset from the control treatment (K), FP23A, FP24C, and FP24A, indicating that FP23B had a significantly greater effect on disease control than these treatments. Meanwhile, the FP23C and FP24B treatments had medium effectiveness values and were included in two groups (b and c), which showed that the two treatments were not significantly different from the treatments in the first or second group. The FP23B treatment was the most effective at suppressing stem rot in vanilla plants. This result means that the optimal effectiveness of the FOBIO biopesticide in suppressing stem rot disease in vanilla plants is at a concentration of 7.5 ml/L, with a shelf life of 12 months. Thus, the FOBIO biopesticide maintains stable performance even after being stored for a fairly long period of time.

Observations in the FP23B treatment showed that flowers emerged or entered the flowering phase after application of the FOBIO biopesticide, whereas in other treatments the plants remained in the vegetative phase. This phenomenon indicates that the application of the FOBIO biopesticide at this concentration and shelf life not only affects vegetative growth but also begins to stimulate the plant's generative phase. This early flowering is associated with the role of antagonistic microorganisms in the FOBIO biopesticide, such as *Trichoderma spp.* , *Bacillus subtilis*, and *Pseudomonas fluorescens*. These microbes are known to produce various phytohormones, especially auxins, cytokinins, and gibberellins, which play important roles in cell division, shoot elongation, and flower initiation (Suryani et al., 2021). The presence of these hormones can accelerate the transition of plants from the vegetative to the

generative phase when environmental and physiological conditions are supportive.

A concentration of 7.5 ml/L of FOBIO biopesticide appears to be optimal, providing a balance between microbial activity and plant physiological needs. Too low a concentration may not sufficiently stimulate antagonistic microbial activity, while too high a dose can cause an imbalance in the microflora around the roots.

Vanilla plant growth was monitored periodically by counting leaves during the FOBIO biopesticide application. The analysis of variance showed that FOBIO biopesticide treatments with different storage periods and concentrations significantly affected the final number of leaves in vanilla plants. Observations were made by counting the leaves on each plant, one by one. A further Least Significant Difference (LSD) test at the 5% level showed that the control treatment (K) was significantly different from all FOBIO biopesticide treatments (FP23A, FP23B, FP23C, FP24A, FP24B, and FP24C). Table 5 shows that the highest average number of leaves was obtained in the FP23C treatment (34.17 leaves), while the lowest value was found in the control treatment (4.60 leaves). The results of further tests showed that the control (K) was significantly different from all FOBIO treatments (Sig. < 0.05); however, the FOBIO treatments (both differences in shelf life and concentration) did not differ significantly (Sig. > 0.05).

Table 3. Mean number of final leaves of vanilla plants on different treatments of biopesticide FOBIO

Treatment	Avg%
K	4.60 ± 5.33a
FP23A	24.21 ± 5.09 b
FP23B	29.09 ± 5.03 b
FP23C	34.17 ± 5.00 b
FP24A	23.30 ± 5.06 b
FP24B	26.57 ± 4.99 b
FP24C	30.37 ± 5.01 b
BNT 5%	12.08

Description: Numbers followed by the same letter indicate no significant difference based on the Least Significant Difference (LSD) test at the 5% level.

The absence of significant differences between FOBIO treatments at various concentrations (5 ml/L, 7.5 ml/L, and 10 ml/L) or storage durations on the number of leaves indicates that the stability of antagonistic microorganisms in FOBIO biopesticides is still well maintained up to a 12-month shelf life. This result is in line with research by Rahmawati *et al. (2021),* which states that microbial viability in solid and liquid biopesticides can survive under certain storage conditions for more than 12 months without a significant decrease in effectiveness.

The significant difference between the control (K) and FOBIO biopesticide treatments indicates that FOBIO biopesticide application increased the number of vanilla leaves compared to untreated plants. This result suggests that the nutrients, microorganisms, or growth regulators present in FOBIO-treated plants stimulate vegetative growth, particularly leaf formation.

The application of the FOBIO biopesticide is effective not only in controlling stem rot but also in supporting the vegetative growth of vanilla plants, especially by increasing leaf number. Treatment with FOBIO, both at 6 and 12 months of storage, has similar effectiveness, providing flexibility for farmers in storing and using biopesticides in the field. The highest average value was observed in the FP23C treatment, namely 34.17%, indicating that the 12-month fobio treatment at a concentration of 10 ml/L of plant was most effective in increasing plant leaf number.

Figure 2 Comparison of the Number of Leaves in Vanilla Plants. a) Beginning of Research, b) End of Research.

4. Conclusion

Based on the results of research conducted on the effects of storage duration and the application of FOBIO biopesticide on stem rot disease caused by *Fusarium oxysporum* in vanilla plants (*Vanilla planifolia* Andrews), the following conclusions can be drawn:

1. FOBIO, with a shelf life of 12 months and used at a concentration of 7.5 mL/L, demonstrated the highest effectiveness (86.67%) and reduced disease intensity to 6.7%, the lowest among all treatments.
2. FOBIO, with a shelf life of six months at a concentration of 10 mL/L, did not show a statistically significant difference; however, it demonstrated the greatest biological tendency to suppress the rate of disease infection.
3. FOBIO, at a concentration of 10 mL/L and a shelf life of 12 months, was the most effective in stimulating vegetative growth, producing the highest average number of leaves (34.17).

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