

Application of Biopriming with Secondary Metabolites of *Beauveria bassiana* to Reduce the Intensity of *Fusarium* Wilt in Chili Pepper Plants

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Abstract

Background: *Fusarium* wilt, caused by *Fusarium* sp., is one of the main obstacles to chili pepper (*Capsicum frutescens* L.) production. Disease control using chemical pesticides and fungicides is considered effective in agricultural fields, but excessive use can result in environmental contamination and health risks, including cancer. This makes the application of biological control an effective strategy in overcoming *Fusarium* wilt disease in chili plants, especially in increasing plant productivity. This study aims to test the effectiveness of biopriming using *Beauveria bassiana* secondary metabolites in suppressing *Fusarium* wilt infection in chili seeds *in vivo*. **Methodology:** This study used a Completely Randomized Design (CRD). Secondary metabolites were applied to chili seeds at four concentration levels (15%, 25%, 35%, and 45%) as well as positive and negative controls. The treated seeds were planted in a medium inoculated with pathogens and observed for nine weeks. Disease intensity was recorded every seven days and analyzed using ANOVA followed by Tukey's Honestly Significant Difference (HSD) test at the 5% level. **Findings:** The results of the variance test 49–63 days after sowing (DAS) showed significant differences between treatments; a further HSD test was conducted at a 5% level. The application of *Beauveria bassiana* secondary metabolites as a biopriming agent on chili seeds is considered to be ineffective. **Contributions:** This study provides the first *in vivo* evidence on the application of *Beauveria bassiana* secondary metabolites as a seed biopriming agent to suppress *Fusarium* wilt in chili plants, thereby advancing preventive biocontrol strategies and contributing to the development of environmentally sustainable plant disease management.

Keywords: *Beauveria bassiana*; Biopriming; *Capsicum frutescens*; *Fusarium* sp.; Secondary Metabolites



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INTRODUCTION

According to [BPS \(2024\)](#) reported that in 2022, East Java province was able to produce 6.467.402 quintals of chili pepper, which decreased to 5,628,161 quintals in 2023. In 2024, there was a slight increase with a production value of 5.689.979,04 quintals. According to the latest BPS data, the production of chili pepper has not yet been able to reach the production value of 2022. Chili pepper is included in the group of commodities that have stable demand in the domestic market, with prices that often experience significant fluctuations due to imbalances in supply and demand ([Rio, 2024](#)). This makes it an important source of income for small-scale farmers.

Chili plants are a type of plant rich in vitamins A and B, making them useful in the food, pharmaceutical, and cosmetic industries ([Jamil et al., 2021](#)). This plant is also susceptible to soil-borne pathogens, such as *Fusarium* wilt, seedling blight, and root rot caused by pathogens such as *Pythium*, *Phytophthora*, *Fusarium*, *Sclerotium*, and *Rhizoctonia* ([Dar et al., 2015](#)). One of the most destructive diseases is *Fusarium* wilt in chili peppers caused by *Fusarium oxysporum*, which causes significant losses every year ([Shen et al., 2013](#)). [Iqbal et al., \(2024\)](#) also mention that *Fusarium oxysporum* is one of the most potentially destructive and deadly pathogens in chili plants.

Fusarium pathogens can survive for a long time in the soil and infect chili plants from the seedling stage to harvest ([Shen et al., 2013](#)). Symptoms of infection include leaf fall, yellowing of leaves, stunted growth, and shortening of stem internodes, which ultimately leads to plant death ([Shaheen et al., 2021](#)). Research conducted by [Dwiastuti et al., \(2015\)](#) shows that *Fusarium* sp. causes green leaves to wilt in the morning and evening, and in severe conditions, this pathogen can infect the plant's root tissue and rhizosphere. Another symptom of chili plants infected with *Fusarium* wilt is that the leaf stalks also begin to droop and eventually the entire plant wilts ([Rusman et al., 2018](#)). The incidence rate of *Fusarium* disease in solanaceous plants, especially chili peppers in Pakistan, is reported to reach 21,9%, resulting in a yield loss of 90.5-115.5 thousand tons ([Bashir et al., 2018](#)).

Disease control using chemical pesticides and fungicides is considered effective in controlling diseases in agricultural fields, but excessive use of chemicals in pesticides and fungicides will result in environmental contamination and health risks, including diseases such as cancer ([Chen & Ying, 2015](#)). This makes the application of biological control an effective strategy in overcoming *Fusarium* wilt disease in chili plants, especially in increasing plant productivity. This method not only supports sustainable agricultural practices but is also environmentally friendly, making it a solution that is in line with ecological principles.

Beauveria bassiana produces bioactive compounds in the form of secondary metabolites that have antibacterial, antifungal, cytotoxic, and insecticidal properties. Research by [Parine et al., \(2010\)](#) shows that this fungus is effective in inhibiting the growth of *Fusarium oxysporum*, the pathogen that causes *Fusarium* wilt in tomato plants, through its antifungal activity. In a study conducted by [Halwiyah et al., \(2019\)](#), *B. bassiana* also demonstrated its ability to inhibit the growth of *Fusarium solani*, a pathogenic fungus, by 29.19% through an antibiosis mechanism as a form of antagonistic interaction. [Ansar & Lakani \(2020\)](#) explained in their discussion that *B. bassiana* has a competitive inhibition mechanism, whereby the fungus will dominate

the space and nutrients until it covers the entire surface of the PSA medium. Antagonistic fungi are able to suppress pathogen development through competition for space and nutrients, antibiosis (producing antibiotics), and parasitism (Ainy et al., 2015). *Beauveria bassiana* is thought to have potential as a biopriming agent to enhance seed resistance to *Fusarium* sp. pathogen infection through its secondary metabolites. *B. bassiana* can induce systemic resistance in plants by activating defense pathways and increasing resistance to pathogens (Proietti et al., 2023).

Recent literature shows that *Beauveria bassiana*, particularly strains BG11 and FRh2, can reduce disease incidence by up to 70% (Raad et al., 2019), making it a potential environmentally friendly biocontrol agent. These findings are in line with the increasing urgency to reduce dependence on chemical fungicides that have a negative impact on the environment and trigger the emergence of resistant pathogen strains (Degani et al., 2022). However, most studies still focus on the direct application of fungi or in vitro testing, while the use of their secondary metabolites as biopriming agents on seeds, especially against *Fusarium* sp. infection in vivo, has been very limited. Therefore, this study offers a novel approach using secondary metabolites of *B. bassiana* as a biopriming agent for chili seeds to suppress *Fusarium* sp. infection from the early stages of plant growth. This study aims to evaluate the effectiveness of this approach in vivo as a sustainable preventive biological control strategy.

METHOD

This research was conducted from May 2025. The production of *Beauveria bassiana* secondary metabolites was carried out at the Surabaya Plantation Seed and Protection Center (BBPPTP), while testing of secondary metabolites in chili seeds was carried out in Waru, Sidoarjo, at a temperature of 25–27 °C and relative humidity of 50–70%. The tools used in the study included test tubes, Petri dishes, measuring cups, Laminar Air Flow (LAF), autoclaves, 250 ml Erlenmeyer flasks, beakers, shakers, centrifuges, 15 ml Eppendorf tubes, vortexes, syringes, 0.2 µm syringe filters, micropipettes and tips, inoculation needles, glass stirrers, Bunsen burners, analytical scales, microscopes, cell phone cameras, and molds. The materials used in the study included *B. bassiana* biological control agent isolates obtained from coffee plantations in Wonosalam and previously applied to *Helopelthis* sp. pests on cocoa plants, *Fusarium* sp. pathogen isolates from corn stalks infected with *Fusarium* sp. pathogens and previously tested with endophytic bacteria *Bacillus* sp, instant Potato Dextrose Agar (PDA), 250 grams of potatoes, 20 grams of sugar, distilled water, aluminum foil, planting media (soil and compost in a 1:1 ratio), seedling trays, spiritus, 70% alcohol, and certified chili seeds.

Research Implementation

Sterilization of Equipment

The method of sterilizing research equipment is crucial to ensure that all tools are free from microorganisms that can affect the research results. One method that is often used is wet sterilization with an autoclave, which uses pressurized steam at a

temperature of 121°C, 1.5 atm for 25 minutes to kill pathogenic microorganisms (Andriani, 2016).

Preparation of Pathogenic Fungal Isolates and Biological Agents

The preparation of *Fusarium* sp. pathogenic fungal isolates, which were collected by Ahmad Adibul Akrom, and *B. bassiana* biological agents, which were collected by the Surabaya Plantation Seed and Protection Center (BBPPTP), was carried out through a rejuvenation process to ensure that both fungi remained viable and had good biological activity. This procedure began with taking fungal cultures from existing collections, then planting them on appropriate culture media, such as Potato Dextrose Agar (PDA) for *Fusarium* and *B. bassiana*. The growing fungal colonies were taken and transferred to new media to multiply the number of isolates after incubation at optimal temperature conditions.

Identification of Fungus Isolation Results

The process of identifying fungal isolates is carried out through microscopic and macroscopic observation. Microscopic observation is performed by examining the shape of fungal conidia using an Olympus CX-33 microscope with a magnification of 400x to 1000x. Halwiyah et al. (2018) explained that microconidia with 0-2 septa are produced from conidiophores with short branches, ovoid-elliptical to cylindrical in shape, straight or slightly curved, and measuring (5.0-12.0) x (2.2-3.5) µm. Akrich et al. (2023) stated that *B. bassiana* fungal spores are round or oval in shape. Macroscopic observations were conducted by observing the color of the colonies using a cell phone camera.

Production of Secondary Metabolites of Beauveria bassiana

The production of secondary metabolites from *Beauveria bassiana* is a process for producing metabolite compounds. Two colonies of 7-day-old *B. bassiana* fungal isolates were introduced into 150 ml of sugar potato extract (EKG) media containing 250 grams of potatoes, 20 grams of sugar, and distilled water in a 250 ml Erlenmeyer flask. The medium containing the fungal isolate was then shaken at a speed of 150 rpm at room temperature for 7 days (Soesanto et al., 2020) and tightly covered with aluminum foil to avoid contamination by other fungi. The secondary metabolites from *B. bassiana* that had been incubated were then transferred to an Eppendorf tube for centrifugation. Separation between the supernatant and pellet was carried out at a speed of 10,000 rpm for 10 minutes. The supernatant was then filtered using a 0.2 µm syringe filter (Sukapiring et al., 2016).

Biopriming of Beauveria bassiana Secondary Metabolites on Chili Seeds

Biopriming of *Beauveria bassiana* secondary metabolites was modified from the best treatment in the study by Deb & Khair (2020) by soaking plant seeds for 24 hours to reduce the incidence of disease caused by seed-borne pathogenic fungi in *B. bassiana* secondary metabolites at concentrations of 15%, 25%, 35%, and 45% in dark conditions. The temperature of the soaking solution used was the normal solution temperature, ranging from 25 to 30°C, with a ratio of 40 seeds/10 ml of secondary

metabolite solution in various concentrations. A total of 240 chili seeds underwent this bioprimering process.

Testing of Beauveria bassiana Secondary Metabolites on Chili Plant Seeds

This treatment consisted of 6 levels of *Beauveria bassiana* secondary metabolite concentration as follows:

BB0 : Secondary metabolite concentration of 100% without pathogen inoculation (as a positive control treatment)

BB1 : 0% secondary metabolite concentration (as a negative control treatment)

BB2 : Secondary metabolite concentration of 15%

BB3 : Secondary metabolite concentration of 25%

BB4 : Secondary metabolite concentration 35%

BB5 : Secondary metabolite concentration 45%

The in vivo treatment used the Completely Randomized Design (CRD) method, which was repeated four times with 10 chili seeds in each repetition, resulting in a total of 240 chili seed units in the experiment.

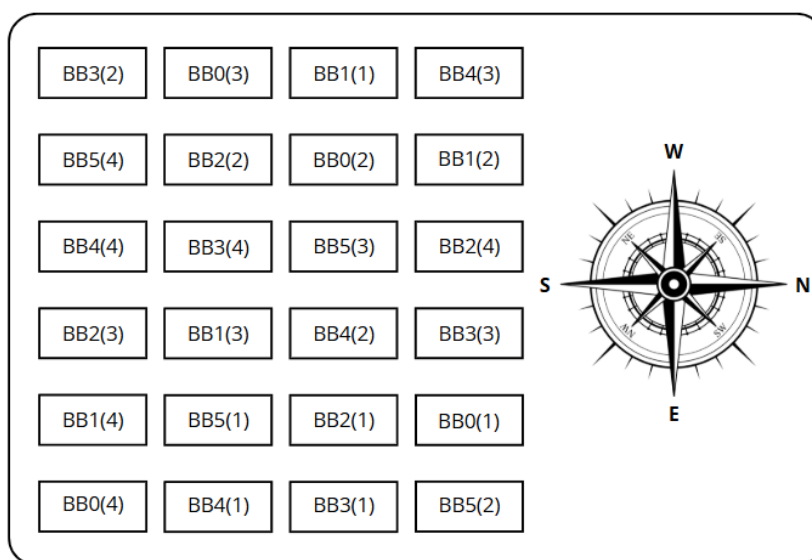


Figure 1. In Vivo Test Layout

The test was conducted by planting each chili seed that had been treated with bioprimering at various levels of secondary metabolites in a planting medium consisting of soil and compost in a 1:1 ratio. The plants were tended to daily, from watering to weeding. The *Fusarium* sp. solution was produced using 1 colony of *Fusarium* sp. with a diameter of 5 mm, which was dissolved in 10 ml of sterile distilled water and shaken for 3 minutes. The *Fusarium* sp. solution was then inoculated at a rate of 1 ml into the growing medium in each seedling tray box and incubated for 7 days to allow the pathogen to develop in the soil. The spore density used for infection was in accordance with the standard of 10^6 mL^{-1} (Khoirunnisa et al., 2022). The planting of chili seeds that had been inoculated with *B. bassiana* secondary metabolites was carried out after

7 days of pathogen incubation in the growing medium in the seedling trays. Observations of disease intensity in chili plants were conducted every 7 days for 9 weeks.

Disease Intensity

The percentage of infected plants was measured from the first day of planting, and observations were recorded every 7 days for 9 weeks. The number of chili seeds observed in this parameter was 240 chili seeds. The calculation of the percentage of infected plants was used to measure the development of symptoms in chili seeds that could grow. [Mugiastuti et al., \(2019\)](#) proposed the following formula for the percentage of infected plants:

$$I = \frac{\sum (n \times v)}{Z \times N} \times 100\%$$

Explanation:

- I : Infection intensity (%)
- n : Number of plants in each attack category i
- v : Scale value for each attack category i
- Z : Scale value of the highest attack category
- N : Number of plants observed

The values used to calculate the percentage score of plant damage caused by *Fusarium* wilt disease based on [Hasanah \(2023\)](#) are as follows:

- 0 : Healthy plants, no symptoms of attack
- 1 : 1 – 25 % of leaves wilted
- 2 : 26 – 50 % of leaves wilted
- 3 : 51 – 75% of leaves wilted
- 4 : 76 – 100% of leaves wilted

Data Analysis

The research data were analyzed using the Analysis of Variance (ANOVA) method. If significant differences were found in the treatment, Tukey's Honestly Significant Difference (HSD) with a significance level of 5% was used in SPSS.

RESULT AND DISCUSSION

Identification of *Fusarium* sp. Pathogenic Fungi

Macroscopically, *Fusarium* sp. pathogen isolates have white colony surfaces when young and gradually turn dark red as the days pass (Figure 2). This is in line with research conducted by [Helena \(2022\)](#), who stated that *Fusarium* fungus isolates have yellowish-brown colonies and white, cotton-like colony surfaces.



Figure 2. Visual of *Fusarium* sp. colony on a 9 cm diameter Petri dish

Microscopic identification of *Fusarium* using an Olympus CX-33 microscope found one of its asexual spores, namely microconidia. This is in accordance with [Nugraheni \(2010\)](#), who stated that *Fusarium* sp. has three types of asexual spores, namely macroconidia, microconidia, and chlamydospores. The microconidia of the fungus found were oval to elongated with blunt ends (Figure 3), which over time would become more pointed and slightly curved to form macroconidia. [Halwiyah et al., \(2018\)](#) explain that microconidia with 0-2 septa are produced from conidiophores with short branches, ovoid-elliptical to cylindrical in shape, straight or slightly curved, and measuring $(5.0-12.0) \times (2.2-3.5) \mu\text{m}$.

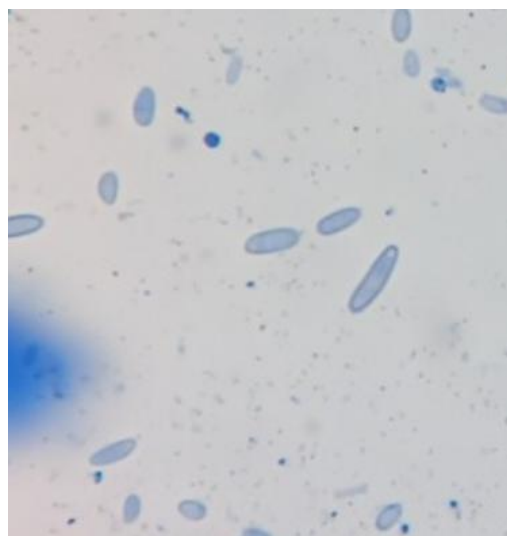


Figure 3. Microconidia *Fusarium* sp. (on 400X magnification)

Identification of *Beauveria bassiana* Antagonistic Fungus

The antagonistic fungus isolate *B. bassiana* used in this study was collected by the Surabaya Plantation Seed and Protection Center (BBPPTP) from coffee plantations in Wonosalam that had previously been treated for *Helopelthis* sp. pests on cocoa plants. The fungal colonies used in this study were macroscopically white and powdery in color and had the characteristic of growing randomly across the Petri dish (Figure 4). Macroscopically, *B. bassiana* fungal colonies on Petri dish media appeared white, spread out, and formed cotton-like clumps (Geremew et al., 2023).

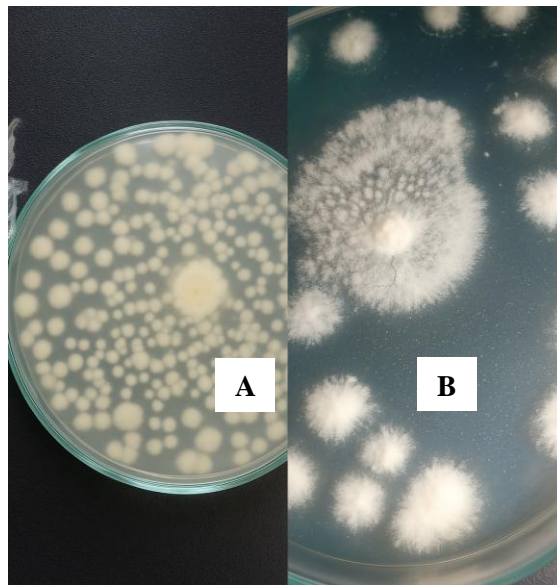


Figure 4. Macroscopic form of *Beauveria bassiana*, bottom view (A); top view (B) on a 9 cm diameter Petri dish.

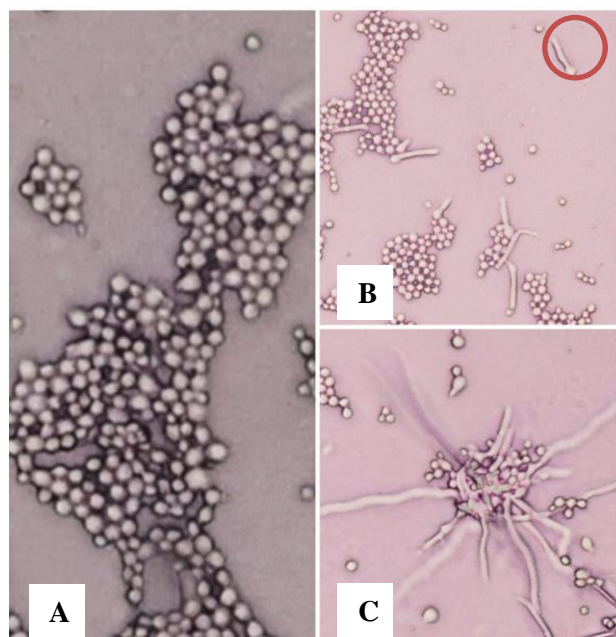


Figure 5. Microscopic morphology of *Beauveria bassiana*, spores (A); germinating spores (B); conidia cluster (C) at 400X magnification.

B. bassiana observed microscopically using an Olympus CX-33 microscope revealed clusters of conidia and fungal spores that were round in shape, which then sprouted into increasingly long hyphae (Figure 5). This is consistent with the opinion of Akrich et al., (2023), who stated that *B. bassiana* fungal spores are round or oval in shape. The hyphae of *B. bassiana* have branches measuring approximately 1-2 μm (Tantawizal et al., 2015).

Testing of *Beauveria bassiana* Secondary Metabolite Biopriming in Reducing the Intensity of *Fusarium* Wilt Disease in Chili Plants

The observation of biopriming testing of *B. bassiana* secondary metabolites in reducing the intensity of *Fusarium* wilt disease in vivo was conducted for 9 weeks (63 days). The observation interval was once a week to observe the symptoms of *Fusarium* sp. attack on chili plants and to perform scoring. The results of the disease intensity observation data are presented in the following graph on figure 6.

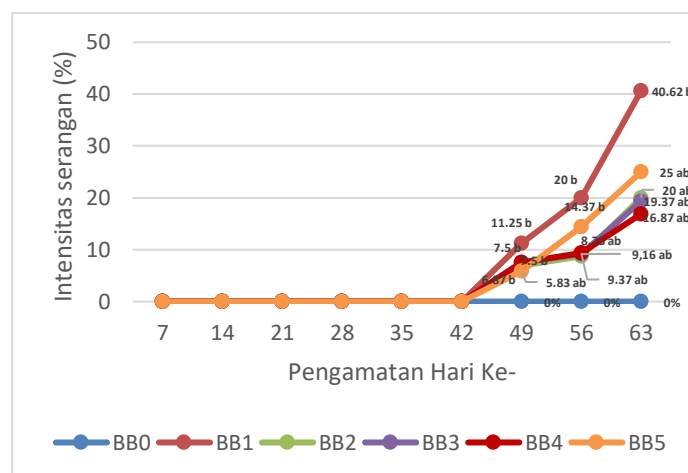


Figure 6. Graph of *Fusarium* sp. disease intensity development

The BB5 treatment at 49 days after sowing (DAS) in the graph above shows that the lowest disease intensity was 5.83%, but this treatment was not significantly different from all other treatments and the control, while the BB1 treatment showed the highest disease intensity of 11.25% (Figure 6). The lowest disease intensity at 56 DAS was found in the BB2 treatment at 8.75%, but this treatment was not significantly different from all treatments and controls, while BB1 showed the highest disease intensity at 20%. The lowest intensity at 63 DAS was found in BB4 at 16.87%, but this result was not significantly different from all treatments and controls, while treatment BB1 showed the highest intensity of 40.62%.

Based on the results of the analysis of variance at 7 DAS to 42 DAS, there was no significant difference between treatments, because at this time the chili plants had not yet shown any symptoms of *Fusarium* sp. wilt disease. Unfavorable environmental factors are suspected to be the cause of the *Fusarium* sp. pathogen not yet developing to infect chili plants so that symptoms did not appear.

Diyasti & Amalia (2021) also stated that plants under environmental stress are more susceptible to pathogens that cause disease. The optimal temperature for *Fusarium* sp.

fungi to grow is between 30-33°C (Susanti et al., 2016). Winanda (2026) explains in his research that the ideal environment for the growth of *Fusarium* sp. is with an acidic soil pH of 5.5 and humidity reaching 54%, causing symptoms to appear more quickly.

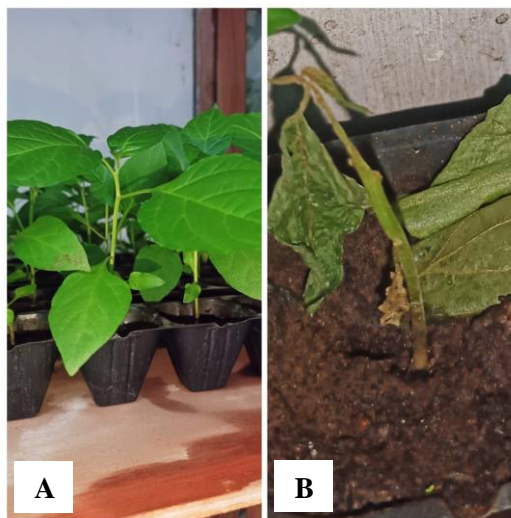


Figure 7. Comparison of disease intensity symptoms in chili plants, healthy chili plants in the control+ treatment (A); chili plants infected with *Fusarium* wilt disease in the 15% concentration treatment (B).

The results of the 49 DAS to 63 DAS variance test showed significant differences between treatments, therefore a further HSD test was conducted at a 5% level. Environmental factors such as weather are suspected to be the cause of the significant differences between treatments at this time, as rainfall intensity was very high during this period, causing symptoms of *Fusarium* sp. infection to appear. High soil moisture and warm temperatures support the growth of *Fusarium* sp. fungi, so infections often occur during the rainy season (Naufal et al., 2021). Shabrina (2010) also stated that unbalanced environmental conditions, including ambient temperature, rainfall, humidity, and soil pH, reduce the ability of biological agents to control plant pathogens.

The symptoms of attack begin with pale green leaves, which over time turn yellow and fall off, causing the plant to wilt (Figure 7). Parihar et al., (2022) also explain that the initial symptoms of *Fusarium* sp. infection are changes in leaf color from pale green to yellow, followed by wilting of the plant, and ultimately death of the entire plant. Another symptom of chili plants infected with *Fusarium* wilt is that the leaf stalks also begin to droop and eventually the entire plant wilts (Rusman et al., 2018). One of the characteristics of *Fusarium* infection is a change in color in the xylem vessels, which turn brown or reddish-brown when the stem is cut (Pamungkas, 2022). This fungus can grow into colonies and spread into the xylem vessels, disrupting the plant's transport system (Wongpia & Lomthaisong, 2010).

CONCLUSION

The application of *Beauveria bassiana* secondary metabolites as a biopriming agent on chili seeds is considered to be ineffective, but a concentration of 35% showed the lowest intensity of *Fusarium* wilt disease at 63 DAS, which was 16.87%, although this value was not significantly different from other treatments or the control. In contrast, the negative control showed the highest disease intensity of 40.62%. These results indicate that *B. bassiana* secondary metabolites have the potential to suppress the development of *Fusarium* wilt disease in the early stages of plant growth, thus offering opportunities for development as a preventive and environmentally friendly disease control strategy. Overall, this study provides scientific evidence of the initial use of *B. bassiana* secondary metabolites as an in vivo biopriming agent for chili seeds against *Fusarium* infection. Further research is recommended to evaluate higher concentrations, optimize application methods, and examine plant defense response mechanisms to improve the effectiveness and consistency of results on a field scale.

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