Bioscientist: Jurnal Ilmiah Biologi
https://e-journal.undikma.ac.id/index.php/bioscientist

June 2026 Vol. 14, No. 2
e-ISSN: 2654-4571
pp. 820-832

Effectiveness of Antagonistic Agents from Several *Bacillus* spp. Isolates against Anthracnose Disease (*Colletotrichum capsici*) in Chili Pepper Fruit (*Capsicum annuum* L.)

[1]Aullyvia Putri Nabiella, [2*]Yenny Wuryandari, [3]Herry Nirwanto
[1,2,3]Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia.
*Corresponding Author e-mail: yennywuryandari@upnjatim.ac.id
Received: April 2026; Revised: May 2026; Accepted: June 2026; Published: June 2026

Abstract: This study aimed to evaluate the antagonistic activity of selected *Bacillus* spp. isolates against *Colletotrichum capsici* and determine the most effective isolate and cell density for suppressing anthracnose in red chili pepper. The study was conducted under in vitro and in vivo conditions. The in vitro assay tested five *Bacillus* spp. isolates, namely Bcz-14, Bcz-16, Bcz-20, Bcz-21, and Bcz-30, at cell densities of 10^9 and 10^{10} CFU/mL. The three most effective treatments from the in vitro assay were subsequently evaluated under in vivo conditions. Data were analyzed using SPSS version 24 through analysis of variance (ANOVA) at a 5% significance level, followed by Tukey's honestly significant difference (HSD) test at 5% when significant differences were detected. The results showed that B1K1 treatment (Bcz-14 at 10^9 CFU/mL), with an inhibition zone of 5.83 mm; B5K1 treatment (Bcz-30 at 10^9 CFU/mL), with an inhibition zone of 5.50 mm; and B2K1 treatment (Bcz-16 at 10^9 CFU/mL), with an inhibition zone of 4.16 mm, exhibited the most stable inhibitory effects against *C. capsici* growth in vitro. The in vivo assay demonstrated that B1K1 treatment (Bcz-14 at 10^9 CFU/mL) was the most effective treatment, resulting in a disease intensity of 26.25% and a control efficacy of 51.16%.
Keywords: Anthracnose; red chili pepper; *Colletotrichum capsici*; *Bacillus* spp.; biological control

How to Cite: Nabiella, A. P., Wuryandari, Y., & Nirwanto, H. (2026). Effectiveness of Antagonistic Agents from Several Bacillus spp. Isolates against Anthracnose Disease (Colletotrichum capsici) in Chili Pepper Fruit (Capsicum annuum L.). *Bioscientist: Jurnal Ilmiah Biologi*, *14*(2), 820–832. https://doi.org/10.33394/bioscientist.v14i2.20692

https://doi.org/10.33394/bioscientist.v14i2.20692

INTRODUCTION

Red chili pepper (*Capsicum annuum* L.) is an important horticultural crop. Horticultural commodities play a key role in supporting the agricultural sector because they have high economic potential and market value. Among horticultural crops, chili occupies the largest cultivated area in Indonesia. According to Umatron et al. (2022), several regions in Indonesia are major producers of red chili, including East Java, West Java, Central Java, and North Sumatra. East Java is one of the largest red chili-producing provinces, with several major production centers, such as Malang, Kediri, Banyuwangi, and Tuban Regencies. Based on data from Statistics Indonesia (BPS) in 2023, East Java produced up to 847,119 quintals of large chili, with Malang Regency contributing the highest production, reaching 161,444 quintals.

However, the demand for chili has not yet been fully met, primarily due to declining red chili production caused by several limiting factors (Sutarni, 2023). One of the major constraints is the attack of plant pests and diseases, commonly referred to as plant-disturbing organisms. Anthracnose is a destructive disease that can reduce both the quality and quantity of red chili production, causing adverse effects for farmers and resulting in economic losses of up to 80%. Plant diseases are among the major causes of crop damage, including anthracnose in chili, which is caused by the fungus *Colletotrichum capsici* (Nurjasmi & Suryani, 2020). Typical symptoms of this disease

include blackish lesions with sunken surfaces, which eventually cause chili fruits to dry, shrink, and become unmarketable.

Anthracnose control by farmers commonly relies on the intensive and repeated application of chemical fungicides at high doses, particularly when disease outbreaks occur. However, such practices may have negative impacts on agricultural products and the environment. Therefore, alternative control strategies are needed, including the use of biological control agents such as antagonistic bacteria. According to Haryani & Tombe (2011), antagonistic bacteria are biological control agents capable of producing compounds that suppress plant pathogens. One group of antagonistic bacteria with potential to control *C. capsici* is *Bacillus* sp. This bacterium can function as a biological control agent because of its ability to compete for nutrients and produce secondary metabolites and extracellular enzymes.

Rani et al. (2022) reported that *Bacillus* sp. tested in vitro at a population density of 10^9 CFU/mL was able to inhibit the growth of *C. capsici*, with an inhibition percentage of 23.04%. Similarly, Hersanti et al. (2021) found that the application of the biological control agents *B. subtilis* and *Lysinibacillus* sp. at a population density of 10^{10} CFU/mL suppressed the growth of *Fusarium oxysporum* f. sp. *lycopersici* (Fol), the causal agent of tomato seedling blight, with an inhibition percentage of 59.49%.

This study used five *Bacillus* spp. isolates, namely Bcz-14, Bcz-16, Bcz-20, Bcz-21, and Bcz-30, from the collection of Prof. Dr. Ir. Yenny Wuryandari, M.P. Based on the findings of Zinidin (2022), the *Bacillus* spp. isolates Bcz-30, Bcz-20, and Bcz-21 inhibited the growth of *R. solanacearum*, producing the largest inhibition zones of 34.67 mm, 34.17 mm, and 34.17 mm, respectively. In addition, Wuryandari et al. (2022) reported that *Bacillus* spp. suppressed the growth of *Fusarium* sp. in chili plants, with isolate Bcz-20 showing the highest inhibition, followed by isolates Bcz-14 and Bcz-16, with inhibition zone diameters of 65.33 mm, 64.00 mm, and 63.67 mm, respectively.

Although these five *Bacillus* spp. isolates have shown potential as antagonistic agents against other pathogens, their effectiveness against *C. capsici* at two population densities has not been fully evaluated. In addition, studies using the detached fruit method on chili fruit remain limited. Therefore, this study was conducted to further evaluate the potential of *Bacillus* spp. in controlling *Colletotrichum capsici*, the causal agent of anthracnose. Specifically, this study aimed to determine the ability of several *Bacillus* spp. isolates to inhibit *C. capsici*, assess their potential to suppress anthracnose development in red chili fruit, and identify the most effective *Bacillus* spp. isolate and population density for controlling anthracnose.

METHOD

Time and Location of the Study

This study was conducted from March to December 2025. The in vitro and in vivo assays were carried out at the Plant Health Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Jawa Timur.

Tools and Materials

The equipment used in this study included Petri dishes, test tubes, a laminar air flow cabinet, inoculating loops, Erlenmeyer flasks, an autoclave, a vortex mixer, measuring cylinders, beakers, micropipettes, micropipette tips, forceps, glass stirring rods, a microscope (Olympus CX 33), glass slides, cover slips, scalpels, an analytical balance, rulers, and pens.

The materials used included pure bacterial isolates of *Bacillus* spp. Bcz-14, Bcz-16, Bcz-20, Bcz-21, and Bcz-30; a pure fungal isolate of *Colletotrichum capsici*; potato dextrose agar (PDA); nutrient agar (NA); sterile distilled water; 70% alcohol; 70%

ethanol; cotton; aluminium foil; tissue paper; rubber bands; straw paper; label paper; and healthy chili fruits.

In Vitro Experimental Design

The in vitro assay was arranged using a factorial completely randomized design. The first factor was the type of *Bacillus* spp. isolate (B), and the second factor was the population density of *Bacillus* spp. (K), consisting of 10^9 CFU/mL (K1) and 10^{10} CFU/mL (K2). Each treatment was replicated three times, resulting in 33 experimental units. The treatments were as follows:

B1K1 = Bcz-14 at a density of 10^9 CFU/mL
B1K2 = Bcz-14 at a density of 10^{10} CFU/mL
B2K1 = Bcz-16 at a density of 10^9 CFU/mL
B2K2 = Bcz-16 at a density of 10^{10} CFU/mL
B3K1 = Bcz-20 at a density of 10^9 CFU/mL
B3K2 = Bcz-20 at a density of 10^{10} CFU/mL
B4K1 = Bcz-21 at a density of 10^9 CFU/mL
B4K2 = Bcz-21 at a density of 10^{10} CFU/mL
B5K1 = Bcz-30 at a density of 10^9 CFU/mL
B5K2 = Bcz-30 at a density of 10^{10} CFU/mL
K = Control without *Bacillus* spp. isolate

In Vivo Experimental Design

The in vivo assay was conducted using a completely randomized design with one factor, namely the *Bacillus* spp. isolate (B). Three isolates were selected based on the highest inhibition values and the standard deviation values as indicators of data stability in the in vitro antagonistic assay. These isolates were Bcz-14, Bcz-30, and Bcz-16, each applied at a population density of 10^9 CFU/mL. Therefore, different treatment codes were used in the in vivo assay.

Each treatment consisted of four replications, and each replication included five red chili fruit samples. Thus, a total of 80 red chili fruits were used. The three isolates with the highest inhibition values in the in vitro assay were then tested in the in vivo assay as follows:

B1K1 = Bcz-14, the isolate with the highest inhibition value, at 10^9 CFU/mL
B2K1 = Bcz-30, the isolate with the second-highest inhibition value, at 10^9 CFU/mL
B3K1 = Bcz-16, the isolate with the third-highest inhibition value, at 10^9 CFU/mL
K = Control without *Bacillus* spp. isolate

Isolation of *Colletotrichum capsici*

Colletotrichum capsici was isolated from chili fruits showing anthracnose symptoms. The surface of symptomatic chili fruits was first sterilized using distilled water, alcohol, and distilled water again. The fruits were immersed for 30 s in distilled water, 60 s in alcohol, and 30 s in distilled water.

After sterilization, the chili fruits were dried, and tissue sections measuring 1 cm were excised from the boundary between symptomatic and healthy areas. The excised tissue pieces were then placed on PDA medium and incubated. After fungal growth appeared, the fungus was purified on PDA medium and incubated for 7 days for observation.

Rejuvenation of *Bacillus* spp. Isolates

The rejuvenation of *Bacillus* spp. isolates was carried out aseptically in a laminar air flow cabinet. The five *Bacillus* spp. isolates, namely Bcz-14, Bcz-16, Bcz-20, Bcz-

21, and Bcz-30, were streaked onto NA slants in test tubes. The isolates were incubated for 24 h at room temperature.

Koch's Postulates Test for *Colletotrichum capsici*

Koch's postulates test was conducted by placing a 5-mm inoculum plug of *C. capsici* on the surface of chili fruits that had been surface-sterilized. The chili fruits were placed in 15-cm-diameter Petri dishes and incubated at room temperature. The fungal isolate was considered virulent when anthracnose symptoms appeared on the chili fruits, characterized by sunken blackish lesions (Sudania et al., 2023).

After Koch's postulates test was conducted to confirm that the isolate obtained was the causal agent of anthracnose, a pathogenicity test was performed. This test was used to determine the ability of the isolate to infect and induce symptoms on the host, thereby allowing its virulence level and the resulting disease intensity to be evaluated.

Preparation of *Bacillus* spp. Bacterial Suspension

Bacillus spp. grown on NA slants and ready for harvesting were prepared by adding 10 mL of sterile distilled water to each test tube. The bacterial growth was then scraped using an inoculating loop. The population densities used were 10^9 CFU/mL (K1), following Putro et al. (2014), and 10^{10} CFU/mL (K2), following Hersanti et al. (2021).

According to Heriyati (2023), suspension preparation was carried out by preparing five test tubes from the culture of each *Bacillus* spp. isolate and adding 10 mL of distilled water to each tube. The initial 10 mL suspension was then serially diluted in 10 test tubes by transferring 1 mL of suspension into 9 mL of distilled water. From each dilution level, 1 mL of suspension was taken and cultured on Petri dishes containing NA medium.

Colony counting was performed within less than 24 h. Colonies on the Petri dishes were counted using the total plate count (TPC) method, and the bacterial population was expressed as colony-forming units (CFU) (Lestari et al., 2016). This calculation was used to determine the desired bacterial population density. The TPC formula was as follows:

$$\text{Number of colonies per plate } x \frac{1}{\text{dilution factor}}$$

Preparation of Chili Fruits

The detached fruit assay was conducted using healthy and fresh chili fruits without visible spots on the surface. Fruits that were not overly ripe were selected to prevent rapid decay during the experiment. The selection of chili fruits at an appropriate maturity stage was intended to ensure fruit durability during the study, so that the experimental process would not be affected by fruit rotting.

In Vitro Inhibition Assay of *Bacillus* spp. against *Colletotrichum capsici*

The inhibition assay was conducted in 9-cm-diameter Petri dishes by inoculating a 7-day-old *C. capsici* isolate together with 24-h-old *Bacillus* spp. cultures. The antagonistic assay followed the dual-culture method described by Flori et al. (2020). Whatman paper discs with a diameter of 0.5 cm were immersed in *Bacillus* spp. suspensions at densities of 10^9 CFU/mL and 10^{10} CFU/mL for 30 min.

The paper discs were then placed at two points near the edge of Petri dishes containing PDA medium, at a distance of 3 cm from the *C. capsici* isolate, which was placed at the center of the Petri dish. The fungal colony plug had a diameter of 0.5 cm and was prepared using a cork borer (Figure 1). In the control treatment, *C. capsici*

was grown without the addition of *Bacillus* spp. isolates. The cultures were then incubated, and growth was observed after 7 days. After 7 days, the normal colony diameter of the fungus (Y) and the inhibited colony diameter of the fungus (X) were measured to determine the inhibition value.

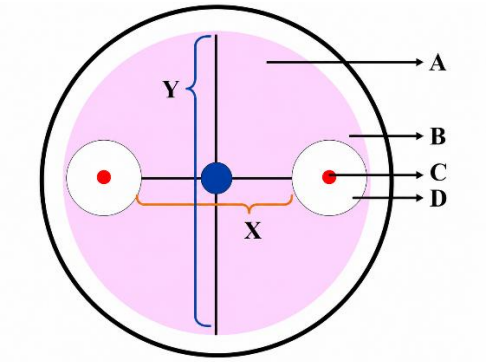

Figure 1. In vitro antagonistic assay of *Bacillus* spp. bacteria against *C. capsici*

In Vivo Assay of *Bacillus* spp. against the Development of Anthracnose Disease Caused by *Colletotrichum capsici* on Chili Fruits

The in vivo assay followed the detached fruit method described by Widianingsih et al. (2025). The surface of chili fruits was sterilized using 0.9% NaCl for 5 min, rinsed with sterile water, and treated with 70% ethanol. After the fruits had dried, they were immersed in a *Bacillus* spp. suspension at a population density of 10^9 CFU/mL for approximately 3 min. In the control treatment, the chili fruits were not immersed in the *Bacillus* spp. suspension.

The treated chili fruits were then wounded using a sterile needle, after which a 5-mm *C. capsici* isolate was placed on the wound site. The inoculated fruits were incubated in sterile 15-cm-diameter Petri dishes lined with straw paper. The chili fruits inoculated with the pathogenic fungus were incubated for 1 week, and symptoms were observed daily.

Observation Parameters

1. Inhibition Value

The parameter observed in the in vitro antagonistic assay was the inhibition value, which was determined by measuring the growth of the pathogen colony in the Petri dish. The inhibition value was calculated using the following formula (Flori et al., 2020):

$$\text{Inhibition value} = \left(\frac{y-x}{2}\right)$$

Note:
Y = normal colony diameter of *Colletotrichum capsica*
X = inhibited colony diameter of *C. capsica*

2. Incubation Period

The observation parameter in the in vivo detached fruit assay was the incubation period, expressed in days. The incubation period refers to the time required for the pathogen to initiate infection, which was determined based on the appearance of the first symptoms on chili fruits after pathogen inoculation. The observed symptoms included small, water-soaked lesions measuring 3–4 cm.

3. Disease Intensity

Disease intensity was also observed as a parameter in the in vivo detached fruit assay. Disease intensity was calculated using the following formula:

$$\text{Disease intensity} = \left\{\frac{\sum (\text{nxv})}{\text{ZxN}}\right\} \text{x}100\%$$

Note:
n = number of fruits in each lesion class
v = score value of each lesion class
N = total number of observed fruits
Z = highest lesion class score

The disease scoring system followed the symptom categories described by Haryadi et al. (2022), as follows:
Score 0: no infection
Score 1: infected chili fruit surface area of 1–25%
Score 2: infected chili fruit surface area of 26–50%
Score 3: infected chili fruit surface area of 51–75%
Score 4: infected chili fruit surface area of 76–100%

4. Effectiveness Value

The effectiveness of *Bacillus* spp. against *Colletotrichum capsici* was determined based on disease intensity and calculated using the following formula described by Suparto et al. (2023):

$$\text{EP} = \frac{IPk - IPp}{IPk} \text{ x } 100\%$$

Note:
EP = effectiveness value
IPk = disease percentage in the control treatment
IPp = disease percentage in the treatment

The effectiveness of the biological control agent was categorized as follows:
EP = 0: not effective
EP = 1–20%: very low effectiveness
EP = >20–40%: low effectiveness
EP = 40–60%: moderately effective
EP = >80%: highly effective

Data Analysis

Data were analyzed using SPSS version 24. A normality test and analysis of variance (ANOVA) were performed to determine the effect of each treatment. When significant differences were detected among treatments, the honestly significant difference test at the 5% level was conducted.

RESULTS AND DISCUSSION

Results of Koch's Postulate Test

Koch's postulate test was conducted using healthy chili fruits and an isolate of *Colletotrichum capsici*. The healthy chili fruits were wounded with a sterile needle and then inoculated by placing a 5-mm-diameter mycelial plug of *C. capsici* onto the wounded fruit surface. The inoculated fruits were incubated in 15-cm-diameter Petri dishes and observed until disease symptoms developed. Symptoms were visible on the fourth day, characterized by sunken black lesions accompanied by orange spore masses around the lesions.

In Vitro Antagonistic Activity of *Bacillus* spp. against *Colletotrichum capsici*

The in vitro antagonism assay was conducted on potato dextrose agar (PDA) using the dual-culture method. The results showed inhibition zones around the colony

of the pathogenic fungus *C. capsici*. Across all treatments, *Bacillus* spp. inhibited the growth of *C. capsici*, although the level of inhibition varied among treatments. In contrast, the control treatment (Figure 2) showed a larger fungal colony diameter than the treatments inoculated with *Bacillus* spp. These findings indicate that *Bacillus* spp. suppressed the growth of *C. capsici*, presumably through an antibiosis mechanism, as indicated by the formation of a clear zone between the pathogen and the antagonist.

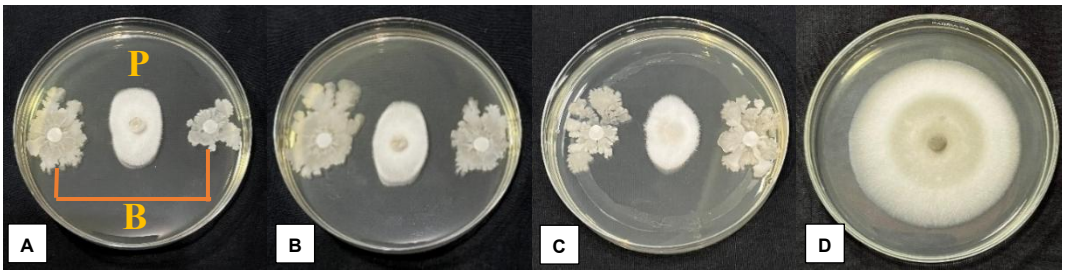

Figure 2. Antagonistic activity of *Bacillus* spp. against *C. capsici*: A) inhibition in treatment B1K1 (*Bcz*-14 at 10^9 CFU/mL); B) treatment B5K1 (*Bcz*-30 at 10^9 CFU/mL); C) treatment B2K1 (*Bcz*-16 at 10^9 CFU/mL); D) control. B: bacterium; P: pathogen

Based on the mean inhibition values and the low standard deviation, which was used as an indicator of data stability, treatments B1K1, B5K1, and B2K1 were the three most effective treatments (Figure 3). These treatments produced high inhibition values with relatively stable data; therefore, they were selected for further in vivo testing. A low standard deviation indicates that the values among replicates are stable and consistent, whereas a high standard deviation indicates greater variability among replicates. According to Harahap et al. (2025), a low standard deviation suggests that most data points are close to the mean, indicating more homogeneous or uniform data, while a high standard deviation indicates that the data are more widely dispersed from the mean.

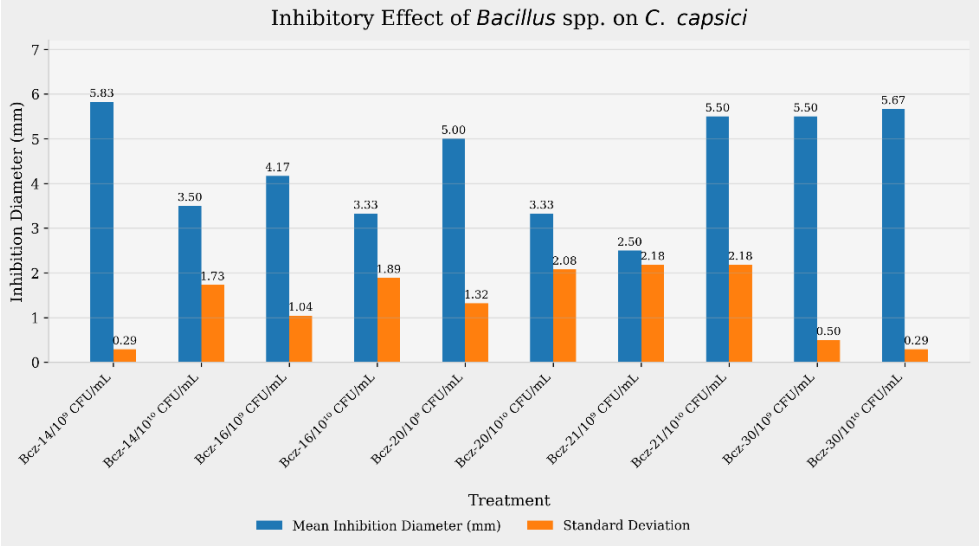

Figure 3. Inhibitory activity of *Bacillus* spp. against *C. capsici* across all treatments

Treatment B1K1 (*Bcz*-14 at 10^9 CFU/mL) showed the highest mean inhibition, with a value of 5.83 mm and a standard deviation of 0.29. This was followed by treatment B5K1 (*Bcz*-30 at 10^9 CFU/mL), which had a mean inhibition of 5.50 mm and a standard deviation of 0.50. Treatment B2K1 (*Bcz*-16 at 10^9 CFU/mL) showed a mean inhibition of 4.16 mm with a standard deviation of 1.04.

A *Bacillus* spp. population density of 10^9 CFU/mL produced better inhibition than 10^{10} CFU/mL. This result may be attributed to excessive bacterial populations, which can increase competition among cells for space and nutrients, thereby reducing the production of antagonistic compounds and limiting metabolic activity. This finding is consistent with Chen et al. (2023), who reported that the mechanisms of antagonistic bacteria include competition and the production of antimicrobial compounds, the effectiveness of which depends on bacterial population conditions. When the population of antagonistic bacteria is too high, competition among bacterial cells may increase and reduce their effectiveness. Similarly, Anjarsari et al. (2022) reported that differences in inhibitory activity among isolates may be influenced by variations in the type and quantity of antimicrobial compounds produced by each isolate.

The antagonistic mechanism observed in this assay was antibiosis, as indicated by the formation of inhibition zones on the Petri dishes. The colony of *C. capsici* was unable to grow close to the colony of *Bacillus* spp. This is in accordance with Butarbutar et al. (2018), who stated that inhibition zones formed in Petri dishes indicate the presence of active compounds produced by *Bacillus* spp. that suppress the growth of pathogenic fungi. Microscopic observations confirmed the effect of these antibiotic compounds on the abnormal hyphal structures of *C. capsici*. The observed abnormalities included swollen hyphae (Figure 4A), coiled hyphae (Figure 4B), and lysed hyphae (Figure 4C). These structural changes were caused by the antibiotic activity of *Bacillus* spp., which disrupted normal fungal growth. According to Bawantari et al. (2020), one mechanism by which biocontrol agents inhibit pathogens is lysis, in which the pathogen hyphae become empty because their cellular contents are utilized by the biocontrol agent as nutrients.

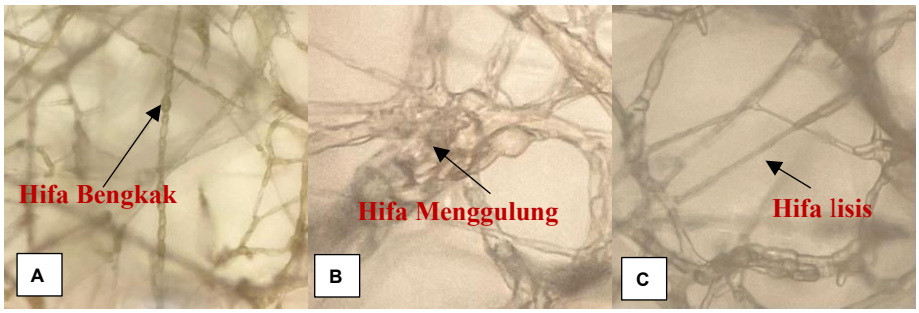

Figure 4. Hyphal abnormalities in *C. capsici*: A) swollen hyphae; B) coiled hyphae; C) lysed hyphae

In Vivo Effect of *Bacillus* spp. on the Suppression of Anthracnose Disease in Chili Fruits

Incubation Period

The application of *Bacillus* spp. to chili fruits under in vivo conditions delayed the incubation period of anthracnose disease. The control treatment had the shortest incubation period, at 2.85 days. The longest incubation period was observed in treatment B1K1 (*Bcz*-14 at 10^9 CFU/mL), at 5.35 days, followed by treatment B2K1 (*Bcz*-30 at 10^9 CFU/mL), at 4.30 days, and treatment B3K1 (*Bcz*-16 at 10^9 CFU/mL), at 3.60 days. Differences in the incubation period among isolate treatments may be attributed to differences in the inhibitory compounds produced by the *Bacillus* spp. isolates. This finding is supported by Apriliani et al. (2022), who reported that variations in incubation period may result from the use of different isolates and differences in the quantity of secondary metabolites capable of suppressing pathogenic fungi.

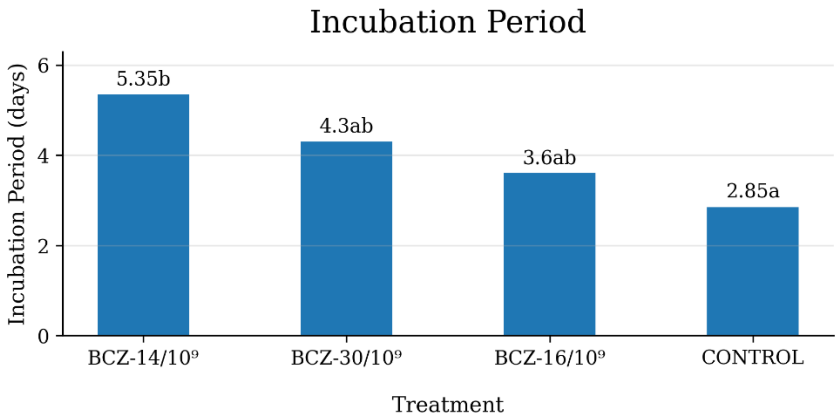

Figure 5. Incubation period of anthracnose disease in chili fruits

The longest incubation period was recorded in treatment B1K1, in which *Bacillus* spp. isolate *Bcz*-14 induced disease symptoms at 5.35 days after inoculation. In comparison, the control treatment showed symptoms at 2.85 days after inoculation. The use of *Bacillus* spp. as an antagonistic agent inhibited *C. capsici* infection in chili fruits, although the delay in symptom appearance was not substantially long. Rani et al. (2022), who also used *Bacillus* sp. as an antagonistic agent to control anthracnose disease, reported inhibition with an average incubation period of 3.28 days, compared with the negative control, in which symptoms appeared after 2.35 days.

Disease Intensity

Disease intensity was calculated by observing the portions of chili fruits that showed symptoms of infection. Based on the in vitro assay, the three treatments with the highest inhibition values were B1K1 (*Bcz*-14 at 10^9 CFU/mL), B2K1 (*Bcz*-30 at 10^9 CFU/mL), and B3K1 (*Bcz*-16 at 10^9 CFU/mL). These treatments were selected for the in vivo assay to evaluate their effectiveness directly on chili fruits. The lowest disease intensity was observed in treatment B1K1, followed by B2K1, while the highest disease intensity among the *Bacillus* treatments was observed in B3K1 (Table 1).

Table 1. Anthracnose disease intensity in chili fruits after treatment with *Bacillus* spp.

Treatment	Disease intensity on day (%)		
	3 DAI	5 DAI	7 DAI
Control	8.75b	31.25b	53.75b
Bcz-14/10^9	0.0a	2.50a	26.25a
Bcz-30/10^9	0.0a	12.50a	31.25ab
Bcz-16/10^9	1.25a	17.50ab	38.75ab

Note: Values followed by the same letter in the same column are not significantly different according to Tukey's HSD test at the 5% level.

The in vivo detached-fruit assay showed that the lowest disease intensity was recorded in treatment B1K1, namely isolate *Bcz*-14 at 10^9 CFU/mL, with an intensity value of 26.25%. Treatment B2K1, namely isolate *Bcz*-30 at 10^9 CFU/mL, showed a disease intensity of 31.25%. Treatment B3K1, namely isolate *Bcz*-16 at 10^9 CFU/mL, showed the highest disease intensity among the *Bacillus* treatments, at 38.75%. In comparison, the control treatment showed a disease intensity of 53.75%.

Figure 6. Anthracnose symptoms on day 7 in the in vivo chili fruit assay: A) treatment B1K1; B) healthy chili fruit

Treatment B1K1, which showed the lowest disease intensity at 26.25%, involved isolate *Bcz*-14 at 10^9 CFU/mL. This treatment reduced anthracnose disease intensity more effectively than the other treatments and the control. This effect may be due to compounds produced by the *Bacillus* spp. isolate, such as antibiotics and secondary metabolites, which were more effective in suppressing the growth of *C. capsici*. This result is consistent with Rani et al. (2022), who reported that, in a detached-fruit assay, *Bacillus* sp. isolate Ba-9 at 10^9 CFU/mL delayed the decay process in red chili fruits infected with *C. capsici*. The treatment produced the lowest disease intensity, at 23.75%, and suppressed pathogen growth by 21.25% compared with the control.

Treatments B2K1 and B3K1 showed relatively high disease intensity. This may be because these isolates produced fewer antagonistic metabolites than isolates associated with lower disease intensity. In addition, differences in bacterial metabolism may affect the ability of isolates to utilize nutrients. Some nutrients may be used primarily for survival rather than for the production of antagonistic metabolites. This is consistent with Saputra (2015), who reported that some bacteria use nutrients for growth, whereas others use nutrients to produce antibiotic compounds.

Effectiveness of *Bacillus* spp. in Suppressing the Growth of *Colletotrichum capsici*

The results showed that *Bacillus* spp. suppressed the growth of *C. capsici* across treatments and inhibited pathogen development on chili fruits, although the level of effectiveness varied. The effectiveness categories were as follows: EP = 0, ineffective; EP = 1–20%, very low effectiveness; EP = >20–40%, low effectiveness; EP = 40–60%, moderately effective; and EP = >80%, highly effective. Table 2 shows that the highest effectiveness was recorded in treatment B1K1, namely isolate *Bcz*-14 at 10^9 CFU/mL, with an effectiveness value of 51.16%, categorized as moderately effective. This was followed by treatment B2K1, namely isolate *Bcz*-30 at 10^9 CFU/mL, with an effectiveness value of 41.86%, also categorized as moderately effective. The lowest effectiveness was observed in treatment B3K1, namely isolate *Bcz*-16 at 10^9 CFU/mL, with an effectiveness value of 27.91%, categorized as less effective. These categories refer to Suparto et al. (2023). Based on these findings, treatments B1K1 and B2K1 were relatively optimal in suppressing and inhibiting *C. capsici* infection in chili fruits. However, treatment B3K1 also suppressed *C. capsici* infection compared with the control treatment, which had an effectiveness value of 0%.

Table 2. Effectiveness of *Bacillus* spp. against *C. capsici*

Treatment	Effectiveness (%)	Category
Control	0a	Ineffective
Bcz-14/10^9	51.16b	Moderately effective
Bcz-30/10^9	41.86b	Moderately effective
Bcz-16/10^9	27.91ab	Less effective

Note: Values followed by the same letter in the same column are not significantly different according to Tukey's HSD test at the 5% level

The differences in effectiveness may be attributed to variation in the ability of each isolate to produce secondary metabolites. Suboptimal production of these metabolites may reduce the ability of the isolates to inhibit *C. capsici*. This is consistent with Elfina et al. (2024), who stated that variation in the inhibitory effectiveness of *Bacillus* sp. isolates is associated with differences in the production level of antifungal metabolites by each isolate. Fadilah (2024) also reported that other factors may influence isolate performance, including the adaptation of *Bacillus* isolates to chili fruit conditions, such as pH, water content, and phenolic compound content. These factors may affect bacterial viability, and *Bacillus* isolates that are able to adapt to chili fruit tissues may maintain antagonistic compound production for a longer period.

CONCLUSION

Based on the in vitro and in vivo evaluations, *Bacillus* spp. isolate Bcz-14 at a population density of 10^9 CFU/mL showed the greatest potential as an antagonistic agent against the pathogenic fungus *Colletotrichum capsici*. In the in vitro assay, isolate Bcz-14 exhibited the highest and most stable inhibitory activity compared with the other isolates, indicating its ability to suppress pathogen growth. Furthermore, in the in vivo assay on red chili fruit, this isolate reduced the development of anthracnose disease, with an inhibition rate of 26.25%. These findings suggest that *Bacillus* spp. isolate Bcz-14 has potential as a biological control agent for managing anthracnose disease in red chili. However, further validation under field conditions is required to confirm its effectiveness and consistency in actual chili cultivation systems.

RECOMMENDATION

Further studies should evaluate the efficacy of this isolate under field conditions by applying it directly to chili plants. Field-scale testing is necessary to determine its effectiveness under real environmental conditions. Direct application trials may also support its potential use as a preventive measure to suppress disease infection from the early stages of plant growth.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to all parties who contributed to this study, particularly the principal supervisor, Prof. Dr. Ir. Yenny Wuryandari, M.P., and the co-supervisor, Dr. Ir. Hery Nirwanto, M.P., for their valuable suggestions and input. The authors also thank their colleagues for their assistance during the research, which enabled the study to be completed successfully.

REFERENCES

Anjarsari, D. T., Prasetyawati, E. T., & Wuryandari, Y. (2022). Inhibitory Test of Bacillus sp. against Phytophthora palmivora Causes Cocoa Fruit Rot Disease. *Nusantara*

Science and Technology Proceedings, 14–21. https://doi.org/10.11594/nstp.2022.2003

Apriliani, A. C., Mujoko, T., & Purnawati, A. (2022). Uji Antagonis Bakteri Endofit Terhadap Patogen Fusarium sp. Asal Tanaman Terung secara In Vitro. *Jurnal Agrohita*, *7*(3), 615–620. https://doi.org/https://doi.org/10.31604/jap.v7i3.7176

Bawantari, N. K. S., Suprapta, D. N., & Khalimi, K. (2020). Uji Antagonistik Bacillus siamensis dan Paenibacillus polymyxa Terhadap Colletotrichum gloeosporioides KLCR2 Penyebab Penyakit Antraknosa pada Buah Cabai Rawit (Capsicum frutescens L.). *E-Jurnal Agroekoteknologi Tropika (Journal of Tropical Agroecotechnology)*, *9*(3).

Butarbutar, R., Marwan, H., & Mulyati, S. (2018). Eksplorasi Bacillus spp. Dari Rizosfer Tanaman Karet (Hevea brasilliensis) dab Potensinya Sebagai Agens Hayati Jamur Akar Putih (Rigidoporus sp.). *Jurnal Agroecotania: Publikasi Nasional Ilmu Budidaya Pertanian*, *1*(2). https://doi.org/https://doi.org/10.22437/agroecotania.v1i2.6339

Chen, X., Zhang, M., Tang, L., Huang, S., Guo, T., & Li, Q. (2023). Screening and characterization of biocontrol bacteria isolated from Ageratum conyzoides against Collectotrichum fructicola causing Chinese plum (Prunus salicina Lindl.) anthracnose. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1296755

Elfina, Y., Sukendi, Efriyeldi, & Sutikno, A. (2024). Ability Test of Bacillus spp. in Inhibiting Ganoderma boninense Pat. the Causal Agent of Oil Palm Basal Stem Rot Disease in Vitro. *Agro Bali*, *7*(2), 575–590.

Fadilah, N. (2024). *Uji Antagonis Bacillus spp. dan Trichoderma sp. terhadap Colletotrichum sp. Penyebab Penyakit Antraknosa pada Buah Cabai (Capsicum annuum)*. https://repository.unja.ac.id/id/eprint/72695

Flori, F., Mukarlina, & Rahmawati. (2020). *Potensi antagonis isolat bakteri Bacillus spp. asal rizosfer tanaman lada (Piper nigrum L.) sebagai agen pengendali jamur Fusarium sp.JDF*. https://doi.org/https://doi.org/10.20956/bioma.v5i1.9923

Harahap, Y. A., Nasution, R. P., & Amir, A. (2025). Peran Ukuran Pemusatan dan Penyebaran Data dalam Analisis Statistika. *Pendas : Jurnal Ilmiah Pendidikan Dasar, 10*(04), 332–350. https://doi.org/https://doi.org/10.23969/jp.v10i04.38955

Haryadi, N. T., Muhlison, W., Muhammad, D., Dirrar, B., Ashar, A., Kalimantan, J., 37, N., Kampus, T., & Boto, J. 68121. (2022). Efektifitas Penanaman Refugia Terhadap Populasi dan Intensitas Serangan Hama Kutu Kebul (Bemisia tabaci) pada Pertanaman Cabai Merah Besar (Capsicum annum L.). *Jurnal Bioindustri*, *4*(2), 2022. https://doi.org/10.33751/ekol.v11i2.256

Haryani, T. S., & Tombe, O. M. (2011). Pemanfaatan Bakteri Antagonis Terhadap Pengendalian Jamur Patogen Fusarium oxysporum dan Phytopthora capsici Secara In Vitro. *Ekologia*, *11*(2), 11–21. https://doi.org/10.33751/ekol.v11i2.256

Heriyati, S. (2023). *Kemampuan Beberapa Isolat Bacillus Spp. Dalam Menghambat Perkembangan Penyakit Layu Fusarium Dan Memacu Pertumbuhan Tanaman Cabai Merah (Capsicum Annuum L.)* [Doctoral dissertation, Upn Veteran Jawa Timur]. https://repository.upnjatim.ac.id/id/eprint/16124

Hersanti, Emilia, N. H., Djaya, L., & Yulia, E. (2021). Bacillus subtilis dan Lysinibacillus sp. (CK U 3) dalam Serat Karbon dan Silika Nano Menekan Pertumbuhan Fusarium oxysporum f.sp. lycopersici. *Jurnal Agrikultura*, *2021*(2), 135–145. https://doi.org/https://doi.org/10.24198/agrikultura.v32i2.33387

Lestari, N. W., Budiharjo, A., & Pangastuti, A. (2016). Bakteri heterotrof aerobik asal saluran pencernaan ikan sidat (Anguilla bicolor bicolor) dan potensinya sebagai

probiotik. *Asian Journal of Tropical Biotechnology*, *13*(1), 9–17. https://doi.org/10.13057/biotek/c130102

Nurjasmi, R., & Suryani, D. (2020). Uji Antagonis Actinomycetes terhadap Patogen Colletotrichum capsici Penyebab Penyakit Antraknosa pada Buah Cabai Rawit. In *Jurnal Ilmiah Respati* (Vol. 11, Number 1). http://ejournal.urindo.ac.id/index.php/pertanian

Putro, N. S., Aini, L. Q., & Abadi, A. L. (2014). *Pengujian Konsorsium Mikroba Antagonis untuk Mengendalikan Penyakit Antraknosa pada Cabai Merah Besar (Capsicum annuum L.)* (Vol. 2).

Rani, S., Prasetyawati, T., Nirwanto, H., Bacillus, P. B., Program, N., Agroteknologi, S., Pertanian, F., & Timur, J. (2022). *Potensi Bakteri Bacillus spp. dalam Menghambat Colletotrichum capsici Penyebab Antraknosa pada Cabai Merah secara In Vitro*.

Saputra, R. (2015). Uji aktivitas antagonistik beberapa isolat Bacillus spp. terhadap penyakit layu bakteri (Ralstonia solanacearum) pada beberapa varietas tomat dan identifikasinya. *Prosiding Sem Nas Masy Biodiv Indon*, 1116–1122. https://doi.org/10.13057/psnmbi/m010525

Sudania, S., Ropalia, R., & Kusmiadi, R. (2023). The Inhibitory Potential of Botanical Fungicides Against Colletotrichum capsici the Causal Agent of Anthracnose on Chili in-Vitro. *CROPSAVER - Journal of Plant Protection*, *6*(1), 40. https://doi.org/10.24198/cropsaver.v6i1.44219

Suparto, H., Ropalia, R., & Kusmiadi, R. (2023). Uji efektivitas pestisida nabati daun mengkudu terhadap pengendalian penyakit antraknosa pada tanaman cabai. *CROPSAVER-Journal of Plant Protection*, *1*(5), 40–48.

Sutarni, S. (2023). Red Chili Production Risk Analysis In Binjai Agung Bekri District, Central Lampung Regency. *Journal of Food System and Agribusiness (JoFSA)*, *7*(2), 166–178. https://doi.org/10.25181/jofsa.v7i2.3258

Umatron, P. P., Gunawan, C. I., & Yusnita, E. A. (2022). Produktivitas Usahatani Cabai Merah di Masa Pandemi. *Jurnal Ekonomi Dan Bisnis*, *11*(2).

Widianingsih, E., Isminingsih, S., Sulistyorini, E., & Saylendra, A. (2025). Uji Efektivitas Rizobakteri dalam Pengendalian Penyakit Antraknosa pada Buah Cabai Merah (Capsicum annum L.) Secara In Vivo. *Jurnal Ilmiah Membangun Desa Dan Pertanian*, *10*(2), 202–211. https://doi.org/10.37149/jimdp.v10i2.1880

Wuryandari, Y., Lestari, S. R., & Kusuma, R. M. (2022). *Kemampuan Antagonistik Bakteri Bacillus spp. Terhadap Patogen Fusarium sp. Penyebab Penyakit Layu Tanaman Cabai (Capsicum annum L.) dan Mekanisme Kerjanya. Fakultas Pertanian*. Universitas Pembangunan Nasional “Veteran” Jawa Timur.

Zinidin, M. (2022). *Eksplorasi Bacillus spp. Pada Rhizosfer Cabai Merah (Capsicum Annuum L.) Dataran Tinggi Dan Potensinya Sebagai Agensia Pengendali Hayati Patogen Ralstonia Solanacearum Secara In Vitro*. Undergraduate thesis, UPN “Veteran” Jawa Timur.