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Effect of Various Concentrations of Antagonistic Rhizobacteria on Germination and Seedling Vigor of Colletotrichum-Infected Chilli Seeds

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Abstract. Red chili pepper (*Capsicum annuum* L.) is an important horticultura commodity in Indonesia. However, chili production is often constrained by anthracnose disease caused by *Colletotrichum* sp., which can infect seeds and reduce seed viability and vigor. The use of antagonistic rhizobacteria from chili rhizosphere is considered a potential biological control method to suppress pathogen infection and improve seed quality. This study aimed to determine the effect of different concentrations of antagonistic rhizobacteria on the viability and vigor of chili seeds infected with *Colletotrichum* sp. The experiment used a non-factorial completely randomized design consisting of five treatment level (control, 10^6, 10^8, 10^{10}, and 10^{12} cfu/ml). The results showed that treatment with antagonistic rhizobacteria had a positive effect in suppressing *Colletotrichum* sp. infection and improving seed performance. The treatment with a concentration of 10^{12} cfu/ml was able to minimize disease incidence to 15.00%, increasing maximum growth potential to 99.00%, increased germination percentage to 94.00%, vigor index to 89.00%, and germination uniformity to 94.00%. The highest growth rate was obtained in the 10^6 cfu/ml treatment, reaching 14.05%. These findings confirm that antagonistic rhizobacteria are effective as environmentally friendly biocontrol agents for improving the viability and vigor of chili seeds infected with *Colletotrichum* sp.

Keywords: chilli seeds, antagonistic bacteria, *Colletotrichum* sp., viability, vigour, biological control

INTRODUCTION

Red chilli pepper (*Capsicum annuum* L.) represents a significant horticultural crop in Indonesia, contributing to both economic value and food production. It is extensively utilised in culinary applications and is recognised for its high vitamin C content, antioxidants, and capsaicin, which contribute to enhanced immunity, metabolic stimulation, and reduced inflammation (Ulya *et al*., 2020). According to the Indonesian Central Statistics Agency, national chilli production fluctuated between 2022 and 2024, with Banten Province experiencing a notable decline during this period (BPS, 2025). A primary factor contributing to reduced chilli yields is anthracnose disease, caused by *Colletotrichum* sp., which infects leaves, stems, fruits, and seeds, resulting in tissue decay, diminished seed quality, and substantial yield losses (Mariana et al., 2021; Paradisa et al., 2021).

Anthracnose disease is commonly managed using synthetic fungicides due to their rapid efficacy and ease of application. However, excessive fungicide use can negatively impact ecosystems, eliminate beneficial microorganisms, and promote the development of resistant pathogens (McLaughlin *et al*., 2023). Infection of seeds by *Colletotrichum* sp. further reduces seed viability and vigour, resulting in abnormal germination and weak seedlings (Rumahlewang, 2024). Therefore, sustainable disease management strategies are required to mitigate the adverse effects of chemical inputs while maintaining high crop yields.

Antagonistic rhizosphere bacteria represent promising biological control agents for managing fungal diseases in horticultural crops. These bacteria suppress pathogens through resource competition, production of antimicrobial compounds, and promotion of plant growth. Previous studies have demonstrated that antagonistic bacteria can enhance the viability and vigour of chilli seeds infected by *Colletotrichum* sp. (Cobbinah *et al*., 2023). Sutarsih (2025) reported that the rhizobacterial isolate BRC 6 (17) exhibited strong inhibition of *Colletotrichum* sp., while Pangestu *et al.* (2022) found that several bacterial strains suppressed other fungi, including *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. Thus, the application of antagonistic rhizobacteria offers an environmentally friendly approach to improving chilli seed quality and controlling anthracnose disease.

RESEARCH METHODOLOGY

This research took place from November 2025 to January 2026 at the Soil and Agroclimatology Laboratory, Department of Agroecotechnology, Faculty of Agriculture, Sultan Ageng Tirtayasa University, Serang, Banten, Indonesia. The study used Kencana variety chilli pepper seeds (*Capsicum annuum* L.), rhizobacterial isolates from the Laboratory of Basic Sciences and Plant Protection, *Colletotrichum* sp. isolates, Nutrient Agar (NA), Potato Dextrose Agar (PDA), distilled water, 70% alcohol, and other lab materials. Equipment included a laminar airflow cabinet, autoclave, incubator, shaker, micropipette, analytical balance, Petri dishes, test tubes, hotplate stirrer, and other standard lab tools.

The experiment was arranged in a Completely Randomised Design (CRD) with five treatment levels and four replications, resulting in 20 experimental units. Treatments included: C0 (control with *Colletotrichum* sp. only), C1 (rhizobacterial suspension at 10^6 cfu/mL + *Colletotrichum* sp.), C2 (10^8 cfu/mL + *Colletotrichum* sp.), C3 (10^{10} cfu/mL + *Colletotrichum* sp.), and C4 (10^{12} cfu/mL + *Colletotrichum* sp.). Each unit contained 25 chilli seeds, totalling 500 seeds. Prior to treatment, seeds were soaked in Colletotrichum sp. suspension for 30 minutes, followed by treatment with the respective rhizobacterial suspensions. Germination was assessed using the rolled paper method in plastic containers under laboratory conditions. The study measured disease incidence, germination percentage, growth rate, maximum growth potential, vigour index, and germination uniformity. Data were analysed using Analysis of Variance (ANOVA) at a 5% significance level. When significant differences were detected, Duncan's Multiple Range Test (DMRT) at 5% was employed to compare treatment means.

DISEASE INCIDENCE

Disease incidence was determined based on the percentage of non-germinated and infected seeds relative to the total number of seeds planted in each experimental unit. Statistical analysis showed that rhizobacterial soaking treatments significantly affected the incidence of *Colletotrichum* sp. on chilli seeds.

Table 1. Average percentage of disease incidence affected by antagonistic bacterial concentrations.

Treatment		Disease Incidence (%)
C0	Control (water + *Colletotrichum* sp. inoculation)	15.00a
C1	Chili rhizobacterial soaking at 10^6 cfu/mL + *Colletotrichum* sp. incoculation	4.00b
C2	Chili rhizobacterial soaking at 10^8 cfu/mL + *Colletotrichum* sp. inoculation	6.00b
C3	Chili rhizobacterial soaking at 10^{10} cfu/mL + *Colletotrichum* sp. inoculation	3.00b
C4	Chili rhizobacterial soaking at 10^{12} cfu/mL + *Colletotrichum* sp. inoculation	1.00b
Average		5.80

Note: Numbers followed by the same lowercase letter indicate a non-significant difference
Based on the 5% DMRT.

The control treatment (C0) exhibited the highest disease incidence at 15.00%, while rhizobacterial treatments reduced disease incidence to 4.00% (C1), 6.00% (C2), 3.00% (C3), and 1.00% (C4). The lowest disease incidence was observed in treatment C4 (10^{12} cfu/mL), indicating that higher rhizobacterial concentrations were more effective at suppressing anthracnose infection. According to Prihatiningsih *et al*. (2020), *Colletotrichum* sp. infection may occur before germination and is characterised by fungal hyphae on seed surfaces, tissue discolouration, plumule and radicle rot, abnormal seedling growth, and seedling death.

The lower disease incidence observed in rhizobacterial treatments demonstrated the antagonistic activity of beneficial bacteria against *Colletotrichum* sp. Increasing rhizobacterial concentrations enhanced bacterial colonisation on seed surfaces, thereby limiting pathogen access to nutrients and ecological niches. This finding is consistent with Alfianto *et al*. (2025), who reported that endophytic bacteria compete with fungal pathogens for nutrients and space while producing antimicrobial compounds that inhibit pathogen growth and development. Consequently, rhizobacterial application effectively reduced pathogen infection and improved seed health.

Treatment C4 (10^{12} cfu/mL) showed the strongest inhibitory effect, reducing disease incidence to only 1.00%. This result indicated that rhizobacteria effectively suppressed fungal growth by producing antifungal secondary metabolites (Unedo *et al.*, 2024). Similarly, Widiantini *et al*. (2018) reported that antagonistic bacteria release inhibitory compounds during interaction with pathogenic fungi. In addition, rhizobacteria can control plant pathogens through multiple mechanisms, including antibiotic production, siderophore secretion, chitinase and β-1,3-glucanase activity, parasitism, and nutrient competition (Glick *et al.*, 2007 in Yanti *et al*., 2021). These mechanisms demonstrate the considerable potential of rhizobacteria as environmentally friendly biological control agents against anthracnose disease in chilli seeds.

GERMINATION PERCENTAGE

Germination percentage represents the proportion of normal seedlings relative to the total number of seeds sown and is commonly used as an indicator of seed viability. Seed viability reflects the ability of seeds to grow and develop into normal seedlings, characterised by healthy plumule and radicle formation (Ridha *et al*., 2017). Analysis of variance showed that chilli rhizobacterial soaking treatments had a highly significant effect on chilli seed germination percentage.

Table 2. Average germination percentage of seeds affected by antagonistic bacterial concentrations.

Treatment		Disease Incidence(%)
C0	Control (water + *Colletotrichum* sp. inoculation)	66.00a
C1	Chili rhizobacterial soaking at 10^6 cfu/mL + *Colletotrichum* sp. incoculation	86.00b
C2	Chili rhizobacterial soaking at 10^8 cfu/mL + *Colletotrichum* sp. inoculation	86.00b
C3	Chili rhizobacterial soaking at 10^{10} cfu/mL + *Colletotrichum* sp. inoculation	87.00bc
C4	Chili rhizobacterial soaking at 10^{12} cfu/mL + *Colletotrichum* sp. inoculation	94.00c
Average		83.80

Note: Numbers followed by the same lowercase letter indicate a non-significant difference Based on the 5% DMRT

The control treatment (C0) produced the lowest germination percentage (66.00%), while rhizobacterial treatments increased germination to 86.00% (C1 and C2), 87.00% (C3), and 94.00% (C4). The highest germination percentage was recorded in treatment C4 (10^{12} cfu/mL), indicating that higher rhizobacterial concentrations were more effective in improving seed germination. According to Hariyono and Nathaniel (2024), germination percentages above 75% are considered to meet seed viability standards.

The low germination percentage observed in the control treatment was associated with the absence of rhizobacterial protection, resulting in higher susceptibility of seeds to *Colletotrichum* sp. infection. Pathogen infection can damage seed tissues and inhibit the germination process, thereby reducing seed viability. In contrast, increasing rhizobacterial concentrations progressively improved germination performance, with treatment C4 showing the best result at 94.00%. This finding suggests that high rhizobacterial concentrations promoted more intensive and uniform bacterial colonisation on seed surfaces during soaking treatment. Jannah (2022) reported that rhizobacteria classified as *Plant Growth Promoting Rhizobacteria* (PGPR) are capable of producing phytohormones such as indole acetic acid (IAA) and gibberellins, which stimulate enzymatic activities, including amylase, protease, and lipase, thereby enhancing seed germination.

The improvement in germination percentage following rhizobacterial treatment was closely related to the dual role of rhizobacteria as biological control agents and plant growth stimulators. According to Afida *et al.* (2025), rhizobacteria produce secondary metabolites, including antibiotics, that inhibit fungal growth by disrupting cell membrane structure and causing abnormalities in *Colletotrichum* sp. hyphae. In addition, rhizobacteria can synthesise plant growth hormones such as auxins, which stimulate root development and accelerate germination processes. Therefore, rhizobacterial soaking treatments not only suppressed pathogen infection but also enhanced the physiological performance of chilli seeds, resulting in improved germination percentages across treatments C1 to C4 compared with the untreated control.

GERMINATION RATE

Germination rate reflects the ability of seeds to germinate normally on a daily basis and is commonly used as an indicator of seed vigour. Seeds that germinate more rapidly generally possess higher vigour and better adaptability under suboptimal environmental conditions (Attar *et al.*, 2023). Germination rate was calculated based on the daily growth of normal seedlings from 1 to 14 days after sowing.

Table 3. Average percentage of seed germination rate affected by antagonistic bacterial concentrations

Treatment		Disease Incidence(%)
C0	Control (water + *Colletotrichum* sp. inoculation)	7.22a
C1	Chili rhizobacterial soaking at 10^6 cfu/mL + *Colletotrichum* sp. incoculation	14.05b
C2	Chili rhizobacterial soaking at 10^8 cfu/mL + *Colletotrichum* sp. inoculation	14.02b
C3	Chili rhizobacterial soaking at 10^{10} cfu/mL + *Colletotrichum* sp. inoculation	13.79b
C4	Chili rhizobacterial soaking at 10^{12} cfu/mL + *Colletotrichum* sp. inoculation	13.95b
Average		12.60

Statistical analysis indicated that rhizobacterial soaking treatments did not significantly affect the germination rate of chilli seeds infected with *Colletotrichum* sp. However, the control treatment (C0) showed the lowest germination rate at 7.22%, whereas rhizobacterial treatments increased germination rates to 14.05% (C1), 14.02% (C2), 13.79% (C3), and 13.95% (C4). These results demonstrated that rhizobacterial application tended to improve seed germination performance compared with the untreated control.

The low germination rate observed in the control treatment was associated with *Colletotrichum* sp. infection, which inhibited seed germination from the early stages of seed development. Infected seeds generally exhibited delayed germination because pathogen invasion disrupted physiological and metabolic activities required for normal seed growth. The highest germination rate was recorded in treatment C1 (10^6 cfu/mL) at 14.05%, indicating that rhizobacterial application promoted faster seed germination. Nevertheless, the overall germination rate remained relatively low compared with the standard vigour criterion of 30% reported by Sadjad (1993) in Lesilolo (2013). This condition suggests that *Colletotrichum* sp. infection still reduced seed quality and vigour despite rhizobacterial treatment. Fitriningtyas (2008) in Pramono *et al*. (2019) stated that higher levels of seed deterioration are closely associated with lower seed vigour, which is reflected by reduced germination rates.

Reduced seed vigour was characterised by seed rot, necrosis in seedlings, abnormal seedling growth, and failure of seeds to germinate properly. The low average germination rate was also influenced by the delayed emergence of seedlings, as most seeds showed rapid growth only at the end of the observation period. According to Tefa *et al*. (2015), low seed vigour is commonly caused by pathogen infection that interferes with seed germination processes. Infection by *Colletotrichum* sp. can damage the endosperm and embryo tissues through cellulase enzyme activity, thereby inhibiting water imbibition and suppressing early metabolic processes essential for germination.

MAXIMUM GROWTH POTENTIAL

Maximum growth potential is a seed viability parameter used to measure the percentage of seeds capable of producing both normal and abnormal seedlings, thereby reflecting the ability of seeds to survive and grow. According to Putri (2026), seeds showing signs of growth indicate the presence of growth potential. Statistical analysis demonstrated that chilli rhizobacterial soaking treatments significantly affected the maximum growth potential of chilli seeds infected with *Colletotrichum* sp.

Table 4. Average percentage of the maximum growth potential of seeds affected by antagonistic bacterial concentrations

	Treatment	Disease Incidence(%)
C0	Control (water + *Colletotrichum* sp. inoculation)	85.00a
C1	Chili rhizobacterial soaking at 10^6 cfu/mL + *Colletotrichum* sp. incoculation	96.00b
C2	Chili rhizobacterial soaking at 10^8 cfu/mL + *Colletotrichum* sp. inoculation	94.00b
C3	Chili rhizobacterial soaking at 10^{10} cfu/mL + *Colletotrichum* sp. inoculation	97.00b
C4	Chili rhizobacterial soaking at 10^{12} cfu/mL + *Colletotrichum* sp. inoculation	99.00b
	Average	94.20

Note: Numbers followed by the same lowercase letter indicate a non-significant difference Based on the 5% DMRT.

The control treatment (C0) showed the lowest value at 85.00%, whereas rhizobacterial treatments increased maximum growth potential to 96.00% (C1), 94.00% (C2), 97.00% (C3), and 99.00% (C4). The low percentage observed in the control treatment indicated that *Colletotrichum* sp. infection inhibited seed germination and reduced seed viability. Suganda et al. (2023) reported that seed soaking with *Colletotrichum* sp. alone allows fungal conidia to attach, germinate, and infect seeds without inhibition, thereby reducing seed viability by more than 15% compared with rhizobacterial treatments.

Rhizobacterial treatments generally produced high maximum growth potential values, ranging from 94.00% to 99.00%, indicating that antagonistic bacteria effectively maintained seed viability despite pathogen infection. The highest value was recorded in treatment C4 (10^{12} cfu/mL) at 99.00%, followed by C3 (97.00%), C1 (96.00%), and C2 (94.00%). According to Fresela *et al.* (2025), maximum growth potential values above 80% are categorised as good seed quality. The high percentages obtained in this study suggest that the seeds possessed excellent genetic and physiological quality, allowing most seeds to germinate optimally even under different rhizobacterial concentrations. Dewi *et al.* (2025) also stated that high maximum growth potential values indicate that seeds still maintain good viability and the ability to germinate under favourable environmental conditions.

Treatment C4 (10^{12} cfu/mL) indicated that the rhizobacterial population had reached an optimal level for supporting seed growth, where further increases in concentration may no longer produce significant additional effects. Overall, all rhizobacterial treatments improved maximum growth potential compared with the control treatment, demonstrating the important role of rhizobacteria in maintaining seed viability under *Colletotrichum* sp. infection. This finding is consistent with Syamsuddin *et al.* (2022), who reported that pre-planting seed treatment using rhizobacteria significantly improved seed viability and maximum growth vigour. Therefore, rhizobacterial application has strong potential as a biological treatment for maintaining seed quality and enhancing seed performance under pathogen stress.

VIGOR INDEX

The vigour index reflects the ability of seeds to germinate and develop into normal seedlings under suboptimal environmental conditions. Seeds with a high vigour index generally exhibit rapid, uniform germination and greater tolerance to unfavourable conditions (Sopian *et al.*, 2021). Statistical analysis showed that chilli rhizobacterial soaking treatments had a highly significant effect on the vigour index of chilli seeds infected with *Colletotrichum* sp.

Table 5. Average percentage of the maximum growth potential of seeds affected by antagonistic bacterial concentrations.

Treatment		Disease Incidence(%)
C0	Control (water + *Colletotrichum* sp. inoculation)	23.00a
C1	Chili rhizobacterial soaking at 10^6 cfu/mL + *Colletotrichum* sp. incoculation	82.00b
C2	Chili rhizobacterial soaking at 10^8 cfu/mL + *Colletotrichum* sp. inoculation	83.00b
C3	Chili rhizobacterial soaking at 10^{10} cfu/mL + *Colletotrichum* sp. inoculation	69.00b
C4	Chili rhizobacterial soaking at 10^{12} cfu/mL + *Colletotrichum* sp. inoculation	89.00b
Average		69.20

Note: Numbers followed by the same lowercase letter indicate a non-significant difference Based on the 5% DMRT.

The highest vigour index was recorded in treatment C4 (89.00%), followed by C2 (83.00%), C1 (82.00%), and C3 (69.00%), whereas the control treatment (C0) showed the lowest value at 23.00%. These results indicated that rhizobacterial application effectively improved seed vigour under pathogen infection conditions.

The high vigour index observed in rhizobacterial treatments was associated with the ability of rhizobacteria to produce plant growth-promoting substances, particularly indole acetic acid (IAA), which supports seed germination and suppresses pathogen development. Harpani et al. (2018) reported that rhizobacteria produce various antibiotic compounds and secondary metabolites with antimicrobial activity capable of inhibiting the growth of *Colletotrichum* sp. In addition, Kolo and Tefa (2016) stated that seed vigour may increase under favourable storage conditions, particularly at low temperatures, because low temperatures suppress physiological deterioration and maintain seed food reserves. These conditions contribute to improved seed performance and germination capacity.

The low vigour index observed in the control treatment indicated that *Colletotrichum* sp. infection severely reduced seed physiological quality in the absence of rhizobacterial protection. Pathogen infection can damage embryo tissues and interfere with physiological processes during germination, resulting in poor seedling growth. Furthermore, warm and humid environmental conditions may enhance pathogen development and accelerate seed deterioration. Dicman (1993) in Adhani *et al.* (2022) explained that anthracnose infection commonly develops under warm and wet conditions, particularly at temperatures around 27°C with high humidity levels reaching 80%. Therefore, rhizobacterial application plays an important role in maintaining seed vigour and reducing the negative effects of *Colletotrichum* sp. infection on chilli seeds.

GERMINATION UNIFORMITY

Germination uniformity refers to the ability of seeds to germinate simultaneously and produce normal seedlings within a relatively uniform period. High germination uniformity indicates strong seed vigour and uniform early plant growth (Perdana *et al.*, 2023). In this study, germination uniformity was evaluated on the 10th and 11th days after sowing, corresponding to the middle stage of germination. Statistical analysis showed that chilli rhizobacterial soaking treatments had a highly significant effect on seed germination uniformity.

Table 6. Average percentage of the maximum growth potential of seeds affected by antagonistic bacterial concentrations

	Treatment	**Disease Incidence(%)**
C0	Control (water + *Colletotrichum* sp. inoculation)	62.00a
C1	Chili rhizobacterial soaking at 10^6 cfu/mL + *Colletotrichum* sp. incoculation	86.00b
C2	Chili rhizobacterial soaking at 10^8 cfu/mL + *Colletotrichum* sp. inoculation	86.00b
C3	Chili rhizobacterial soaking at 10^{10} cfu/mL + *Colletotrichum* sp. inoculation	79.50b
C4	Chili rhizobacterial soaking at 10^{12} cfu/mL + *Colletotrichum* sp. inoculation	94.00b
	Average	81.50

Note: Numbers followed by the same lowercase letter indicate a non-significant difference
Based on the 5% DMRT

The highest uniformity percentage was observed in treatment C4 (94.00%), followed by C1 (86.00%), C2 (86.00%), and C3 (79.50%), while the control treatment (C0) showed the lowest value at 62.00%. These results indicated that rhizobacterial application improved seed vigour and promoted more synchronised germination.

The high germination uniformity observed in rhizobacterial treatments demonstrated that treated seeds possessed strong vigour and high growth potential. According to Megasari *et al.* (2024), high germination uniformity reflects strong absolute vigour because seeds that germinate uniformly generally exhibit better growth performance. Furthermore, Zulmi *et al*. (2024) stated that germination uniformity values ranging from 40%–70% are considered acceptable seed quality standards, whereas values above 70% indicate very high seed vigour. Therefore, the germination uniformity percentages obtained in treatments C1, C2, C3, and C4 confirmed that rhizobacterial treatments effectively maintained seed physiological quality and improved the ability of seeds to germinate simultaneously under pathogen infection conditions.

The low germination uniformity observed in the control treatment was associated with *Colletotrichum* sp. infection, which disrupted seed physiological processes and reduced seed vigour. The pathogen infected the seed coat tissues, endosperm, and embryo structures, causing tissue damage and irregular seedling growth. Although treatment C3 showed a lower percentage than C1 and C2, the statistical analysis indicated no significant difference among these treatments. Germination uniformity is closely related to the physiological quality of seeds, reflecting the ability of seeds to survive and grow under both optimal and suboptimal conditions. Hariyono and Nathaniel (2024) explained that high physiological seed quality indicates that seeds possess the capacity to grow and develop properly even under unfavourable environmental conditions.

CONCLUSION AND RECOMMENDATIONS

Based on the results of this study, the application of antagonistic rhizobacteria effectively suppressed Colletotrichum sp. infection and improved the viability and vigor of chilli seeds. Rhizobacterial treatment at a concentration of 10^{12} cfu/mL resulted in the lowest disease incidence of 1.00% compared with 15.00% in the control treatment. In addition, rhizobacterial application increased germination percentage up to 94.00% and maximum growth potential up to 99.00%. Improvements were also observed in seed vigor parameters, with the highest vigor index reaching 89.00%, germination uniformity 94.00%, and germination rate 14.05%. These findings indicate that rhizobacteria isolated from the chilli rhizosphere have considerable potential as biological control agents for anthracnose disease while simultaneously improving chilli seed quality.

Based on the aforementioned conclusions, the use of antagonistic bacteria derived from the chili pepper rhizosphere can be applied as a pre-planting treatment, particularly at concentrations of 10^{10}–10^{12} cfu/mL, to suppress disease incidence and improve seed quality.

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