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Biocontrol Potential of *Pseudomonas* sp. LB14 Against Anthracnose Disease in Chili Pepper (*Capsicum annuum* L.)

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Abstract. Anthracnose caused by *Colletotrichum gloeosporioides* is a major postharvest disease of chili peppers (*Capsicum annuum* L.) that leads to severe quality deterioration during storage. Biological control using *Pseudomonas* sp. offers a sustainable alternative to chemical fungicides. This study evaluated the in vivo effectiveness of endophytic isolate *Pseudomonas* sp. LB14 in suppressing anthracnose development and its influence on postharvest fruit quality in red and green chili peppers during a 7-day storage period. The novelty of this study lies in comparing biocontrol performance between red and green chili under postharvest conditions. A Completely Randomized Design (CRD) was applied with three treatments: negative control (untreated), positive control (*C. gloeosporioides*), and biocontrol treatment (*C. gloeosporioides* + *Pseudomonas* sp. LB14). Lesion diameter, fruit weight, and weight loss were assessed as key indicators of disease severity and postharvest quality. Lesion data were analysed using Kruskal-Wallis and Dunn's tests, while weight and weight-loss data were evaluated using ANOVA and multivariate analysis. Results showed that *Pseudomonas* sp. LB14 effectively suppressed lesion development, particularly in red chili peppers, where lesion formation was completely inhibited throughout storage. In green chili peppers, LB14 significantly delayed disease progression, with significant differences observed only at later storage stages (6–7 dpi), indicating a disease-suppressive rather than curative effect. In contrast, LB14 treatment did not significantly reduce fruit weight loss in either pepper type, and weight loss patterns were largely influenced by pepper type and storage duration rather than treatment effects. *Pseudomonas* sp. LB14 demonstrates strong potential as a postharvest biocontrol agent by effectively limiting anthracnose progression and preserving fruit integrity, particularly in red chili peppers.

1. Introduction

Chili pepper (*Capsicum annuum* L.), including red and green varieties, is one of the most widely consumed horticultural commodities in Indonesia and plays an important role in national food security, nutrition, and economic stability [1]. However, maintaining postharvest quality remains a major challenge due to the fruit's high susceptibility to microbial infection, which leads to rapid deterioration and economic losses along the supply chain [2].

Among these diseases, anthracnose is recognized as one of the most destructive constraints affecting chili production and storage [3]. The disease is primarily caused by *Colletotrichum*

species, particularly *Colletotrichum gloeosporioides*, which induces sunken and expanding necrotic lesions on fruit surfaces and leads to severe postharvest losses [4]. Infection can occur during preharvest stages and continue to develop during storage and transportation, making anthracnose a persistent challenge in postharvest management systems [5].

Current control strategies for anthracnose rely largely on chemical fungicides [6-7]. However, their continuous usage has raised serious concerns, including the emergence of fungicide-resistant pathogen populations, environmental pollution, and potential health risks due to chemical residues on food products [8]. These drawbacks highlight the need for alternative disease control strategies that are both effective and environmentally sustainable.

In this context, biological control using antagonistic bacteria has emerged as a promising approach [9]. Among various candidates, species within the genus *Pseudomonas* have been extensively studied and are well known for their strong biocontrol capabilities. [10-13]. These bacteria can suppress plant pathogens through multiple mechanisms, including the production of enzymes, metabolites [11] and biosurfactant such as rhamnolipids that inhibit fungal infection by damaging to the germinating spore [10], competition for nutrients and ecological niches, as well as the induction of systemic resistance in host plants. In addition, *Pseudomonas* spp. are recognized for their rapid growth, metabolic versatility, and ability to colonize diverse plant environments, making them highly effective as biocontrol agents in both preharvest and postharvest systems.

The *Pseudomonas* isolate used in this study (LB14) was obtained from the internal tissues of *Aloe vera*, indicating its origin as an endophytic bacterium. Although endophytic microorganisms are known to produce bioactive compounds, in this study the emphasis is placed on the biocontrol potential of *Pseudomonas* sp. LB14 rather than its ecological origin. Previous *in vitro* studies have demonstrated its antifungal activity against plant pathogens [14], suggesting its potential application as a biological control agent.

Despite the well-documented biocontrol properties of *Pseudomonas*, the *in vivo* effectiveness of *Pseudomonas* sp. LB14 against *C. gloeosporioides* on chili peppers under postharvest conditions has not been fully evaluated. Furthermore, comparative information regarding its performance on different fruit types, such as red and green chili peppers, remains limited. Therefore, this study aims to evaluate the *in vivo* efficacy of *Pseudomonas* sp. LB14 as a biological control agent against *C. gloeosporioides* on red and green chili peppers (*Capsicum annuum* L.). This study provides insights that support sustainable postharvest disease management through reduced fungicide use, lower environmental impact, and improved food safety in accordance with global sustainability goals [15].

2. Material and methods

2.1 Rejuvenation of pathogen and biocontrol agent

The fungal pathogen isolate of *Colletotrichum gloeosporioides* was obtained from the Indonesian Oil Palm Research Center, Medan. The isolate was cultured on Potato Dextrose Agar (PDA) medium and incubated at room temperature for 5 days. The biocontrol agent isolate *Pseudomonas* sp., LB14 previously isolated from aloe vera (*Aloe vera* (L.) Burm.f.) based on the study by Venny [14], was rejuvenated on Nutrient Agar (NA) medium and incubated at room temperature for 24 hours.

2.2 Preparation pathogen suspension and biocontrol agent suspension

Spores of *Colletotrichum gloeosporioides* were harvested from actively growing cultures using sterile distilled water with a sterile wire loop and transferred into a 50 mL Erlenmeyer flask. The suspension was filtered through Whatman No. 1 filter paper to remove mycelial fragments and hyphae. Spore concentration was determined using a hemocytometer and adjusted to a final concentration of 1×10^6 spores/mL by dilution with sterile distilled water [16].

For preparation of the bacterial suspension, one loopful of *Pseudomonas* sp. LB14 culture was transferred into 10 mL of sterile distilled water and vortexed. The bacterial cell density was measured using a spectrophotometer and adjusted to an optical density of 0.5 (1×10^8 CFU/mL) based on the McFarland standard [17].

2.3 Preparation of chili pepper fruits

Fresh green and red chili fruits (*Capsicum annum* L.) were selected and screened. The chili fruits were free from physical damage and showed no visible symptoms of disease. The chili fruits were washed under running water to remove surface debris. The fruits were then disinfected with 0.5% NaOCl solution for 5 minutes and rinsed with sterile distilled water. Subsequently, the fruit surfaces were sterilized using 70% ethanol [18].

2.4 In vivo evaluation of *Pseudomonas* sp. against *C. gloeosporioides* on chili fruits

The in vivo assay was conducted the previous studies with modification [17,19]. The chili fruits were divided into two groups based on fruit color (red and green chili peppers). Each group was subjected to three treatments:

- Negative control (-): without application of *Pseudomonas* sp. and *C. gloeosporioides*
- Positive control (+): inoculated with *C. gloeosporioides* only
- Biocontrol treatment: inoculated with *Pseudomonas* sp. LB14 and *C. gloeosporioides*.

Each treatment consisted of five replicates, with one fruit used per replicate, resulting in a total of 30 experimental units (5 replicates $\times$ 3 treatments $\times$ 2 fruit types).

Artificial wounds (one wound per fruit) were made on the fruit surface using a sterile inoculation needle. Artificial wounding was applied to ensure infection, as *C. gloeosporioides* typically penetrates through injured tissues. A volume of 20 μ L of *C. gloeosporioides* suspension (1.25×10^6 conidia/mL containing 0.02% Tween 80 v/v to enhance the spore dispersion) was injected into the wound. Subsequently, the suspension of the biocontrol agent *Pseudomonas* sp. was sprayed evenly over the fruit surface and allowed to air-dry. All treated fruits were placed in perforated closed containers and incubated for 7 days. The 7-day incubation period was selected because anthracnose symptoms typically develop within 5–7 days under postharvest conditions, allowing reliable assessment of lesion progression. All fruits were incubated at 25–27°C in darkness area to facilitate anthracnose development, following standard postharvest pathogen incubation conditions. Disease development was evaluated by measuring lesion diameter, fruit weight and fruit weight loss.

2.5 Statistical analysis

Lesion diameter data that did not meet normality assumptions (Shapiro–Wilk test, $p < 0.05$) were analyzed using Kruskal–Wallis and Dunn’s post hoc tests. Fruit weight and weight loss data were analyzed using one-way ANOVA followed by Duncan’s Multiple Range Test. Multivariate analysis (MANOVA with Wilks’ Lambda test statistic) was performed to explore relationships among variables. All analyses were conducted at a 95% confidence level ($\alpha = 0.05$) using SPSS software.

3. Results and discussion

3.1 Effect of *Pseudomonas* sp. LB14 on lesion development

In red chili peppers, lesions caused by *Colletotrichum gloeosporioides* became visible at 3 dpi and increased progressively until 7 dpi (Table 1; Fig. 1a). This pattern indicates that the pathogen was able to adapt, colonize, and progressively develop within red chili tissues during postharvest storage, leading to continuous disease progression [4]. In contrast, red chili fruits treated with *Pseudomonas* sp. LB14 showed no lesion development throughout the storage period. The

absence of significant differences across time points indicates complete suppression of disease progression from the earliest stages of infection, suggesting strong antagonistic activity of LB14 that prevented the pathogen from establishing measurable lesions [20]. This result is consistent with the findings Sandani et al. [32] who showed that *Burkholderia* and *Pseudomonas* effectively suppressed the mycelial growth of *C. truncatum* and spore germination in in vitro and in vivo assays and Lahkar et al. [33], who reported that biosurfactants produced by *Pseudomonas aeruginosa* were highly effective in reducing disease severity at their maximum concentration, even outperforming a commercial fungicide.

Statistical analysis using the Kruskal–Wallis test supported these observations, as lesion diameter data failed to meet normality and homogeneity assumptions. The nonparametric analysis showed no significant difference in lesion diameter across time points in red chili peppers, consistent with complete suppression of disease development. In green chili peppers, significant differences were detected only at 6 and 7 dpi, and Dunn's test confirmed that lesion distribution patterns differed between treatments at later stages of infection. These results indicate that differences in disease progression became statistically detectable only when lesion expansion exceeded early-stage variation.

Table 1. Lesion diameters of red and green chili (mm).

Fruit Types	Treatment	Days post infection (dpi)							
		0	1	2	3	4	5	6	7
Red Chili	<i>Colletotrichum gleosporoides</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.42 ± 3.18	2.96 ± 4.13	4.42 ± 6.18	5.10 ± 7.20
	<i>Pseudomonas</i> sp. LB14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Control Effect (%)	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00
Green Chili	<i>Colletotrichum gleosporoides</i>	0.00 ± 0.00	0.92 ± 1.28	1.12 ± 1.54	4.14 ± 5.19	4.32 ± 5.90	6.20 ± 6.50	8.12 ± 6.98	9.90 ± 7.51
	<i>Pseudomonas</i> sp. LB14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 0.76	0.46 ± 1.03	0.84 ± 1.88	0.88 ± 1.97	0.40 ± 0.89
	Control Effect (%)	0.00	100.00	100.00	91.79	89.35	86.45	89.16	95.96

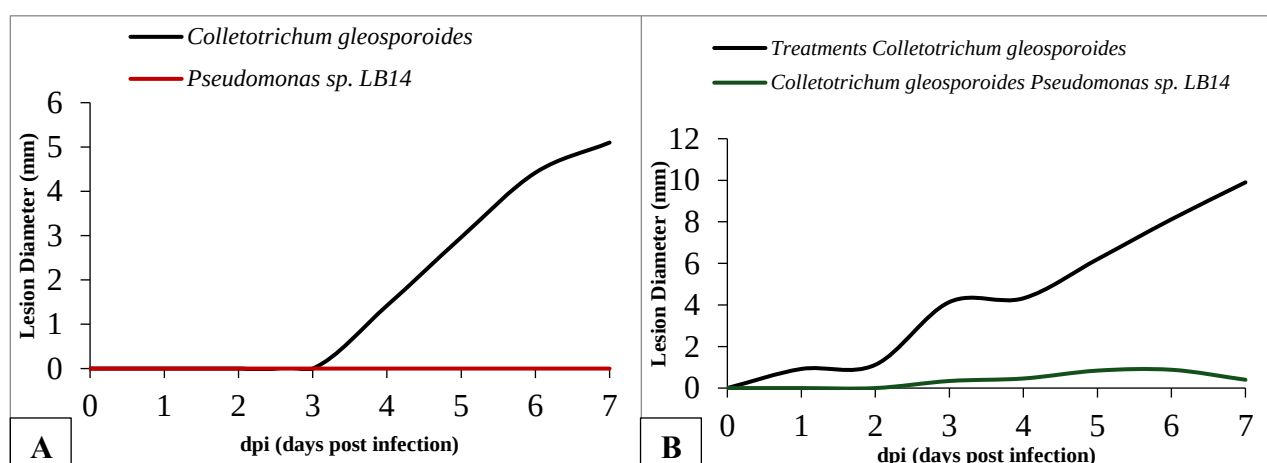


Figure 1. Lesion diameter of (a) red chili and (b) green chili peppers infection.

In green chili peppers, lesion diameter increased continuously in the pathogen control, indicating active disease progression during storage (Table 1; Fig. 1b). Application of

Pseudomonas sp. LB14 significantly reduced lesion expansion at 6 and 7 dpi, demonstrating its ability to delay disease progression rather than completely inhibit infection. This delayed suppression suggests that the biocontrol activity of LB14 became more effective during later stages of pathogen colonization [21-23].

The contrasting responses between red and green chili peppers highlight the role of host-related factors in determining biocontrol efficacy. The strong control effect (100% in red peppers and 95.96% in green peppers at the end of storage) indicates direct antagonism of *Pseudomonas* sp. LB14 against *C. gloeosporioides*, likely through antibiosis or competition [20]. Moreover, differences between red and green peppers suggest that host-related factors such as tissue maturity, structural rigidity, secondary metabolites, or colonization dynamics may influence the performance of the biocontrol agent. Red peppers appear to provide more conducive conditions for LB14-mediated suppression, whereas green peppers exhibit slower or less complete inhibition [24, 25]. These findings demonstrate that *Pseudomonas* sp. LB14 effectively suppresses anthracnose development during postharvest storage, with efficacy influenced by chili type and disease progression dynamics.

3.2 Fruit quality parameters (weight and weight loss)

Fruit weight and weight loss were evaluated as secondary quality parameters to assess treatment effects on postharvest quality rather than direct antifungal activity. Across both red and green chili peppers, fruit weight decreased and weight loss increased progressively over storage time, indicating that physiological water loss and respiration were the dominant drivers of quality deterioration during storage [26-29]. Statistical analysis showed that treatment effects on weight were limited and mostly restricted to the early storage period (0–2 dpi), while no consistent significant differences were observed during later stages of storage.

3.2.1 Red chili fruit weight and weight loss

One-way ANOVA confirmed that weight data met normality and variance homogeneity assumptions, as validated by Levene's test. A significant treatment effect was detected only at 0 dpi ($F = 6.897$, $p < 0.05$), while no significant differences occurred from 1–7 dpi. Although fruits treated with *Pseudomonas* sp. LB14 maintained slightly higher final weights than those inoculated with *C. gloeosporioides*, this pattern was strongly influenced by initial weight variation. The positive control exhibited the lowest final weight (1.07 ± 0.27 g), whereas LB14-treated fruits remained slightly heavier (1.17 ± 0.13 g) after 7 days. The untreated control consistently showed the highest weights (1.39 ± 0.27 g). These patterns support that treatment-related differences in weight were primarily attributable to variability present at the start of storage rather than true treatment effects.

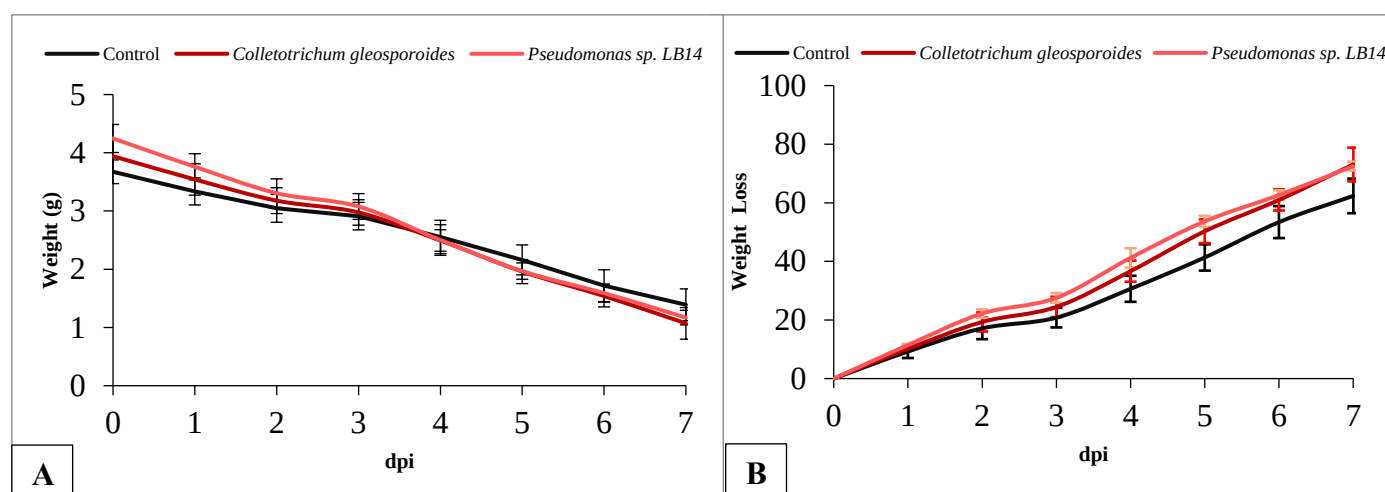
Weight loss data also met ANOVA assumptions, except at 6 dpi. Weight loss increased significantly over time, and treatment effects emerged starting at 3 dpi. However, *Pseudomonas* sp. LB14 did not reduce weight loss compared to the pathogen control. By day 7, LB14-treated red peppers exhibited 72.5% weight loss, similar to fruits inoculated only with *C. gloeosporioides* (73.1%), indicating that LB14 did not mitigate moisture loss during storage. The untreated control consistently showed the lowest weight loss (62.3%) and differed significantly from LB14-treated fruits throughout late storage. These results highlight that weight loss dynamics were more strongly influenced by fruit physiology and storage duration than by biocontrol treatment.

3.2.2 Green chili fruit weight and weight loss

A similar trend was observed in green chili peppers (Table 3; Fig. 3). Significant treatment effects on weight were detected only during the early storage period (0–2 dpi), consistent with the initial

Table 2. Daily weight and weight losses of red chili for 7 days post infection

Parameter	Treatment	Days post infection (dpi)							
		0	1	2	3	4	5	6	7
Daily Weight (g)	<i>Colletotrichum gloeosporioides</i>	3.94	3.54	3.18	2.98	2.49	1.96	1.54	1.07 ± 0.27
		±	±	±	±	±	±	±	
		0.28	0.27	0.22	0.22	0.18	0.21	0.19	
	Control	3.67	3.34	3.05	2.91	2.55	2.16	1.72	1.39
		±	±	±	±	±	±	±	±
		0.20	0.23	0.24	0.24	0.29	0.26	0.27	0.27
Weight Loss (%)	<i>Pseudomonas</i> sp. LB14	4.25	3.76	3.31	3.08	2.50	1.97	1.59	1.17
		±	±	±	±	±	±	±	±
		0.24	0.22	0.25	0.22	0.26	0.14	0.16	0.13
	<i>Colletotrichum gloeosporioides</i>	0.00	10.17	19.31	24.44	36.66	50.27	60.97	73.06
		±	±	±	±	±	±	±	±
		0.00	0.56	3.23	3.47	3.60	4.06	3.61	5.76
Weight Loss (%)	Control	0.00	9.24	17.13	20.80	30.63	41.33	53.40	62.34
		±	±	±3.67	±	±	±	±	±
		0.00	2.23		3.34	4.44	4.49	5.46	5.90
	<i>Pseudomonas</i> sp. LB14	0.00	11.46	22.20	27.55	41.19	53.66	62.62	72.50
		±	±	±	±	±	±	±	±
		0.00	0.38	1.45	1.67	3.29	1.88	1.62	1.52

**Fig. 2** (A) Daily weight of red chili peppers (B) Weight loss of red chili peppers

weight variability among treatments. At later stages (4–7 dpi), no significant differences were observed, indicating that treatment did not influence weight retention during storage. By the end of the storage period, fruits inoculated with *Colletotrichum gloeosporioides* showed the lowest mean weight, while LB14-treated fruits exhibited slightly higher values; however, these differences were not statistically meaningful.

Weight loss progression also showed minimal treatment influence. A significant effect was observed only at 1 dpi, after which weight loss rates did not differ among treatments. Although fruits treated with *C. gloeosporioides* tended to display the highest weight loss at 5–7 dpi and LB14-treated fruits showed slightly higher loss during early storage, these variations were not significant. Control fruits consistently exhibited the lowest weight loss values. The results indicate that LB14 did not alter postharvest physiological processes related to water loss in green chili

peppers, supporting the conclusion that its primary role lies in disease suppression rather than quality preservation through moisture retention.

Multivariate analysis (MANOVA) integrating both pepper types and all treatments further supported these findings. Significant main effects of treatment and pepper type were detected for both weight and weight loss, with green and red peppers showing inherently different physiological responses across storage time. Treatment effects were significant only during the early storage period (0–2 dpi), consistent with the influence of initial weight variability. No significant interaction between pepper type and treatment was observed for either variable, indicating that their effects were independent. These results confirm that fruit type contributed more strongly to weight and weight loss dynamics than biocontrol treatment, reinforcing that LB14 influences disease development but does not substantially affect postharvest physiological water loss [30-31].

Table 3. Daily weight and weight losses of green chili for 7 days post infection

Parameter	Treatment	Days post infection (dpi)							
		0	1	2	3	4	5	6	7
Daily Weight(g)	<i>Colletotrichum gleosporoides</i>	3.61 ± 0.30	3.09 ± 0.32	2.62 ± 0.27	2.36 ± 0.36	1.72 ± 0.38	0.93 ± 0.25	0.79 ± 0.23	0.52 ± 0.19
	Control	2.87 ± 0.19	2.38 ± 0.23	2.05 ± 0.24	1.89 ± 0.22	1.46 ± 0.26	1.03 ± 0.28	0.80 ± 0.23	0.66 ± 0.16
	<i>Pseudomonas</i> sp. LB14	3.51 ± 0.17	2.80 ± 0.20	2.35 ± 0.21	2.13 ± 0.22	1.58 ± 0.33	1.06 ± 0.28	0.84 ± 0.28	0.71 ± 0.28
Weight Loss (%)	<i>Colletotrichum gleosporoides</i>	0.00 ± 0.00	14.56 ± 1.76	27.66 ± 2.81	35.07 ± 4.92	52.79 ± 6.90	74.47 ± 5.14	78.39 ± 4.89	85.95 ± 4.35
	Control	0.00 ± 0.00	17.17 ± 3.11	28.71 ± 4.21	34.50 ± 4.00	49.37 ± 6.31	64.53 ± 7.94	72.41 ± 6.72	77.25 ± 4.35
	<i>Pseudomonas</i> sp. LB14	0.00 ± 0.00	20.10 ± 3.72	33.05 ± 4.79	39.32 ± 5.24	55.00 ± 9.04	69.79 ± 7.64	76.14 ± 7.56	79.88 ± 6.48

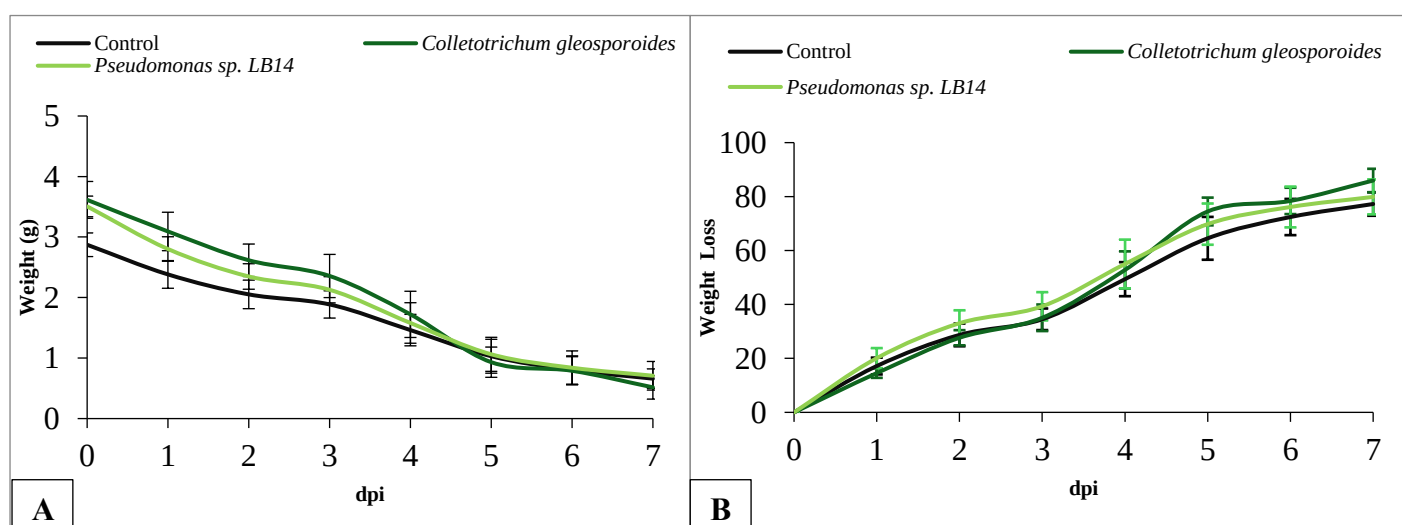


Fig. 3 (A) Daily weight of green chili peppers (B) Weight loss of green chili peppers

4. Conclusions

Pseudomonas sp. LB14 effectively suppressed anthracnose development caused by *Colletotrichum gloeosporioides* during postharvest storage, with complete inhibition in red chili peppers and reduced lesion progression in green peppers. Despite its strong biocontrol activity, LB14 did not significantly affect fruit weight or weight loss, which were primarily determined by intrinsic fruit physiology and storage conditions. This study highlights the potential of *Pseudomonas* sp. LB14 as a sustainable alternative to chemical fungicides for postharvest anthracnose management. Future work should include field-scale validation and formulation development to support practical adoption of LB14 as a biocontrol agent.

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