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RESEARCH PAPER

Application of biocontrol products Bio P60 and Bio T10 as single or in combination in suppressing chili fruit anthracnose in the field

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ABSTRACT

This study aimed to evaluate the effectiveness of single or combined applications of Bio P60 and Bio T10 products in suppressing anthracnose disease in chili pepper under field conditions. The research was conducted at an altitude of 1200 m above sea level using a randomized block design with five treatments: control, chemical fungicide, Bio P60, Bio T10, and a combination of Bio P60 and Bio T10, each replicated five times. Observed variables included incubation period, disease incidence, disease intensity, area under disease progress curve (AUDPC), infection rate, plant height, number of leaves, time of first flower, time of first fruit, number of fruits, fruit weight per plant, harvest weight per plot, and qualitative phenolic compound content. The results showed that the combined application of Bio P60 and Bio T10 was the most effective, delaying the incubation period, suppressing disease intensity, and reducing AUDPC by 13.71%, 69.34%, and 47.06%, respectively, compared to the control. The combination treatment also enhanced plant growth and yield, increasing plant height, number of fruits, fruit weight per plant, and harvest weight per plot by 27.38%, 62.65%, 90.85%, and 82.99%, respectively. Furthermore, the application of Bio P60, Bio T10, and their combination increased phenolic compound content qualitatively in chili pepper plants.

Key words: Anthracnose, Bio P60, Bio T10, chili pepper, secondary metabolites

INTRODUCTION

Chili is a highly important commodity in the daily lives of Indonesian people. The demand for chili in Indonesia continues to increase along with population growth. In 2021, chili pepper production in Indonesia reached 1.39 million tons (Statistics Indonesia, 2021). However, this figure represents a decrease compared to the 2020 production of 1.5 million tons, marking a decline of 8.09%. However, this figure represents a decrease compared to the 2020 production of 1.5 million tons, marking a decline of 8.09%.

One of the major pathogens causing losses in chili production is the fungus *Colletotrichum capsici*, the causal agent of anthracnose. Anthracnose can lead to shoot dieback in mature plants and fruit infections, significantly reducing chili yields (Saxena et al., 2016). Yield losses due to anthracnose can reach up to 50%, and in the absence of proper control measures, losses may rise to 100%, particularly during the rainy season (Ciofini et al., 2022).

Currently, anthracnose control primarily relies on the intensive use of synthetic fungicides. However, excessive application of these chemicals can have long-term negative effects on soil health, the environment, and the wellbeing of farmers and consumers (Noel et al., 2022; Deresa & Diriba, 2023). Alternative control methods, such as the use of botanical pesticides, have been explored (Ilondu, 2011). However, these approaches have several limitations, including the need for frequent applications due to volatility, limited availability in some areas, and the inability of non-polar compounds to penetrate plant tissues (Soesanto et al., 2019b).

Environmentally friendly alternatives include the use of antagonistic microbes. Several studies have been investigated the use of antagonistic microbes for controlling chili anthracnose (Oo & Oh, 2016; Saxena et al., 2016; Suprapta, 2022). However, the application of spore-based biological agents in the field faces challenges, such as abiotic stress and inconsistent spore production (Azubuike et al., 2016; Hojnik et al., 2019).

Therefore, new strategies are needed, including

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the use of secondary metabolites produced by biological control agents (Soesanto et al., 2020). These metabolites represent a promising alternative to spore-based agents. The antagonistic bacterium *Pseudomonas fluorescens* P60 and the fungus *Trichoderma harzianum* T10 have each been formulated into biological products known as Bio P60 and Bio T10, respectively (Soesanto et al., 2019a). However, the application of Bio P60 and Bio T10, either individually or in combination, to control chili anthracnose in the field has not yet been studied. The objective of this study was to evaluate the potential of single or combined applications of Bio P60 and Bio T10 in reducing anthracnose incidence, enhancing plant height, and increasing fruit yield in chili pepper.

MATERIALS AND METHODS

Research Site. This research was conducted in an anthracnose-endemic area at Bansari Village, Bansari Subdistrict, Temanggung Regency, Central Java, at an altitude of 1200 m above sea level, with a tropical climate, temperatures ranging around 20–30 ºC, and annual rainfall approximately 2000 mm/year (Temanggung Regency Government, 2021). Laboratory research was carried out at the Plant Protection Laboratory, Faculty of Agriculture, Jenderal Soedirman University. This research was conducted over seven months, from December 2022 to July 2023.

Preparation of Bio P60 and Bio T10 Formulations. Preparation of the Bio P60 formulation began with the rejuvenation of *P. fluorescens* P60 isolate was inoculated into 1 L of King's B broth and shaken at 135 rpm at room temperature for 2–3 days (Soesanto et al., 2013). Secondary metabolites of *P. fluorescens* P60 were produced using a medium consisting of 200 g of catfish meat, 80 g of shrimp paste, and 20 L of water. The medium was boiled, filtered, placed in sterile jerry cans, allowed to cool, and then inoculated with 40 mL of *P. fluorescens* P60 liquid culture. It was then shaken at 135 rpm at room temperature for 3 days, and the cell density was determined to be 10^8 cells mL^{-1} (Soesanto et al., 2010).

Preparation of Bio T10 began by growing *T. harzianum* T10 on sterile broken corn and incubating it for 7 days at room temperature (25–27 ºC) (Soesanto et al., 2019a). The medium for producing secondary metabolites of *T. harzianum* T10 consisted of 6 L of coconut water, 250 g of rice flour, and 60 g of sugar. This mixture was boiled, filtered, and placed into sterile jerry cans, and allowed to cool. *T. harzianum* T10 from 3–4 plastic bags (150–200 g) was dissolved, filtered, and added to the propagation medium. The mixture was shaked at 135 rpm for 7 days at room temperature, and the conidia density was determined to be 10^8 conidia mL^{-1}.

Preparation of Planting Area. The planting area, with andosol soil covering 200 m^2, was amended 10–20 g of chicken manure and 5–10 g of chemical fertilizer (NPK 15: 15: 15) per plant. Beds were formed with a width of 70 cm and length adjusted to the land size, then covered with plastic mulch.

Cultivation. Chili seedlings aged approximately 30 days after sowing were transplanted using a spacing of 40 cm × 40 cm. One seedling was planted per planting hole.

Experimental Design. A randomized block design (RBD) was used with five treatments and five replicates, resulting in 25 experimental units. The treatments included: (i) Control (sprayed with 50 mL of water per plant), (ii) Chemical fungicide (a.i. mancozeb 80%) at the recommended dose, (iii) Bio P60, (iv) Bio T10, (v) Combination of Bio P60 and Bio T10 (1:1, v/v).

Bio P60, Bio T10, or their combination were applied in two stages:
First stage: 5 mL L^{-1} solution at 50 mL per plant on days 52, 56, 60, 64, and 68 after planting (DAP),
Second stage: 10 mL L^{-1} solution at 100 mL per plant on days 72, 76, 80, 84, 88, 92, and 96 DAP.
Inoculation and infection of anthracnose pathogens occurred naturally in the field.

Maintenance. Maintenance of chili plants included manual watering as needed, fertilization according to dosage and growth stage, manual pest and weed control, and replanting of dead seedlings.

Observation. The observed parameters included incubation period, disease incidence, disease intensity, AUDPC (Area Under Disease Progress Curve), infection rate, plant height, and number of leaves. Yield components included time to first flowering, number of flowers, time to first fruiting, number of fruits per plant, fruit weight per plant, fruit weight per plot, and qualitative analysis of phenolic compounds.
Incubation period. Incubation period was recorded from the onset of the first anthracnose symptoms on chili fruits.
Disease incidence. Disease incidence was calculated using the formula (Rodrigues et al., 2019):

$$IS = \left(\frac{A}{B}\right) \times 100\%$$

IS = Disease Incidence;
A = Number of affected plants;
B = Total number of observed plants.

Disease intensity. Disease intensity was calculated using the formula (Gusmarini et al., 2014):

$$DI = \frac{\sum (n \times v)}{N \times V} 100\%$$

DI = Disease Intensity (%);
n = Number of fruits in each category;
v = Score of each disease category.
N = Total number of observed fruits;
V = Highest score in the scale.

Scale based on fruit anthracnose pathogen attack interval, according to Gusmarini et al. (2014): 0 = No symptoms; 1 = Fruit with 1–20% anthracnose symptoms; 2 = Fruit with 21–40% anthracnose symptoms; 3 = Fruit with 41–60% anthracnose symptoms; 4 = Fruit with 61–80% anthracnose symptoms; 5 = Fruit with 81–100% anthracnose symptoms.

Control effectiveness. Control effectiveness was calculated using the formula:

$$NE = \left(\frac{I_1 - I_2}{I_1}\right) \times 100\%$$

NE = Control effectiveness;
I_1 = Disease intensity in control;
I_2 = Disease intensity in treatment.

According to Rahayuniati & Mugiastuti (2012), control effectiveness is categorized as follows: CE > 69%: Very good; CE = 50–69%: Good, CE = 30–49%: Fair, CE < 30%: Poor.

AUDPC. AUDPC was calculated using the formula (Simko & Piepho, 2012):

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{Yi+1 + Yi}{2}\right] \times \left(Ti+1 - Tl\right)$$

AUDPC = Disease progression curve (%-days);
Y_i, Y_{i+1} = Observation data;
T_{i+1} = Observation time;
T_i = 1st observation time.

Infection rate. Infection rate (r) was calculated as:

$$r = \frac{2,3}{t_2 - t_1}\left[\log\left(\frac{X_t}{1 - X_t} - \log\left(\frac{X_0}{1 - X_0}\right)\right)\right]$$

r = Infection rate;
X_0 = Proportion of disease at the beginning of observation (t = 0);
X_t = Proportion of disease at time t;
t = Time of observation.

Growth components included plant height and number of leaves. Yield components included time to first flowering, number of flowers, time to first fruiting, number of fruits per plant, fruit weight per plant, and fruit weight per plot. In addition, qualitative phytochemical analysis was performed for phenolic compounds, especially tannins (Bele et al., 2010), glycosides (Rufai et al., 2016), and saponins (Ribeiro et al., 2013; Vidal et al., 2018).

Data Analysis. Data were analyzed with the F-test at a 5% significance level. If significant difference were found, further analysis was conducted using the Duncan's Multiple Range Test (DMRT) at $\alpha \leq 0.05$. The results of phytochemical content tests were analyzed descriptively.

RESULTS AND DISCUSSION

The Effect of Bio P60, Bio T10, and their Combination on Pathosystem Component

Incubation Period. The analysis of the incubation period showed that the combined application of Bio P60 and Bio T10 significantly delayed sympton onset by 13.71%, or about 13 days longer than the control (Table 1). The single application of the chemical fungicide, Bio P60, and Bio T10 delayed the incubation period by 7.85%, 11.15%, and 11.73%, respectively, compared to the control. The shorter incubation period in the control group was likely due to the absence of any inhibitory activity against the pathogen. Anthracnose infection occurred naturally, with symptoms first appearing on chili fruits in the control group (Figure 1).

The faster incubation period observed in the control is thought to result from factors such as the aggressiveness of the pathogen and the lack of inhibition (Susi & Laine, 2017). Soesanto et al. (2010) also found that the *P. fluorescens* P60 formulation was able to delay the incubation period of bacterial wilt in tomatoes. This study confirms that single and combined applications of Bio P60 and Bio T10 can delay *C. capsici* infection.

Bio P60 and Bio T10 may enter plant tissues via nutrient and water transport systems, stimulating resistance due to their polar (water-based) characteristics (Soesanto et al., 2010; Soesanto et al., 2019a). The secondary metabolites of *P. fluorescens* P60 include the antibiotic 2,4-diacetylphloroglucinol, which can enhance resistance by increasing phenolic compounds in plants. Meanwhile, *T. harzianum* T10 produces chitinase, β-1,3-glucanase, and cellulase enzymes that degrade pathogen cell walls (Ting & Chai, 2015). Soesanto et al. (2020) demonstrated that secondary metabolites from *T. harzianum* T10 and T213 isolates can delay disease onset. This study further supports that combining secondary metabolites from multiple antagonists enhances efficacy in field conditions (Alexander et al., 2021).

Disease Incidence. The application of single (Bio P60 or Bio T10), combined secondary metabolites, as well as the fungicide Mancozeb (80%), significantly reduced disease incidence compared to the control (Table 1). The combined treatment reduced disease incidence by 18%. This reduction may result from mechanisms such as cell wall lysis and intracellular parasitism mediated by microbial secondary metabolites.

P. fluorescens produces salicylic acid and peroxidase enzymes (Prakash et al., 2021), which activate plant resistance genes through induced systemic resistance (ISR) (Ryals et al., 1996). Peroxidases strengthen plant cell walls by pcatalyzing the polymerization of lignin precursors (Shigeto & Tsutsumi, 2016). Additionally, higher levels of phenolic compounds were detected in treated plants (Table 4), indicating increased biochemical resistance.

The superior performance of the combined application is likely due to the synergistic effects of multiple antimicrobial compounds. Saikia & Chetia (2024) suggested that combinations of antibiotics often outperform single agents. Compounds present in the secondary metabolites of antagonistic microbes have been shown to suppress plant pathogens effectively (He et al., 2021). Moreover, these compounds may also promote plant growth. *P. fluorescens* produces auxins such as IAA (Dorjey et al., 2017), while *T. harzianum* is recognized as a plant growth-promoting fungus (PGPF) due to its ability to produce phytohormones (Oskiera et al., 2015; Kumar et al., 2022).

Disease Intensity and Control Effectiveness. As shown in Table 1, the combined application significantly reduced disease intensity by 67.67%, compared to 54.88% and 53.38% for Bio P60 and Bio T10, respectively. This indicates that the combination is more effective than either treatment alone or the synthetic fungicide. The cumulative effects of the combined treatment likely enhanced efficacy through synergistic interactions (Niu et al., 2020). Multiple-strain biological control agents (MSBCAs), consisting of two strains—such as fungi and bacteria— where one or both have biocontrol activity, have proven to

Table 1. Pathosystem component of chili pepper anthracnose

Treatments	Incubation period (DAP)	Disease incidence (%)	Disease intensity (%)	AUDPC (%-days)	Infection rate (%/day)	Control effectiveness (%)
Control	94.04 a	100 b	66.50 c	362.86 d	0.057 c	-
Mancozeb 80%	101.42 b	96 a	35.50 b	254.52 c	0.013 a	46.61
Bio P60	104.52 bc	96 a	30.00 b	224.60 b	0.024 b	54.88
Bio T10	105.08 bc	94 a	31.00 b	234.39 bc	0.006 a	53.38
Combination	106.94 c	82 a	21.50 a	192.08 a	0.004 a	67.67

The disease intensity and incidence data were transformed to Arsin V(x+0.5). Numbers in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 95% confidence level, DAP = days after planting.

Figure 1. Anthracnose symptoms on chili pepper fruit.

be more efficient. For example, a consortium of *T. asperellum* and *Bacillus amyloliquefaciens* was more effective against *Botrytis cinerea* (grey mold disease) than either strain alone.

Bio P60, which contains secondary metabolites of *P. fluorescens* P60, can protect plants against pathogens through multiple mechanisms, such as hydrolytic enzymes, induced systemic resistance (ISR), and antibiotics like 2,4-diacetylphloroglucinol (DAPG) (Vinale et al., 2012). Vinale et al. (2012) also reported that the diverse range of secondary metabolites produced by *Trichoderma* species contributes significantly to their beneficial biological activities. This is supported by the research from Weller et al. (2012), which highlighted the importance of DAPG in ISR; mutants unable to produce DAPG could not trigger resistance, indicating that antibiotics are the key ISR-activating components.

Bio T10, containing secondary metabolites of *T. harzianum* T10, operates through an antibiosis mechanism marked by the production of bioactive compounds such as peptaibols, pyrones, viridins, koningins, gliotoxins, gliovirins, and viridiol (Guzmán-Guzmán et al., 2023). *Trichoderma* sp. can induce long-term upregulation of salicylic acid-responsive genes more quickly than pathogen infections, such as those caused by *B. cinerea*. Pretreatment with *Trichoderma* modulates salicylic acid-dependent gene expression and, after infection, activates defense genes through the jasmonic acid signaling pathway, thereby enhancing ISR over time (Tucci et al., 2011).

Based on the calculation, the highest control effectiveness was observed in the combined application (67.67%). Meanwhile, the single application of Bio P60 and Bio T10 chieved effectiveness values of 54.88% and 53.38%, respectively. The synergistic effect of combining microbial secondary metabolites enhances their efficacy compared to individual applications (Niu et al., 2020). Based on these values, the use of Bio P60, Bio T10, or their combination in managing chili pepper anthracnose in the field falls into the "good" category, as the effectiveness ranges from 50–69%. According to Sathua et al. (2017), efficacy values ≥ 50% are considered "good."

***Area Under Disease Progress* (AUDPC)**. The AUDPC results (Table 1) showed that the combined application reduced disease progression by 47.06% compared to the control. The fungicide, Bio P60, and Bio T10 reduced AUDPC values by 29.85%, 38.12%, and 34.85%, respectively. The superior performance of the combined application indicates its enhanced ability to inhibit *C. capsici* development.

This inhibition is likely due to the chitinase enzymes produced by both antagonists, which degrade fungal cell walls (Lee & Kim, 2015; Mohiddin et al., 2021). According to Langner & Göhre (2016), chitinase enzyme can inhibit and degrade chitin in the fungal cell walls, leading to fungal cell lysis. Additionally, the accumulation of phenolic compounds may activate phenylalanine ammonia-lyase (PAL), further enhancing resistance (Li et al., 2023). AUDPC is positively correlated with disease intensity, as stated by Simko & Piepho (2012).

Infection Rate. Bio T10 and the combined treatment significantly suppressed the infection rate by 89.47% and 92.98%, respectively (Table 1), while Bio P60 reduced it by 57.89%. This suppression is likely due to increased phenolic compound levels, which act as natural toxins without affecting plant growth (Soesanto et al., 2010). Phenolic presence was confirmed qualitatively (Table 4).

The Effect of Bio P60, Bio T10, and their Combination on Growth Components

Plant Height. Table 2 showed that the combined treatment increased plant height by 27.38% compared to the control, while Bio T10 alone increased height by 14.26%. The increase in crop height from the combined application of Bio P60 and Bio T10 is likely due to the synergistic action of their secondary metabolites, which

Table 2. Chili pepper growth component

Treatments	Plant height (cm)	Number of leaves
Control	61.34 a	137.47 a
Mancozeb 80%	62.58 ab	141.31 a
Bio P60	63.11 ab	145.02 a
Bio T10	70.08 bc	151.67 a
Combination	78.13 c	164.36 a

Numbers in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

work together more effectively than when applied individually. *P. fluorescens* can act as a plant growth-promoting rhizobacteria (PGPR), producing plant hormones such as indole acetic acid (IAA) (Ardebili et al., 2011). In addition, *Trichoderma* species are known to produce IAA and indole compounds such as indole-3-ethanol, indole-3-acetaldehyde, and indole-3-carboxaldehyde as part of their metabolism processes (Contreras-Cornejo et al., 2011).

Number of Leaves. Although no significant differences were observed among treatments (Table 2), combined application tended to result in a higher number of leaves. The control group had the fewest leaves, likely due to nutrient limitations or lack of hormonal stimulation. According to Perry et al. (2022), low doses of microbial metabolites may reduce hormone activity, thereby affecting leaf development. Hayat et al. (2010) also emphasized that rhizosphere bacteria can enhance plant growth through hormonal signaling.

The Effect of Bio P60, Bio T10, and their Combination on Yield Components

Time to First Flowering. The results of data analysis showed no significant difference in flowering time between the control and the treated groups, although there was a tendency for the combined application to induce earlier flowering (Table 3). This is likely due to the low concentration of secondary metabolites, resulting in insufficient growth hormone content to optimally promote flowering. The concentration of secondary metabolites in Bio P60 and Bio T10 should ideally be more than 5 mL L^{-1} each. As is well known, secondary metabolites contain bioactive compounds, including plant hormones. Additionally, the physiological effect of phytohormones on flowering can vary across different parts of the plant canopy, influencing the timing of flowering (Gasperini & Howe, 2024). According to Vinale et al. (2012), *Trichoderma* secondary metabolites at high doses may actually inhibit plant growth.

First Appearance of Fruit. The application of single (Bio P60 or Bio T10) and combined (Bio P60 and Bio T10) secondary metabolites, and chemical fungicide did not have a significant effect on the timing of the first fruit appearance, although the combined application tended to produce fruit earlier (Table 3). This is consistent with the flowering time analysis results, suggesting that the low content of bioactive compounds in these secondary metabolites may not be sufficient to accelerate fruit development. In general, the application of secondary metabolites can enhance plant growth due to the presence of growth regulators. These compounds can aid in nutrient uptake, improve stress resistance, and promote overall plant health (Erb & Kliebenstein, 2020).

Number of Fruits. The results of fruit number parameters showed significantly different between the combined application of secondary metabolites and the control (Table 3). The combined application of Bio P60 + Bio T10 was able to increase the number of fruits by 67.65% compared to the control, which is in line with the pathosystem component data above (Table 1). This is because the more diverse content of bioactive compounds gave the best effect. According to Kanchana et al. (2014), the combination of PGPR *P. fluorescens* and *B. subtilis* can affect the number of fruits on chili pepper. Furthermore, according to Adnan et al. (2019), *Trichoderma* sp. has a high reproductive ability, efficiency in nutrient utilization, responsiveness to pathogens and the ability to spur plant growth and defense.

Fruit Weight Per Plant and Harvest Weight Per Plot. The results of data analysis of fruit weight per plant and harvest weight per plot showed a highly significant difference in the combined application and a significant difference in other treatments compared to the control

Table 3. Chili pepper yield component

Treatments	First flowering time (DAP)	First developing fruit (dap)	Number of fruits per crop	Fruit weight per crop (g)	Harvest weight per plot (8 m^2) (g)
Control	59.60 a	69.62 a	34.26 a	29.30 c	240.40 c
Mancozeb 80%	61.24 a	68.40 a	42.28 a	44.38 b	355.00 b
Bio P60	60.30 a	69.92 a	43.28 a	44.71 b	357.70 b
Bo T10	59.60 a	69.62 a	43.08 a	46.65 b	373.18 b
Combination	56.96 a	67.76 a	57.44 b	55.92 a	439.92 a

Numbers in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 95% confidence level, DAP = days after planting.

(Table 3). The combined application of Bio P60 + Bio T10 increased fruit weight per plant by 90.85% and harvest weight per plot by 82.99% compared to the control. Meanwhile, the single application of chemical fungicide, Bio P60, and Bio T10 significantly increased the number of fruits per plant by 51.46%, 52.59% and 59.21%, respectively, and increased harvest weight per plot by 47.67%, 48.79%, and 50.23%, respectively, compared to the control. The superior effect of the combined treatment is attributed to the synergistic action of multiple secondary metabolites, which do not work individually but rather in a balanced concentration that supports the growth and development of chili plants. This aligns with the findings of Hayat et al. (2010) and Niu et al. (2020), who reported that combining beneficial microbial isolates can enhance the effects achieved by a single isolate as PGPR.

Phenolic Compound Analysis. Bio P60, Bio T10, and their combination increased the levels of saponins, tannins, and glycosides compared to the control (Table 4). Bio P60 and Bio T10, which contain secondary metabolites, can function as PGPR that benefit plants by not only stimulating the production of phytohormones but also enhancing plant resistance to pathogens (Hayat et al., 2010). This biochemical resistance is characterized by an increase in phenolic compound content. The application of single and combined secondary metabolites resulted in the highest levels of phenolic compounds observed qualitatively. The accumulation of phenolic compounds can enhance plant resistance (Wallis & Galarneau, 2020).

Polyphenol oxidase activity, which is induced by pathogen attacks, can lead to an increase in quinone concentrations—compounds known to be cytotoxic (Zhang & Sun, 2021). In relation to plant disease resistance, polyphenol oxidase catalyzes the oxidation of phenolic compounds into quinones, which are more toxic to pathogenic microbes than the phenolics themselves. Although phenolic compounds are naturally present in plants, their baseline levels are often insufficient (Babenko et al., 2019). The increase in phenolic content can be triggered by the presence of external organisms, such as the application of secondary metabolites from antagonistic microbes (Wallis & Galarneau, 2020). High phenolic content in plants contributes to stronger resistance against phytopathogen (Kulbat, 2016; Wallis & Galarneau, 2020). Chili pepper plants in the control treatment, which showed low phenolic content (Table 4), also exhibited high disease intensity—consistent with the high disease incidence and severity observed in the control group (Table 1).

CONCLUSION

The combined application of Bio P60 and Bio T10 demonstrated superior effectiveness against anthracnose, as evidenced by a delayed incubation period, reduced disease intensity, and lower AUDPC values—by 13.71%, 69.34%, and 47.06%, respectively — compare to the control. It also increased control effectiveness to 67.67%. Furthermore, the combined treatment enhanced plant growth and yield by increasing plant height, number of fruits, fruit weight per plant, and harvest weight per plot by 27.38%, 62.65%, 90.85%, and 82.99%, respectively, compared to the control. In addition, the application of Bio P60, Bio T10, and their combination qualitatively increased phenolic compounds (tannins, saponins, and glycosides) in chili pepper plants.

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Table 4. Result of phenolic compound analysis qualitatively

Treatments	Saponin	Tannin	Glycosides	Number of plus
Control	+	++	++	5
Mancozeb 80%	++	++	++	6
Bio P60	+++	+++	+++	9
Bio T10	+++	+++	+++	9
Combination	+++	+++	+++	9

+ = low; ++ = medium; +++ = high; - = not found.

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AUTHORS' CONTRIBUTIONS

LS and EM conceived and planned the research. LS drafted the manuscript. LS and MWRS conducted the literature review. FH and NWAL collected the data. EM and FH performed data analysis. All authors contributed to reviewing the research design, data interpretation, and manuscript development. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there is no competing interest regarding this publication.

REFERENCES

Adnan M, Islam W, Shabbir A, Khan KA, Ghramh HA, Huang Z, Chen HYH, & Lu GD. 2019. Plant defense against fungal pathogens by antagonistic fungi with *Trichoderma* in focus. *Microb Pathog* 129: 7–18. https://doi.org/10.1016/j.micpath.2019.01.042

Alexander A, Lo RKS, & Chong KP. 2021. The effectiveness of selected biological control agents in controlling *Ganoderma boninense*. *J. Sustain. Sci. Manag.* 16(6): 128–137. http://doi.org/10.46754/jssm.2021.08.011

Ardebili ZO, Ardebili NO, & Hamdi SMM. 2011. Physiological effects of *Pseudomonas fluoresecens* CHAO on tomato (*Lycopersicon esculentum* Mill.) plants and its possible impact on *Fusarium oxysporum* f.sp *lycopersici*. *AJCS*. 5(12): 1631–1638.

Azubuike CC, Chikere CB, & Okpokwasili GC. 2016. Bioremediation techniques–classification based on site of application: Principles, advantages, limitations and prospects. *World J. Microbiol. Biotechnol.* 32: 180. https://doi:10.1007/s11274-016-2137-x

Babenko LM, Smirnov OE, Romanenko KO, Trunova OK, & Kosakivska IV. 2019. Phenolic compounds in plants: Biogenesis and functions. *Ukr. Biochem. J.* 91(3): 5–18. https://doi.org/10.15407/ubj91.03.005

Bele AA, Jadhav VM, & Kadam VJ. 2010. Potential of tannnins: A review. *Asian J. Plant Sci*. 9(4): 209–214. https://doi.org/10.3923/ajps.2010.209.214

Ciofini A, Negrini F, Baroncelli R, & Baraldi E. 2022. Management of post-harvest anthracnose: Current approaches and future perspectives. *Plants.* 11(14): 1856. https://doi.org/10.3390/plants11141856

Contreras-Cornejo HA, Macías-Rodríguez L, Beltrán-Peña E, Herrera-Estrella A, & López-Bucio J. 2011. *Trichoderma*-induced plant immunity likely involves both hormonal- and camalexin-dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungi *Botrytis cinerea*. *Plant Signal. Behav*. 6(10): 1554–1563. https://doi.org/10.4161/psb.6.10.17443

Deresa EM & Diriba TF. 2023. Phytochemicals as alternative fungicides for controlling plant diseases: A comprehensive review of their efficacy, commercial representatives, advantages, challenges for adoption, and possible solutions. *Heliyon.* 9(3): e13810. https://doi.org/10.1016/j.heliyon.2023.e13810

Dorjey S, Dolkar D, & Sharma R. 2017. Plant growth promoting rhizobacteria Pseudomonas: A review. *Int. J. Curr. Microbiol. App. Sci.* 6(7): 1335–1344. https://doi.org/10.20546/ijcmas.2017.607.160

Erb M & Kliebenstein DJ. 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: The blurred functional trichotomy. *Plant Physiol.* 184(1): 39–52. https://doi.org/10.1104/pp.20.00433

Gasperini D & Howe GA. 2024. Phytohormones in a universe of regulatory metabolites: Lessons from jasmonate. *Plant Physiol*. 195(1): 135–154. https://doi.org/10.1093/plphys/kiae045

Guzmán-Guzmán P, Kumar A, de los Santos-Villalobos S, Parra-Cota FI, Orozco-Mosqueda MC, Fadiji AE, Hyder S, Babalola OO, & Santoyo G. 2023. Trichoderma species: Our best fungal allies in the biocontrol of plant diseases—A review. *Plants.* 12(3): 432. https://doi.org/10.3390/plants12030432

Gusmarini M, Dirmawati SR, Nurdin M, & Akin HM. 2014. Pengaruh beberapa jenis ekstrak tumbuhan terhadap penyakit antraknosa pada tanaman cabai besar (*Capsicum annuum* L.) di

lapangan [Effect of several types of plant extracts on anthracnose disease in large chili plants (*Capsicum annuum* L.) in the field]. *J. Agrotek Trop*. 2(2): 197–201. https://doi.org/10.23960/jat.v2i2.2084

Hayat R, Ali S, Amara U, Khalid R, & Ahmed I. 2010. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol*. 60: 579–598. https://doi.org/10.1007/s13213-010-0117-1

He DC, He MH, Amalin DM, Liu W, Alvindia DG, & Zhan J. 2021. Biologcal control of plant diseases: An evolutionary and eco-economic consideration. *Pathogens*. 10(10): 1311. https://doi.10.3390/pathogens10101311

Hojnik M, Modic M, Ni Y, Filipič G, Cvelbar U, & Walsh JL. 2019. Effective fungal spore inactivation with an environmentally friendly approach based on atmospheric pressure air plasma. *Environ. Sci. Technol*. 53(4): 1893–1904. https://doi.10.1021/acs.est.8b05386

Ilondu EM. 2011. Evaluation of some aqueous plant extracts used in the control of pawpaw fruit (*Carica papaya* L.) rot fungi. *J. Appl. Biosci*. 37: 2419–2424.

Kanchana D, Jayanthi M, Usharani G, Saranraj P, & Sujitha D. 2014. Interaction effect of combined inoculation of PGPR on growth and yield parameters of chili var K1 (*Capsicum annuum* L.). *Int. J. Microbiol. Res*. 5(3): 144–151.

Kulbat K. 2016. The role of phenolic compounds in plant resistance. *Biotechnol. Food Sci*. 80(2): 97–108.

Kumar A, Shrivastava D, Dixit M, Meena SK, Suman, & Prasad A. 2022. *Trichoderma*: A plant growth promoting fungi. *Gorteria Journal.* 34(6): 60–76.

Langner T & Göhre V. 2016. Fungal chitinases: Function, regulation, and potential roles in plant/pathogen interactions. *Curr. Genet*. 62: 243–254. https://doi.org/10.1007/s00294-015-0530-x

Lee YS & Kim KY. 2015. Statistical optimization of medium components for chitinase production by *Pseudomonas fluorescens* strain HN1205: Role of chitinase on egg hatching inhibition of root-knot nematode. *Biotechnol. Biotechnol. Equip*. 29(3): 470–478. https://doi.org/10.1080/13102818.2015.1010702

Li G, Song C, Manzoor MA, Li D, Cao Y, & Cai Y. 2023. Functional and kinetics of two efficient phenylalanine ammonia lyase from *Pyrus bretschneideri. BMC Plant Biol*. 23: 612. https://doi.org/10.1186/s12870-023-04586-0

Mohiddin FA, Padder SA, Bhat AH, Ahanger MA, Shikari AB, Wani SH, Bhat FA, Nabi SU, Hamid A, Bhat NA, Sofi NR, Waza SA, Hamid B, Parveen S, Hussain A, Bhat AN, Ali OM, Dar MS, & Latef AAHA. 2021. Phylogeny and optimization of *Trichoderma harzianum* for chitinase production: Evaluation of their antifungal behaviour against the prominent soil borne phyto-pathogens of temperate India. *Microorganisms.* 9(9): 1962. https://doi.org/10.3390/microorganisms9091962

Niu B, Wang W, Yuan Z, Sederoff RR, Sederoff H, Chiang VL, & Borriss R. 2020. Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. *Front Microbiol.* 11: 1–16. https://doi.org/10.3389/fmicb.2020.585404

Noel ZA, Longley R, Benucci GMN, Trail F, Chilvers MI, & Bonito G. 2022. Non-target impacts of fungicide disturbance on phyllosphere yeasts in conventional and no-till management. *ISME Commun*. 2(1): 19. https://doi.org/10.1038/s43705-022-00103-w

Oo MM & Oh SK. 2016. Chili anthracnose (*Colletotrichum* spp.) disease and its management approach. *Korean J. Agric. Sci*. 43(2): 153–162. https://doi.org/10.7744/kjoas.20160018

Oskiera M, Szczech M, & Bartoszewski G. 2015. Molecular identification of *Trichoderma* strains collected to develop plant growth-promoting and biocontrol agents. *J. Hortic. Res*. 23(1): 75–86. https://doi.org/10.2478/johr-2015-0010

Perry EK, Meirelles LA, & Newman DK. 2022. From the soil to the clinic: The impact of microbial secondary metabolites on antibiotic tolerance and resistance. *Nat. Rev. Microbiol.* 20: 129–142. https://doi.org/10.1038/s41579-021-00620-w

Prakash N, Vishunavat K, Khan GT, & Prasad P. 2021. SA, ABA and *Pseudomonas fluorescens* elicit defense responses in tomato against Alternaria blight. *J. Plant Biochem. Biotechnol*. 30: 13–25. https://doi.org/10.1007/s13562-020-00564-x

Rahayuniati RF & Mugiastuti E. 2012. Keefektifan *Bacillus* sp. dan *Pseudomonas fluorescens* dalam mengendalikan *Fusarium oxysporum* f.sp. *lycopersici* dan *Meloidogyne* sp. penyebb penyakit layu pada tomat secara *in vitro*] [Effectivity of *Bacillus* sp. and *Pseudomonas fluorescens* to control *Fusarium oxysporum* f.sp. *lycopersici* and *Meloidogyne* sp. causing tomato wilt in vitro. *Jurnal Pembangunan Pedesaan*. 12(1): 65–70.

Ribeiro BD, Barreto DW, & Coelho MAZ. 2013. Application of foam column as green technology for concentration of saponins from sisal (*Agave sisalana*) and juá (*Ziziphus joazeiro*). *Braz. J. Chem. Eng.* 30(4): 701–709. https://doi.org/10.1590/S0104-66322013000400002

Rodrigues GB, Rezende AM, Sobrinho GGR, & de Novaes QS. 2019. Etiology, occurrence and epidemiology of a begomovirus disease in passionflower in the South West of Bahia. *Sci. Agric.* 76(4): 337–343. https://doi.org/10.1590/1678-992X-2017-0272

Rufai Y, Isah Y, & Isyaka MS. 2016. Comparative phyto-constituents analysis from the root bark and root core extractives of *Cassia ferruginea* (Schrad D. C) plant. *Sch. J. Agric. Vet. Sci.* 3(4): 275–283.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, & Hunt MD. 1996. Systemic acquired resistance. *Plant Cell.* 8(10): 1809–1819. https://doi.org/10.1105%2Ftpc.8.10.1809

Saikia S & Chetia P. 2024. Antibiotics: From mechanism of action to resistance and beyond. *Indian J. Microbiol*. 64: 821–845 https://doi.org/10.1007/s12088-024-01285-8

Sathua SK, Reddy MSS, Sulagitti A, & Singh RN. 2017. Bio-efficacy of various insecticides and botanicals against chili thrips (*S. dorsalis* Hood) and their comparative cost: Benefit analysis in chili crop. *J. Entomol. Zool. Stud*. 5(2): 130–134.

Saxena A, Raghuwanshi R, Gupta VK, & Singh HB. 2016. Chili anthracnose: The epidemiology and management. *Front. Microbiol*. 7: 1527. https://doi.org/10.3389/fmicb.2016.01527

Shigeto J & Tsutsumi Y. 2016. The role of plant peroxidases in cell wall formation and modification. *Mokuzai Gakkaishi*. 62(4): 91–100. https://doi.org/10.2488/jwrs.62.91

Simko I & Piepho HP. 2012. The area under the disease progress stairs: Calculation, advantage, and application. *Phytopathology.* 102(4): 381–389. https://doi.org/10.1094/PHYTO-07-11-0216

Soesanto L, Mugiastuti E, & Rahayuniati RF. 2010. Kajian mekanisme antagonis *Pseudomonas fluorescens* P60 terhadap *Fusarium oxysporum* f.sp. *lycopersici* pada tanaman tomat *in vivo* [Antagonistic mechanisms study of *Pseudomonas fluorescens* P60 on *Fusarium oxysporum* f.sp. *lycopersici* of tomato in vivo]. *J. Trop Plant Pests Dis.* 10(2): 108–115. https://doi.org/10.23960/j.hptt.210108-115

Soesanto L, Mugiastuti E, & Rahayuniati RF. 2013. Aplikasi formula cair Pseudomonas fluorescens P60 untuk menekan penyakit virus cabai merah [Liquid formula application of *Pseudomonas fluorescens* P60 for suppressing viral diseases in chili peppers]. *Jurnal Fitopatologi Indonesia.* 9(6): 179–185. https://doi.org/10.14692/jfi.9.6.179

Soesanto L, Hiban A, & Suharti WS. 2019a. Application of Bio P60 and Bio T10 alone or in combination against stem rot of pakcoy. *J. Trop. Hortic.* 2(2): 38–44. https://doi.org/10.33089/jthort.v2i2.20

Soesanto L, Kustam, & Mugiastuti E. 2019b. Application of Bio P60 and Bio T10 in combination against phytophthora wilt of papaya. *Biosaintifika.* 11(3): 339–344.

Soesanto L, Mugiastuti E, Suyanto A, & Rahayuniati RF. 2020. Application of raw secondary metabolites from two isolates of *Trichoderma harzianum* against anthracnose on red chili pepper in the field. *J. Trop. Plant Pests Dis*. 20(1): 19–27. https://doi.org/10.23960/j.hptt.12019-27

Statistics Indonesia. 2021. *Vegetbales*. https://www.bps.go.id/indicator/55/61/1/produksi-tanaman-sayuran.htm. Accessed 25 January 2025.

Suprapta DN. 2022. Biocontrol of anthracnose disease on chili pepper using a formulation containing *Paenibacillus polymyxa* C1. *Front. Sustain. Food Syst*. 5: 782425. https://doi.org/10.3389/fsufs.2021.782425

Susi H & Laine AL. 2017. Host resistance and pathogen aggressiveness are key determinants of coinfection in the wild. *Evolution.* 71(8): 2110–2119. https://doi.org/10.1111/evo.13290

Temanggung Regency Government. 2021. *Statistical*

Data 2021. https://e-statistik.temanggungkab.go.id/frontend/datastatistik/2021. Accessed 12 December 2022.

Ting ASY & Chai JY. 2015. Chitinase and β-1,3-glucanase activities of *Trichoderma harzianum* in response towards pathogenic and non-pathogenic isolates: Early indications of compatibility in consortium. *Biocatal. Agric. Biotechnol.* 4(1): 109–113. https://doi.org/10.1016/j.bcab.2014.10.003

Tucci M, Ruocco M, De Masi L, De Palma M, & Lorito M. 2011. The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. *Mol. Plant Pathol*. 12(4): 341–354. https://doi:10.1111/j.1364-3703.2010.00674.x

Vidal NP, Adigun OA, Pham TH, Mumtaz A, Manful C, Callahan G, Stewart P, Keough D, & Thomas RH. 2018. The effects of cold saponification on the unsaponified fatty acid composition and sensory perception of commercial natural herbal soaps. *Molecules.* 23(9): 2356. https://doi.org/10.3390/molecules23092356

Vinale F, Sivasithamparam K, Ghisalberti EL, Ruocco M, Woo S, & Lorito M. 2012. *Trichoderma* secondary metabolites that affect plant metabolism. *Nat. Prod. Commun.* 7(11): 1545–1550. https://doi.org/10.1177/1934578X1200701133

Wallis CM & Galarneau ERA. 2020. Phenolic compound induction in plant-microbe and plant-insect interactions: A meta-analysis. *Front. Plant Sci*. 11: 580753. https://doi.org/10.3389/fpls.2020.580753

Weller DM, Mavrodi DV, van Pelt JA, Pieterse CMJ, van Loon LC, & Bakker PAHM. 2012. Induced systemic resistance in Arabidopsis thaliana against Pseudomonas syringae pv. tomato by 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens. Phytopathology*. 102(4): 403–412. https://doi.org/10.1094/PHYTO-08-11-0222

Zhang J & Sun X. 2021. Recent advances in polyphenol oxidase-mediated plant stress responses. *Phytochemistry*. 181: 112588. https://doi.org/10.1016/j.phytochem.2020.112588