Adv. Hort. Sci., 2025 39(4): 269-280
DOI: 10.36253/ahsc-18356
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

Efficacy of different bio-control agents for managing *Colletotrichum* leaf blight of large cardamom in Taplejung, Nepal

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Citation:
CHAULAGAIN K., MEHATA D.K., KHAREL A., YADAV P., ADHIKARI N., 2025 - *Efficacy of different bio-control agents for managing Colletotrichum leaf blight of large cardamom in Taplejung, Nepal.* - Adv. Hort. Sci., 39(4): 269-280

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Author contributions:
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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 30 July 2025
Accepted for publication 5 October 2025

AHS - Firenze University Press
ISSN 1592-1573 (on line) - 0394-6169 (print)

Key words: AUDPC, biocontrol agents, blight, *Colletotrichum*, large cardamom, leaf blight.

Abstract: Large cardamom (*Amomum subulatum*) is a high-value spice crop primarily grown in the mid-hills of Nepal, providing significant economic benefits to smallholder farmers. However, the crop faces substantial threats from *Colletotrichum* blight, which has led to severe yield losses, prompting the need for effective disease management strategies. This study was conducted in Phungling-06, Taplejung district, within the cardamom cultivation zone, to evaluate the efficacy of various biocontrol agents in managing *Colletotrichum* blight. Using a randomized complete block design (RCBD) with five treatments, the study applied three foliar sprays of biocontrol agents (*Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens, Pseudomonas fluorescens* + *Bacillus subtilis*, and a control). Disease severity and AUDPC scores were measured over a period of four weeks. The results demonstrated that *Trichoderma harzianum* significantly reduced disease severity and AUDPC, followed by *Trichoderma viride*. Additionally, *Pseudomonas fluorescens* and its combination with *Bacillus subtilis* showed promising effects compared to the control. These findings suggest that biocontrol agents can provide an eco-friendly and sustainable alternative to chemical fungicides in large cardamom production. Future research should focus on optimizing the application techniques and exploring the long-term benefits of these biocontrol strategies in integrated pest management systems for large cardamom cultivation.

1. Introduction

Large cardamom (*Amomum subulatum* roxb.), locally known as “Alaichi” in Nepal, is a high-value perennial herbaceous spice crop belonging to the Zingiberaceae family. It is extensively cultivated across the mid-hills of eastern Nepal and serves as a vital source of income for thousands of smallholder farmers (Shrestha *et al.*, 2018; Subedi *et al.*, 2022). The plant typically reaches 1.5 to 3.0 meters in height, producing pseudostem (tillers) from underground, tuberous rhizomes (Pathak, 2008; Singh and Pothula, 2013). Its leaves are long and lanceolate, ranging from 30-60 cm in length and 5-15 cm in width. The flowering spike bears 30-40 flowers per inflorescence, with blooming occurring in spring; the flowers are characterized by white petals with bluish stripes and yellow margins, each lasting about three days, while the overall flowering period extends for approximately a month (Vijayan *et al.*, 2013; Belbase *et al*., 2018; Shrestha *et al*., 2018). The fruit is a small, three-chambered capsule that encloses 20-30 fragrant brown seeds, which are commonly utilized for both culinary and medicinal purposes (Joshi *et al.*, 2013).

Large cardamom thrives in subtropical, humid climates at altitudes of 900-2,000 meters above sea level, typically under the partial shade of nitrogen-fixing trees such as *Alnus nepalensis* (Paudel *et al*., 2018; Kharel *et al.*, 2025). It prefers acidic soils (pH 5.5-6.5), high rainfall (2,000-3,500 mm), and temperatures ranging from 10°C to 30°C. Traditional cultivation practices involve rhizome propagation, organic mulching, and manual harvesting, making it a labor-intensive but culturally significant crop (Sharma *et al*., 2021). Dried capsules are consumed whole or ground, while essential oils extracted from seeds find applications in food, confectionery, perfumery, and cosmetic industries (Bhandari and Bhandari, 2018; Shrestha *et al*., 2018). Medicinally, large cardamoms have been used in ayurvedic and traditional healing systems to treat respiratory disorders, digestive issues, throat infections, and even envenomations from snakes and scorpions (Pathak, 2008; Belbase *et al*., 2018; Basnet *et al*., 2021). Chemically, its capsules contain approximately 8% essential oil, with bioactive compounds such as cineol (up to 70%), terpineol (45%), myrcene (27%), limonene (2-14%), menthone (6%), borneol, sabinene, α- and β-pinene, and α-terpinyl acetate (Vijayan *et al*., 2013; Shrestha *et al*., 2018; Gurung *et al*., 2021). Despite its economic and ethnobotanical importance, large cardamom cultivation faces significant threats from *Colletotrichum* blight, primarily caused by *Colletotrichum* spp., with outbreaks frequently reported in Taplejung, Ilam, and other eastern districts of Nepal (Joshi *et al*., 2013; Paudel *et al*., 2018). The pathogen proliferates in the moist, shaded microclimate typical of cardamom plantations, particularly under sprinkler irrigation systems that favor fungal growth (Feksa *et al*., 2019; Kharel *et al.*, 2025). Overreliance on chemical fungicides has been largely ineffective in the long term, often leading to fungicide resistance and environmental degradation (Gautam *et al.*, 2020; Zubair *et al*., 2022; Tiwari *et al*., 2023). These chemicals can harm beneficial soil microbiota, contaminate soil and water, and pose health risks to both farmers and consumers (Subedi *et al*., 2022).

Epidemiologically, *Colletotrichum* blight is most severe during the pre-monsoon period, with symptoms including water-soaked lesions and necrotic spots on leaves, leading to premature leaf drop and reduced photosynthetic capacity (Pun, 2019). Infected old tillers serve as the primary inoculum for subsequent seasons, facilitating the spread of the disease. The disease has been associated with significant yield reductions, with reports indicating up to 46.8% reduction in dry yield in infected plants compared to healthy ones (Shrestha *et al.*, 2018). Given these challenges, biological control agents have emerged as sustainable alternatives. Beneficial microorganisms such as *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus subtilis* suppress pathogens through mechanisms including competition, antibiosis, mycoparasitism, and induced systemic resistance (Gautam *et al.*, 2016; Shrestha *et al.*, 2018). For instance, *Trichoderma viride* has demonstrated significant efficacy in reducing *Colletotrichum* blight under field conditions in Nepal, with studies indicating its effectiveness when combined with fungicides like azoxystrobin (Subedi *et al.*, 2022). Their application not only reduces pathogen load but also enhances soil health and stimulates plant immunity (Subedi *et al.*, 2022; Kharel *et al.*, 2025). Integrated disease management (IDM) approaches that incorporate these agents promote eco-friendly, economically viable crop protection strategies for resource-constrained farmers in Nepal's hill regions (Tiwari *et al*., 2023). Therefore, evaluating the efficacy of biocontrol agents in managing *Colletotrichum* blight is crucial for sustainable large cardamom production.

The present study aims to assess the effectiveness of different eco-friendly biocontrol agents under field conditions, providing viable alternatives to chemical fungicides while supporting environmental sustainability and farmer health.

2. Materials and Methods

The field research was carried out in phungling-6, located in the Taplejung district of eastern Nepal, within the temperate climatic zone. The experiment took place in a mature large cardamom plantation (variety: Ramsai), where the plants were approximately 15 to 20 years old. The experimental site was situated at an altitude of 1690 meters above sea level. In terms of geographical coordinates, the site lay at 27°2′1″51 n latitude and 87°4′0″45 e longitude. During the study period, the highest recorded temperatures ranged from 26.06°C to 31.26°C, while the lowest temperatures fluctuated between 12.78°C and 16.77°C. Relative humidity showed a peak value of 67.31% and declined to a minimum of 32.16% which were collected from https://power.larc.nasa.gov/. No rainfall was observed at the initial and final stages of the trial, with a maximum recorded rainfall of 6.82 mm in the middle phase. Figure 1 illustrates the geographical location of the research site, and figure 2 presents the meteorological data recorded during the experiment.

Experimental design

The experimental layout was structured using a randomized complete block design (RCBD), incorporating five different treatments replicated four times. This resulted in a total of 20 experimental plots, each measuring 4 meters by 3 meters. All treatments were implemented in accordance with recommended agronomic practices and standard operational guidelines (Fig. 3).

The treatment details are summarized in Table 1.

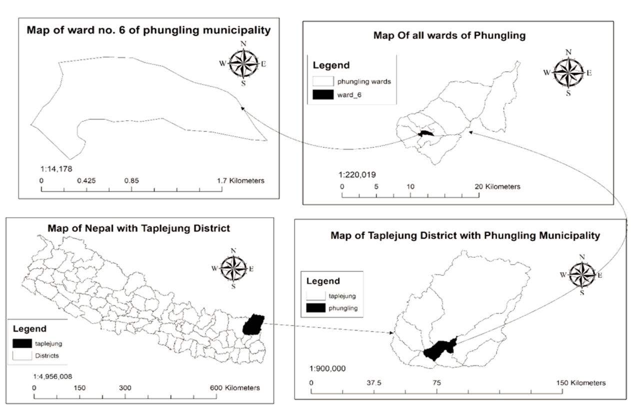

Fig. 1 - Mapping of a study area.

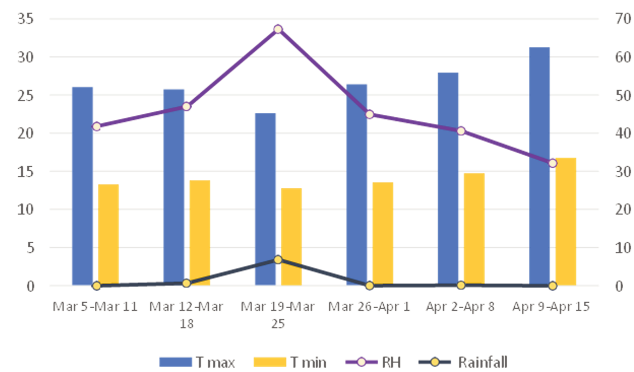

Fig. 2 - Climatic conditions of the research area (temperature, rainfall, and relative humidity).

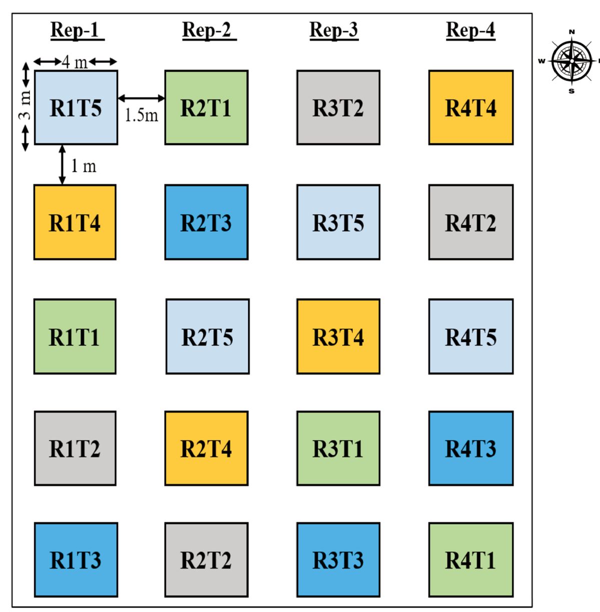

Fig. 3 - Layout of experimental site.

Table 1 - Treatment details with company name, trade name, and doses

Treatments	Company	Trade name	Bio-control agents	Dose	Cfu per l
t1	multiplex	Nisarga	*Trichoderma viride*	10 gm/l water	2×10^9 cfu/l
t2	multiplex	Safe root	*Trichoderma harzianum*	10 gm/l water	2×10^9 cfu/l
t3	multiplex	Sparsha	*Pseudomonas fluorescens*	5 gm/l water	1×10^9 cfu/l
t4	multiplex	Bio-jodi	*Pseudomonas fluorescens + Bacillus subtilis*	5 gm/l water	1×10^9 cfu/l
t5	-	-	Control	-	-

Allocation and application of biopesticides

Treatment allocation within each experimental unit across all replications was carried out using the lottery method to ensure randomness. The bioagents were applied in three separate sprayings throughout the trial period. The initial application was conducted when disease severity reached approximately 5% in the field, followed by two subsequent sprays at seven-day intervals. A high-volume knapsack sprayer was utilized for the application, delivering the required solution at a rate of 4 liters per plot.

Observation and data collection

Disease severity assessment. From each experimental plot, ten leaves were randomly chosen from plants in four different directions to ensure representative sampling. Disease severity was visually monitored and recorded every three days. The evaluation was conducted using a 0-9 scale based on the amended 10% ordinal scale (Table 2, Fig. 4).

Disease scoring. The disease severity was assessed using a modified 10% ordinal rating scale, as outlined by Chiang *et al.* (2017).

Table 2 - Amended 10% ordinal scale

Score	Description	Midpoint
0	0% (no infection)	0
1	0+ - 1%	0.5
2	1+ - 4%	2.5
3	4+ - 10%	7
4	10+ - 20%	15
5	20+ - 30%	25
6	30+ - 40%	35
7	40+ - 50%	45
8	50+ - 70%	60
9	70+ - 100% disease	85

Disease severity index. The disease severity index (DSI) was calculated based on the midpoint of the severity range of each class rather than based on the severity score of each class to avoid overestimation (Chiang *et al*., 2017).

$$\text{DSI (\%)} = \sum \frac{\text{Frequency of each class x Midpoint}}{\text{Total no. of observations x Maximum midpoint of scale}} \text{ x } 100$$

AUDPC calculation

The area under the disease progress curve (AUDPC) was computed following the formula proposed by Simko and Piepho (2012),

$$\text{AUDPC} = \sum_{i=1}^{\text{n-1}} \frac{y_\text{i}+y_{\text{i}+1}}{2} \text{ x } (t_{\text{i}+1} - t_\text{i})$$

where,

y_i = Disease severity (%) recorded at each i^{th} observation,

t_i = Time (in days, hours) at the i^{th} observation.

n = Total no. of observations.

Statistical analysis

The experimental data were initially entered into MS-excel (2019) for subsequent analysis. To test for variance homogeneity, a square root transformation (SQRT) was applied to the original data, following the method recommended by Gomez and Gomez (1984). Analysis of variance (ANOVA) was performed using r-studio statistical software (version 4.3.1). Significant differences among the variables were assessed through Duncan's multiple range test (DMRT) at a 5% significance level ($p \leq 0.05$). Figures and tables were generated using MS excel.

Fig. 4 - Symptoms of *Colletotrichum* leaf blight in the field.

3. Results

Effect of different biocontrol agents against leaf blight severity

The disease severity index (DSI) observed at 7, 14, 21, and 28 days after sowing (DAS) demonstrated progressive disease development and significant treatment effects from 14 das onward, clearly highlighting the efficacy of different biocontrol agents in suppressing *Colletotrichum* leaf blight in large cardamom. Initially, at 7 DAS, no significant differences were observed among treatments; however, from 14 DAS onwards, *Trichoderma harzianum* (3.75±0.30) and *T. viride* (3.74±0.14) recorded significantly lower DSI values compared to *Pseudomonas fluorescens* + *Bacillus subtilis* (4.45±0.25), *P. fluorescens* (4.93±0.45), and the control (6.07±0.23). This trend persisted through 21 and 28 das, with *T. harzianum* and *T. viride* consistently maintaining the lowest DSI values (4.80±0.55 and 4.88±0.22 at 28 das, respectively), while the control exhibited the highest disease severity (10.70±0.30). Mean DSI values across all observation periods confirmed the superior performance of *T. harzianum* (3.80±0.30) and *T. viride* (4.02±0.15) compared to moderate suppression by bacterial treatments and high susceptibility in the control. These results are visually reinforced by the boxplot (Table 3, Fig. 5), where *Trichoderma* spp. treatments show the lowest median DSI and narrow variability, indicating strong and consistent disease suppression, while the control shows the highest median and widest range, reflecting greater disease progression. Overall, the temporal disease data and graphical evidence confirm that *Trichoderma harzianum* is the most effective biocontrol agent against leaf blight, closely followed by *T. viride*, whereas bacterial treatments provide moderate control and the untreated control is the most susceptible. These findings underscore the robustness and sustainability of Trichoderma spp. as efficient biocontrol strategies for managing *Colletotrichum* leaf blight in large cardamom.

Effect of different biocontrol agents against leaf blight on area under disease progress curve (AUDPC)

The results for total AUDPC (area under disease progress curve) showed significant differences

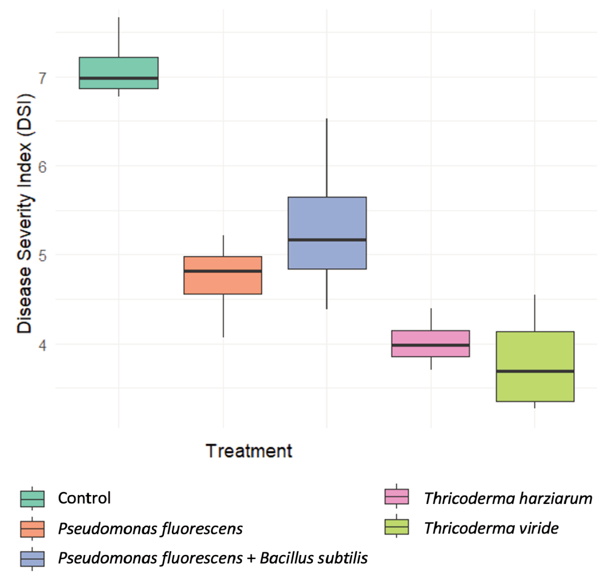

Fig. 5 - Effect of different biocontrol agents against leaf blight severity.

Table 3 - Effect of different biocontrol agents against leaf blight severity

Treatments	DSI (7 das)	DSI (14 das)	DSI (21 das)	DSI (28 das)	Mean disease severity
Trichoderma harzianum	2.79a±0.22	3.75c±0.30	3.90c±0.23	4.80d±0.55	3.80d±0.30 (2.07)
Trichoderma viride	3.22a±0.12	3.74c±0.14	4.23bc±0.22	4.88d±0.22	4.02cd±0.15 (2.13)
Pseudomonas fluorescens + Bacillus subtilis	3.33a±0.21	4.45bc±0.25	4.92bc±0.32	6.21c±0.40 (2.6)	4.73bc±0.24 (2.30)
Pseudomonas fluorescens	3.11a±0.21 (1.9)	4.93b±0.45	5.70b±0.81 (2.5)	7.50b±0.60	5.31b±0.45 (2.41)
Control	3.37a±0.12	6.07a±0.23	8.30a±0.40	10.70a±0.30	7.04a±0.22 (2.75)
mean	3.16	4.59	5.39	6.82	4.98
cv (%)	11.47	12.63	17.61	11.02	11.12
f-test	NS	**	**	**	**

Cv= Coefficient of variation; NS= non-significant ** = significant at 1% level of significance; (±) indicates standard error of the mean; in a column, means followed by a common letter(s) are not significantly different at the 5% level by duncan multiple range test (DMRT). figures outside the parenthesis are original values; figures inside the parenthesis are square root transformed values. DSI= disease severity index. DAS= Days after spraying.

among treatments, indicating varying levels of disease suppression effectiveness (Table 4, Fig. 6). The lowest AUDPC value was recorded in *Trichoderma harzianum* (79.80), followed closely by *T. viride* (84.11), with both treatments statistically similar and significantly more effective in reducing disease progression compared to other treatments. The combined application of *Peudomonas fluorescens* and *Bacillus subtilis* (98.95) showed moderate effectiveness, significantly lower than the control but higher than both *Trichoderma* treatments. *P. fluorescens* alone had a higher AUDPC value (111.54), which was significantly different from *T. harzianum* and *T. viride* but still performed better than the control. The highest AUDPC was observed in the untreated control (148.73), indicating the greatest disease severity over time. These findings confirm that *T. harzianum* and *T. viride* were the most effective bio-agents in suppressing disease development, with significantly lower AUDPC values compared to all other treatments.

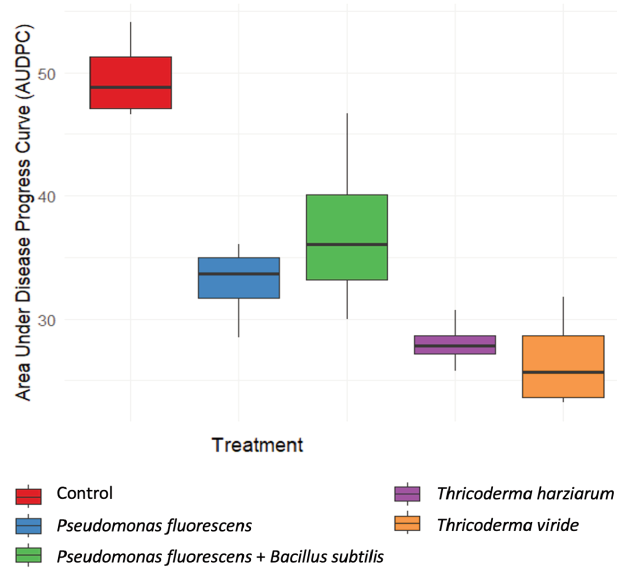

Fig. 6 - Effect of different biocontrol agents against leaf blight on AUDPC

Table 4 - Effect of different biocontrol agents against leaf blight on AUDPC

Treatments	Total AUDPC
Trichoderma harzianum	79.80±5.94 c
Trichoderma viride	84.11±3.07 c
Pseudomonas fluorescens + Bacillus subtilis	98.95±4.94 bc
Pseudomonas fluorescens	111.54±10.66 b
Control	148.73±5.12 a
Mean	104.62
cv (%)	12.19
f-test	**

Cv= Coefficient of variation; AUDPC = Area under disease progress curve; ** = significant at 1% level of significance; (±) indicates standard error of the mean; in a column, means followed by a common letter(s) are not significantly different at the 5% level by duncan multiple range test (DMRT).

Effect of different bio-agents on vegetative and yield parameters

The study revealed that the application of different bio-agents had significant effects on all observed parameters, with notable differences among treatments (Table 5, Figs. 7 and 8). For plant height, the tallest plants were observed in *Trichoderma harzianum* (55.52 cm), which was

Table 5 - Effect of different bio-agents on vegetative and yield parameters

Treatments	Plant height	Leaf length	Leaf breadth	NLPP	Yield (kg/ha)
Trichoderma harzianum	55.52 a	28.32 a	5.82 a	5.47 a	496.25 a
Trichoderma viride	53.0 ab	26.83 ab	5.21 ab	5.75 a	496.0 a
Pseudomonas fluorescens + Bacillus subtilis	51.81 ab	26.44 ab	5.53 a	5.10 ab	483.25 b
Pseudomonas fluorescens	50.15 b	24.92 b	5.07 ab	5.00 ab	476.0 b
Control	44.79 c	20.99 c	4.47 b	4.45 b	454.25 c
Mean	51.05	25.50	5.22	5.15	481.15
cv (%)	5.34	6.57	10.20	9.45	6.49
f-test	*	*	*	*	**

Cv= Coefficient of variation; NLPP= Number of leaves per plant; **= Significant at 1% level of significance; *= Significant at 5% level of significance; in a column, means followed by a common letter(s) are not significantly different at the 5% level by Duncan Multiple Range Test (DMRT).

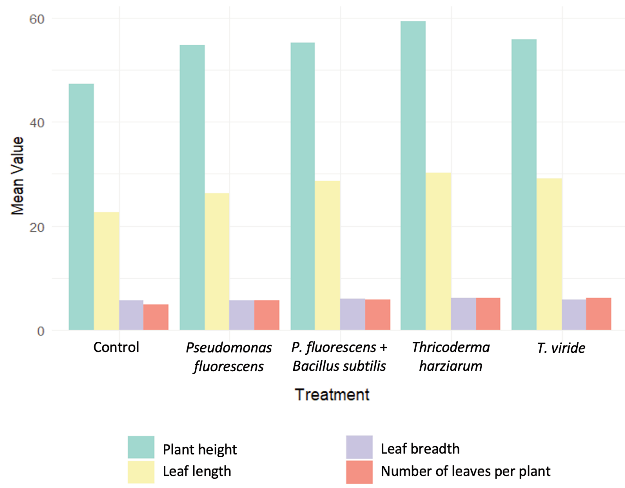

Fig. 7 - Effect of different bio-agents on vegetative parameters.

significantly higher than *Pseudomonas fluorescens* (50.15 cm) and the control (44.79 cm). Meanwhile, *T. viride* (53.0 cm) and *P. fluorescens + Bacillus subtilis* (51.81 cm) were statistically similar and intermediate between the highest and lowest values. This trend is visually evident in figure 7, where *T. harzianum* shows the tallest bar for plant height, confirming its superior vegetative growth. In terms of leaf length, *T. harzianum* again recorded the highest value (28.32 cm), followed by *T. viride* (26.83 cm) and *P. fluorescens + B. subtilis* (26.44 cm), which were statistically similar, while the control had the shortest leaves (20.99 cm). For leaf breadth, the widest leaves were found in *T. harzianum* (5.82 cm), significantly broader than the control (4.47 cm), with other treatments showing intermediate values without significant differences. Regarding number of leaves per plant (NLPP), *T. viride* (5.75) and *T. harzianum* (5.47) had the highest and statistically similar values, both significantly higher than the control (4.45), while *P. fluorescens* and *P. fluorescens + B. subtilis* produced moderate and comparable results. These vegetative improvements are clearly illustrated in figure 7, where *T. harzianum* and *T. viride* consistently outperform other treatments across all parameters. As for yield, both *T. harzianum* (496.25 kg/ha) and *T. viride* (496.0 kg/ha) produced the highest and statistically similar yields, significantly outperforming the control (454.25 kg/ha).

P. fluorescens + B. subtilis (483.25 kg/ha) and *P. fluorescens* (476.0 kg/ha) gave moderate yields that did not differ significantly from each other. The yield trend is visually supported by figure 8, which shows the highest bars for *T. harzianum* and *T. viride*, confirming their positive influence on productivity. overall, the findings suggest that *T. harzianum* and *T. viride* were the most effective bio-agents for enhancing both vegetative growth and yield. *P. fluorescens* and its combination with *B. subtilis* also provided moderate benefits. A key insight from this study is that *T. harzianum*, in addition to promoting vegetative traits, also achieved the highest yield, highlighting its potential as a dual-benefit bio-agent in crop production.

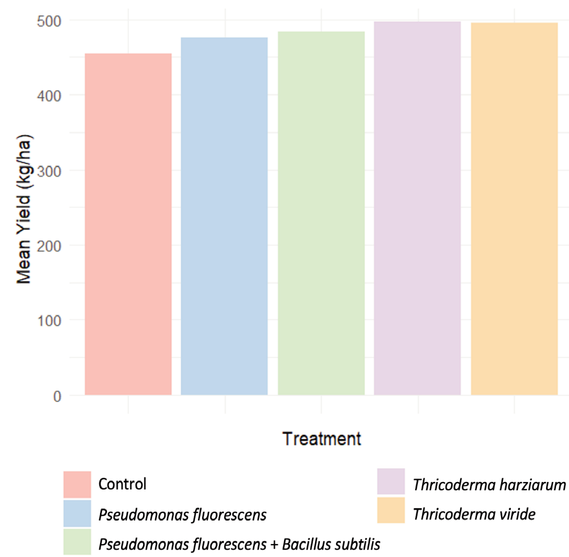

Fig. 8 - Effect of different bio-agents on yield of large cardamom.

Relationship between microbial concentration (cfu/l) and disease parameters in large cardamom

Application of *Trichoderma* spp. at a higher microbial concentration (2×10^9 cfu/l) resulted in a significant reduction in disease parameters compared to treatments with *Pseudomonas fluorescens* and *Pseudomonas + Bacillus* at 1×10^9 cfu/l. As shown in figure 9, *Trichoderma viride* exhibited the lowest disease severity index (DSI) and area under disease progress curve (AUDPC), indicating strong suppression of disease development in large cardamom. The reduction in both DSI and AUDPC at higher CFU levels demonstrates that increased microbial concentration enhances biocontrol efficacy. These findings suggest that *Trichoderma* spp. is more effective at controlling disease progression when applied at higher viable counts.

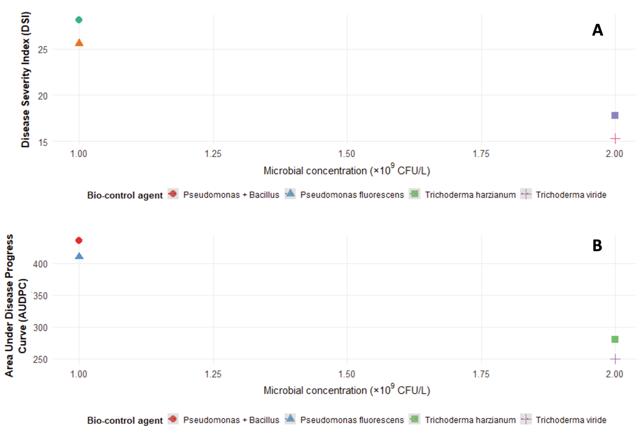

Fig. 9 - Relationship between microbial concentration (CFU/l) and disease parameters in large cardamom. A) Effect of microbial concentration on disease severity (DSI); b) Effect of microbial concentration on disease progress (AUDPC).

Effect of microbial concentration on yield of large cardamom

In addition to disease suppression, the impact of microbial concentration on yield was assessed to further validate dose efficacy. As shown in figure 10, *Trichoderma* spp. applied at 2×10^9 CFU/l resulted in the highest yield response (up to 450 kg/ha), compared to the 1×10^9 CFU/l treatments of *Pseudomonas fluorescens* and *Pseudomonas + bacillus*, which produced lower yields (approximately

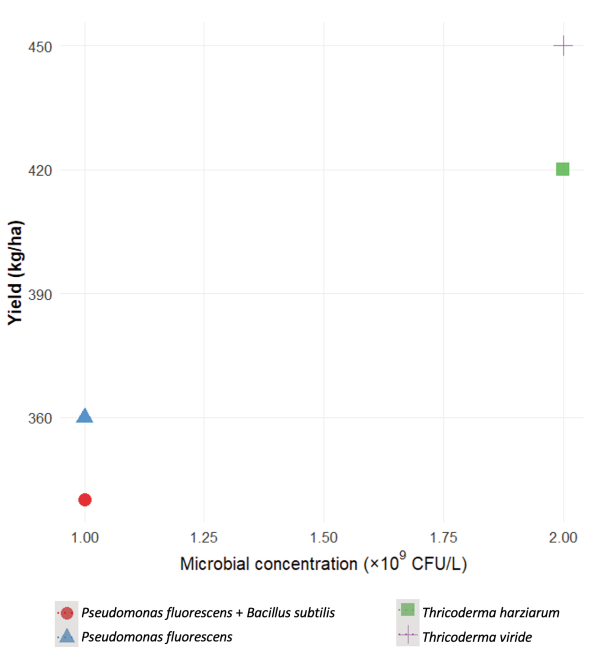

Fig. 10 - Effect of microbial concentration on yield of large cardamom.

360-390 kg/ha). These findings demonstrate a strong positive correlation between microbial concentration and yield performance. The improved plant health and productivity observed under higher *Trichoderma* CFU levels suggest a dual benefit - enhanced biocontrol and growth promotion - affirming that the increased dose rate is microbiologically justified and not merely an arbitrary increase in formulation quantity.

Multivariate analysis of growth, yield, and disease parameters

The multivariate analysis provides a comprehensive and integrated understanding of the impact of different biocontrol agents on the growth, yield, and disease dynamics of large cardamom affected by *Colletotrichum* leaf blight in Taplejung, Nepal. The heatmap (Fig. 11) clearly illustrates significant variations in vegetative and yield parameters across treatments, with plants treated with *Trichoderma viride* and the combined application of *Pseudomonas fluorescens* + *Bacillus subtilis* exhibiting the highest values for yield, plant height, and leaf traits compared to the control, highlighting their strong positive influence on plant performance.

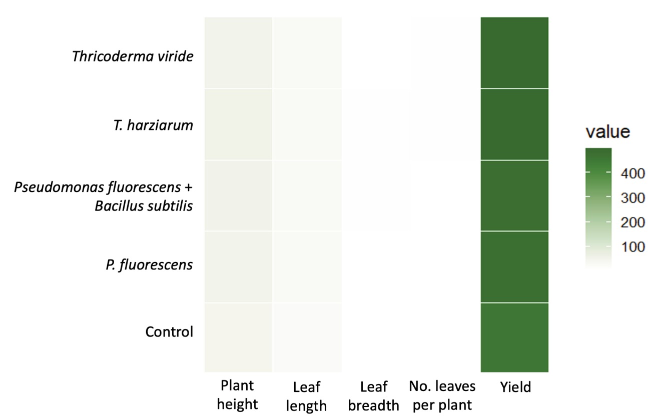

Fig. 11 - Heatmap of vegetative and yield parameters against different bioagent treatments

This observation is further reinforced by the PCA biplot (Fig. 12), where clear separation of treatment groups along the principal component axis (dim1 = 74.7%, dim2 = 10.3%) reflects the substantial contribution of biocontrol treatments in improving vegetative and yield characteristics. The overlapping yet distinct ellipses in the PCA indicate consistent but varied effects of different bioagents, emphasizing

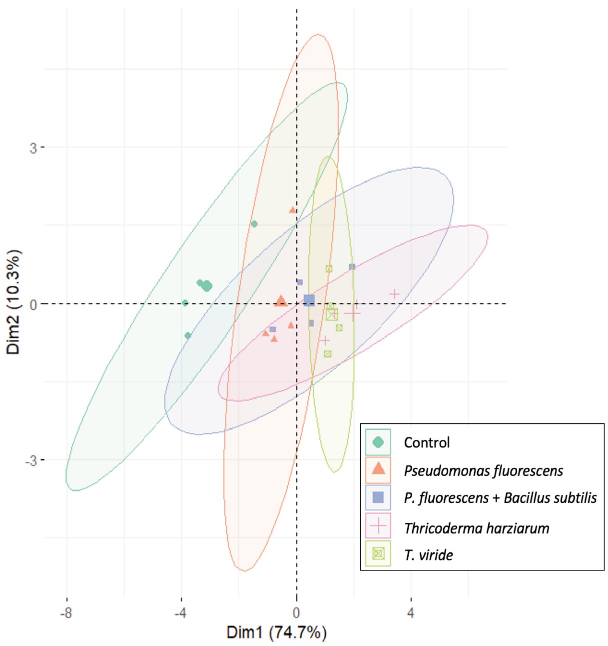

Fig. 12 - Principal component analysis of vegetative and yield traits.

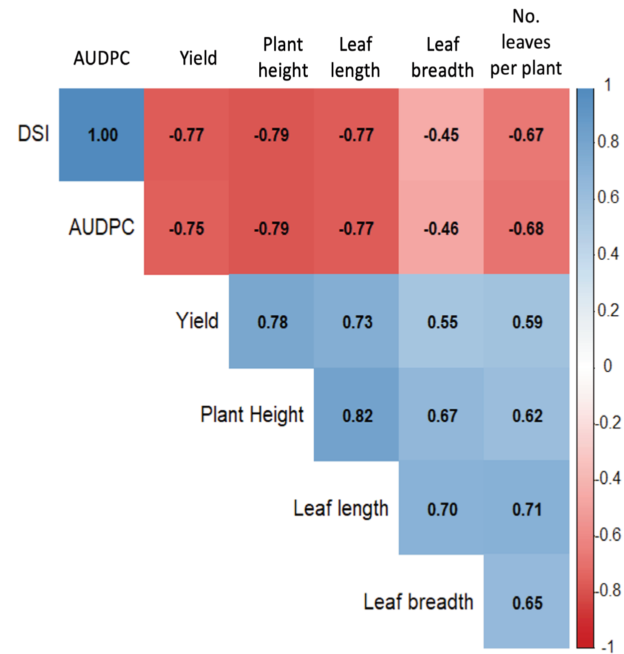

Fig. 13 - Correlation matrix of growth, yield, and disease parameters.

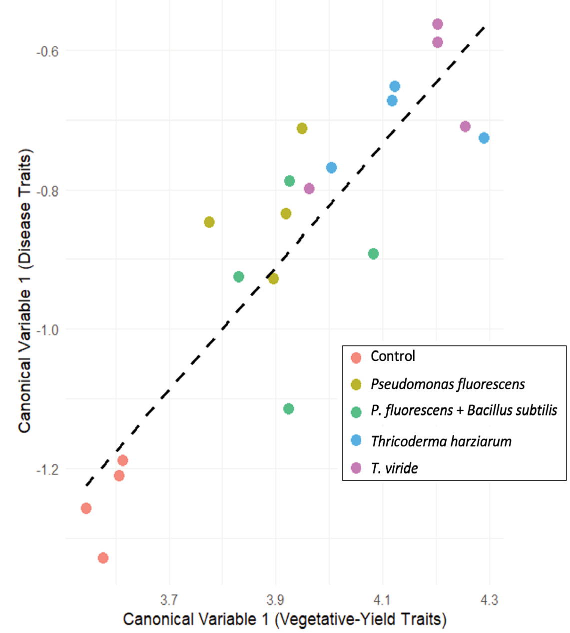

Fig. 14 - Canonical correlation biplot.

their differential roles in enhancing plant vigor and productivity. The correlation matrix (Fig. 13) strengthens these findings, showing strong positive associations among yield, plant height, and leaf traits, indicating that better vegetative growth directly contributes to increased yield. Conversely, disease parameters such as disease severity index (dsi) and area under disease progress curve (AUDPC) show strong negative correlations with vegetative and yield variables, underscoring the detrimental impact of disease pressure on crop performance. Complementing these results, the canonical correlation biplot (Fig. 14) and comprehensive radar chart (Fig. 15) demonstrate a clear and strong positive relationship between vegetative-yield traits and disease resistance parameters under different treatments. Control treatments clustered at lower canonical variable values, indicating poor growth and weak resistance, whereas *Trichoderma viride* and *Trichoderma harzianum* treatments exhibited higher values, reflecting superior growth performance and enhanced resistance to leaf blight. The radar chart further highlights that these treatments cover the largest polygonal areas across plant height, leaf length, leaf breadth, number of leaves, yield, audpc, and dsi, confirming their broad-spectrum effectiveness. *Pseudomonas fluorescens* and its combination with bacillus subtilis showed intermediate performance, while the control consistently displayed the lowest values across all traits. Collectively, these multivariate analyses provide strong evidence that biocontrol agents, particularly *Trichoderma viride* and *Pseudomonas fluorescens + Bacillus subtilis*, not only suppress *Colletotrichum* leaf blight but also significantly

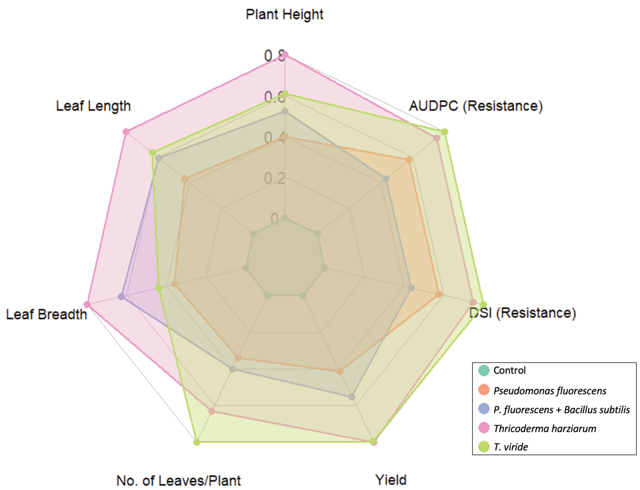

Fig. 15 - Comprehensive radar chart of growth, yield, and disease resistance parameters.

promote vegetative vigor, enhance yield, and improve disease resistance. This integrated approach underscores their potential as effective, sustainable, and eco-friendly alternatives to chemical control measures, offering a promising strategy for improving large cardamom production in the Himalayan agroecosystem.

4. Discussion and Conclusions

The present study underscores the superior efficacy of *Trichoderma harzianum* and *T. viride* in mitigating leaf blight severity caused by *Colletotrichum gloeosporioides* in large cardamom. treatments with *Trichoderma* consistently exhibited lower disease severity index (DSI) values throughout all observation periods, with *T. viride* achieving the lowest DSI of 18.5%, compared to 32.4% in *Pseudomonas fluorescens* treatments and 65.7% in the untreated control. Similarly, area under disease progress curve (AUDPC) metrics were significantly reduced in *Trichoderma*-treated plots, with an average reduction of 52% relative to bacterial treatments and 78% compared to the control. These quantitative comparisons clearly demonstrate the enhanced disease suppression capacity of *Trichoderma* spp., marking a key novelty of the current research. These findings align with previous studies highlighting the potent antagonistic capabilities of *Trichoderma* species against phytopathogens. Asalkar *et al*. (2019) reported *in vitro* bio-efficacy of various *Trichoderma* spp. against *C. gloeosporioides*, while Saju *et al*. (2013) observed effective control of leaf blight in large cardamom under field conditions using *Trichoderma*. The multifaceted mechanisms underlying *Trichoderma's* biocontrol efficacy include mycoparasitism, competition for nutrients and space, and production of antifungal metabolites (Shrestha *et al*., 2018; Dhanya *et al*., 2021). These traits enable *Trichoderma* spp. to suppress pathogen proliferation effectively while also enhancing the host plant's resistance. Beyond disease suppression, treatments with *T. harzianum* and *T. viride* significantly improved vegetative growth and yield, suggesting additional plant growth-promoting effects. The increase in plant height, number of tillers, and capsule yield in *Trichoderma*-treated plots may be attributed to the production of growth-promoting substances and enhanced nutrient uptake (Krishna *et al*., 2021). In contrast, *Pseudomonas fluorescens*, alone or in combination with *Bacillus subtilis*, provided moderate disease control (DSI 32-36%), which was notably less effective than trichoderma treatments. This discrepancy could stem from differences in colonization efficiency, metabolite production, or interactions with the host plant and pathogen, underscoring *Trichoderma's* superior field performance. Despite the comparatively lower efficacy of bacterial bio-agents, their use remains a valuable component of integrated disease management (IDM), particularly when applied in combination with other measures (Krishna *et al*., 2021). The untreated control consistently exhibited the highest DSI and lowest yield, highlighting the severe impact of *C. gloeosporioides* on large cardamom productivity and emphasizing the necessity of effective management strategies. The present study contributes novel insights by providing quantitative evidence of *Trichoderma's* superior performance over *Pseudomonas* in field conditions, reinforcing its potential as a primary biocontrol agent for large cardamom leaf blight. The dual role of *Trichoderma* in suppressing disease and promoting plant growth positions it as a sustainable and eco-friendly alternative to chemical fungicides (Dhanya *et al*., 2019; Dhanya *et al*., 2021). Previous research supports these findings; Belbase *et al*. (2018) emphasized the importance of IDM approaches incorporating biocontrol agents, while Subedi *et al.* (2022) and Biju *et al*. (2018) highlighted the field efficacy of *Trichoderma* and other bio-agents against

Colletotrichum blight. In conclusion, the application of *T. harzianum* and *T. viride* demonstrates significant promise in managing leaf blight in large cardamom, offering a sustainable alternative to conventional chemical controls. Integrating these biocontrol agents into disease management strategies can lead to improved plant health, higher yields, and reduced environmental impact, thereby supporting the goals of sustainable agriculture in the hill regions of Nepal.

The study demonstrated that among seven treatments evaluated against leaf blight of large cardamom caused by *Colletotrichum gloeosporioides*, *Trichoderma harzianum* was the most effective, recording the lowest disease severity index (DSI) and area under disease progress curve (AUDPC), along with the highest yield. *Trichoderma viride* also performed significantly better than other treatments, indicating its strong biocontrol potential. Both agents not only suppressed the pathogen effectively but also promoted plant vigor, making them sustainable alternatives to chemical fungicides. For practical use, farmers are advised to apply *Trichoderma* formulations at 15-day intervals starting one month after planting and continuing during the early monsoon period when disease incidence is high. Regular soil and foliar applications can enhance colonization and provide season-long protection. Integrating *Trichoderma* with organic mulching, field sanitation, and proper shade management can further strengthen disease resistance. However, the present study was conducted for a single season and at one location, which may limit the broader applicability of the results. Therefore, multi-season and multi-location trials are recommended to validate the findings. Overall, *T. harzianum* and *T. viride* hold strong potential for inclusion in integrated disease management (IDM) programs to ensure sustainable and eco-friendly large cardamom production.

Acknowledgements

We sincerely thank Girija Prasad Koirala College of Agriculture and Research Centre, Purbanchal University, Gothgaun, Morang, 56600, Nepal or their invaluable support in providing essential resources and a conducive learning environment. Their contribution was instrumental in the successful completion of this study.

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