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Biological and Sustainable Interventions for Banded Leaf and Sheath Blight in Maize

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ABSTRACT

Banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* f. sp. *sasakii*, severely limits maize production affecting food security and smallholder livelihoods in Nepal. This study assessed eco-friendly management strategies combining biological control agents (BCAs) and cultural practices to control BLSB and enhance maize yield. A field experiment with 17 treatments combining seed, soil, and foliar applications of *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis*, botanical extracts, and leaf sheath removal was set. The combined seed, soil, and foliar application of the three BCAs significantly reduced disease incidence (DI %) (0.83%), percent disease index (PDI) (11.85%), and area under disease progress curve (AUDPC) (807.40), achieving 85.04% disease control compared to untreated control (DI%- 89.58%, PDI- 79.25%, AUDPC- 2062.96). This integrated treatment also produced the highest grain yield (3.48 t/ha), thousand-grain weight (194.23 g), cob length (14.60 cm), and diameter (4.63 cm). Other treatments including botanical extracts and leaf stripping were less effective. These results demonstrate that integrating multiple BCAs across seed, soil, and foliar applications offers a sustainable and environmentally safe approach to managing BLSB in Nepalese maize, providing an effective alternative to chemical fungicides for improving smallholder productivity.

Keywords: Banded leaf and sheath blight, biological control, maize, *Rhizoctonia solani*, sustainable management

INTRODUCTION

Maize (*Zea mays* L.) plays a vital role in ensuring food security, supporting rural livelihoods, and maintaining economic stability in Nepal. It ranks as the second most extensively cultivated cereal after rice and remains an essential sustenance for more than 90% of rural households (FAO 2022). It is cultivated across various agro-ecological zones, extending from the terai plains to the mid-hills (Paudel et al. 2020; Sharma 2023). However, maize production is severely constrained by several biotic stresses, among which banded leaf and sheath blight (BLSB), caused by the fungal pathogen *Rhizoctonia solani* f. sp. *sasakii* (AG1-IA), poses a significant challenge. The disease is especially widespread in mild, humid maize-producing areas, where it has the potential to induce significant productivity reductions, ranging from 11% to over 40% under conducive conditions (Sharma et al. 2021; Sharma et al. 2024). BLSB affects the leaf sheaths and foliage of maize plants, resulting in distinctive banded lesions, plant lodging, early senescence, and ultimately diminished kernel filling (Kumar et al. 2019). In Nepal, the prevalence of the disease has increased owing to climate variability, mono-cropping practices, and insufficient disease management, thereby jeopardizing the livelihoods of subsistence farmers who account for over 70% of the country's agricultural production (MoALD 2021).

Chemical fungicides have been the mainstay of traditional BLSB control techniques. These pose questions about food safety, infection resistance, and environmental sustainability even though they could offer instant control (Chand et al. 2022). Furthermore, the cost and accessibility of chemical inputs further restrict their widespread and long-term usage in smallholder-dominated systems like those in Nepal. Due to growing pathogen resistance, environmental pollution, and high costs for farmers with limited resources, the current dependence on synthetic fungicides like carbendazim and mancozeb has demonstrated minimal performance (Devkota 2020). Moreover, chemical interventions often disrupt beneficial soil microbiota, exacerbating ecological imbalances (Kumar et al., 2021). These difficulties highlight the critical need for economical, sustainable solutions that are adapted to Nepal's agro-ecological and socioeconomic environment. Promising solutions include cultural practices and biological control agents (BCAs). Antagonistic microbes, including *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, have demonstrated suppression of *R. solani* through competition, antibiosis, and induced systemic resistance in maize (Singh et al., 2019). While foliar sprays may slow the spread of disease, seed treatment and soil application of BCAs can create early plant protection.

Complementary cultural strategies such as optimum planting density, crop rotation, and residue management can minimize inoculum density and humidity-driven disease transmission (Shrestha et al. 2020).

Despite the progress, there are still few integrated studies assessing these tactics across Nepal's many agroclimatic zones, which restricts the use of evidence-based policy and farmer acceptance. Developing and assessing complete management packages that combine biological control with suitable cultural practices and are adapted to Nepal's various agro-ecological contexts is imperative. By examining environmentally acceptable, locally adaptive BLSB solutions, this study fills up these gaps. The goal of this research is to create a scalable paradigm for sustainable disease management by combining biocontrol agents with culturally viable methods.

MATERIALS AND METHODS

Isolation, identification and mass multiplication of the pathogen

In April 2021, samples of leaves and sheaths exhibiting characteristic BLSB symptoms were taken from the National Maize Research Program (NMRP) maize crop in Rampur, Chitwan district. To avoid bacterial contamination, the pathogen was isolated in potato dextrose agar (PDA) (200 g potato, 20 g D-glucose, and 20 g agar combined with 50 ppm of streptomycin sulphate before pouring). A green, healthy section and small, symptomatic leaf samples measuring about 5 mm were cut, and they were first cleaned with 1% sodium hypochlorite for a minute before being cleaned three times with sterile distilled water.

The chopped pieces were then dried using sterile tissue paper with the use of forceps, put on water agar (WA) (20 g agar in 1 liter of distilled water) for hyphae growth, and incubated at 25°C for two to three days. By examining the pathogen's morphological and cultural development, the pathogen was confirmed. For later usage in the investigation, the pure culture was stored in PDA slants at 4°C ± 1°C (Phulara et al. 2025).

The mass culture of *R. solani* was prepared in barley grains by the method described by Ahuja and Payak (1978). Barley grains were soaked in distilled water with 2% sucrose overnight. Soaked seeds (40 g) were filled in an Erlenmeyer flask (250 ml), plugged with a non-adsorbent cotton plug, and autoclaved two times at 121°C, 15 pounds per square inch (psi) for two consecutive days. The 3 days old pure culture of pathogen was homogenized in sterile water pouring 5 ml in each flask. The fungus was allowed to grow for 10 days by incubating at 26°C ± 2°C with intermittent shaking for uniform coverage.

Experimental design, treatment details and agronomic practices

The experiments to evaluate the ecofriendly management strategies on BLSB disease of maize and grain yield were conducted from June 2021 to September 2021 in the research farm located at AFU, Rampur (27°40′ N latitude, 84°19′ E longitude, 228 meter above sea level). Maize seeds of a susceptible variety, Rampur composite was sown and raised by following standard agronomic practices. The soil of the research farm was sandy loam type, and prior to land preparation, farmyard manure was incorporated at the rate of 8 t per hectare. During the final land preparation, carbofuran 3% CG at the rate of 2 kg a.i./ha was applied to protect the maize seedlings from insects and pests. Chemical fertilizers were applied at the rate of 120:60:40 kg NPK/ha through urea, diammonium phosphate (DAP) and murate of potash (MOP). Half dose of nitrogen and full dose of phosphorous and potash was applied as a basal dose. Remaining half dose of nitrogen was applied in two split doses; first split during earthing up and the second at the time of flowering. Weeding was done at knee high stage and before tasseling and earthing up was done successively. Thinning was done 15 days of sowing to maintain optimum plant density in the plot. Irrigation was provided at critical stage of growth and development i.e., knee high stage and tasseling stage.

Experimental setup was done in randomized complete block design (RCBD). There were 3 replications with 17 treatments. Altogether, there were 51 plots. Each plot was 3 m long and 1 m wide. Block to block distance was 1 m and plot to plot distance was 0.5m. Commercial formulation of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were used as bio-control in different combinations and methods. Botanical extract such as garlic clove extract and *Cannabis* extract were used as treatment along with stripping (removal of lower 2 or 3 leaf sheath) to manage the disease. Treatment details of the experiment are listed in Table 1. For soil treatment, dosage was calculated and the mixture was incubated for seven days in shade area. In every two days interval, mixture was turned for uniform mixing. Seed was treated on the day of sowing for 30 minutes. Soil was also treated on the day of sowing. Four grains were placed in between leaf sheath and stalk of the 32 days old maize plant at knee high stage as the part of artificial inoculation. High humidity was maintained by spraying water for the development of the disease. After seven days, leaves and sheaths of the plant developed a characteristic symptom of concentric bands and rings that are discolored, brown, tan or grey in colour (Phulara et al. 2025).

Table 1 Details of the treatments used in eco-friendly management strategies of BLSB

S.N. (Treatment)	Seed treatment	Soil treatment	Foliar spray (thrice at 10 days interval)
1	*Trichoderma* at 10 ml per kg		0.2% of *Trichoderma* formulation per L
2	*Pseudomonas at 10* ml per kg		0.2% of *Pseudomonas* formulation per L
3	*Bacillus* at 10 ml per kg		0.2% of *Bacillus* formulation per L
4		*Trichoderma* at the rate of 5 L per 200 kg compost per ha	0.2% of *Trichoderma* formulation per L
5		*Pseudomonas* at the rate of 5 L per 200 kg compost per ha	0.2% of *Pseudomonas* formulation per L
6		*Bacillus* at the rate of at the rate of 5 L per 200 kg compost per ha	0.2% of *Bacillus* formulation per L
7	*Trichoderma* at 10 ml per kg	*Trichoderma* at the rate of 5 L per 200 kg compost per ha	0.2% of *Trichoderma* formulation per L
8	*Pseudomonas* at 10 ml per kg	*Pseudomonas* at the rate of 5 L per 200 kg compost per ha	0.2% of *Pseudomonas* formulation per L
9	*Bacillus* at 10 ml per kg	*Bacillus* at the rate of at the rate of 5 L per 200 kg compost per ha	0.2% of *Bacillus* formulation per L

10	*Trichoderma* + *Pseudomonas* at 5 ml + 5 ml per kg	*Trichoderma* + *Pseudomonas* at the rate of 2.5 L + 2.5 L per 200 kg per ha	*Trichoderma* + *Pseudomonas* at 1 ml + 1 ml per L
11	*Pseudomonas* + *Bacillus* at 5 ml + 5 ml per kg	*Pseudomonas* + *Bacillus* at the rate of 2.5 L + 2.5 L per 200 kg per ha	*Pseudomonas* + *Bacillus* at 1 ml + 1 ml per L
12	*Bacillus* + *Trichoderma* at 5 ml + 5 ml per kg	*Bacillus* + *Trichoderma* at the rate of 2.5 L + 2.5 L per 200 kg per ha	*Bacillus* + *Trichoderma* at 1 ml + 1 ml per L
13	*Trichoderma* + *Pseudomonas* + *Bacillus* at 3.3 ml + 3.3 ml + 3.3 ml per kg	*Trichoderma* + *Pseudomonas* + *Bacillus* at the rate of 1.75 L + 1.75 L + 1.75 L per 200 kg per ha	*Trichoderma* + *Pseudomonas* + *Bacillus* at 1 ml + 1 ml + 1 ml per L
14	5% garlic extract		10% garlic extract
15	5% *Cannabis* extract		10% *Cannabis* extract
16	Stripping of lower 3 leaves along with leaf sheath		
17	Untreated control		

Disease assessment

Ten plants were chosen at random from each plot and tagged prior to inoculation. After five days of foliar spraying, the first data was collected, and then data were collected every ten days after that. Rating scale was assessed by using the 1-9 scale of (Ahuja and Payak 1983) as in Table 2.

Table 2 Disease rating scale of banded leaf and sheath blight of maize

Rating scale	Degree of infection
1	Disease on one leaf sheath only; few small, non-coalescent lesions present.
2	Disease on two sheaths; lesions large and coalescent
3	Disease up to four sheaths, lesions many and always coalescent.
4	As in scale 3 + rind discolored with small lesions.
5	Disease on all sheaths except two internodes below the ear.
6	Disease up to one internode below the ear shoot; rind discoloration on many internodes with large depressed lesions
7	Disease up to internode bearing the ear shoot but shank not affected.
8	Disease on the ear; husk leaves show bleaching, bands and caking among them-selves as also of silk fibers; abundant fungal growth between and on kernel rows; kernel formation normal except their being lusterless; ear size less than normal; some plant prematurely dead.
9	In addition to scale 8, shrinkage of stalk; reduced ear dimensions; wet rot and disorganization of ear; kernel formation absents or rudimentary; premature dead plants common; abundant sclerotia production on husk leaves, kernels, ear tips or silk.

Estimation of disease incidence percentage (DI%)

The percentage for disease incidence was calculated by using the formula of Wheeler (1969).

$$Disease\ incidence\ \% = \frac{No.of\ infected\ plants}{Total\ no\ of\ observed\ plants}\ X\ 100\%$$

Estimation of percent disease index (PDI)

The disease score was converted into the severity percentage by using the formula of Wheeler (1969).

$$Disease\ severity\ \%$$

$$= \frac{(Sum\ of\ all\ numerical\ ratings)}{No.of\ plants\ observed\ X\ maximum\ reading\ of\ scale\ (i.e.9)} X\ 100\%$$

Assessment of percent reduction of disease over control (PDC)

The percentage disease control was calculated with the help of following formula given by Shivankar and Wangikar (1993).

$$Disease\ control\ \% = \frac{PDI\ in\ control - PDI\ in\ treatment}{PDI\ in\ control} X\ 100\%$$

Estimation of area under disease progress curve (AUDPC)

The AUDPC was calculated using the formula given by Campbell and Madden (1990).

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where, "t" is the time of each reading, "y" is the percent disease severity at each reading, and "n" is the number of readings.

Assessment on yield and yield attributing characters

Table 3 Assessment of different yield and yield attributing traits of maize

Character	Code	Stage of observation
Plant height	PH	Measured 3 weeks before harvest from the ground line up to the first tassel branch
Number of cobs per plant	CN	Number of cobs per plant was counted at the time of harvesting.
Cob length	CL	Cob length was measured using a ruler scale.
Cob diameter	CD	Cob diameter was measured using a digital vernier caliper.
Thousand grain weight	TGW	Manually 1000 seeds were counted and their weight was measured using a high precision digital balance.

Grain yield was estimated by following the formula mentioned by Tandzi and Mutengwa (2020).

$$Grain\ yield\ (ton/ha) = \frac{FEW\ (kg/plot) \times (100 - HMC) \times S\ \times 10000}{(100 - DMC) \times NPA\ \times 1000}$$

In this case,

FEW= Fresh ear weight, HMC= Moisture content at harvest, DMC= Desired moisture content i.e., 12.5%, S = Shelling coefficient i.e., 0.8 and NPA= Net plot area in m^2

Statistical data analysis

Microsoft Excel was used to enter and save the experiment's data. R and its packages, such as agricolae and ggplot2, were used to conduct an analysis of variance (ANOVA) and a post hoc test (DMRT) at a 5% level of significance in order to correctly interpret the data.

RESULTS

Isolation and identification of the pathogen

After three days, an effused white colony with a thread-like structure was seen. The colony's color shifted from buff to brown after ten to twelve days. Mycelial mass tends to consolidate with time, i.e., after three weeks, to form the resting structure, sclerotia, which were initially grey-white. They then developed into structures like mustard seeds that were brown to black in color. They were rather flattened and sub-globose. The morphological growth characteristics of the pathogen in the PDA medium resembled the pathogen *Rhizoctonia solani* as described by Piparava et al (2024). It had the normal features of multinucleate cells, right-angle branches, branches at the distal septa of cells, dolipore septa, and constriction at the junction of these hyphae.

Effects of ecofriendly management strategies on disease parameters

There was a very high significant difference ($p<0.001$) in DI %, PDI, PDC and AUDPC between the treatments. Lowest DI (20.83 %) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis*. It was followed by the combination of *Trichoderma viride* and *Pseudomonas fluorescens* (45.83%) which was statistically at par with *Trichoderma viride* (47.91%), *Pseudomonas fluorescens* (56.25%). Lowest PDI (11.85) was observed in the seed, soil and foliar treatment with the combination of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis,* which was statistically at par with the combination of *Trichoderma viride* and *Pseudomonas fluorescens* (20), *Trichoderma viride* (25.92), *Pseudomonas fluorescens* (31.11),

combination of *Trichoderma viride* and *Bacillus subtilis* (31.85), combination of *Pseudomonas* fluorescens and *Bacillus subtilis* (32.59) and soil plus foliar treatment of *Trichoderma viride* (32.59), however, combination of all the three biocontrol agents was found to be superior as it is least affected by the pathogen.

Highest PDC (85.04) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis.* Lowest AUDPC (807.40) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis,* which was statistically at par with the combination of *Trichoderma*. *viride* and *Pseudomonas* fluorescens (833.33), *Trichoderma viride* (903.70) and *Pseudomonas fluorescens* (925.92) alone. The highest AUDPC (2062.96) was observed in untreated control which was statistically at par with seed and foliar treatment of garlic clove extract (2003.70) and *Cannabis* leaf extract (1940.74) as shown in Table 4.

Table 4 Effect of treatments on different disease parameters of BLSB (*Rhizoctonia solani* f. sp. *sasakii*) of maize (Rampur Composite) at Rampur, Chitwan, 2021

SN	Treatments	DI %	PDI	PDC	AUDPC
1	Tv (SdT @ 10 ml/kg and FS @ 0.2%)	75^{c}	40.74^{def}	48.59	1385.18^{de}
2	Pf (SdT @ 10 ml/kg and FS @0.2%)	77.08^{bc}	40.74^{def}	48.59	1500^{cd}
3	Bs (SdT @ 10 ml/kg and FS @0.2%)	85.41^{abc}	62.96^{abc}	20.56	1807.40^{b}
4	Tv (ST @ 5 L/ha and FS @ 0.2%)	62.5^{d}	32.59^{efg}	58.87	1277.77^{e}
5	Pf (ST @ 5 L/ha and FS @ 0.2%)	64.58^{d}	37.03^{ef}	53.27	1311.11^{e}
6	Bs (ST @ 5 L/ha and FS @ 0.2%)	83.33^{abc}	60.74^{abcd}	23.36	1640.74^{c}
7	Tv (SdT @ 10 ml/kg + ST @ 5 L/ha and FS @ 0.2%)	47.91^{e}	25.92^{fg}	67.28	903.70^{g}
8	Pf (SdT @ 10 ml/kg + ST @ 5 L/ha and FS @ 0.2%)	56.25^{de}	31.11^{efg}	60.74	925.92^{g}
9	Bs (SdT @ 10 ml/kg + ST @ 5 L/ha and FS @ 0.2%)	81.25^{abc}	57.77^{bcd}	27.10	1577.77^{c}

10	Tv + Pf (SdT @ 5 ml + 5 ml/kg + ST @ 2.5 L + 2.5 L/ha and FS @ 1 ml + 1 ml/L)	45.83^{e}	20^{fg}	74.76	833.33^{g}
11	Pf + Bs (SdT @ 5 ml + 5 ml/kg + ST @ 2.5 L + 2.5 L/ha) and FS @ 1 ml + 1 ml/L	62.5^{d}	32.59^{efg}	58.87	1118.51^{f}
12	Bs + Tv (SdT @ 5 ml + 5 ml/kg + ST @ 2.5 L + 2.5 L/ha and FS @ 1 ml + 1 ml/L)	60.41^{d}	31.85^{efg}	59.81	1077.78^{f}
13	Tv + Pf + Bs (SdT @ 3.3 ml + 3.3 ml + 3.3 ml/kg + ST @ 1.75 L + 1.75 L + 1.75 L/ha and FS @ 1 ml + 1 ml + 1 ml/L)	20.83^{f}	11.85^{g}	85.04	807.40^{g}
14	*Cannabis* extract (SdT @ 5% and Fs @ 10%)	87.5^{ab}	62.96^{abc}	20.56	1940.74^{ab}
15	Garlic clove extract (SdT @ 5% and Fs @ 10%)	87.5^{ab}	72.59^{ab}	8.41	2003.70^{a}
16	Stripping of lower 2 leaves and sheaths	81.25^{abc}	50.37^{cde}	36.44	1544.44^{c}
17	Untreated control	89.58a	79.25^{a}		2062.96^{a}
	SEM (±)	2.66	2.94		57.6
	LSD (p<0.05)	10	18.6		143
	F-test	***	***		***
	CV (%)	8.77	25.3		6.17
	Grand mean	68.8	44.2		1395

Note: Tv, Pf, Bs denotes *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* respectively, SdT, ST and FS denotes seed treatment, soil treatment and foliar spray respectively, *** denotes significance at p < 0.001, CV = Coefficient of variation, SEM (±) = Standard error of mean, LSD = Least significant difference, Treatment means within a column followed by the different letter were significantly different at p = 0.05 level by DMRT

Effects of ecofriendly management strategies on yield and yield attributing characters of maize

There was a very high significant difference (p<0.001) in yield and yield attributing characters between treatments. Highest yield (3.48 t/ha) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* followed by the combination of *Trichoderma viride* and *Pseudomonas fluorescens* (3.21 t/ha), *Trichoderma* viride (3.14 t/ha). Similarly, highest TGW (194.23 g) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis.* It was followed by the combination of *Trichoderma viride* and *Pseudomonas fluorescens* (188.03 g) which was statistically at par with *Trichoderma viride* (187.60 g). Lowest TGW (172.76 g) was observed in untreated control as shown in Table 5.

Highest number of cobs per plant (1.5) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*. Lowest number of cobs per plant (0.76) was observed in untreated control which was statistically at par with seed and foliar treatment with garlic clove extract (0.83) and *Cannabis* leaf extract (0.83)

Widest (4.63 cm) was observed in the seed, soil and foliar treatment with the combination of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* followed by the combination of *Trichoderma viride* and *Pseudomonas fluorescens* (4.45 cm) and *Trichoderma viride* alone (4.37 cm). *Trichoderma viride* was statistically at par with *Pseudomonas fluorescens,* the combination of *Trichoderma viride* and *Bacillus subtilis* (4.34 cm) and the combination of *Pseudomonas* fluorescens and *Bacillus* subtilis (4.33 cm). Longest (14.60 cm) was observed in seed, soil and foliar treatment with the combination of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* which was followed by the combination of *Trichoderma viride* and *Pseudomonas fluorescens* (14.42 cm) and *Trichoderma viride* alone (14.35 cm).

Table 5 Effect of treatments on yield and yield attributing character of maize (Rampur Composite) against BLSB (*Rhizoctonia solani* f. sp. *sasakii*) at Rampur, Chitwan, 2021

SN	Treatments	Yield (t/ha)	TGW (g)	CN	CD (cm)	CL (cm)

1	Tv (SdT @ 10 ml/kg and FS @ 0.2%)	2.80^{g}	183.56^{de}	1de	4.30^{efg}	14.27^{defg}
2	Pf (SdT @ 10 ml/kg and FS @0.2%)	2.78^{gh}	183.12^{ef}	0.93^{ef}	4.30^{efg}	14.26^{efg}
3	Bs (SdT @ 10 ml/kg and FS @0.2%)	2.5^{ij}	181.2^{g}	0.85^{gh}	4.27^{fg}	14.23^{g}
4	Tv (ST @ 5 L/ha and FS @ 0.2%)	2.88^{ef}	183.76^{cd}	1de	4.32^{de}	14.29^{cdef}
5	Pf (ST @ 5 L/ha and FS @ 0.2%)	2.81^{fg}	183.66^{cd}	1de	4.31^{def}	14.27^{defg}
6	Bs (ST @ 5 L/ha and FS @ 0.2%)	2.51^{i}	181.36^{g}	0.85^{gh}	4.28^{fg}	14.25^{fg}
7	Tv (SdT @ 10 ml/kg + ST @ 5 L/ha and FS @ 0.2%)	3.14^{c}	187.60^{b}	1.11^{c}	4.37^{c}	14.35^{c}
8	Pf (SdT @ 10 ml/kg + ST @ 5 L/ha and FS @ 0.2%)	2.97^{d}	184.13^{c}	1.01^{d}	4.35^{cd}	14.32^{cd}
9	Bs (SdT @ 10 ml/kg + ST @ 5 L/ha and FS @ 0.2%)	2.73^{h}	182.93^{f}	0.91^{fg}	4.28^{fg}	14.25^{fg}
10	Tv + Pf (SdT @ 5 ml + 5 ml/kg + ST @ 2.5 L + 2.5 L/ha and FS @ 1 ml + 1 ml/L)	3.21^{b}	188.03^{b}	1.26^{b}	4.45^{b}	14.42^{b}
11	Pf + Bs (SdT @ 5 ml + 5 ml/kg + ST @ 2.5 L + 2.5 L/ha) and FS @ 1 ml + 1 ml/L	2.90^{de}	183.33^{cd}	1de	4.33^{cde}	14.30^{cdef}
12	Bs + Tv (SdT @ 5 ml + 5 ml/kg + ST @ 2.5 L + 2.5 L/ha and FS @ 1 ml + 1 ml/L)	2.92^{de}	184.1^{cd}	1.01^{d}	4.34^{cde}	14.31^{cde}
13	Tv + Pf + Bs (SdT @ 3.3 ml + 3.3 ml + 3.3 ml/kg + ST @ 1.75 L + 1.75 L + 1.75 L/ha and FS @ 1 ml + 1 ml + 1 ml/L)	3.48^{a}	194.23^{a}	1.5^{a}	4.63^{a}	14.60^{a}
14	*Cannabis* extract (SdT @ 5% and Fs @ 10%)	2.49^{ij}	181.16^{g}	0.83^{hi}	4.26^{g}	14.22^{g}
15	Garlic clove extract (SdT @ 5% and Fs @ 10%)	2.44^{j}	180.16^{h}	0.83^{hi}	4.25^{g}	14.22^{g}
16	Stripping of lower 2 leaves and sheaths	2.75^{gh}	183.1^{ef}	0.91^{fg}	4.30^{efg}	14.26^{efg}

17	Untreated control	1.22^{k}	172.76^{i}	0.76^{i}	4.15^{h}	14.13^{h}
	SEM (±)	0.065	0.59	0.02	0.014	0.014
	LSD (p<0.05)	0.065	0.49	0.06	0.04	0.04
	F-test	***	***	***	***	***
	CV (%)	1.44	0.16	4.06	0.57	0.21
	Grand mean	2.74	183	0.98	4.32	14.3

Note: Tv, Pf, Bs denotes *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* respectively, SdT, ST and FS denotes seed treatment, soil treatment and foliar spray respectively, *** denotes significance at p < 0.001, CV = Coefficient of variation, SEM (±) = Standard error of mean, LSD = Least significant difference, Treatment means within a column followed by the different letter were significantly different at p = 0.05 level by DMRT

DISCUSSION

The findings of this study confirm that integrated biological control through combined seed, soil, and foliar application of *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis* substantially reduces the incidence and severity of banded leaf and sheath blight (BLSB) in maize. These biocontrol agents work through various mechanisms, including competition with the pathogen, production of antifungal metabolites, and induced systemic resistance in the host plant, consistent with prior research demonstrating their efficacy against *Rhizoctonia solani* (Naglot et al. 2015; Sivasakthi et al. 2014). The superior disease suppression observed in this study compared to botanical extracts and mechanical interventions highlights the advantages of microbial antagonists for targeted pathogen management under field conditions. Furthermore, reduction in disease symptoms led to significant improvements in yield and yield components, reinforcing the importance of integrated disease management approaches in sustaining crop productivity and food security in maize-growing regions (Hooda et al. 2017; Khokhar et al. 2021).

The adoption of biological control agents as alternatives to chemical fungicides addresses critical concerns related to environmental safety, pathogen resistance, and resource accessibility for smallholder farmers. Chemical fungicides, although effective initially, often lead to resistance development and ecological imbalances (Ons et al. 2020). In contrast, the use of beneficial microbes offers a sustainable, cost-effective strategy aligned with agro ecological principles, promoting soil health and biodiversity while maintaining

disease control efficacy (Deguine et al. 2023). This study's integration of multiple biocontrol agents applied through diverse modes optimizes protective effects throughout the crop growth stages and provides a scalable model for sustainable BLSB management in Nepal and similar agro ecological systems, supporting long-term agricultural resilience and farmer livelihoods.

CONCLUSION

The current research presents strong evidence that using a combination of biological control agents like *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis* via seed, soil, and foliar methods, provides an effective and sustainable approach for controlling banded leaf and sheath blight in maize. The integrated treatment significantly reduced both disease incidence and severity while greatly improving important yield traits, outperforming traditional botanical extracts and mechanical methods. These results highlight the promise of combined biocontrol strategies as eco-friendly alternatives to chemical fungicides, supporting sustainable farming and enhancing the productivity and livelihoods of smallholder farmers. The study advocates for wider implementation of integrated biological management systems, customized to various agro-ecological settings, to reduce pathogen effects and strengthen maize production resilience.

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AUTHORS' CONTRIBUTION

B Phulara: Project designing, data collection, analysis and original draft preparation
S Sharma: Data collection, manuscript reviewing and editing
HK Manandhar: Reviewing the research design, data interpretation
RK Yadav: Research conceptualization
H Khati: Reviewing and editing manuscript

CONFLICTS OF INTEREST

The authors have declared that there is no conflict of interest regarding the publication of this manuscript.

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