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Research Article

Evaluation of management practices for lentil wilt (*Fusarium oxysporum* f.sp. lentis) at Rampur, Chitwan, Nepal

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ABSTRACT

Lentil wilt is caused by *Fusarium oxysporum* f. *sp. lentis*, a major limiting factor in lentil production in Nepal. This study aimed at finding better and sustainable ways to manage it through combined biological and chemical methods. The study was done from September 2016 to March 2017 at Agriculture and Forestry University, Chitwan, Nepal. Eleven native *Trichoderma* isolates were tested in the lab using a dual culture method, and two fungicides, carbendazim and propiconazole, were tested for their inhibitory effects via the poison food method at concentrations of 10, 50, and 100 ppm. Field trials were conducted with seed treatment, foliar spray, and combined application in randomized complete block design with three replications. The plot size was 3 m × 3 m. Study results showed that *Trichoderma* isolated from Rampur and Tarahara had the highest pathogen inhibition percentage which reduces fungal growth up to 70% and 63%, respectively. Chemical carbendazim inhibited wilt mycelial growth by 86% at 10 ppm and fully at 50 ppm, while chemical propiconazole inhibited on 100 ppm fully. In the field condition, wilt disease severity was recorded lowest in the plots which were treated with combined seed and foliar applications of these chemicals but carbendazim-treated plots have the highest yield. This study highlights that combining biological control agents with chemical fungicides offers effective way to manage lentil wilt and yields. Future work should focus on reducing chemical usage by integrating resistant lentil varieties and optimized biological treatments.

Keywords: Carbendazim, Lentil, Propiconazole, Trichoderma, Wilt

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INTRODUCTION

Lentil, as an important grain legume of Nepal, take up about a tenth of all cultivated land and the most widely grown grain legume of Nepal. Lentil makes up more than 60% of the area and production among all grain legumes grown here (AICC, 2017). It is not just a key food for many people, but also gives farmers a way to earn money, especially since lentil exports have become a bigger part of Nepal's economy in recent years. According to trade reports, lentil is now one of the country's top twelve export products, and Nepal supplies a noticeable share of the world's lentil market (Prasain, 2016; International Trade Centre, 2010). Lentil

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cultivation is mostly concentrated in the lowland region of Nepal as a winter crop after rice harvest. The increasing consumer demand, improved package of production, and expanding export potential offering increasing area and production of lentil in recent years (Shrestha *et al.*, 2011).

Despite the economic and nutritional importance, lentil average national yields remain below potential due to various biotic and abiotic constraints. Lentil is vulnerable to numerous diseases caused by fungi, bacteria, and viruses, among which Fusarium wilt—caused by *Fusarium oxysporum* f. sp. *lentis*—is one of the most destructive. The pathogen is seedborne and soilborne, persists for many years in the soil, and occurs across all lentil-growing regions of Nepal. The disease can lead to severe epidemics under favourable environmental conditions and, in extreme cases, might leads to complete crop failure. Several past studies show disease can reduce yields by more than 30% (Nazneen *et al.*, 2024). Other fungal diseases such as collar rot, Stemphylium blight, botrytis grey mold, and anthracnose also threatening the lentil production, but Fusarium wilt is the most widespread and damaging constraint for growers in Nepal.

Research in Nepal has largely emphasized single component strategies (biological or chemical) rather than integrated or combined approaches to manage lentil wilt. There is limited information available on the comparative performance of native *Trichoderma* isolates and the interaction between biological and chemical (fungicides) under both laboratory and field conditions. The combined use of biocontrol agents and fungicides may offer a sustainable and effective means for managing lentil wilt.

This study addresses such research gaps by evaluating native *Trichoderma* isolates alongside two widely used chemical fungicides under both laboratory and field conditions at Rampur, Chitwan. The objective was to develop effective and environmentally responsible management practices capable of reducing Fusarium wilt severity and improving lentil productivity in Nepal.

MATERIALS AND METHODS

Study Sites and Experimental Materials

The field experiment was conducted at the Agronomy Farm of the Agriculture and Forestry University (AFU), Chitwan, Nepal, from September 2016 to March 2017. Laboratory assays were performed in the Department of Plant Pathology, AFU. The lentil variety 'Shital', commonly cultivated in the Terai region, was used for the field study.

Laboratory Experiments

Isolation and Maintenance of the Pathogen

Fusarium oxysporum f. sp. *lentis* was isolated from wilt-infected lentil plants collected from the field. Infected tissues were surface-sterilized first, plated on Potato Dextrose Agar (PDA), and incubated at 25 ± 2°C at lab. Pure cultures were maintained on PDA slants. Pathogenicity of the isolated fungus was confirmed through periodic inoculation of healthy lentil stems followed by re-isolation to satisfy Koch's postulates.

Multiplication of *Trichoderma* Isolates

Eleven *Trichoderma* isolates maintained in the Plant Pathology Laboratory were revived and sub-cultured on PDA. These isolates represented different geographical locations and were used in dual culture assays to evaluate the antagonistic potential.

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Dual Culture Assay

Antagonism between *Trichoderma* isolates and the wilt pathogen was assessed using dual culture following a Completely Randomized Design (CRD). A 5-mm mycelial disc of each *Trichoderma* isolate (3-day-old culture) and a 5-mm disc of *F. oxysporum* (7-day-old culture) were placed on opposite sides of 9-cm PDA plates. Plates were incubated at 25 ± 2°C until the pathogen and antagonist colonies met. Radial growth inhibition was calculated using the formula (Arora & Upadhyay, 1978):

$$\text{Percent growth inhibition} = \frac{C-T}{C} \times 100$$

where
C = pathogen radial growth in control plates (cm),
T = pathogen radial growth in dual treated plates (cm).

In Vitro Fungicide Assay

Two commonly used fungicides—Bavistin (carbendazim 50 WP) and Tilt (propiconazole 25 EC)—were evaluated using the poison food technique. Stock solutions (250 ml) and working solutions (150 mL) were prepared, and PDA was amended with fungicides at 10, 50, and 100 ppm by adding the fungicide to molten agar cooled to 40°C. Plates (20 mL/plate) were inoculated at the center with a 5-mm mycelial disc of *F. oxysporum* and sealed with parafilm. All treatments were replicated four times in a CRD and incubated at 25 ± 2°C for 8 days. Percent inhibition was calculated using Vincent's formula (1927):

$$\text{Percent inhibition (I)} = \frac{C-T}{C} \times 100$$

where,
C = Growth of test fungus in control (cm),
T = Growth of test fungus in treatment (cm).

Field Experiment

Experimental Design

A split-plot design with 16 treatments and three replications was used. The main-plot factor consisted of control agents (carbendazim, propiconazole, *Trichoderma* TRA-isolate, and *Trichoderma* TTA-isolate), while the subplot factor included application methods (seed treatment, foliar spray, combined application, and water control). The details of treatment structure is given in Table 1. Randomization was performed within main and subplot levels.

Table 1: Treatment structure used in this study

Treatments	Main plot factor (Control agent)	Sub plot factor (Application method)
T1	Carbendazim	Seed treatment
T2	Carbendazim	Foliar spray
T3	Carbendazim	Seed treatment+ Foliar spray
T4	Carbendazim	Control (Water)
T5	Propiconazole	Seed treatment
T6	Propiconazole	Foliar spray
T7	Propiconazole	Seed treatment+ Foliar spray
T8	Propiconazole	Control (Water)
T9	*Trichoderma*-T1 (TRA- isolate)	Seed treatment
T10	Trichoderma-T1 (TRA- isolate)	Foliar spray
T11	*Trichoderma*-T1 (TRA- isolate)	Seed treatment+ Foliar spray
T12	*Trichoderma*-T1 (TRA- isolate)	Control (Water)

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Treatments	Main plot factor (Control agent)	Sub plot factor (Application method)
T13	*Trichoderma*-T2 (TTA- isolate)	Seed treatment
T14	*Trichoderma*-T2 (TTA- isolate)	Foliar spray
T15	*Trichoderma*-T2 (TTA- isolate)	Seed treatment+ Foliar spray
T16	*Trichoderma*-T2 (TTA- isolate)	Control (Water)

Disease Assessment

Ten plants were randomly selected and tagged in each plot. Disease assessment began 80 days after sowing, when wilt symptoms first appeared, and continued at 10-day intervals for three consecutive observations. Disease severity was scored using the 1–9 scale proposed by Bayaa *et al.* (1997):

- **1** = no symptoms (highly resistant)
- **3** = yellowing of basal leaves (resistant)
- **5** = yellowing of ~50% foliage (moderately resistant)
- **7** = flaccidity and partial drying (susceptible)
- **9** = complete or partial wilting and drying (highly susceptible)

Statistical Analysis

Data from laboratory assays, disease scoring, and yield measurements were entered and organized in Microsoft Excel and analyzed using R Studio (R version 3.0.3) with the agricolae package (version 1.1-8). Analysis of variance (ANOVA) was performed for all parameters, and treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level. Descriptive statistics and graphical summaries were generated using Microsoft Excel 2013.

RESULTS AND DISCUSSION

In Vitro Evaluation

Effect of *Trichoderma* on Mycelial Growth of *Fusarium oxysporum* f. sp. *lentis*

The dual culture assay revealed significant variation in the antagonistic potential of eleven *Trichoderma* isolates against *F. oxysporum* f. sp. *lentis*. During the first five days of incubation at 25 ± 2°C, all *Trichoderma* isolates exhibited faster colony growth compared to the pathogen. The *Trichoderma* (TRA) isolate from Rampur, Chitwan, demonstrated the highest inhibition of pathogen radial growth (69.83%), followed by the *Trichoderma* (TTA) isolate from Tarahara, Sunsari (62.93%) (Table 2). Several isolates showed moderate inhibition (20–50%), while others were less effective.

Table 2: Percent mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lentis* by *Trichoderma* on 5th day in dual culture on PDA at 25±2^{0}C

Trichoderma isolates	Radial growth inhibition (%)
TPK	18.96^{g}±1.99
TMA	49.13^{c}±1.72
TSG	41.37^{d}±2.81
TJU	29.31^{e}±1.99
TIL	20.68^{fg}±0.00
TNP	30.17^{e}±1.72
TRA	69.82^{a}±1.72
TTA	62.93^{b}±1.72
TLU	42.24^{d}±1.72

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Trichoderma isolates	Radial growth inhibition (%)
PALPA I	20.68^{fg}±2.81
PALPA II	22.41^{f}±1.99
HSD	3.2
p-value	< 2e-16 ***
CV(%)	5.54

Mean value in the column with different superscripts are significantly different (p≤ 0.01) according to Tukey's HSD tes

The observed differences in antagonistic activity are consistent with the previous study findings indicating that the efficacy of *Trichoderma* is strongly influenced by local environmental conditions and origin of isolates (Choudhary & Mohanka, 2012; Purushottam *et al.*, 2014; Kaur *et al.*, 2022). Native isolates often perform better than exotic strains due to their greater adaptability to local soil and climatic conditions. However, the successful establishment and efficacy in soil could be influenced by physicochemical and biological factors of the rhizosphere.

Effect of Fungicides on Mycelial Growth

Both carbendazim and propiconazole significantly inhibited the radial growth of *F. oxysporum* f. sp. *lentis* at all tested concentrations. Carbendazim found highly effective even at low concentrations (86.38% inhibition at 10 ppm) as compared to propiconazole (Table 3). These results corroborate earlier studies reporting the superior fungitoxic activity of carbendazim against Fusarium wilt (Maheshwari *et al.*, 2008; Dahal & Shrestha, 2018; Singh *et al.*, 2023). The stronger inhibition of carbendazim at lower concentrations suggests its potential for efficient disease management with minimal chemical application.

Table 3: Effect of fungicides against radial mycelia growth on PDA and percent inhibition of *Fusarium oxysporum* f. sp. *lentis* on 7th day at 25^0C

Fungicides	Radial growth inhibition (%)
Carbendazim 10 ppm	86.38^{b}±1.89
Carbendazim 50 ppm	100^{a}
Carbendazim 100 ppm	100^{a}
Propiconazole 10 ppm	52.5^{d}±2.46
Propiconazole 50 ppm	76.38^{c}±1.89
propiconazole 100 ppm	100^{a}
HSD	2.13
p-Value	< 2e-16 ***
CV(%)	1.72

Mean value in the column with different superscripts are significantly different (p≤ 0.01) according to Tukey's HSD test

Field Evaluation

Effect of Control Agents on Wilt Severity

Significant differences were observed among treatments at 80, 90, and 100 days after sowing (DAS). At 80 DAS, propiconazole-treated plots recorded the lowest disease severity as compared to other treated plots. By 100 DAS, seed and foliar treatment with carbendazim offers the lowest disease severity, followed closely by propiconazole and *Trichoderma* isolates (Tables 4–6).

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Table 4: Interaction effect of control agents and different application methods on lentil wilt severity at 80 DAS at Rampur, Chitwan, Nepal during 2017

Treatments	Seed treatment	Foliar spray	Both (seed+foliar)	None
Carbendazim	$37.04^{abcd}\pm0.74$	$34.32^{abcdef}\pm0.85$	$28.39^{ef}\pm0.85$	$40.74^{abc}\pm1.48$
Propiconazole	$32.84^{cdef}\pm4.76$	$38.27^{abcd}\pm7.00$	$26.91^{f}\pm0.85$	$33.33^{bcdef}\pm3.1$
Trichoderma (TRA-isolate)	$41.23^{ab}\pm0.85$	$42.22^{a}\pm1.48$	$39.25^{abcd}\pm1.48$	$40.24^{abcd}\pm0.85$
Trichoderma (TTA-isolate)	$34.32^{abcdef}\pm.60$	$32.35^{def}\pm0.85$	$35.30^{abcde}\pm1.71$	$38.27^{abcd}\pm3.72$
Mean	35.94			
p-Value	0.001537^{**}			
HSD	8.37			
CV(%)	7.50			

Mean value in the column with different superscripts are significantly different (p≤ 0.05) according to Tukey's HSD test

Table 5: Interaction effect of control agents and different application methods on lentil wilt severity at 90 DAS at Rampur, Chitwan, Nepal during 2017

Treatments	Seed treatment	Foliar spray	Both (seed+foliar)	Control
Carbendazim	$52.099^{ab}\pm1.71$	$50.61^{abcd}\pm4.52$	$40.74^{e}\pm1.48$	$55.55^{a}\pm1.48$
Propiconazole	$41.72^{de}\pm7.30$	$47.16^{abcde}\pm5.60$	$41.23^{e}\pm0.85$	$49.62^{abcde}\pm6.78$
Trichoderma (TRA-isolate)	$51.11^{abc}\pm2.96$	$53.58^{a}\pm3.08$	$48.14^{abcde}\pm1.48$	$47.65^{abcde}\pm6.68$
Trichoderma (TTA-isolate)	$43.20^{bcde}\pm2.26$	$43.20^{bcde}\pm1.71$	$42.22^{cde}\pm1.48$	$51.60^{ab}\pm3.42$
Mean	47.46			
p-Value	0.00123^{**}			
HSD	8.98			
CV(%)	6.09			

Mean value in the column with different superscripts are significantly different (p≤ 0.05) according to Tukey's HSD test

Table 6: Interaction effect of control agent and application methods on lentil wilt severity at 100 DAS at Rampur, Chitwan, Nepal during 2017

Treatments	Seed treatment	Foliar spray	Both (seed+foliar)	None
Carbendazim	$60.98^{bcde}\pm0.85$	$60.00^{bcdef}\pm2.96$	$52.34^{f}\pm1.13$	$66.41^{ab}\pm2.26$
Propiconazole	$55.06^{ef}\pm4.27$	$65.43^{abc}\pm2.26$	$56.04^{ef}\pm4.76$	$65.92^{abc}\pm2.56$
Trichoderma (TRA- isolate)	$62.46^{bcde}\pm0.85$	$60.98^{bcde}\pm3.08$	$58.02^{cdef}\pm2.26$	$64.93^{bcd}\pm2.26$
Trichoderma (TTA- isolate)	$65.92^{abc}\pm1.48$	$65.92^{abc}\pm1.48$	$57.03^{def}\pm2.96$	$73.33^{a}\pm2.96$
Mean	61.93			
p-Value	0.00485^{**}			
HSD	8.11			
CV(%)	6.82			

Mean value in the column with different superscripts are significantly different (p≤ 0.05) according to Tukey's HSD test

Combined application (seed treatment + foliar spray) consistently reduced disease severity more effectively than individual methods. Seed treatment protects germinating seedlings by metabolizing germination stimulatory compounds of pathogen, whereas foliar application suppresses pathogen proliferation (Howell, 2002). Similar notion is highlighted by Tripathi *et al.* (2021), Rehman *et al.* (2013), Sreeja (2014), and Dolatabadi *et al.* (2011). Integrated or

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combined application of such methods could offer maximum protection and superior efficacy in disease management. Among the two isolates, the *Trichoderma*-TTA isolate from Tarahara found more effective, particularly under combined application methods, which highlights the importance of selecting region-specific biocontrol strains.

Effect of Control Agents on Yield

Lentil yield varied significantly among treatments (Table 7). Carbendazim-treated plots recorded the highest yield as compared to other treated plots. Maximum yield was obtained when control agents were applied through both seed and foliar treatment with carbendazim. The yield enhancement observed with such treatments might be due to multiple mechanisms such as including growth promotion via increased synthesis of phytohormones (auxins, gibberellins, cytokinins), improved nutrient uptake, and production of siderophores or antibiotics that suppress deleterious rhizosphere organisms. Similar result and discussions are found on the study of Patra and Biswas (2016) and, Sharma and Joshi (2024). Similar observations have been reported in lentil and chickpea, where biocontrol agents improved both disease suppression and yield (Kumar *et al.*, 2013; Sallam *et al.*, 2008; Sahaiduzzaman, 2015).

Table 7: Interaction effect of control agents and different application methods on final yield of lentil at Rampur, Chitwan, Nepal during 2017

Treatments	Seed treatment	Foliar spray	Both (seed+foliar)	None
Carbendazim	868.17^{ab}±32.24	699.45^{cde}±41.86	954.67^{a}±30.49	393.17^{g}±22.72
Propiconazole	750.28^{cd}±34.90	634.76^{ef}±90.19	778.17^{bcd}±20.36	449.17^{g}±39.96
Trichoderma (TRA- isolate)	581.91^{f}±28.46	404.35^{g}±10.81	629.94^{ef}±12.41	392.95^{g}±12.64
Trichoderma (TTA-isolate)	729.45^{cde}±18.57	681.63^{def}±17.59	799.52^{bc}±22.46	385.82^{g}±20.15
Mean	633.34			
p Value	1.42e-06***			
HSD	114.94			
CV(%)	5.84			

Mean value in the column with different superscripts are significantly different (p≤ 0.01) according to Tukey's HSD test

CONCLSION

This study highlights the efficacy of both chemical and biological means of Fusarium wilt management of lentil at Rampur, Chitwan condition. Native *Trichoderma* isolates demonstrated strong antagonistic activity which emphasize the importance of local adaptation for effective biocontrol agent. Carbendazim remains the effective chemical means to suppress pathogen growth even at low concentrations as compared to propiconazole for similar inhibition. Field evaluations showed that combined seed and foliar treatment with carbendazim, propiconazole, or the *Trichoderma* TTA isolate effectively reduced disease severity. Carbendazim offers the highest yield as compared to other. Integrated application consistently outperformed single-method treatments and contribute for maximizing yield along with disease control. The integration of biocontrol agents, resistant cultivars and optimized chemical applications would be the recommended future management strategies to minimize chemical inputs and promote sustainable lentil production.

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Authors' Contributions

Marahatta S. arranged the research concept, data analysis and wrote the manuscript. Shrestha S, Manandhar H.K. and Aryal L. had contributed in designing of the research and provided guidance in writing the manuscript. Whereas, Jnawali A.D. assisted in the field research observations and data collection.

Conflict of Interest

The authors declare that there are no potential conflicts of interest regarding the research, authorship, and/or publication of this manuscript. Authors have independently contributed to the study and its reporting.

Ethics Approval Statement

This study involved field-based and lab based research and did not include human or animals. The lab-based study was performed in accordance with institutional, national, and international guidelines for laboratory safety and biosafety. Field-based research was conducted in accordance with environmental and biosafety guidelines.

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