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BIOCONTROL EFFICACY OF NATIVE *Trichoderma* STRAINS ISOLATED FROM FARM AND FOREST SOIL AGAINST COLLAR ROT (*Sclerotium rolfsii*) OF LENTIL

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ABSTRACT

Lentil, *Lens culinaris* is an important pulse crop of Nepal which is susceptible to collar rot disease caused by *Sclerotium rolfsii* during its early growth stages. The fungus attacks the collar region of the plant, causing rotting of the stem resulting in stunted growth, wilting and reduced productivity. Additionally, it weakens the plant, making them more susceptible to other diseases and stresses such as drought. The pathogen can survive in the soil for extended periods by producing sclerotia. *Trichoderma* species are known for their antagonistic ability against diverse plant pathogens, including *S. rolfsii*. This study was conducted to identify promising native *Trichoderma* isolates for controlling collar rot of lentil during 2020 and 2021 in Chitwan, Nepal. A total of 104 *Trichoderma* isolates collected from farm and sal, *Shorea robusta* forest soil and examined in vitro by dual culture technique against *S. rolfsii* in a completely randomized design. Of them, 30 isolates were selected to study biocontrol potential under a screen house by inoculating both the pathogen and *Trichoderma* onto lentil seedlings. Subsequently, four promising *Trichoderma* isolates were selected representing different agro-ecological regions for the field trial in a randomized complete block design to assess their ability to control *S. rolfsii* in field conditions. Results showed that *Trichoderma* isolates T_{73}, Forest soil (96.96%); T_{74}, Forest soil (94.49%) and T_{62}, Darchula (88.92%) had shown strong inhibition of *S. rolfsii* growth, with forest soil isolates exhibiting higher antagonistic potential. In the screen house assay, all *Trichoderma* isolates increased seed germination, enhanced plant growth, and reduced disease incidence as compared to the control. *Trichoderma* isolates T_{49}, Bhaktapur, and T_{31}, Banke showed the highest percentage of disease control and percentage yield increase in the field conditions. These findings highlight the consistent potential of *Trichoderma* for biocontrol of collar rot diseases in lentil.

Keywords: Collar rot, Disease incidence, Lentil, *Sclerotium rolfsii, Trichoderma*

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INTRODUCTION

Lentil (*Lens culinaris* Medik.) holds significant importance as a pulse crop in Nepal. It can be grown successfully relatively dry areas with low soil nutrients. However, lentil cultivation is challenged by various abiotic and biotic factors causing both qualitative and quantitative yield reduction in field conditions. Temperature, relative humidity, and soil characteristics are the key factors affecting pests and diseases infestation and thereby on the productivity. In Nepal, severe lentil diseases like vascular wilt (*Fusarium oxysporum* f.sp. *lentis* Vasudeva & Srinavasan), Stemphylium blight (*Stemphylium botryosum* Walr.), collar rot (*Sclerotium rolfsii* Sacc.), rust (*Uromyces fabae* Pers.), and Botrytis grey mold (*Botrytis cinerea* Pers.), causing substantial grain yield losses, especially in the Terai as well as in the hill regions (Pandey et al., 2000). Vascular wilt and collar rot are prevalent across major lentil-growing areas. Collar rot, a major disease-causing yield loss up to 60% in farmer's fields (Darai et al., 2010), is increasingly affecting leguminous crops such as lentil, chickpea (*Cicer arietinum* L.), bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) etc. in various districts of Nepal (Adhikari et al., 2022a). The pathogens prolonged saprophytic survival in the soil makes chemical management less effective and economical. Addition of compost and straw of either oat or corn, as suggested by Bulluck and Ristaino (2002), enhance beneficial soil microbe populations that can reduce *Sclerotium rolfsii* incidence. Also, bioagents such as *Trichoderma* offer an alternative for the management of soil-borne diseases since they produce biologically active compounds. *Trichoderma* spp. offer biocontrol against plant pathogens through various mechanism such as competition, enhance plant growth and development, inducing systemic resistance, antibiosis, or mycoparasitism (Harman et al., 2004; Harman, 2000). Several *Trichoderma* species secrete both volatile and non-volatile chemical compounds that counteract plant pathogenic fungi (Kumar & Ashraf, 2017). *Trichoderma* species not only control plant pathogens but also promote root development and improve nutrient assimilation in plants (Harman et al., 2004). According to Vinale et al. (2008) secondary metabolites like Koninginins, 6-pentyl-α-pyrone, and harzianic acid, produced by *Trichoderma,* enhance plants overall growth. According to Benítez et al. (2004) *Trichoderma* spp. play a role in making phosphates, micronutrients, and mineral cations like iron, manganese, and magnesium more soluble by producing organic acids, such as gluconic, citric acids, and fumaric acids. This in turn, makes these nutrients available for plant metabolism. Also, these bio-control agents can prompt local or systemic resistance in plants by initiating early defense response triggered by the secondary metabolites produced by them (Contreras-Cornejo et al., 2016).

Although *Trichoderma* effect on various plant diseases has been investigated, limited research has explored the effect of *Trichoderma* isolated from diverse agricultural land and sal forest on *S. rolfsii*. The study is primarily focused to identify *Trichoderma* isolates that are effective against *S. rolfsii* under field conditions.

MATERIALS AND METHODS

Isolation of *Trichoderma* spp. from soil samples

Trichoderma were isolated from farm and sal (*Shorea robusta* Gaertn.) forest soil using *Trichoderma* selective medium (TSM). The ingredients used in the medium were $MgSO_4.7H_2O$ (0.2g), K_2HPO_4 (0.9g), KCl (0.15g), NH_4NO_3 (1.0g), Glucose (3.0g), Agar-agar (20g), Distilled water (1 liter), Streptomycin sulphate (0.02g), Captan (0.2g) and Rose Bengal (0.15g). The plates were incubated at 25ºC and examined daily for the growth of *Trichoderma* colonies for 5 days. The entire process was carried out under laminar flow station. Appearance of *Trichoderma* colonies were observed on 5th day of inoculation. Each colony that appeared in the TSM plate were transferred to PDA tubes.

Isolation of pathogen

Lentil roots with disease symptoms were washed and cut into 1 cm sized sections. The cut root samples were surface sterilized by dipping in 1% sodium hypochlorite for 1 min, rinsed with sterile distilled water, and plated on moist chamber. The plates (9 cm diameter) were incubated at 25 ± 1ºC in an incubator until sclerotia were produced (5 days) and the sclerotia produced were transferred to PDA to get pure culture. The pure culture was maintained at 4ºC.

Screening of Trichoderma isolates against S. rolfsii

One hundred four isolates of *Trichoderma* isolated from various farm land and sal (*Shorea robusta* Roth.) forest soil were subjected to *in vitro* testing using dual culture to assess their interactions with *S. rolfsii* (Adhikari et al., 2022b). The study was carried out in CRD with three replications. Inhibition percent of pathogen mycelial growth was calculated by: P = [(Growth of pathogen in control - growth of pathogen with the antagonist)/Growth of pathogen in control] × 100.

Biocontrol of collar rot of lentil under field conditions

Four *Trichoderma* isolates such as T_{40}, Jumla; T_{87}, Forest soil; T_{31}, Banke and T_{49}, Bhaktapur were selected to evaluate their efficacy against collar rot of lentil (var. Simrik) under field conditions. The isolates were selected in such a way that they represent three agro-ecological zones (mountain, mid-hills and terai) and one forest environment based on their their biocontrol efficacy *in vitro*. The field experiment was conducted in Mangalpur, Chitwan (27°39'58"N 84°22'07"E) during cropping season of 2020 and 2021. The experiment was conducted in two-factorial randomized complete block design (RCBD) with three replications. The individual plot size was 1.0 x 2.0 square meter, 1.0 m space was kept between the plots and 10 cm from row to row. Sowing of lentil was done on 24th November 2019 and 4th November 2020. In the first year of experiment, disease incidence was low and thus in second year the sowing date was shifted early to get the maximum disease incidence in the experimental plots. Recommended dose of fertilizers was applied prior to seed sowing. Urea, DAP and MOP were applied in the field at the rate of 6, 9 and 7 g per

plot, respectively. *Trichoderma* isolates were applied as seed treatment at the rate of 1.0 ml conidial suspension (10^8 CFU/ml) per gram seed and soil treatment at the rate of 1.0 lit conidial suspension (10^8 CFU/ml) per plot. Each experimental plot was inoculated with mass culture of *S. rolfsii* at the rate of 10 g per plot. Disease incidence was recorded and calculated based on the following formula.

$$\text{Disease incidence (\%)} = \frac{\text{No.of infected plants}}{\text{total no.of plants observed}} \times 100$$

Similarly, percent disease control (PDC) was calculated using the following formula.

$$\text{PDC (\%)} = \frac{\text{Disease in treated plot} - \text{Disease in control plot}}{\text{Disease in control plot}} \times 100$$

The crop was harvested manually by uprooting individual plant. Yield was recorded and expressed as kg/ha.

$$\text{PYI(\%)} = \frac{\text{Yield in treated plot} - \text{Yield in control plot}}{\text{Yield in control plot}} \times 100$$

Statistical analysis

The data recorded were tabulated in Microsoft Excel and analyzed using R-studio version 4.1.0. Mean comparison was done by Duncan's Multiple Range Test (DMRT) at 1 and 5% levels of significance. Count data were subjected to square root transformation (Gomez &Gomez, 1984). Graphs were prepared by using MS Excel 2016 and Minitab 14.

RESULTS AND DISCUSSION

Inhibition percentage of *Trichoderma* isolates

The mean percentage inhibition of *Trichoderma* isolated from agricultural land and Forest soil varied significantly. Only four isolates viz., T_{62}, Darchula (88.92 %); T_{63}, Darchula (87.93%); T_{30}, Palpa (86.16%); and T_{53}, Makwanpur (85.11%)isolated from agricultural land showed inhibition percentage of more than 85%. Majority of *Trichoderma* isolates of agricultural land exhibited inhibition percentage between 60-80%. While *Trichoderma* isolated from forest soil showed inhibition percentage between 60-99% except for one isolate (Figure 1).

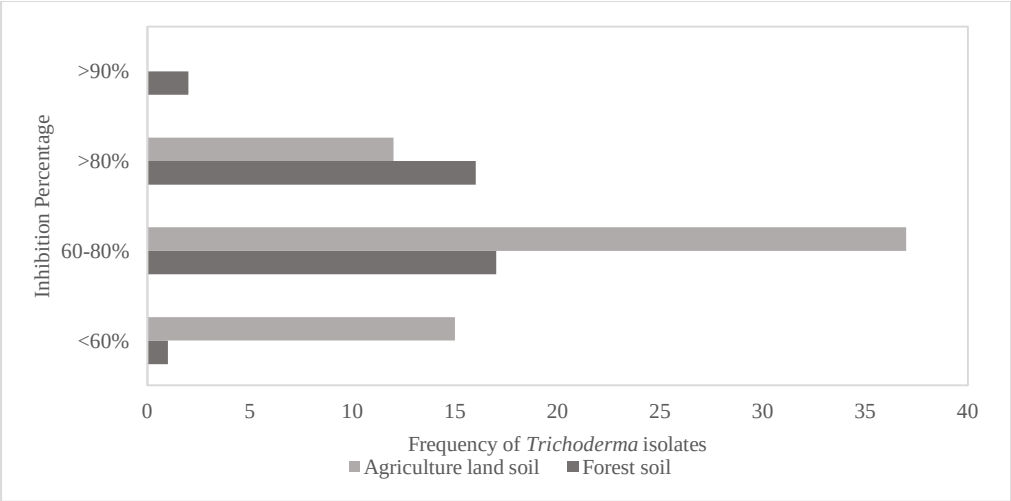

Figure 1. Percentage inhibition exhibited by Trichoderma isolated from agricultural land and forest soil

About thirty *Trichoderma* isolates showed inhibition percentage above 80% against *S. rolfsii*. Similar results have been reported by various researchers Kamel et al. (2020); Adhikari et al. (2022); Das et al. (2023); Kangjam et al. (2023). According to Vinale et al. (2008) *Trichoderma* employ mycoparasitism and antibiosis against the fungal pathogen. During mycoparasitic interactions with the pathogen, *Trichoderma* produces range of enzymes that break down the cell wall. The enzymes include chitinase, cellulose, glucanase etc. causing the cell wall to rupture and release oligomers (Kubicek et al., 2001; Howell, 2003; Woo et al., 2006; Vinale et al., 2008). However, the efficacy of different *Trichoderma* isolates to suppress the plant pathogen is inconsistent. Different strains of the same *Trichoderma* species can exhibit varied levels of pathogen inhibition, according to Anees et al. (2010) and Scherm et al. (2009). Consequently, antagonism is not an inherent trait of a species. Those particular strains that can promptly and effectively activate genes associated with the antagonistic activities in the presence of a host tend to be more potent antagonists (Scherm et al., 2009).

In this study, the majority of *Trichoderma* isolated from forest soil exhibited higher inhibition percentage as compared to those from agriculture soil. Within the forest ecosystem, both *Trichoderma* and *S. rolfsii* co-evolve and adapt together. Unlike the disturbances present in agriculture soil, forest soil remains relatively undisturbed, potentially leading to more potent *Trichoderma* strains that could be virulent towards the pathogen. According to Buckling and Rainey (2014) antagonistic coevolution plays a critical role in population dynamics of prey and parasite and evolution of virulent parasite.

Biocontrol of collar rot of lentil in the field

The *Trichoderma* isolates used in the experiment significantly (P≤0.05) affected grain yield of the crop. The highest grain yield (1621.66 kg/ha) was recorded in soil application of *Trichoderma* isolate T_{31}, Banke in the first year (Table 1). Similarly, Percentage yield increment was highest in T_{31}, Banke isolate (39.05%) followed by T_{40}, Jumla (30.72%) and T_{87}, Forest soil (30.48%) in first year. In the second year of the experiment, soil application of T_{49}, Bhaktapur isolate showed higher grain yield (1743.44 kg/ha) as compared to other isolates and control. Percentage yield increment (PYI) was highest in T_{49}, Bhaktapur isolate (87.41%) followed by T_{31}, Banke isolate (79.15%) as soil treatment.

Table 1. Effect of *Trichoderma* isolates grain yield and PYI of lentil under field conditions, Mangalpur, Chitwan, 2020/21

Treatments	First year (2020)		Second year (2021)	
	Yield (kg/ha)	PYI (%)	Yield(kg/ha)	PYI (%)
T_{40}, Jumla, seed treatment	1426.67^{a}	44.35	1532.63^{ab}	64.75
T_{40}, Jumla, soil treatment	1355.00^{ab}	37.10	1219.76^{bc}	31.12
T_{87}, Forest soil, seed treatment	1281.66^{ab}	29.68	1248.96^{abc}	34.26
T_{87}, Forest soil, soil treatment	1421.66^{a}	43.84	1434.39^{ab}	54.19
T_{31}, Banke, seed treatment	1296.66^{ab}	31.20	1317.74^{abc}	41.65
T_{31}, Banke, soil treatment	1621.66^{a}	64.08	1666.54^{ab}	79.15
T_{49}, Bhaktapur, seed treatment	1348.33^{ab}	36.43	1336.87^{abc}	43.71
T_{49}, Bhaktapur, soil treatment	1333.33^{ab}	34.91	1743.44^{a}	87.41
Control	988.33^{b}	-	930.26^{c}	-
S.Em±	120.07		149.67	
F- test	*		*	
LSD (=0.05)	359.97		448.72	
CV (%)	15.82		18.76	

Note: PYI: Percentage Yield Increase

The *Trichoderma* isolates used in the study were significantly (P≤0.05) effective to control collar rot of lentil under field conditions. In the first year, *Trichoderma* isolate T_{31}, Banke, exhibited higher percent disease control (65.20%) as compared to other isolates (Table 2). The appearance of disease was observed 15 days after seedling emergence (DAE). At 22 DAE, the highest disease index was recorded in those plots where *Trichoderma* isolate T_{40}, Jumla (13.33%) was applied as a soil treatment. The control plot exhibited significantly highest disease index (37.77%) in 29 DAE than *Trichoderma* treated plots. However, at 36 DAE highest disease index was observed

in control (50.00%), followed by T_{49}, Bhaktapur isolate (38.88%) and T_{40}, Jumla isolate (38.88%) as a seed treatment. The highest AUDPC value was noted in control (894.44) followed by seed application of T_{40}, Jumla isolate (661.11) and lowest in T_{31}, Banke (303.33) as seed treatment.

Table 2. Effect of *Trichoderma* isolates on collar rot incidence of lentil in the field condition, Mangalpur, Chitwan, 2020

Treatments	First year (2020)					Second year (2021)				
	DI			PDC (%)	AUDPC	DI			PDC (%)	AUDPC
	22 DAE	29 DAE	36 DAE			22 DAE	29 DAE	36 DAE		
T_{40}, Jumla, seed treatment	8.88	16.66^{ab} (0.49)	38.88^{a} (0.67)	27.40	661.11	21.11^{b}	28.89^{b}	34.44^{b}	48.48	455.00
T_{40}, Jumla, soil treatment	13.33	16.66^{ab} (0.39)	22.22^{b} (0.48)	47.22	478.33	20.00^{b}	33.33^{b}	42.22^{b}	35.72	622.22
T_{87}, Forest soil, seed treatment	6.66	14.44^{b} (0.27)	21.11^{b} (0.47)	56.34	392.78	23.33^{b}	26.67^{b}	33.33^{b}	49.75	583.33
T_{87}, Forest soil, soil treatment	1.11	14.44^{b} (0.27)	22.22^{b} (0.48)	55.32	353.89	27.77^{b}	33.33^{b}	38.89^{b}	41.22	610.55
T_{31}, Banke, seed treatment	2.22	8.89^{b} (0.21)	18.89^{b} (0.44)	57.65	303.33	23.33^{b}	30.00^{b}	35.56^{b}	46.72	501.66
T_{31}, Banke, soil treatment	7.77	11.11^{b} (0.33)	17.78^{b} (0.43)	65.20	334.44	21.11^{b}	31.11^{b}	35.56^{b}	46.72	773.88
T_{49}, Bhaktapur, seed treatment	5.55	24.44^{ab} (0.51)	38.88^{a} (0.67)	26.78	645.56	20.00^{b}	28.89^{b}	36.67^{b}	45.69	523.33
T_{49}, Bhaktapur, soil treatment	6.66	15.55^{ab} (0.39)	23.33^{b} (0.48)	51.23	431.67	21.11^{b}	25.56^{b}	32.22^{b}	50.79	501.61
Control	11.11	37.77^{a} (0.66)	50.00^{a} (0.78)	-	894.44	44.44^{a}	62.22^{a}	66.67^{a}	-	812.77
S.Em±	5.53	0.08	0.057			4.41	4.04	4.14		
F- test	ns	*	**			*	***	***		
LSD (=0.05)		0.263	0.17			13.24	12.12	12.42		
CV (%)	13.62	18.30	18.05			18.00	18.02	18.16		

Note: Figures in the parentheses are square root transformation values. DI: Percentage Disease Index, DAE: Days After Emergence, PDC: Percentage Disease Control, AUDPC: Area Under Disease Progress Curve.

However, in the second year, collar rot incidence was observed 7 days after the emergence of lentil seedlings. The disease index was significantly high in control plot at 22 (44.44%), 29 (62.22%) and 36 (66.67%) DAE than in *Trichoderma* treated plots. The higher percent disease control was recorded in soil treatment with *Trichoderma* isolate T_{49}, Bhaktapur (50.79%) followed by *Trichoderma* isolate T_{87},

Forest soil (49.75%) as seed treatment. The highest AUDPC value was recorded in control (812.77) followed by soil application of T_{31}, Banke isolate (773.88).

Results showed that *Trichoderma* isolates significantly enhanced seed germination, reduced plant mortality and reduced disease incidence over the control. The findings aligned with Arya et al. (2021); Hasna et al.(2020); Kashem et al. (2011). According to Khattabi et al. (2004), the effectiveness of *Trichoderma* isolates as antagonists in natural soil is indicated by their competitiveness and *in vitro* activity.

In field condition, collar rot incidence was low in first year as compared to second year. In first year, lentil was grown in the fallow land where there was no crop in previous years. When agricultural land are abandoned for a long period, the abundance of pathogenic fungi decrease over time (Hannula et al., 2017). Pathogen was inoculated in the first year, as a result, population of the pathogen increased in the field. These sclerotia and mycelium present in soil might have caused more disease incidence in second year. Paparu et al. (2020) found weak but positive correlations between the number of sclerotia produced and Southern blight of common bean. This may be one of the reason in difference in percentage disease incidence between two years. Similarly, another reason may be the sowing date and agrometeorological factor. In first year, lentil was sown in late November and in second year in the first week of November. Mishra et al. (2021) also reported maximum disease incidence of *Sclerotinia* stem rot in lentil sown in 5^{th} and 15^{th} November. According to Roy et al. (2021), collar rot occurrence tends to be lower in crops sown later (during the initial week of December) in certain regions of Eastern India.

Since *Trichoderma* was applied as seed treatment and soil treatment, there was no significant difference between two application methods for disease incidence and percentage disease control. However, soil treatment had shown better results over seed treatment. Similar findings were also documented by Hasna et al. (2020) and Kashem et al. (2011), who observed diminished disease incidence in chickpea and lentil respectively, when *T. harzianum* was introduced into the soil. According to Hasna et al. (2020), *Trichoderma* sp. is inherently a soil inhabitant, which allows it to establish and proliferate more quickly in soil compared to the seed surface. In contrary, Biswas and Sen (2000) reported that seed treatment with conidial suspension proved more effective in decreasing disease incidence than soil application. Despite the promising outcomes of *Trichoderma* soil treatment in controlling soil borne plant pathogens, the significance of seed treatment remains paramount. Juliatti et al. (2019) emphasized that seed microbiolization is a crucial method of bio-control agent application, as it demands a smaller quantity of biological material in comparison to the amount needed for soil application.

CONCLUSION

Trichoderma isolates, especially those obtained from forest soil, demonstrate significant potential for biocontrol against the fungal pathogen *S. rolfsii* causing collar rot in lentils. The study indicates that *Trichoderma* isolates from forest soil generally exhibited higher inhibition percentages compared to those from agricultural land, potentially due to co-evolutionary adaptations within undisturbed forest ecosystems. These isolates, when applied as soil treatments, showed substantial improvements in grain yield and disease control in lentil crops, suggesting their effectiveness as biocontrol agents in agricultural practices. However, the efficacy of *Trichoderma* isolates varied, highlighting the importance of strain selection for optimal biocontrol outcomes.

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