

## Research Article

# Efficacy of *Trichoderma* isolates against *Sclerotium rolfsii* causing collar rot of lentil

Laxman Aryal<sup>1\*</sup>, Suraj Baidya<sup>2</sup> and Subash Subedi<sup>3</sup>

<sup>1</sup>Nepal Agricultural Research Council, National Wheat Research Program, Bhairahawa, Rupandehi, Nepal

<sup>2</sup>Nepal Agricultural Research Council, National Plant Pathology Research Centre, Khumaltar, Lalitpur, Nepal

<sup>3</sup>Nepal Agricultural Research Council, Oilseed Research Program, Nawalpur, Sarlahi, Nepal

\*Corresponding author's email: [laxmanaryal.ag@gmail.com](mailto:laxmanaryal.ag@gmail.com)

\*ORCID: <https://orcid.org/0000-0001-5588-565X>

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## ABSTRACT

Lentil collar rot disease caused by *Sclerotium rolfsii* Sacc. is an important disease causing significant yield loss in Nepal. Seven *Trichoderma* spp. isolates collected from different regions of Nepal were tested for their capacity to inhibit mycelial growth of *Sclerotium rolfsii* in in-vitro condition. Experiment was conducted in Completely Randomized Design with four replications in the plant pathology laboratory of Grain Legumes Research Program, Khajura, Banke during 2020. Dual culture method was performed to assess the efficacy of *Trichoderma* isolates. *Trichoderma* spp. isolates inhibited mycelial growth of *S. rolfsii* to various degrees ranging from 48.33-72.47% after 120 hours of inoculation. Maximum colony inhibition of *S. rolfsii* (72.47%) was obtained by Kapilvastu isolate followed by Nepalgunj isolate (67.72%). Minimum colony inhibition of *S. rolfsii* (48.33%) was obtained by Mangalpur, Chitwan isolate followed by Rampur, Chitwan isolate (49.33%). Among seven *Trichoderma* isolates, two isolates (Kapilbastu and Nepalgunj) showed good antagonistic activity against *S. rolfsii* and were also highly competitive in in-vitro condition. These isolates would therefore be useful in biological disease management.

**Keywords:** collar rot, colony inhibition, lentil, *Sclerotium rolfsii*, *Trichoderma*

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## INTRODUCTION

*Sclerotium rolfsii* (Sacc.) is considered to be devastating pathogen against several crops worldwide with an extensive host range, which includes more than 500 species in 100 families worldwide (Agrios, 2005; Cilliers *et al.*, 2000). The pathogen *S. rolfsii* may cause damping-off, collar rot and stem rot on host plant with the help of sclerotial germination, which may measure 1-3 mm with mustard seed-like appearance on surfaces of the affected plant. The pathogen may exploit host plant internally as well as externally with white cottony-like mycelium formation resulting poor growth of the infected plant. The pathogen is

very common in tropical, subtropical and warm temperate region in the world (Hemanth *et al.*, 2016). Collar rot affects plant at initial stage of the crop dominantly in the early seedling stage (Njambere & Chen, 2011). Because of prolific growth and necrotroph nature, it can damage the crop in short period of time. This pathogen has ability to produce persistent sclerotia which survives in soil and can incite disease in the next year. Under conducive conditions it can cause 55-95% mortality of the crop at seedling stage (Gurha & Dubey 1982). The *S. rolfsii* and its capability of producing excessive sclerotia may persist in soil for several years (Singh *et al.*, 2012). Hence management of *S. rolfsii* causing collar rot of lentil is difficult to achieve chemically, thus prove ineffective and costly to growers. In this context bio agents can be used as an alternative source for controlling soil-borne diseases since they comprise a rich source of bioactive substance (Jegathambigai *et al.*, 2010). Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. is well documented as effective biological control agents of plant diseases (Sain & Pandey, 2016). Sanchez *et al.* (2006) reported *Trichoderma* species can inhibit the growth of plant pathogens especially fungi through competition for nutrients, enzymes, substrate, oxygen and space.

The dual culture technique can be used for the evaluation of the antagonistic capacity of *Trichoderma* isolates against *Sclerotium rolfsii* pathogen of lentil. The antagonistic potential of *Gliocladium virens* and *Trichoderma harzianum* against *S. rolfsii* based on antagonism in-vitro in dual culture as colony degradation tests, hyphal interaction, antibiosis and parasitism of sclerotia and observed a positive response as evaluated by (Jomduang & Sariah, 19). The present study was conducted to evaluate the efficacy of trichoderma isolates collected from different agro-ecological regions of Nepal against *S. rolfsii* pathogen causing collar rot of lentil.

## MATERIALS AND METHODS

### Study site

In-vitro study was carried out in Plant Pathology lab of Grain Legume Research Program (GLRP) at Khajura, Banke, Nepal during 2020. A total of seven *Trichoderma* isolates PDA tubes were collected from Directorate of Agricultural Research, Khajura, Banke.

**Table 1: List of *Trichoderma* isolates collected (with their designation) from Directorate of Agricultural Research, Khajura, Banke**

S.N	<i>Trichoderma</i> Isolates	Designation
1.	Dang	T-D
2.	Rampur, Chitwan	T-RC
3.	Raniban, Kathmandu	T-RK
4.	Kapilbastu	T-K
5.	Saptari	T-S
6.	Mangalpur, Chitwan	T-MC
7.	Nepalgunj	T-N

### Culture maintenance of *S. rolfsii*

Infected lentil seedling was brought from field. Root and collar region of the infected seedling was cut into small pieces and surface sterilized in 1% sodium hypochlorite solution with 3 serial washing in distill water. Surface sterilized root pieces was placed in PDA and incubated in 25±1°C. After 6 days PDA plate was fully covered with white mycelium growth

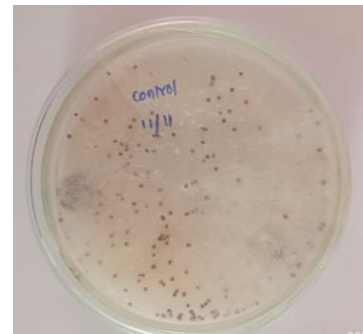
of *S. rolf sii*.



**Figure 1:** Collar rot disease sample of lentil



**Figure 2:** Sclerotia from disease sample



**Figure 3:** Mycelial growth of Sclerotia

### **Culture maintenance of *Trichoderma* isolates**

PDA plate of *Trichoderma* isolates were multiplied in PDA petridish measuring 9 cm for antagonistic study of *Trichoderma* against *S. rolf sii* in dual culture method.

### **Dual culture of *Trichoderma* isolates and *S. rolf sii***

Mycelial disks (5 mm diameter) taken from the margins of 6-day-old colonies of *Trichoderma* isolates and *S. rolf sii* were cut with the help of cork borer and were transferred to Petri dishes containing 20 ml PDA. One isolate of *Trichoderma* and one of *S. rolf sii* were placed simultaneously on opposite sides of each dish, 5 cm apart. The control was prepared with only *S. rolf sii*. In-vitro experiment was conducted in CRD design with 4 replications. Mycelial growth of *S. rolf sii* was assessed by measuring the colony diameter in two perpendicular directions after 24 hr, 48 hr, 72 hr, 96 hr and 120 hr of incubation at 30°C. Percent inhibition (I) of mycelial growth of *S. rolf sii* in the presence of *Trichoderma* spp. was determined according to the method of Camporota (1985).

$$I = (C1 - C2 / C1) \times 100$$

Where, C1 is the linear growth of *S. rolf sii* in control Petri dishes, and C2 is the linear growth of *S. rolf sii* in dual culture with the *Trichoderma* isolate.

### **Statistical analysis**

Experiment was carried out in CRD design. Data were statistically analyzed using R studio and mean comparisons were made using Least significant difference (LSD).

## **RESULTS AND DISCUSSION**

### **Effect of *Trichoderma* isolates on the linear diameter colony growth of *Sclerotium rolf sii***

*Trichoderma* isolates collected from different origin showed significant ( $p \leq 0.01$ ) effect on the linear diameter colony growth of *Sclerotium rolf sii* pathogen at 24, 48, 72, 96 and 120 hours after inoculation in the growth media. Kapilvastu isolate worked effectively among all the tested isolates and showed lowest colony growth of *S. rolf sii* at 24, 48, 72, 96 and 120 hours after inoculation in growth media. After 120 hours when control plate fully colonized with *S. rolf sii* pathogen colony growth reading was stopped. In the final reading, the lowest colony growth of the pathogen due to antagonistic effect of *Trichoderma* was obtained by Kapilvastu isolate (2.47 cm) followed by Nepalgunj isolate (2.90 cm). Maximum colony

growth (4.65 cm) of the *S. rolfsii* pathogen was obtained by Mangalpur, Chitwan isolate followed by Rampur, Chitwan isolate (4.57 cm) (Table 2).

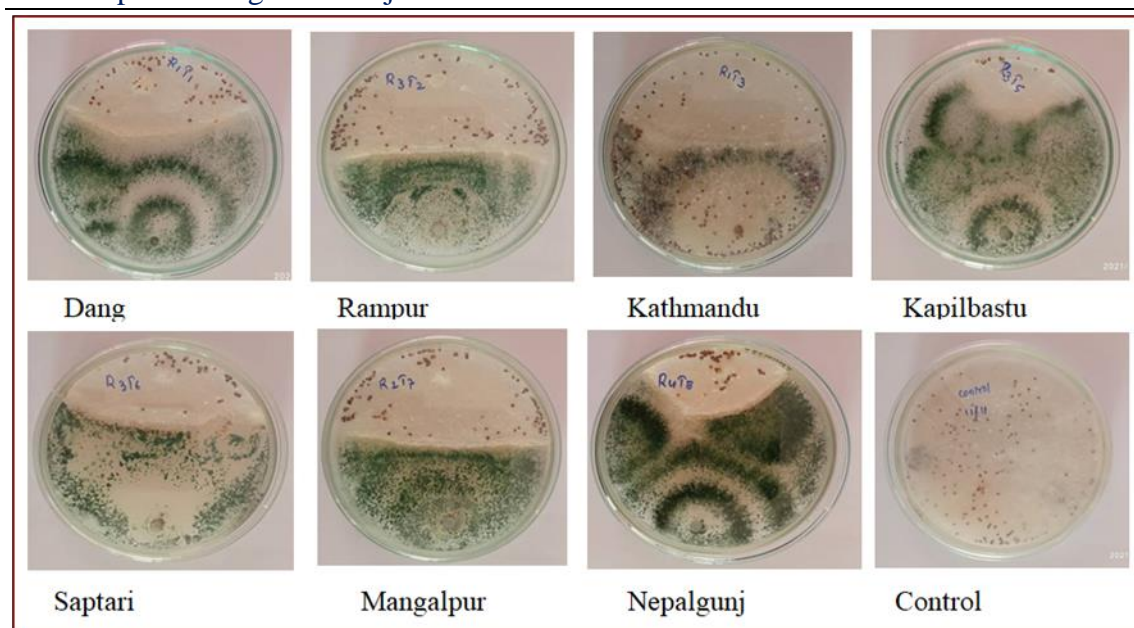
**Table 2: Effect of Trichoderma isolates on the linear diameter colony growth of *Sclerotium rolfsii***

S.N	Trichoderma Isolates	Linear diameter colony growth (cm)				
		24 hour*	48 hour*	72 hour*	96 hour*	120 hour*
1	T-D	0.46 <sup>cd</sup>	1.71 <sup>cd</sup>	2.25 <sup>b</sup>	2.50 <sup>c</sup>	3.02 <sup>cd</sup>
2	T-RC	0.96 <sup>a</sup>	1.82 <sup>ab</sup>	2.70 <sup>a</sup>	3.92 <sup>a</sup>	4.57 <sup>a</sup>
3	T-RK	0.89 <sup>b</sup>	1.76 <sup>bc</sup>	2.37 <sup>b</sup>	2.90 <sup>b</sup>	3.82 <sup>b</sup>
4	T-K	0.38 <sup>e</sup>	1.62 <sup>e</sup>	1.88 <sup>c</sup>	2.11 <sup>d</sup>	2.47 <sup>e</sup>
5	T-S	0.48 <sup>c</sup>	1.75 <sup>bc</sup>	2.27 <sup>b</sup>	2.75 <sup>b</sup>	3.25 <sup>c</sup>
6	T-MC	0.93 <sup>ab</sup>	1.88 <sup>a</sup>	2.77 <sup>a</sup>	3.85 <sup>a</sup>	4.65 <sup>a</sup>
7	T-N	0.42 <sup>d</sup>	1.67 <sup>de</sup>	1.92 <sup>c</sup>	2.24 <sup>d</sup>	2.90 <sup>d</sup>
	LSD	0.03**	0.07**	0.26**	0.21**	0.25**
	CV%	0.38	0.05	0.15	0.24	0.22

\*Average of four replication, LSD: Least significant difference, CV: Coefficient of variation. Figures in the column superscript having common letter(s) do not differ significantly at 5% levels by LSD. LSD value with double asterisk indicates highly significant difference between the treatments.

### Percent inhibition of *Sclerotium rolfsii* pathogen colony in dual culture

All the tested Trichoderma isolates significantly ( $p \leq 0.01$ ) inhibited the colony growth of the *S. rolfsii* pathogen in in-vitro condition. Among the tested Trichoderma isolates of different origin, Kapilvastu isolate had maximum colony inhibition percent at 24 hours (79.38%), 48 hours (58.54%), 72 hours (68.33%), 96 hours (70.29%) and 120 hours (72.47 %) (Table 3). Similarly minimum colony inhibition percent at 24 hours (52.26 %) was possessed by Rampur, Chitwan isolate, at 48 hours after inoculation by Mangalpur, Chitwan isolate (51.91%), at 72 hours after inoculation by Mangalpur, Chitwan (53.43%) followed by Rampur, Chitwan (54.69%), at 96 hours after inoculation by Rampur, Chitwan (44.90%) followed by Mangalpur, Chitwan (45.92%) and at 120 hours after inoculation by Mangalpur, Chitwan (48.33%) followed by Rampur, Chitwan (49.33%).



**Figure 4: Antagonistic effect of Trichoderma isolates against *S. rofsii* pathogen in in-vitro condition**

Maximum colony inhibition after 120 hours of inoculation was observed in Kapilvastu isolate (72.47%) followed by Nepalgunj isolate (67.72%). After 120 hours of inoculation, the control plate was fully colonized with mycelium and reading was stopped (Table 3).

**Table 3: Percent inhibition of *Sclerotium rofsii* colony in dual culture with Trichoderma isolates**

S.N	Trichoderma Isolates	Linear colony growth inhibition%				
		24 hour*	48 hour*	72 hour*	96 hour*	120 hour*
1	T-D	75.26 <sup>bc</sup>	56.25 <sup>bc</sup>	62.24 <sup>a</sup>	64.88 <sup>b</sup>	66.38 <sup>bc</sup>
2	T-RC	48.67 <sup>e</sup>	53.50 <sup>de</sup>	54.69 <sup>c</sup>	44.90 <sup>d</sup>	49.33 <sup>e</sup>
3	T-RK	52.26 <sup>d</sup>	55.10 <sup>cd</sup>	60.15 <sup>b</sup>	59.26 <sup>c</sup>	57.50 <sup>d</sup>
4	T-K	79.38 <sup>a</sup>	58.54 <sup>a</sup>	68.33 <sup>a</sup>	70.29 <sup>a</sup>	72.47 <sup>a</sup>
5	T-S	74.20 <sup>c</sup>	55.29 <sup>cd</sup>	61.82 <sup>b</sup>	61.37 <sup>c</sup>	63.88 <sup>c</sup>
6	T-MC	50.53 <sup>d</sup>	51.91 <sup>e</sup>	53.43 <sup>c</sup>	45.92 <sup>d</sup>	48.33 <sup>e</sup>
7	T-N	77.26 <sup>b</sup>	57.27 <sup>ab</sup>	67.70 <sup>a</sup>	68.53 <sup>a</sup>	67.72 <sup>b</sup>
	LSD	2.11**	1.81**	4.46**	3.007**	2.81**
	CV%	0.20	0.042	0.098	0.16	0.14

\*Average of four replication, LSD: Least significant difference, CV: Coefficient of variation. Figures in the column superscript having common letter(s) do not differ significantly at 5% levels by LSD. LSD value with double asterisk indicates highly significant difference between the treatments.

Vey *et al.* (2001) reported that there are large varieties of volatile secondary metabolites produced by Trichoderma such as ethylene, hydrogen cyanide, aldehydes and ketones which play an important role in controlling the plant pathogen. In this study Nepalgunj isolates showed 66.17% colony inhibition of *S. rofsii* (Table 3). This finding was almost similar with the results that were reported by Pandey and Devkota (2020). After 120 hours of inoculation, control plate of 9 cm diameter was fully covered with mycelial growth of *S. rofsii* but the growth of *S. rofsii* pathogen in Nepalgunj and Kapilbastu isolate was 2.47cm and 2.90 cm,

respectively (Table 2). Among the seven *Trichoderma* isolates studied, mycelium growth inhibition of *S. rolfsii* ranged from 48.33% to 72.47%. Among which two isolates (Kapilvastu and Nepalgunj) showed good antagonistic activity against *S. rolfsii* and were also highly competitive in in-vitro condition (Figure 4). It is also found that the microorganisms that naturally survive in the soil are having more or less similar potential antagonistic effect on the various crop disease caused by various pathogens (Howel, 2003).

Subedi *et al.* (2019) also reported the effectiveness of native *Trichoderma* isolates against different soil borne pathogens of lentil. Khattabi *et al.* (2004) also found varied antagonistic effectiveness of the *T. harzianum* isolates collected from the same locations Doukkala region of Morocco. Highest mycelium colony inhibition by *Trichoderma* isolates from Nepalgunj and Kapilvastu might be due to higher production of nonvolatile antibiotics which were active against *S. rolfsii* pathogen by disrupting the mycelial growth. Moreover lentil is grown more in western terai of Nepal and root rot problem is evident in those areas, so the native isolates collected from Nepalgunj and Kapilvastu might have antagonistic effect against *S. rolfsii*.

## CONCLUSION

It is clear that *Trichoderma* isolates are an effective means of biological control of the diseases caused by *S. rolfsii*. However, further study should be carried out to isolate more number of *Trichoderma* from different parts of Nepal and explore the antagonistic property so that *Trichoderma* spp with most efficient antagonistic value could be used in field condition for the biological control of the diseases caused by *Sclerotium rolfsii*.

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## Authors' contribution

L. Aryal designed and conducted the experiment, recorded and analyzed data and wrote the manuscript. S. Baidya gave idea in conducting the experiment. S. Subedi helped in editing the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the study, and approved it for publication.

## Conflicts of Interest

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

## REFERENCES

- Agrios, G.N. (2005). Plant Pathology. (5<sup>th</sup> ed.) San Diego, CA: Academic Press. pp.599-600.
- Camporota, P. (1985). Antagonisme in vitro de *Trichoderma* spp. vis-à-vis de *Rhizoctonia solani* Kühn. *Agronomie*, 5, 613-620.
- Cilliers, A. J., Herselman, L., & Pretorius, Z.A. (2000). Genetic variability within and among mycelia compatibility groups of *Sclerotium rolfsii*. *South Africa Phytopathology*, 90, 1026- 1031.
- Gurha, S. N., & Dubey, R.S. (1982). Occurrence of possible sources of resistance in chickpea (*Cicer arietinum* L.) against *S. rolfsii* Sacc. *Madras Agriculture Journal*, 70, 63- 64.

- Hemant, G., Kumar, P.K.R., Niharika, P.S., & Kolli, S.K. (2016). Fungicides effect on soil micro flora in Tekkali Mandal, Srikakulam (Dist.). *International Journal of Research and Development in Pharmacy and Life Sciences*, 5, 2245-2250.
- Howel, C.R. (2003). Mechanisms employed by *T. harzianum* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease*, 87, 4–10
- Jegathambigai, V., Wijeratnam, W., & Wijesundera, R.L.C. (2010). Effect of *Trichoderma* sp. on *Sclerotium rolfsii*, the causative agent of collar rot on *Zamioculcas zamiifolia* and an on farm method to mass produce *Trichoderma* species. *Plant Pathology*, 9, 47-55.
- Jomduang, J., & Sariah, M. (1997). Antagonistic effect of Malaysian isolates of *Trichoderma harzianum* and *Gliocladium virens* on *Sclerotium rolfsii*. *Pertanika Journal of Tropical Agricultural Science*, 20, 35-42
- Khattabi, N., Ezzahiri, B., Louali, L., & Oihabi, A. (2004). Antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii*: screening of efficient isolates from Morocco soils for biological control. 2004. *Phytopathology Mediterranean*, 43, 332-340
- Njambere, E., & Chen, W. (2011). Compendium of Chickpea and Lentil Diseases and Pests. St Paul, MN: The American Phytopathology Society, pp.13-15.
- Pandey, P., & Devkota, R. (2020). In Vitro Evaluation of Efficacy of *Trichoderma* isolates against *Sclerotium rolfsii*. 2020. *Journal of Experimental Research*, 8, 1-7
- Sain, S.K., & Pandey, A.K. (2016). Spectrum of three isolates of *Trichoderma harzianum* Rifai against important fungal diseases of tomato. 6<sup>th</sup> International Conference Plant Pathogens and People. Feb.23-27, New Delhi, India
- Sanchez, V., Rebolledo, O., Picaso, R.M., Cardenas, E., Cordova, J., & Samuels, G.J. (2006). In vitro antagonism of *Theieloviopsis paradoxa* by *Trichoderma longibrachiatum*. *Mycopathologia*, 163, 49-58.
- Singh, S.R., Singh, S.K., & Pandey, R.K. (2012). Integrated management of collar rot of lentil caused by *Sclerotium rolfsii*. *International Journal of Plant Protection*, 5, 386-390.
- Subedi, S., Neupane, S., Shrestha, J., & Ghimire, T.N. (2019). Efficacy of botanicals, bio-control agents and fungicides against wilt root rot complex of lentil. *Agriways*, 7, 69-74.
- Vey, A., Hoagland, R.E., & Butt, T.M. (2001). Toxic Metabolites of Fungal Biocontrol Agents. *Fungi as biocontrol agents*, 3, 11.