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In-vitro efficacy of *Trichoderma* isolates on *Sclerotium rolfsii* causing collar rot of chili

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Abstract. *Yadav D, Adhikari A, Dhuingana B, Gurung H, Khatri N, Pandit S. 2022. In-vitro efficacy of* Trichoderma *isolates on* Sclerotium rolfsii *causing collar rot of chili. Asian J Agric 6: 97-102.* The experiment was conducted in the Nepal polytechnic institute plant pathology laboratory to study the in-vitro efficacy of *Trichoderma* isolates on *Sclerotium rolfsii* Sacc. collar of chili, Bharatpur, Chitwan, Nepal by dual culture technique. The experiment was carried out in a completely randomized design (CRD) with four replications. The *Trichoderma* isolates, namely Kapilvastu isolate, Kavre isolates, Salyan isolates, Lalitpur isolates, and Taplejung isolates, were used in the experiment. The mycelium growth was measured at 2 DAI, 4 DAI, 6 DAI, 8 DAI, and 10 DAI. Also, the number of sclerotia, days to sclerotia, and width of the browning area at the interception region of interception were measured in 10 DAI. All the *Trichoderma* isolates significantly affect mycelium growth and the number of sclerotia formed. Among all the *Trichoderma* isolates, Kavre isolates show a good result with (74.44%) followed by Salyan isolates (74.22%) and Lalitpur isolates (73.55%) inhibition in the mycelium growth and several sclerotia (9.6~10) also formed. The lowest number of sclerotia was observed in Salyan isolates, which was three days, followed by Kapilvastu isolate, i.e., 20 days. The antagonist Kavre isolate can be used as a bio-control agent against *S. rolfsii* of chili in Nepal.

Keywords: Sclerotia, *Sclerotium rolfsii*, *Trichoderma* isolates

INTRODUCTION

Chili (*Capsicum annuum* L.) is mainly grown in almost all planes throughout the country of Nepal, whereas the green pepper is grown relatively at little high elevations, also where the climate is relatively mild. Chili belongs to the family Solanaceae, locally known as Khursani. Chili is a critical vegetable commodity with future business opportunities, containing excessive calories, protein, fat, carbohydrates, calcium, and vitamins A, B1, and C (Piay et al. 2010; Yanti et al. 2016). The chilies are used in green as well as dry in powdered form. It's a wealthy supply of vitamins A and C, among most vegetables, for spices, pickles, and sauces. In Nepal, the green chili production was 120,462 tons under a 12,134-ha cultivated area yielding 9,927 kg/ha (FAO 2019). The dry production of chili was 67,167 tons under a 10,690-ha cultivated area yielding 6,283 kg/ha (FAO 2019). *Sclerotium rolfsii* Sacc. is an important disease that has spread globally and causes diseases in many economically important crops (Khan et al. 2020; Jabeen et al. 2021). The soil-borne fungus is a subgroup of Deuteromycotina, which contains more than 500 species of cultivated and wild plants in tropical and subtropical areas (Javid et al. 2022). Many fungal, bacterial, and viral diseases affect the crop, resulting in huge economic losses. Among fungal infections, *S. rolfsii*, responsible for southern blight in chilies, is the most common (Javaid et al. 2020; Sharf et al. 2021). The *S. rolfsii* causing collar rot in chili may occur in any plant growth stage (Haque et al. 2001). Allowing the leaves and the growth of dark brown sores in the collar area along the soil line are symptoms of the disease, which leads to the complete drying of the plant (Mahadevakumar et al. 2018). The rot-affected plant has a fungal infection in the column region, just above the soil level, in the form of a griddle. With white mycelium, the grilling rises to the top. Wilting begins within 3-5 days, and when the green canopy is removed, the entire plant will dry out (Daunde et al. 2018).

The *S. rolfsii*, the polyphagous fungus, has a host range of more than 500 species in about 100 families, including nuts, green beans, lima beans, onions, garden beans, peppers, potatoes, sweet potatoes, tomatoes, and tomatoes watermelons worldwide, causing great loss (Aycock 1966). The *S. rolfsii* grows well in highly active areas and thus thrives well near the underground. Natural conditions conducive to fungus and disease growth are high temperatures (27 to 35°C), humid conditions, and acidic soils (Mullen 2000). Initially, this fungal infection could cause a decrease of up to 53.4% in yield quality and some pepper plants (Fery and Dukes 2011). Most of the diseases, collar rot or pepper rot, due to *S. rolfsii* occur in almost all pepper growing regions. It reasons a decrease in yield to 16-80% (Daunde et al. 2018). The pathogen is found to cause various symptoms like crown rot, root rot, stem rot, rotting of pseudo bulbs, wilt, and the gradual death of the plants, etc. (Agrios 2005). The pathogen is very common in tropical, subtropical, and warm temperate regions. Collar rot is prominent in one-month-old seedlings. Under field

conditions, the pathogen has been reported to cause a 30 to 60% reduction in the yield of chickpeas. Because of the prolific growth of and ability to produce persistent sclerotia, it is contributing to a high degree of economic losses. Under conducive conditions, it can cause 55-95% mortality of the crop at the seedling stage (Gurha and Dubey 1982). Since chemicals are hazardous to soil and users, biological control agents are the best alternative to toxic chemicals. Among the biocontrol agents, *Trichoderma* spp. was the most effective against many soil-borne pathogens (Eziashi et al. 2006). Dennis and Webster (1971) described the antagonistic properties of *Trichoderma* in antibiotic production and hyphal interactions. The species of *Trichoderma* are capable of hyper-parasitizing pathogenic fungi and are highly efficient antagonists (Iqbal and Mukhtar 2020). Sanchez et al. (2006) reported that *Trichoderma* species could inhibit the growth of plant pathogens, especially fungi, through competition for nutrients, enzymes, substrate, oxygen, and space.

Trichoderma is a fungus found in all soils and is the most commonly occurring fungus. Many species of this genus can be identified as opportunistic virulent plant symbionts (Harman et al. 2004). That refers to the capability of several *Trichoderma* species to form harmonious endophytic relationships with numerous plant species (Bae et al. 2011). *Trichoderma* spp. is known as mycoparasites of some plant pathogens. For example, the *T. harzianum* Rifai colonizes *S. rolfsii* hyphae, disrupts mycelial growth, and kills the organism (Ali et al. 2020). *Trichoderma* can potentially suppress the boom of pathogenic fungi (Jegathambigai et al. 2010; Khan and Javaid 2020; Khan et al. 2021). It has also been reported that *Trichoderma* species with different mechanisms, such as lysis of sclerotia, inhibit the growth of mycelial *S. rolfsii* with flexible metabolites that produce and parasites hyphal styles of disease agent (Shaigan et al. 2008). The genome of many *Trichoderma* spp. edited and publicly available at the Joint Genome Institute (Mycocosm 2021). Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. is well-documented as an effective biological control agent for plant diseases (Sain and Pandey 2016). Therefore, the present research was carried out to evaluate the native *Trichoderma* spp. against *S. rolfsii*, causing collar rot of chili.

MATERIALS AND METHODS

Sample collection

Diseases of plants infected with *S. rolfsii* of chili wilt were identified in the field by specific symptoms, i.e., the development of browning discoloration in the root surface of chili. First, the pure culture of *S. rolfsii* was acquired from the AFU Rampur, Chitwan, Nepal, and Laboratory. Then the samples were preserved at 4°C centigrade in the refrigerator for isolation of *S. rolfsii*.

Media preparation

Potato dextrose agar media was prepared with the composition of 200 g peeled potato agar, 20 g of dextrose, and 20 g for one liter of the final volume of water. The media was autoclaved at 121℃ and 15 psi for 15 to 20 minutes and allowed to cool to bring around 50 to 60°C at room temperature before pouring sterilizing the glass wares under the hot air oven.

Prepared PDA powder was also used with the composition of 42 g for one liter of the final volume of water. The media was sterilized in an autoclave at 15 psi (125°C) for 15-20 minutes. The sterilized media was allowed to cool at 50 to 60°C, pouring the sterilized media into the Petridis.

Collection and isolation of the pathogen

Diseases plants infected with *S. rolfsii* of chili wilt were identified in the field by specific symptoms. Pure culture of *S. rolfsii* was acquired from the AFU Rampur, Chitwan, and Laboratory. Then the samples were preserved at 4°C centigrade in the refrigerator for isolation of *S. rolfsii*. Potato Dextrose Agar media was prepared and autoclaved at 121°C and 15 psi for 15 to 20 minutes and allowed to cool to bring around 50 to 60℃ to room temperature before pouring sterilized the glass wares under the hot air oven. The pure culture of the segregated areas was repaired following hyphal tip methods and later transferred to a new PDA site.

Dual culture technique

Culture discs (5 mm) of *Trichoderma* and the pathogen taken from the edges of the cultures grow vigorously and are evenly spaced approximately seven inches from 9 mm Petri plates containing a 20 mL PDA. With each treatment, a minimum of four responses were maintained, and controls were maintained by placing a pathogen disc only in the center (Singh et al. 2019). Petrol plates are then heated to 24 ± 2ºC. The pathogen's growth and Trichoderma's ability to prevent the pathogen were documented from time to time. The reduction of the growth percentage (Pi) of the experimental pathogen is calculated when the growth of *S. rolfsii* is filled with control plates using the following formula (Vincent 1947).

$$\mathrm{Pi} = \frac{C-T}{C} \times 100$$

Where
Pi : Percent growth reduction of test pathogen
C : Radial growth of test pathogen in control (mm)
T : Radial growth of test pathogen in treatment (mm)

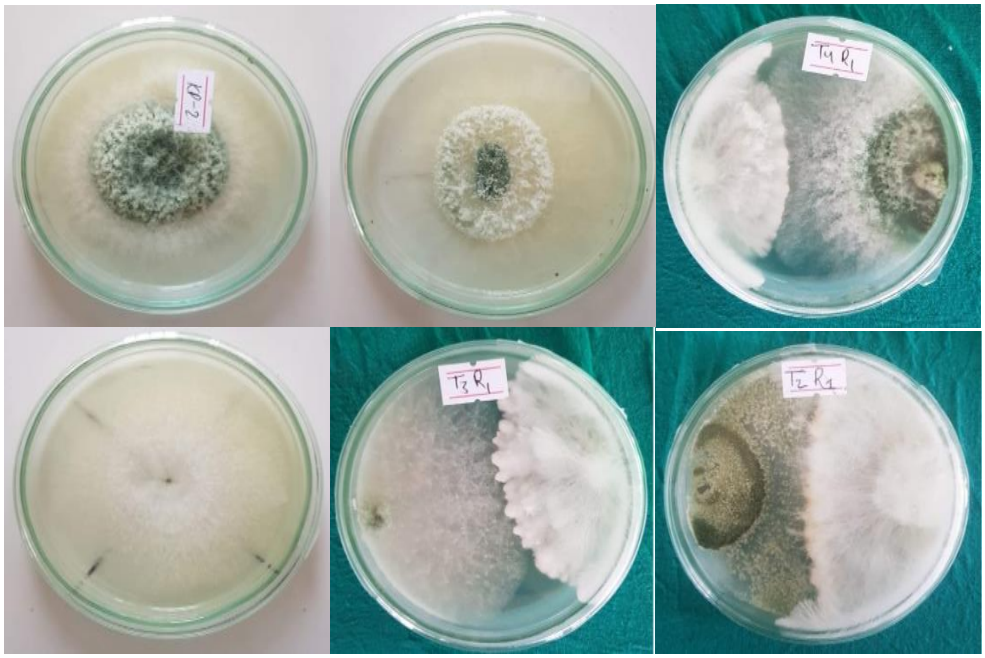

Figure 1. *Trichoderma* isolates and species selected for study

Statistical analysis

All recorded information was processed into the R-studio, and analysis was performed using R 4.0.4 (R Core Team (2013) and the Agricola 1.1-8 version package (De Mendiburu (2014). The data entry was done to develop an ANOVA table, and different treatments were compared by Duncan's multiple range test and the least significant difference at a 5% significance level. All the figures and graphs were prepared using Microsoft excel 2013.

RESULTS AND DISCUSSION

The radial mycelium growth of *S. rolfsii* for all treatments varied significantly at 2, 4, 6, 8, and 10 DAI (P≤0.001) (Figure 1). The highest inhibition percentage of mycelial growth was found in Kavre isolate, i.e., 52.41, 69.11, 69.77, 72.00, 74.44 after 2, 4, 6, 8, and 10 days of incubation, respectively. Whereas Salyan isolates, Taplejung isolates, Kavilvastu isolates, and Lalitpur isolates were found at par (Table 1).

This result is supported by Elad et al. (1984), and Bastakoti et al. (2017) stated *Trichoderma* species could produce extracellular lytic enzymes responsible for their antagonistic activity. They also reported a similar positive effect on *Trichoderma* species. Therefore, it can be assumed that *T. harzianum* attacks the pathogen's mycelium by penetrating its cell wall in a certain location (Chet et al. 1981). In field condition, *Trichoderma viride* Pers. reduce disease incidence by 75.54% (Jegathambigai et al. 2010). It is supported by Pandey and Gaire (2019), Rampur rice field isolate (0.42) and Tarhara, Sunsari isolates (0.55) were found to be better performance against *S. rolfsii* under the width of the browning region at the point of interaction. The present findings corroborate the results of Rasu et al. (2013). They screened different *Trichoderma* spp. against *S. rolfsii*, among all *T. asperellum* (TTH1) exhibited 64.40% of mycelial growth inhibition. Further, Darvin et al. (2013) recorded 56.25% of mycelial growth inhibition of *S. rolfsii* through *T. harzianum*.

The days of sclerotia formed in the dual culture of *S. rolfsii* for all treatments varied significantly at 10 DAI (P≤0.001)(Table 2). Lalitpur isolate takes a long period to form sclerotia, i.e., 9.6~10 days statistically at par with Salyan isolate (9.6~10 days), Kavre isolates (8.8~9 days), Kapilvastu isolates (8.8~9 days), and Taplejung isolate (8.8~9 days). In contrast, early sclerotia formation was observed in control with 5.8~6 days. *Trichoderma* isolates killed 62-100% of the sclerotia within 25 days of inoculation (Dos and Dhingra 2011). This result is supported by Ordóñez et al. (2015); the process of forming sclerotia ends on the 9th day when they were easy to detach from the culture medium and had a black coloration. Mycelium was hyaline, thin-walled, sparsely septate hyphae. Several others showed similar morphological characteristics of the pathogen's sclerotia (Singh and Thapliyal 1998; Swart et al. 2003; Jeeva et al. 2005).

Table 1. Inhibition percentage of *Sclerotium rolfsii* against *Trichoderma* isolates

Treatment (*Trichoderma* isolates)	Inhibition percentage (%)				
	2DAI	4DAI	6DAI	8DAI	10DAI
Taplejung isolate	50.9 [a] (45.50)	65.33[a](53.94)	67.55[a](55.29)	69.33[a](56.38)	70.44 [a] (57.08)
Salyan isolate	51.25 [a] (45.69)	69.11[a](56.25)	69.55[a](56.54)	71.78[a](57.95)	74.22 [a](59.51)
Lalitpur isolate	50.52 [a] (45.28)	67.78[a](55.40)	68.66[a](55.95)	71.78[a](57.92)	73.55 [a] (59.08)
Kavre isolate	52.41 [a] (46.36)	69.11[a](56.24)	69.77[a](56.67)	72 [a] (58.09)	74.44 [a] (59.67)
Kapilvastu isolate	50.89 [a] (45.49)	68.22[a](55.66)	68.66[a](55.94)	70.44[a](57.04)	71.55 [a] (57.75)
Control (*Sclerotium rolfsii* Sacc.)	0 [b] (2.56)	0 [b] (2.56)	0 [b] (2.56)	0 [b] (2.56)	0 [b] (2.56)
Mean	42.69(38.48)	56.62(46.68)	57.40(47.16)	59.25(48.32)	60.74(49.27)
CV	3.15	4.15	4.35	4.51	4.25
LSD	1.58***	2.53***	2.68***	2.85***	2.73***
SEm(±)	0.77	1.22	1.29	1.38	1.32

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

Table 2. Days to sclerotia formed in dual culture

Treatment (*Trichoderma* isolates)	Days to sclerotia formation
Taplejung isolate	(8.8 [a])
Salyan isolate	(9.6 [a])
Lalitpur isolate	(9.6 [a])
Kavre isolate	(8.8 [a])
Kapilvastu isolate	(8.8 [a])
Control (*Sclerotium rolfsii* Sacc.)	(5.8 [b])
Mean	8.57
CV	11.07
LSD	1.24***
SEm(±)	0.6

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

Table 3. Number of sclerotia formed in dual culture

Treatment (*Trichoderma* isolates)	No. of sclerotia
Taplejung isolate	24.8 [c] (5.01)
Salyan isolate	2.8 [d] (1.80)
Lalitpur isolate	62 [b] (7.84)
Kavre isolate	27 [c] (5.26)
Kapilvastu isolate	20 [c] (4.56)
Control (*Sclerotium rolfsii* Sacc.)	501 [a] (22.38)
Mean	106.43(7.81)
CV	8.78
LSD	0.89***
SEm(±)	0.43

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

The number of sclerotia formed in the dual culture of *S. rolfsii* for all treatments varied significantly at 10 DAI (P≤0.001)(Table 3). The lowest number of sclerotia was observed in the Salyan isolate (2.8~3), followed by the Kapilvastu isolate (20), statistically at par with the Taplegunj isolate (24.8~25) and Kavre isolates (27), followed by Lalitpur isolate (62) whereas the maximum number of sclerotia observed in control (501). The ability of *T. viride* to control *S. rolfsii* infection has been attributed to the ability of *Trichoderma* to parasitize the sclerotia (Jegathambigai et al. 2010).

Table 4. Width of the browning area at the region of interception in dual culture

Trichoderma isolates	Width of the browning area at the region of interception in dual culture (*Sclerotium rolfsii*)
Kapilvastu isolates	0.78 [c]
Kavre isolates	0.96 [b]
Lalitpur isolates	1.14 [a]
Salyan isolates	0.56 [d]
Taplejung isolates	1.06 [ab]
Mean	0.9
CV	13.52
LSD	0.16***

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

Decreased concentrations were less inhibitory to the growth of *S. rolfsii*. Kapil and Kapoor (2005) tested the volatile and non-volatile metabolites of bioagents viz. *T. harzianum*, *T. viride*, and *T. atroviride* significantly reduced the mycelial growth and germination of *Sclerotinia sclerotiorum.* This result is supported by Mishra et al. (2011) reported the efficacy of culture filtrate of *T. viride* Tr 8 against *M. phaseolina* and other soil-borne pathogens under in vitro conditions that also suppressed the growth of test pathogen.

The number of sclerotia formed in the dual culture of *S. rolfsii* for all treatments varied significantly at 10 DAI (P≤0.001)(Table 4). The maximum width of the browning area at the interception region in dual culture was observed in the Lalitpur isolate (1.14 cm). Followed by Lalitpur isolate (1.14 cm), Kavre isolates (0.96 cm), Kapilvastu isolates (0.78 cm), and the minimum width of the browning area at the region of interception in dual culture was observed in Salyan isolate (0.56 cm). Rampur rice field isolate (0.42 cm) and Tarhara, Sunsari isolate (0.55 cm) were found to be better performing against *S. rolfsii* under the width of the browning region at the point of interaction, Supported by (Pandey and Gaire 2019). A similar result was shown by Pandey and Devkota (2020) as they stated

that the average growth of mycelium of *S. rolfsii* on PDA plates treated with liquid culture filtrate (LCF) of *Trichoderma* species was maximum. The width of the area was 0.775 cm. (Darvin et al. 2013) reported that *T viride* isolate completely inhibited the mycelial growth of *S. rolfsii* through poisoned food technique.

In conclusion, *S. rolfsii*, the polyphagous fungus, has a wide host range of vegetables. Therefore, it is an important pathogen to be controlled by integrated pest management. Using botanicals and bio-agents provides an alternative to synthetic pesticides with the advantage of minimizing the cost of cultivation and avoiding health hazards. *Trichoderma* is an eco-friendly fungus, nonhazardous to soil and human beings. From the in vitro findings, it can be suggested that among all the *Trichoderma* isolates, Kavre isolates, on average, shows a good result in controlling this. Therefore, it is recommended that the biological agent Kavre isolate be suggested to the farmers to manage the collar rot of chili.

REFERENCES

Agrios GN. 2005. Plant pathology. 5th Edition. In: Root and Stem Rots Caused by Basidiomycetes. Elsevier Academic Press, USA.

Ali A, Javaid A, Shoaib A, Khan IH. 2020. Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. Egypt J Biol Pest Control 30: 102. DOI: 10.1186/s41938-020-00305-1.

Aycock R. 1966. Stem Rot and Other Diseases Caused by *Sclerotium rolfsii*: or the Status of Rolfs' Fungus After 70 Years. North Carolina State Univ., US.

Bae H, Roberts DP, Lim HS, Strem MD, Park SC, Ryu CM, Melnick RL, Bailey BA. 2011. Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. Molecular Plant-Microbe Interactions: MPMI 24 (3): 336-351. DOI: 10.1094/MPMI-09-10-0221.

Bastakoti S, Belbase S, Manandhar S, Arjyal C. 2017. *Trichoderma* species as biocontrol agent against soil borne fungal pathogens. Nepal J Biotechnol 5 (1): 39-45. DOI: 10.3126/njb.v5i1.18492.

Chet I, Harman GE, Baker R. 1981. *Trichoderma* hamatum: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb Ecol 7 (1): 29-38. DOI: 10.1007/BF02010476.

Darvin G, Venkatesh I, Reddy NG. 2013. Evaluation of *Trichoderma* spp. against *Sclerotium rolfsii* in vitro. Intl J Appl Biol Pharm Technol 4 (4): 268-272.

Daunde AT, Apet KT, Suryawanshi AP, Khandare VS. 2018. Prevalence of collar rot of chili caused by *Sclerotium rolfsii* Sacc. under the agro-climatic zones of Marathwada region of Maharashtra. J Pharmacogn Phytochem 7 (4): 1905-1908.

de Mendiburu F. 2014. Agricolae version 1.1-8 Practical Manual. La Molina National Agrarian University, Lima, Peru.

Dennis C, Webster J. 1971. Antagonistic properties of species groups of *Trichoderma*. III. Hyphal interaction. Trans Brit Mycol Soc 57 (3): 363-369. DOI: 10.1016/S0007-1536(71)80050-5.

Dos AFD, Dhingra OD. 2011. Pathogenicity of *Trichoderma* spp. on the sclerotia of *Sclerotinia sclerotiorum*. Canad J Bot 60 (4): 472-475. DOI: 10.1139/B82-064.

Elad Y, Barak R, Chet I. 1984. Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*. Soil Biol Biochem 16 (4): 381-386. DOI: 10.1016/0038-0717(84)90037-3.

Eziashi EI, Uma NU, Adekunle AA, Airede CE. 2006. Effect of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium. Afr J Biotechnol 5 (9): 703-706. DOI: 10.3923/pjbs.2006.1987.1990.

FAO. 2019. Food and Agriculture Organization of the United Nations Statistics Division. (Accessed 23 February 2021). http://www.fao.org/faostat/en/data/QC/visualize/.

Fery RL, Dukes PD. 2011. Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: Genetic characterization of two sources of resistance. Intl J Agron 2011: 652404. DOI: 10.1155/2011/652404.

Gurha SN, Dubey RS. 1982. Occurrence of possible sources of resistance in chickpea (*Cicer arietinum* L.) against *Sclerotium rolfsii* Sacc. Madras Agric J 70: 63-64.

Haque MMM, Siddique MAB, Meah MB, Islam MN. 2001. Control of foot rot of brinjal through chemicals and organic soil amendments. Sci J Biol Sci 1 (10): 946-948. DOI: 10.3923/jbs.2001.946.948.

Harman GE, Howell CR. Viterbo A, Chet I, Lorito M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2 (1): 43-56. DOI: 10.1038/nrmicro797.

Iqbal U, Mukhtar T. 2020. Evaluation of biocontrol potential of seven indigenous *Trichoderma* species against charcoal rot causing fungus, *Macrophomina phaseolina*. Gesunde Pflanzen 72 (2): 195-202. DOI: 10.1007/s10343-020-00501-x.

Jabeen N, Javaid A, Shoaib A, Khan IH. 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel*. J Plant Pathol 103 (3): 901-913. DOI: 10.1007/s42161-021-00874-6.

Javaid A, Afzal R, Shoaib A. 2020. Biological management of southern blight of chili by *Penicillium oxalicum* and leaves of *Eucalyptus citriodora*. Intl J Agric Biol 23 (1): 93-102. DOI: 10.17957/IJAB/15.1263.

Javid M, Khan FN, Arif U. 2022. Income and price elasticities of natural gas demand in Pakistan: A disaggregated analysis. 2022: 106203. DOI: 10.1016/j.eneco.2022.106203.

Jeeva ML, Hegde V, Makeshkumar T, Nair RR, Edison S. 2005. *Dioscorea alata*, a new host of *Sclerotium rolfsii* in India. Plant Pathol 54 (4): 574-574. DOI: 10.1111/j.1365-3059.2005.01204.x.

Jegathambigai V, Wilson Wijeratnam RS, Wijesundera RLC. 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 Pathol 9 (2): 47-55. DOI: 10.3923/PPJ.2010.47.55.

Khan IH, Javaid A, Ahmed D. 2021. *Trichoderma viride* controls *Macrophomina phaseolina* through its DNA disintegration and production of antifungal compounds. Intl J Agric Biol 25 (4): 888-894. DOI: 10.1080/00275514.2021.1990627.

Khan IH, Javaid A, Al-Taie AH, Ahmed D. 2020. Use of neem leaves as soil amendment for the control of collar rot disease of chickpea. Egypt J Biol Pest Control 30: 98. DOI: 10.1186/s41938-020-00299-w.

Khan IH, Javaid A. 2020. In vitro biocontrol potential of *Trichoderma pseudokoningii* against *Macrophomina phaseolina*. Intl J Agric Biol 24 (4): 730-736.

Mahadevakumar S, Chandana C, Deepika YS, Sumashri KS, Yadav V, Janardhana GR. 2018. Pathological studies on the southern blight of China aster (*Callistephus chinensis*) caused by *Sclerotium rolfsii*. Eur J Plant Pathol 151 (4): 1081-1087. DOI: 10.1007/s10658-017-1415-2.

Mishra BK, Mishra RK, Mishra RC, Tiwari AK, Yadav RS, Dikshit, A. 2011. Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. Arch Appl Sci Res 3 (2): 361-369.

Mullen J. 2000. Southern Blight, Southern Stem Blight, White Mold. The Plant Health Instructor. DOI: 10.1094/PHI-I-2001-0104-01.

Mycocosm 2021. https://mycocosm.jgi.doe.gov/mycocosm/home, accessed on 19 December 2021

Ordóñez-Valencia C, Ferrera-Cerrato R, Quintanar-Zúñiga RE, Flores-Ortiz CM, Guzmán GJM, Alarcón A, García-Barradas O. 2015. Morphological development of sclerotia by *Sclerotinia sclerotiorum*: A view from light and scanning electron microscopy. Annals Microbiol 65 (2): 765-770. DOI: 10.1007/s13213-014-0916-x.

Pandey A, Devkota S. 2020. Prospects and challenges of sugarcane development in Nepal: Production, market and policy. Am J Agric Biol Sci 15 (1): 98-106. DOI: 10.3844/ajabssp.2020.98.106.

Pandey P, Gaire SP. 2019. Effect of different media on mycelium growth of *Sclerotium rolfsii* Sacc. in vitro condition. Intl J Environ Agric Biotechnol 4 (4): 1195-1198. DOI:10.22161/IJEAB.4445.

Piay SS, Tyasdjaja A, Ermawati Y, Hantoro FRD. 2010. Budidaya dan Pascapanen Cabai Merah (*Capsicum annuum*). Balai Pengkajian Teknologi Pertanian Jawa Tengah, Badan Penelitian dan Pengembangan Pertanian. [Indonesian]

R Core Team. 2013. R A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Rasu T, Sevugapperumal N, Thiruvengadam R, Ramasamy S. 2013. Biological control of sugarbeet root rot caused by *Sclerotium rolfsii*. Intl J Biol Ecol Environ Sci 2: 7-10.

Sain SK, Pandey AK. 2016. Efficacy of three isolates of Trichoderma harzianum Rifai against major fungal pathogens of tomato. Phytopathology 69 (4): 510-515.

Sanchez V, Rebolledo O, Picaso RM, Cardenas E, Cordova J, Samuels GJ. 2006. In vitro antagonism of *Theieloviopsis paradoxa* by *Trichoderma longibrachiatum*. Mycopathologia 163: 49-58. DOI: 10.1007/s11046-006-0085-y.

Shaigan S, Seraji A, Moghaddam SAM. 2008. Identification and investigation on antagonistic effect of *Trichoderma* spp. on tea seedlings white foot and root rot (*Sclerotium rolfsii* Sacc.) in vitro condition. Pak J Biol Sci 11 (19): 2346-2350. DOI: 10.3923/PJBS.2008.2346.2350.

Sharf W, Javaid A, Shoaib A, Khan IH. 2021. Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis*. Egypt J Biol Pest Control 31: 16. DOI: 10.1186/s41938-021-00364-y.

Singh U, Thapliyal PN. 1998. Effect of inoculum density, host cultivars and seed treatment on the seed and seedling rot of soybean caused by *Sclerotium rolfsii*. Indian Phytopathol 51 (3): 244-246.

Singh UB, Malviya D, Singh S, Kumar M, Sahu PK, Singh HV, Kumar S, Roy M, Imran M, Rai JP, Sharma AK, Saxena AK. 2019. *Trichoderma harzianum*-and methyl jasmonate-induced resistance to bipolaris sorokiniana through enhanced phenylpropanoid activities in bread wheat (*Triticum aestivum* L.). Front Microbiol 10: 1697. DOI: 10.3389/fmicb.2019.01697.

Swart WJ, Tesfaendrias MT, Terblanche J. 2003. First report of *Sclerotium rolfsii* on kenaf in South Africa. Plant Dise 87 (7): 874-874. DOI: 10.1094/PDIS.2003.87.7.874A.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 159 (4051): 850. DOI: 10.1038/159850B0.

Yanti Y, Astuti FF, Habazar T, Nasution CR. 2016. Screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili. Biodiversitas 17: 1-9. DOI: 10.13057/biodiv/d180101.