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Anjali Arya
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Prashant Mishra
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Amit Yadav
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Arjun Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Ankit Kumar
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Corresponding Author:
Anjali Arya
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Collar rot disease of Lentil caused by *Sclerotium rolfsii* and its management

Anjali Arya, Prashant Mishra, Amit Yadav, Arjun Singh and Ankit Kumar

Abstract
Several diseases are known to infect lentil (*Lens culinaris*) during its growth stages. Among them, collar rot caused by *Sclerotium rolfsii,* is very common in all the major lentil growing areas. The characteristic symptoms include white fungal strands (mycelia or hyphae) around collar region of the infected plant parts and on the soil surrounding the plant. The disease causes appreciable loss in yield. Therefore, this study was conducted with the objectives on the efficacy of fungicides, bio-agents and comparative study combining various management tactics against *Sclerotium rolfsii*. Among the bio-agents the maximum inhibition was shown by *Chaetomium globosum*(83.21%) followed by *Pseudomonas fluorescens*(76%) and *Trichoderma viridae*(71.86%). Complete mycelial inhibition was recorded with fungicides Curzate M and Nativo at the concentration (100 ppm) after 96 hrs of inoculation. During the assessment of Integrated Disease Management least percent disease incidence, maximum yield was recorded in *Trichoderma* and *Pseudomonas* bioprimed seeds followed by soil application with neem cake and *Trichoderma harzianum*.

Keywords: *Sclerotium rolfsii,* trichoderma, pseudomonas

Introduction
Lentil (*Lens culinaris* L.), an important pulse crop is probably the oldest of grain legumes to be domesticated (Bahl *et al*., 1993). Lentil is known by many names in different parts of the world *viz*., Massour, Renuka, Mangu/Margu, Masura, Mangalaya etc. (Kay, 1979). In India, it is also grown as an intercrop with barley, linseed, mustard and autumn planted sugarcane. In North-eastern part of country, lentil is also cultivated as utera crop with rice. Lentil is relatively tolerant to drought and is grown throughout the world. About 17 diseases have been recorded in lentil of which 12 are caused by fungi, 2 by nematode and 2 by viruses and 1 by mycoplasma (Baker and Rashid, 2007). Among the fungal diseases, collar rot of Lentil caused by *Sclerotium rolfsii* is of wide economic importance.

Collar rot disease of Lentil
Collar rot disease on lentil crop is caused by *Sclerotium rolfsii*, a very important polyphagus pathogenic fungus causing substantial losses in quality and productivity of yield. *Sclerotium rolfsii* Sacc. is a soil-borne pathogenic fungus and has a wide host range of over 500 species (Punja and Grogan, 1988) [23]. The fungus can attack the crop during any time from seedling to flowering stage and are comparatively more destructive at the seedling stage. A typical characteristic feature of the fungus includes formation of sclerotia on the plant parts including stems and roots, on completion of its life cycle. Extensive crop damage, lack of high levels of host resistance, and the general difficulty of managing diseases caused by Sclerotium have been the impetus for sustainable research on this pathogen.

Materials and Methods
Collection and purification of the diseased specimens
Lentil plants showing the typical symptoms of Collar rot were collected from farmer’s field and from university at ‘Crop Research Centre’ Chirori (Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut) during the crop season of 2018-19. The specimens were brought to the laboratory and critically examined and studied for the symptoms of the disease and isolation of the pathogen. The part of collar region showing typical symptoms of the disease was cut into small pieces which were surface sterilized with 0.1% sodium hypochlorite solution for one minute. Such pieces were washed thoroughly in sterile distilled water three times to remove the traces of sodium hypochlorite solution, and then aseptically transferred to sterilized potato dextrose agar (PDA) plates.

These plates were incubated at 27±1 °C for three days for growth of the fungus. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions.

Mass multiplication of the pathogen

For the mass multiplication of pathogen, wheat grains were used. The wheat grains were soaked in water for one day, from which two hundred and fifty grams of wheat were taken in each of four 500 ml conical flasks.

These wheat grains were sterilized at 121 ^{O}C temperature and 1.1 kg/cm^2 for 15 min. Two to three 5 mm mycelial bits were added to each of conical flasks under aseptic conditions and kept for incubation at 28^{0}C for 30 days. The flasks were agitated regularly to obtain a uniform growth all over the flasks.

Morphology of the pathogen

The fungus grew up to 90 mm in 3 days on potato dextrose agar (PDA) medium. The pathogen *S. rolfsii* forms fan like white colonies on the PDA Plates.

The mycelium was hyaline, much branched and hyphae were thin walled, septate. The colonies appeared as pure white to dull white mycelial growth and formed sclerotial bodies after 6-7 days of incubation. Sclerotia were small, mustard shaped, white, round bodies with clamp in the beginning, later becoming light to dark brown with shine and measuring 1.0 to 1.15 mm in size. (Fig.1.)

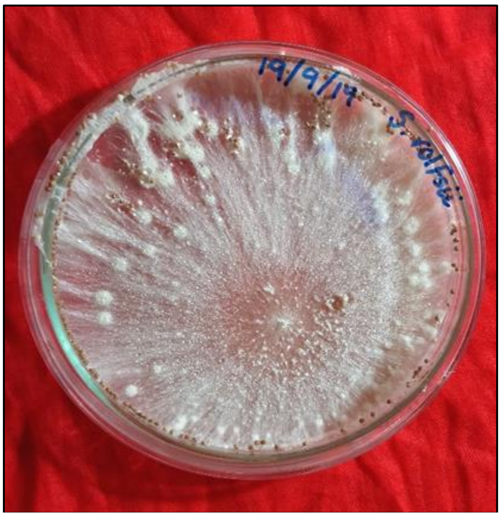

Fig 1: Fan like growth of *S. rolfsii* colony with development of sclerotia.

Symptomatology

Symptoms appeared around seedling to flowering stage and are comparatively more destructive at seedling stage. Affected plants showed various types of symptoms *viz*., yellowing, drooping, drying and shedding of leaves, the collar region of infected plant showed dark and extensive rotting with mycelial growth.

The characteristic symptoms include white fungal strands (mycelia or hyphae) around collar region of the infected plant parts and on the soil surrounding the plant. The pathogens cause damping-off of seedlings, brown and necrotic lesions girdle the stem near ground level resulting in yellowing of leaves and drying of plants.

Fig 2: White mycelial growth of *S. rolfsii* around the collar region of lentil plant.

Management of collar rot of Lentil

In recent years, considerable success has been achieved by introducing antagonists to soil (or) infection court (Papavizas and Lewis 1981; Mukhopadhy and Kaur, 1990) [21, 14]. The use of bio-primed seeds might be considered as a safe, cheap and easily applied biocontrol method against these soil borne plant pathogens (El-Mougy and Abdel-Kader, 2008) [16]. Plant defense enzymes play a vital role in mitigating pathogen-induced stress during the biological control by Biocontrol agents. Biocontrol agents (BCAs) that have been used in agriculture across the globe, provide systemic resistance to plants infested by various fungal phytopathogens (Surekha *et al*., 2014) [4]. An integrated approach by including the fungicides with different bi-oagents and plant products appears to be a possible solution for effective and economic management of Collar Rot Disease.

Evaluation of Bio-agents on growth of pathogen, *In vitro.*

Five different Bio-agents (*T. viridae, T. harzianum, C. globosum, B. subtilis, P. fluorescens*) were tested against the pathogen using Dual Culture Technique. From the present investigation, the maximum inhibition of the Pathogen was recorded with *Chaetomium globosum* i.e. 83.21% followed by *Pseudomonas fluorescens* i.e. 76.0% and Trichoderma viride i.e. 71.86% (Table.1.). Uikey *et al*. (2019) [27] reported similar results where maximum inhibition (46.66%) of *Sclerotium spp*. in treatment where *Chaetomium* was used. Banakar *et al*. (2017) [2] evaluated the efficacy of five bio-agents and observed that *Trichoderma viride* and *Trichoderma harzianum* recorded 61% and 44% inhibition respectively. However, *B. subtilis* did not show any inhibition of mycelial growth of *S. rolfsii*.

The success of Trichoderma as biocontrol agents (BCAs) is due to their high reproductive capacity, ability to survive under unfavorable conditions, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms.

Table 1: Evaluation of bio-agents on the radial growth of *S. rolfsii in vitro*

S. No.	Name of Antagonist	96 hrs	
		Avg. radial growth (mm)	% Inhibition
T1	*Trichoderma viride*	25.33	71.86
T2	*Trichoderma harzianum*	25.50	71.67
T3	*Chaetomium globosum*	15.11	83.21
T4	*Bacillus subtilis*	26.33	70.74
T5	*Pseudomonas florescence*	21.60	76.00
Control		90.00	0.00
C.D.(0.05)		2.770	
S.E.(m)		0.889	

In vitro screening of fungicides against the _Sclerotium rolfsii_ causing collar rot of Lentil.

Fungicides are an important component of integrated disease management. Use of fungicides as foliar spray or as seed treatment provides a potential tool to protect crops from ravages of plant diseases. In the present investigation, eight fungicides Bavastin 50% W.P., Equation Pro, Curzate M, Pulsor, Nativo, Contaf 5% E.C., Tilt 25% E.C., Kocide were evaluated *in vitro*. against *Sclerotium rolfsii* at 100 ppm concentration using Poisoned Food Technique. Among the fungicides evaluated, overall maximum 100% inhibition of the mycelial growth was recorded with Curzate M and Nativo (Table.2.). The result was more or less in agreement with Rajendra Prasad *et al.* (2017) [22] where Cymoxanil + Mancozeb (Curzate M) and Tebuconazole + Trifloxysrobin (Nativo) recorded maximum inhibition (100%) of *S. rolfsii.*

Fungicides that are toxic to fungi may cause mycelium to cease growing, change metabolic processes or be killed, spores may fail to germinate or be killed (Neely, 1969) [18].

Table 2: Effect of different fungicides on radial growth *S. rolfsii* at 100 ppm concentration

Treatment	96 hrs	
	Mycelial growth (mm)	%M Inhibition
Bavistin50% WP	0.00	100
Equation Pro	13.33	85.18
Curzate M	0.00	100
Pulsor	0.00	100
Nativo	0.00	100.0
Contaf 5% EC	7.6	95.50
Tilt 25% EC	0.00	100
Kocide	0.00	100
Control	90.00	
C.D. (0.05)	1.187	
S.E.(m)	0.396	

Integrated disease management of Collar Rot of Lentil under Artificial inoculation condition

Keeping in view the current requirement and trend of sustainable development in agriculture, the present study was undertaken for the management of Collar rot of lentil caused by *Sclerotium rolfsii* by combining Fungicides, Biocontrol agents and organic matters. The percent disease incidence (PDI) was calculated by using the formula devised by Mathur *et al.* 1972.

$$\text{PDI} = \frac{\text{Infected Plants}}{\text{Total No. of plants observed}} \text{X } 100$$

Almost all treatments significantly increased the Germination Percentage, decreased the Percentage of Disease Incidence and hence increased the yield and test weight. The lowest percent disease incidence (6.66%) was recorded in (T1) Seed treatment with *Trichoderma harizanum* (@5g/kg) + *Pseudomonas fluorescens.* (@5 g kg^{-1}) with yield of 15.08 gm/pot i.e. maximum, followed by (T7) Soil application of Neem Cake (25 g kg-^{1}) + *Trichoderma harizanum*(@5 gkg^{-1}) where P.D.I. (8.88%) and yield of 14.06 g/pot was recorded. Compared to PDI of 37.77%, germination (91.83%) and yield of 10.35 gm/pot as found in Control.

The maximum percent Disease incidence (40%) was observed in (T4) Soil application of Mustard oilcake (@ 50g/kg soil) (Table. 3.). These results are nearly in agreement with Tetali *et al.*, (2016) who reported the use of *Trichoderma viride* along with neem cake, produced the higher germination percentage, shoot length and significantly reduced the per cent disease incidence. Ganeshan *et al.* (2007) [10] reported Pseudomonas strains to able to significantly control a number of fungal, bacterial and nematode diseases in cereals, horticultural crops, oil seeds and others.

Table 3: Percentage disease incidence and yield obtained

S. No.	Treatment	PDI (%)	Yield (gm/pot)
T1	Seed treatment with *Trichoderma harizanum* (@5g kg^{1}) +*Pseudomonas fluorescens.* (@5 g kg^{-1})	6.66	15.08
T2	Soil application of *Trichoderma harzianum* (@5g kg^{-1}) + FYM (@100g/pot)	15.55	12.67
T3	Soil application of *Trichoderma harizanum* (@5 g kg^{-1}) + Press mud (@50g kg^{-1})	22.21	11.23
T4	Soil application of Mustard Cake (@ 50g kg^{-1})	40	9.83
T5	Soil application of Carbendazim (@2g kg^{-1}) + *Trichoderma harizanum* (@5 g kg^{-1})	13.33	13.40
T6	Soil application of Neem Cake (50 g kg^{-1})	15.55	13.76
T7	Soil application of Neem Cake (25 g kg-^{1}) + *Trichoderma harizanum* (@5 g kg^{-1})	8.88	14.06
T8	Soil application of *Trichoderma harizanum* (@5g kg^{-1})	17.77	14.56
	Control	37.77	10.35
	C.D.	7.683	0.987
	S.E.(m)	2.566	0.330

Discussion

Pulse crops have remained as a mainstay of Indian agriculture for centuries. They are rich in high value protein and forms an integral part of daily diet of the predominantly vegetarian population in India. Because of their ability to fix atmospheric Nitrogen, these crops are important in cropping systems and achieving the goal of sustainable crop production. The diseases of lentil not only reduce yield but also deteriorate seed quality. Collar rot of Lentil is one of the major diseases responsible for hampering the production of lentil.

In recent years, considerable success has been achieved by introducing antagonists to soil. The use of bio-primed seeds might be considered as a safe, cheap and easily applied biocontrol method against these soil borne plant pathogens (El-Mougy and Abdel-Kader, 2008) [16]. Biocontrol agents (BCAs) that have been used in agriculture across the globe, provide systemic resistance to plants infested by various fungal phytopathogens (Surekha *et al*., 2014) [4]. An integrated approach by including the fungicides with different bi-oagents and plant products appears to be a possible solution for effective and economic management of Collar Rot Disease.

Conclusion

Collar rot disease caused by *Sclerotium rolfsii*, is a serious threat to lentil and its control has acquired very limited success. Present investigation was carried out with a view to ascertain the cultural factors responsible for the growth of the *Sclerotium rolfsii* and management option to minimize the disease.

Fungicides like Curzate M, Nativo, Carbendazim and Thifluzamide have proved to be highly effective in inhabiting the growth of pathogen *in vitro* at 100ppm concentration. Among the tested bio-agents, *Chaetomium globosum* was highly effective followed by *Pseudomonas fluorescens* inhibiting the mycelial growth of the pathogen *in vitro*. Integrated management with *Trichoderma*, *Pseudomonas* and plant products like neem cake was found to be very effective. Therefore, further studies must be conducted to explore the possibility of the use of the antagonists for the biological control of the diseases caused by *Sclerotium rolfsii*.

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