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Cultural, morphological and pathogenic variability among the isolates of *Sclerotium rolfsii* Sacc. Causing collar rot of lentil in Madhya Pradesh and interaction with chemical fungicides

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Abstract
Lentil occupies a unique position in the world of agriculture, as a pulse crop the majority of world production comes from Canada (68%), followed by India (33%). Collar rot of lentil disease caused by *Sclerotium rolfsii* Sacc. is very important and a polyphagus pathogen causing substantial losses in quality and productivity of yield. The lentil plants showing characteristic collar rot symptoms were collected from different lentil growing districts of Madhya Pradesh. Variability was observed among the isolates of *S. rolfsii* in their mycelial characters, sclerotial characters and pathogenic characters. It was found that all the six isolates collected from different districts of central zone of Madhya Pradesh were showing significant difference in their growth rate, colony type, mycelial appearance, and color of sclerotia, pattern sclerotia production, days taken for sclerotial production and number of sclerotia per plate. Pathogenic variability was also observed in all six isolates from less severe to more severe. *In vitro* evaluation of five fungicide mixtures revealed that, Azoxystobulin + Difenconozole, Hexaconozole and Azoxystobulin was found most effective against the *Sclerotium rolfsii* Sacc. at all the tested concentrations.

Keywords: Lentil, *Sclerotium rolfsii* Sacc. Morphology, cultural and pathogenic variability

Introduction
Lentil (*Lens culinaris* M.) is a high value, edible pulse crop; it is the oldest crop in the world of Agriculture as it has been grown since 8000 years ago and originated in the fertile crescent of the Near East and dates back to the beginning of agriculture itself (Sabaghpour *et al*., 2004) [16]. It belonging to the family Leguminosae is a cool season legume crop and a bushy annual grown for its seeds. The name came from its characteristic lens shaped seeds. Lentil provides a balance of essential amino acids for human nutrition. It also supplies protein for animals, a member of the legume family, which improves soil fertility by fixing and utilizing atmospheric nitrogen through symbiotic relationship with *Rhizobium* at the root nodule of the crop. Lentil also contains a broad range of beneficial plant compounds called phytochemicals (Sarker and Kumar, 2011). Lentil occupies a unique position in the world of agriculture, as a food crop the majority of world production comes from Canada (68%), followed by India (33%). Among Rabi pulses, lentil is next to chickpea and it is cultivated In India, with a 1.59 million ha of harvest area, producing 0.94 million tones with an average yield of 591kg per ha and accounts up to 7% of the total pulses production in the country (Annonymous 2019) [2]. The consumption of lentils is more in India than any other country in the world and produces more than 50 varieties in different states. In India, major lentil producing states are Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal and Uttarakhand. Madhya Pradesh ranks first with an area of about 6.13 lakh ha and an annual production of 4.16 thousand (Kumar *et al*., 2019) [8].
The average national productivity of 675 kg/ha in India is much less than its yield potential, mainly due to biotic and abiotic stresses. Among various biotic stresses, fungal pathogens are important because of their ability to infect all parts of the plant at all stages of the growth. Mainly the soil borne pathogen causing wilts and root rots have been a matter of worry for sustainable production of pulses for a couple of years. Collar rot of lentils caused *Sclerotium rolfsii* (Dey *et al*., 1993) [5] is a common and most serious disease of lentils. The pathogen is a soil inhabitant, very aggressive in nature, attacks the collar portion of the plant, which ultimately leads to its death. Germinated seedlings were killed within 7 days after emergence.

There are several reports of *S. rolfsii* which have significant variations in morphological behavior (Sharma *et al.*, 2002) [18]. Variability is the property of an organism to change its characters from one generation to the other. There is a need to study and know the variability of fungus in culture, isolated from different districts of central zone of Madhya Pradesh. The control practices of stem rot disease include cultural methods such as plant rotation, deep soil processing and weed control as well as soil solarization, use of antagonistic microorganisms or fungicides treatments after sowing on the plant rows (Damicone & Jackson, 1994) [4].

Despite many achievements in modern agriculture, management of diseases is not being successful still now. Being a soil borne pathogenic fungus, the most effective way to control *Sclerotium rolfsii* Sacc. is seed treatment with chemicals. There is lack of information available about variability in cultural, morphological and pathogenicity of S. *rolfsii* isolates from different lentil growing areas of Madhya Pradesh. So it is imperative to take up the study along its interaction with some chemical fungicides

Materials and Methods

Collection and Isolation of Pathogen

An intensive roving survey was conducted during Rabi season, 2020-21 in the central zone of Madhya Pradesh. Lentil plants showing characteristic collar rot symptoms were evident as yellowing collapsed. The affected roots showed rotting at the collar region and downward with the whitish mycelium in earlier stages of infection, rapeseed like sclerotia can be observed attached to mycelium around the collar region, at seedling to flowering stage were collected from different lentil growing districts (Satna, Sagar, Katni, Damoh, Panna and Jabalpur) of Madhya Pradesh.

The infected collar rot lentil samples were brought to the Laboratory of Plant Pathology, JNKVV, Jabalpur, and Madhya Pradesh. The roots of diseased plants showing symptoms were washed thoroughly with tap water, small pieces of infected roots were cut with the help of sterilized blades (Rangaswami and Mahadevan, 1999) [15]. These pieces were surface sterilized with 1:1000 mercuric chloride (HgCl2) solution for one minute followed by three washings with sterilized distilled water to remove traces of HgCl2. The pieces were then dried on blotter paper to remove excess water and then transferred aseptically to Petri plates containing PDA medium. Inoculated Petri plates were incubated at 25± 1 ^{0}C for five days and examined at frequent intervals to see the growth of the fungus developing from different pieces. As and when fungal colonies appeared they were transferred to PDA slant. Auxenic culture of the pathogen was obtained by a single hyphal tip method and maintained on PDA slants throughout the present investigation. The pathogen was identified as *S. rolfsii* based on mycelial and sclerotial characters described by Barnett and Hunter (1972) [3].

Cultural, morphological and pathogenic variability

Isolates of *S. rolfsii* collected from different districts of Madhya Pradesh (Satna, Sagar, Katni, Damoh, Panna and Jabalpur) were studied for their Cultural, morphological and pathogenic variability, growth rate and sclerotia formation etc., using PDA media. All isolates of *S. rolfsii* were grown in Petri plates containing PDA medium. The mycelial disc of 4 mm diameter of each isolate was inoculated at the centre of plate and replicated four times. The inoculated plates were incubated at 27±1 ^{0}C for 15 days. Radial growth of each colony in two directions at right angles was measured. Visual observations on sclerotia formation were recorded. A total of 8 morphological characters based on mycelial (mycelial growth, colony color, and appearance) and sclerotia parameters (sclerotia color, maturity days, number of sclerotia and their arrangement on surface of media) were recorded at 7 and 15 days of incubation, respectively for each isolate**.** Pathogenic variability was studied by growing lentil seeds in poly bags containing soil inoculated with different isolates *S. rolfsii* and Pathogencity was recorded by inoculating each isolate separately in a poly bag containing sterilized soil and sowing of lentil seeds. Pathogenicity recorded after 15 days of inoculation based on severity of disease caused by each isolate.

In-vitro Evaluation fungicides

In-vitro efficacies of five fungicides (Hexaconazole, Azoxystrobin, Azoxystrobin + Difenconazole, COC and Propiconazole) at different concentrations (250ppm, 500ppm, 750ppm and 1000ppm) were evaluated against the highly severe isolate of *S. rolfsii by* poisoned food technique (Nene and Thapliyal, 1993) [12]. The 100ml PDA medium was used for each different concentration and 20ml of this medium was plated in 9 cm Petri plates, four replications and a control was maintained for each concentration of fungicide, plate without fungicide served as control. A 7 mm mycelial disc of five days old pathogen was inoculated at the centre and incubated at 27± 1°C until full growth was observed in control. Per cent inhibition in radial growth over control was calculated using the formula: I = (C-T)/C X 100 where, I = Per cent inhibition in growth of test pathogen, C = Radial growth of pathogen in control, T = Radial growth of pathogen in treatment (Nene and Thapliyal, 1993) [12].

Result and Discussion

Collection, isolation and identification

The isolates collected from different lentil growing districts of Madhya Pradesh were identified as *S. rolfsii* based on their cultural, morphological and sclerotial character. Mycelium was first silky white in color later turned to dull white with radial spreading given a fan like appearance. Microscopic examination of the fungal culture revealed the aerial hyaline, thin walled, septate hyphae with profusely branched mycelium showing clamp connections, when fungus attained maturity small mycelial knots were formed which later turned to mustard seed like sclerotia which were deep brown or brownish black, shiny, hard and spherical to irregular in shape. Similar reports were given by Subramanian (1964) [20], Barnett and Hunter (1972) [3], Mahmood *et al.*, (1976) [9], Singh (1987) [19], Mirza and Aslam (1993) [10], Mohan *et al.* (2000). Different cultural, morphological and pathogenic variability of six isolates were studied based on mycelial, sclerotial and severity of collar rot disease on lentil plants.

Cultural, morphological and pathogenic variability

From the present investigation it was found that all six isolates of *S. rolfsii* exhibit significant differences in their mycelial characters, sclerotial characters and pathogenic variability. A wide range of variation was observed in the growth rate of *S. rolfsii* isolates within 96 hrs incubation, ranging from very fast growth to very slow growth. The

highest mycelial growth (62.95mm) was observed in Isolate SrM2 from Sagar followed by isolate SrM1(51.57mm) from Satna and lowest mycelial growth was observed in isolate SrM4(37.89mm) from Damoh (table 1). It was found that SrM1 and SrM3 were significantly far from each other at 24 hrs and 48 hrs. Isolate SrM1 and SrM1 were significantly far apart at 48hrs. Significant increases in the growth of isolates were observed from 24 hrs to 96hrs. All isolates of *S. rolfsii* produced light white to extra white colonies and also showed a difference in their mycelial appearance In Petri plates and (table2). Variation was observed in Sclerotial color from light brown to dark brown, isolates SrM1and SrM6 produced dark brown sclerotia, reddish brown sclerotia was produced by SrM2 and SrM5 isolates and light brown color produced by SrM3 and SrM4 (table3). Pathogenic variability was observed in all isolates ranging from less severe to highly severe. Isolate SrM2 (34.4%) was found more severe in causing collar rot disease and isolate SrM4 (17.6%) was found less severe (table4). Similar result was observed by Rakholiya *et al.* (2011) [14] studied variability of 30 isolates of *S. rolfsii* and reported considerable variability in mycelial and sclerotial dimensions.

The cultural, morphological and pathogenic characters of *S. rolfsii* isolates tested were highly variable. The variability among isolates observed in the study could be attributed to physico-metabolic differences among isolates arising from different crop production systems and also some biochemical variability to adapt to their ecological and environmental conditions. Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Harlton *et al.* 1995; Okabe *et al.* 1998) [7, 13]. In India, Sharma *et al.* (2002) [18] studied variability among 26 isolates of *S.rolfsii* collected from various hosts/soil samples and localities. Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. The significant variation in culture characteristics, mycelial morphology and pathogenicity amongst test isolates indicated that *S. rolfsii* can best be characterized by a combination of culture characteristics, morphology and virulence on host plants. The differences in sclerotial forming capacity among isolates could be a useful parameter for characterizing isolates, due to the fact that the number of sclerotia formed among fungal isolates was significant.

In-vitro Evaluation fungicides

In vitro evaluation of five fungicide mixtures revealed that, Azoxystobulin + Difenconozole, was found most effective against Sclerotium *rolfsii* with highest inhibition per cent of (93.51) followed by Azoxystobin (89.58%) and lowest inhibition per cent was recorded in COC (26.75%). It was observed that as the concentration of fungicide increased the mycelial growth of test pathogen decreased and inhibition percent increased. (Table 5) It was also found that Azoxystobulin + Difenconozole was effective against *Sclerotium rolfsii* at all tested concentrations (250ppm, 500ppm, 75oppm and 100ppm). Shepard *et al.* (1986) reported that hexaconazole at 10 mg l^{-1} completely inhibited the *S. rolfsii*. Other systemic fungicides Azoxystrobin and Difenconazole were found most effective against *S. rolfsii* in percent inhibition of mycelial growth. Copper oxy chloride did not show any effect on per cent inhibition of mycelium even up to 2000 ppm. In the present investigation the pathogen *S. rolfsii* showed susceptible to Azoxystrobin and Difenconazole, here the percent inhibition of mycelial growth is more. The tolerance of these fungi towards the fungicides is correlated with increasing ability to synthesize extracellular melanin under fungicidal stress (Amany *et al.*, 2003) [1]. In *S. rolfsii* the outer layer of sclerotia contains two to four layers of melanized rind. Melanin may be the inhibitory factor playing the role, natural occurrence or the induction of melanin pigment secretion may be a mode of pathogen defense against the toxic effect of the fungicide. Melanized cells posses increased resistance to environmental stress (Fogarty and Tobin, 1996) [6].

Table 1: Mycelial Growth *S. rolfsii* isolates at different time intervals

Treatments	24hrs	48hrs	72hrs	96hrs	Mean
SRM1	13.567	34.467	73.700	84.567	51.575
SRM2	27.000	57.067	78.167	89.600	62.958
SRM3	13.000	31.600	68.967	75.400	47.242
SRM4	18.333	23.367	45.633	64.233	37.892
SRM5	18.400	35.200	59.167	73.333	46.525
SRM6	21.533	28.667	42.000	75.333	41.883
Mean	18.639	35.061	61.272	77.078	
Factors	C.D.	SE(d)	SE(m)		
S. rolfsii isolates	0.490	0.243	0.171		
Hours	0.400	0.198	0.140		
Isolates×Hours	0.979	0.485	0.343		

Table 2: Mycelial characters of *S. rolfsii* isolates on PDA medium

Isolates	Districts	Growth rate	Colony color	Colony appearance
SRM1	Satna	fast	Light white	Cottony, Dense at centered, aggregated
SrM2	Sagar	Very Fast	Dull white	Compact growth, fluffy at edges and dense at centered
SrM3	Katni	Moderately fast	Dirty white	fluffy at centered upright growth habit
SrM4	Damoh	Very Slow	Dull white	Compact growth
SrM5	Panna	slow	Extra white	Fluffy, upright growth colony
SrM6	Jablpur	Moderately fast	Light white	Fluffy, suppressed thin strands

Average of 4 replications and significance difference at 5% level

Table 3: Sclerotial characters of *S. rolfsii* isolates on PDA medium

Isolates	Districts	Color of Sclerotia	Pattern of sclertial production	Maturity (d)*	No. of sclerotia* / Plate	Weight (mg)*
SrM1	Satna	Dark brown	Regular	12	45.60	5.6
SrM2	Sagar	Reddish brown	Circular	10	201.2	12.5
SrM3	Katni	Light brown	Irregular	10	67.00	4.6
SrM4	Damoh	Light brown	Near corner of plate	15	195.0	7.5
SrM5	Panna	Reddish brown	Upper surface of plate	13	149.0	8.3
SrM6	Jablpur	Dark brown	circular	12	67.5	9.4

Table 4: Pathogenic variability in different isolates of *S. rolfsii*

Isolates	Severity percent (*)	Pathogenicity
SRM1	23.07	Less severe
SRM2	33.95	Highly severe
SRM3	23.70	Moderately severe
SRM4	17.97	Verey less severe
SRM5	20.77	Less severe
SRM6	26.97	Moderately severe
C.D.	1.81	
SE(m)	0.59	
SE(d)	0.84	
C.V.	4.89	

*Average of 4 replication and significance difference at 5% level

Table 5: *In vitro* evaluation of different fungicides against *S. rolfsii*

Treatments	250ppm	500ppm	750ppm	1000ppm	Mean
Hexaconozole	67.07	74.50	80.43	84.50	76.62
Azoxystobin	81.10	88.13	93.60	96.57	89.85
A+Difenconozolde	87.73	92.37	95.40	98.56	93.51
COC	23.47	24.50	27.50	31.57	26.75
Propiconazole	2.063	45.63	51.60	57.33	43.80
Control	0.00	0.00	0.00	0.00	4.70
Mean	54.13	49.85	58.09	61.22	
Factors	C.D.	SE(d)	SE(m)		
Fungicides	7.01	3.47	2.45		
concentration	5.73	2.83	2.00		
Fungicides×concentration	14.03	6.95	4.91		

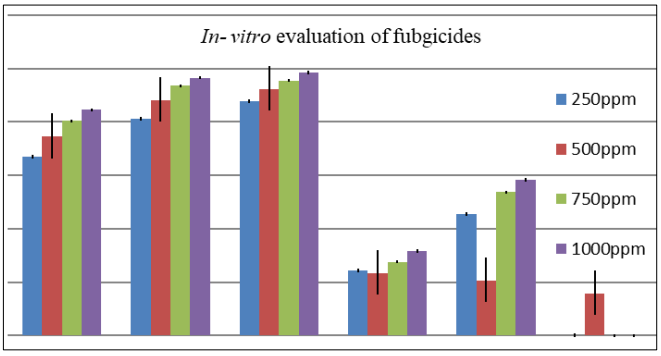

Fig 1: Bar diagram representing of *in- vitro* evaluation of fungicides against *S. rolfsii* at different concentrations. The error bars indicate standard error of three independent replication

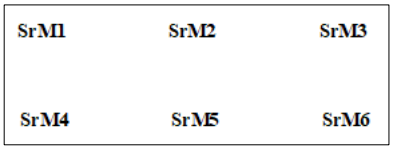

Plate 1: Morphological and cultural variability *of S. rolfsii* isolates

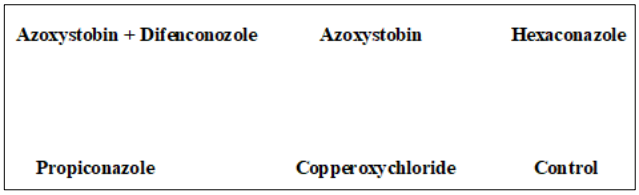

Plate 2: *In vitro* evaluation of different fungicides against *S. rolfsii*

Conclusion

From the present study it can be concluded that variation in morphological, cultural and pathogenicity of *S. rolfsii* isolates collected from different districts of central zone of Madhya Pradesh might be due to the variation in geographical cropping system and their adaptability to the different soil factors, weather parameters and their biochemical variability to various cropping system. It can be recommended that use of fungicide mixtures for seed treatment at lower concentration was effective for management of *S. rolfsii* along with integrated disease management practices to avoid crop loss and to increase the yield of lentil

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