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Pathogenicity Study of *Sclerotium rolfsii* Isolates on Popular Lentil Varieties in Net House Condition

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ABSTRACT

Background: Collar rot of lentil caused by *Sclerotium rolfsii* became a major problem for early sown lentil in North-eastern plain zones of India, as it associated with high yield losses every year. The disease cause huge loss in yield due to which, area under this crop is consistently decreasing. In global perspective, losses of 10-20 million dollars associated with *S. rolfsii* with yield depletion ranging from 1-60% in fields. The resistance and susceptibility of a potential host plant depends on the virulence of isolates of *S.rolfsii.* So the objectives of this study is to observe the potentiality of different isolates those were collected from major lentil growing districts of West Bengal and tested their virulence on three popular varieties of lentil. However the pathogenecity of *S.rolfsii* in this region never been tested before. This information could be a strong background for effective management of pathogen for future research.

Methods: Collar rot infected samples were collected from different geographical locations of West Bengal during (Nov-Dec, 2018). And then the collected samples were isolated in Department of Plant Pathology, BCKV, Nadia, W.B. and were confirmed by their morphological characters.

Result: The isolates from different geographical locations showed differences in their virulence. Among the all isolates SRC2 considered as most virulent as it causes highest diseases incidence as well as mortality percentage in all selected varieties of lentil. Two varieties, HUL-57 and BM-6 not so much popular among the farmers but can be consider as resistant to collar rot in case of early sowing condition.

Key words: Collar rot, Different isolates, Lentil varieties, Morphological variation, Pathogenicity test, Sclerotia, *Sclerotium rolfsii*.

INTRODUCTION

Sclerotium rolfsii Sacc. is a soil-borne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt, foot rot and collar rot on more than 500 plant species including almost all the agricultural and horticultural crops (Fernando *et al*. 2004; Clarkson *et al*. 2004; Del Rio *et al*. 2007; Sten *et al*.2017). According to Agrios (2005), it causes diseases to a wide variety of plants. Among the diseases of lentil seventeen diseases have been recorded to cause severe loss in yield among them twelve diseases are caused by fungi, two are caused by nematode and two by viruses where one is caused by mycoplasma (Baker and Rashid, 2007). The most important fungal diseases of lentil are Alternaria blight, Anthracnose, Ascochyta blight, Black root rot, Black streak root rot, Botrytis graymold, Cercospora leaf spot, Collar rot, Downy mildew, Dry root rot, Fusarium wilt, Helminthosporium leaf spot, Leaf yellowing and Phoma leaf spot *etc* (Taylor *et al*.2007). Among all the above diseases of lentil, collar rot or root rot is most destructive disease which is caused by *S.rolfsii* (Smolinska and Kowalska,2018),occurs in almost every lentil growing region in warm areas with high soil moisture (30-40%) and high temperature (25ºC) at the seedling stage. The pathogen survives well in soil as sclerotia in the presence of sufficient organic matter even under adverse climatic conditions (Wu *et al*.,2008) *S. rolfsii* is a non-specialized soil borne fungal pathogen of worldwide importance and has a host range of over 500 species (Xu *et al*., 2008). It is too difficult to remove the pathogen from infected field due to its diverse nature of survival as sclerotia production and their ability to persist in the soil for several years except use of PGPR those are potential biocontrol agent too (Das *et al*., 2017; Singh *et al*., 2012).

So, collar rot of lentil became a major problem for early sown lentil in North-eastern zones of India, as it associated

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with high yield losses every year. The disease cause huge loss in yield due to which, area under this crop is consistently decreasing. On a global perspective, estimated that the losses of 10-20 million dollars associated with *S. rolfsii* have been recorded with yield depletion ranging from 1-60% in fields (Liamngee Kator *et al*. 2015). The resistance and susceptibility of a potential host plant depends on the isolates of *S. rolfsii* (Sennoi *et al.,*2010; Xie *et al*., 2014). So the objectives of this study is to observe the potentiality of different isolates those were collected from major lentil growing districts of West Bengal and tested the ability to cause the disease on three popular varieties of lentil. However the pathogenecity of *S. rolfsii* in West Bengal condition never been tested before. This information could be a strong background for effective management of pathogen for further researchers. With this aim, this experiment also helps to find out whether the susceptibility and tolerance level of the most popular varieties depends on virulence of the isolated collected from geographically different locations.even future pathotype analysis of the isolates can be possible with this basic study.

MATERIALS AND METHODS

Collection and isolation of *S.rolfsii*

Collar rot infected lentil plants were collected from different fields situated in Nadia, Hoogly, Purba Burdwan, Birbhum, Murshidabad and Jalpaiguri in West Bengal during (Nov-Dec, 2018). And then the collected samples were isolated in Department of Plant Pathology, BCKV, Nadia, W.B. and were confirmed by their morphological characters. The collar region showing typical symptoms of rotting was cut into small pieces. These pieces were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds. Such pieces were washed thoroughly in sterile distilled water twice to thrice to remove trace of mercuric chloride and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. They were incubated at 27±1°C for four days for the growth of the fungus. Later, loop full of fungal growth was transferred to PDA slants. The fungus was further purified by hyphal tip method under aseptic conditions. Total 8 isolates of *S. rolfsii* were purified and used for further studies.

Cultural and morphological study of the fungal pathogen

The experiment was conducted in order to study the variation in the morphological characters of eight isolates of *S rolfsii*. For this purpose, 20 ml of potato dextrose agar was poured into petri plates. Mycelial disc of seven days old culture of the respective isolates was placed at the centre of the plate. Three replications were maintained at room temperature 27±1°C for four days and colony character like pigmentation, radial growth and concentric rings were recorded. To get mature sclerotial bodies, the cultures were further incubated up to 15-20 days. The growth and morphological characters of the isolates *viz*., colony morphology, mycelial growth rate, sclerotial number and colour were observed.

Preparation of artificial inoculums

About 80 g of maize meal (Saw dust) was taken into 250 ml conical flasks and sterilized them in autoclave for 20 minutes at 15 psi. A 5 mm diameter mycelium disc from an actively growing culture of *S. rolfsii* on potato dextrose agar (PDA) medium was transferred into each conical flask. The flasks were incubated at room temperature 27 ± 1°C for 15 to 20 days for growing of mycelium and maturation of sclerotia. After maturation of sclerotia, then it was stored in a refrigerator for further use.

Pathogenicity test

Sterilized soil was artificially infested with sclerotia of *S.rolfsii* @ 1 sclerotia/g of soil and transferred into 8 cm diameter thermopole pots 150 g soil per pot. Sterilized soil without infested with *S. rolfsii* served as control. Seeds of three lentil varieties (Moitree, BM-6 and HUL-57) were sown in separate sets @ 10 seeds per pot. There were three replications of each treatment and the pots were randomized on a net house bench.

Estimation of Diseases Incidence % and mortality percentage:

Diseases Incidence (DI %):

Total number of healthy plants and infected plants were counted and accordingly PDI was calculated.

$$DI\ \% = \frac{\text{Total number of infected plants}}{\text{Total number of healthy plants}} \times 100$$

Mortality percentage (%):

Percent mortality was calculated by using the following formula,

$$\text{Mortality percentage (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of seedlings}} \times 100$$

RESULTS AND DISCUSSION

Survey and collection of diseases samples from different locations

A survey was conducted during Rabi season (Nov-Dec 2018) in major lentil growing districts in West Bengal during seedling to flowering stages. Collar rot infected lentil plants were collected during the survey and brought to the laboratory for isolation of *S.rolfsii*. (Table 1). Eight number of isolates were collected from different geographical locations and at the time of sample collection the disease incidence were recorded from those infected field and given in the Table 1.The maximum disease incidence percentage of collar rot of lentil was recorded in Chakdaha (Nadia) and Ramchandrapur (Murshidabad) (8.6%) followed by Katwa (Purba Burdwan) (7.6%), Balagarh (Hoogly) and Sriniketan (Birbhum) (7.0%). The lowest disease incidence of collar rot (5.6%) was recorded in Saguna near Gayeshpur (Nadia) and Maynaguri (Jalpaiguri KVK).

In-vitro virulence test of the isolates

Mycelial growth rate

The mycelial growth rate of eight isolates of *S. rolfsii* were recorded and isolate SRC4 showed highest mycelial growth per day (40.67 mm) followed by SRC2, SRC5 and SRC8, which recorded 37.58 mm and 37.33 mm growth per day respectively. The isolate SRC6 was recorded lowest mycelial growth per day (29.83 mm) and their differences were statistically significant (Table 2).

Among the eight isolates of *S. rolfsii* showed morphological variation, three isolates *viz*., SRC3, SRC6 and SRC8 were with fluffy colony growth appeared on the petri plates and four isolates *viz*., SRC1, SRC2, SRC4 and SRC5 were compact sclerotial colony appeared in this present study (Table 3).

Number of sclerotia

All the eight isolates SRC_1 to SRC_8 produced different number of sclerotia and their differences were statistically significant. The number of sclerotia which were varied from 58 to 201 sclerotia / plate at 15 DAI. Most of the isolates showed their different number of sclerotial bodies and little beat differences in the colour those were measured (Table 2). The colour of sclerotia were varied from light brown to dark brown at maturity period in each isolates.

This is in confirmation with the report of Komathi (2002) who reported that the highly virulent strains exhibited very rapid growth and produced huge number of sclerotia in the culture. The pathogen *S. rolfsii* produced sclerotia at the edges of the petri plates from 15 days to 25 days after inoculation at room temperature 27 ± 1°C in BOD incubator. Sclerotia production varied in all the eight isolates. Among the eight isolates, SRC1 produced (>190 numbers) of sclerotia, while other isolates formed minimum numbers of sclerotia (<180/ plate). The total number of sclerotia was more in isolate SRC1 (201) followed by SRC5 (171) and SRC4 (158). The minimum number of sclerotia was observed in isolate SRC6 (29) followed by SRC7 (33), SRC1 and SRC3 (36). The sclerotia was small and round in shape like that mustard seeds. The sclerotial colour of each isolates were normally observed in light brown to dark brown at maturity (Table 2).

Pathogenecity study in *in vitro* study

Disease incidence percentage

The incidence percentage of different isolates at 10 and 14 days after inoculation in plants were different in different varieties and their differences were statistically significant. These eight isolates were also tested on three different lentil varieties *viz*., Moitree, BM-6 and HUL-57 for their pathogenicity and it was observed that all the eight isolates were pathogenic on three lentil varieties though their pathogenicity were different on different varieties and their differences were statistically significant. Among the isolate SRC2 was found more virulent than other isolates and it causes maximum incidence percentage (36.67%) followed by SRC1, SRC4, SRC6 and SRC7 (30.0%) and minimum in SRC8 (18.33%) irrespective of varieties used. It recorded

Table 1: Details description of diseased plant sample collection and GPS Location on different isolates.

Isolation code	Place of collection	Latitude	Longitude	Disease incidence % at 25 DAS
SRC1	Farmers field, Chakdaha, Nadia.	23.08° (N)	88.52° (E)	8.6%
SRC2	Ramchandrapur, Murshidabad, KVK,	24.18° (N)	88.27° (E)	8.6%
SRC3	Farmers field ,Saguna Nadia.	22.59° (N)	88.29° (E)	5.6%
SRC4	Farmers field, Balagarh, Hoogly.	23.12° (N)	88.46° (E)	7.0%
SRC5	C block farm, Kalyani, Nadia.	22.98° (N)	88.48° (E)	6.6%
SRC6	Farmers field, Purba Burdwan.	23.24° (N)	87.85° (E)	7.6%
SRC7	Palli Siksha Bhavana, (Institute of Agriculture) Sriniketan, Birbhum.	23.67° (N)	87.72° (E)	7.0%
SRC8	Jalpaiguri, KVK	26.52° (N)	88.73° (E)	5.6%

Table 2: Morphological characteristics of different isolates of *Sclerotium rolfsii*

Isolate	Colony type	Colour of sclerotia	No. of sclerotia / Plate
SRC-1	Compact growth	Reddish brown	201.66
SRC-2	Compact growth	Dark brown	150.33
SRC-3	Fluffy growth	Dark brown	132.66
SRC-4	Compact growth	Reddish brown	158.00
SRC-5	Compact growth	Light brown	171.00
SRC-6	Fluffy growth	Reddish brown	153.66
SRC-7	Fluffy growth	Dark brown	58.00
SRC-8	Fluffy growth	Reddish brown	109.00
	C.D. at 5%		42.59
	SEM(±)		13.90

Table 3: Mycelium Growth rate of eight isolates of *Sclerotium rolfsii* on PDA medium at different DAI.

Isolates	Growth rate on PDA media at DAI (mm/day)				Mean DAI
	DAY1	DAY2	DAY3	DAY4	
SRC1	2.33	21.33	34.00	87.33	36.25
SRC2	5.67	26.00	32.00	86.67	37.58
SRC3	6.67	18.33	31.67	88.00	36.17
SRC4	8.33	28.00	38.33	88.00	40.67
SRC5	3.00	25.67	32.33	89.33	37.58
SRC6	3.00	13.33	23.00	80.00	29.83
SRC7	3.67	14.00	27.67	86.67	33.00
SRC8	6.00	24.67	31.33	87.33	37.33
Mean Isolates	4.83	21.42	31.29	86.66	4.83
		SEM(±)	**C.D at 5%**		
Isolates		0.551	1.561		
DAI		0.390	1.104		
Isolates x DAI		1.102	3.122		

Average of three replications*DAI= Days after inoculation.

Table 4: Pathogenicity test on disease incidence percentage at different days after inoculation and effect of different isolates on three popular lentil varieties.

Isolates	Disease incidence% at DAI					
	Moitree		BM6		HUL57	
	10 DAI	14 DAI	10 DAI	14 DAI	10 DAI	14 DAI
SRC1	20.00	50.00	20.00	30.00	30.00	30.00
SRC2	50.00	50.00	40.00	40.00	20.00	20.00
SRC3	20.00	30.00	10.00	20.00	20.00	20.00
SRC4	30.00	30.00	40.00	40.00	20.00	20.00
SRC5	40.00	40.00	20.00	20.00	10.00	20.00
SRC6	30.00	50.00	30.00	30.00	20.00	20.00
SRC7	40.00	40.00	30.00	30.00	20.00	20.00
SRC8	20.00	30.00	10.00	20.00	10.00	20.00
Control	0.00	0.00	0.00	0.00	0.00	0.00
Table of SEM(±), SE(d). C.D. at 5%						
Factors		C.D. at 5%		SE(d)		SE(m)±
Factor (ISOLATES)		0.758		0.382		0.270
Factor (VARIETY)		0.464		0.234		0.165
Interaction (Isolates X Variety)		1.313		0.661		0.468
Factor(DAI)		0.379		0.191		0.135
Interaction (Isolates X DAI)		1.072		0.540		0.382
Interaction (variety X DAI)		0.657		0.331		0.234
Interaction (Isolates X Variety X DAI)		1.857		0.935		0.661

that the maximum collar rot incidence of 50.00%, 40.00% and 20.00% in Moitree, BM-6 and HUL-57 respectively.The isolate SRC8 was less virulent among the isolates. Whereas, among the varieties, Moitree produced maximum infection (35.62%) followed by BM-6 (26.87%) and minimum in HUL-57 (20.0%) irrespective of isolates and their difference in disease incidence were statistically significant (Table 4).

Mortality percentage

The mortality percentage of plants were also important selection criteria to measure the pathogenic virulence of the isolates. The mortality percentage of the three different varieties were recorded by pot inoculation of eight different isolates at 25 and 29 days after inoculation. The interaction between isolates and varieties were statistically significant. Among the isolates, SRC7 caused maximum mortality (50.94%) followed by SRC2 (49.72%) and minimum in SRC8 (21.55%) followed by SRC1 (33.67%) and their differences were statistically significant irrespective of varieties used. Among the varieties Moitree showed maximum mortality (65.83%) followed by HUL-57 (50.0%) and minimum in BM-6 (37.0%) and their differences were statistically significant. From the above result it was noticed that all the isolates of *S. rolfsii* isolated from eight different locations produced

Table 5: Pathogenicity test on mortality percentage at different days after inoculation and effect of different isolates on three popular lentil varieties.

Isolates	Mortality% at DAI					
	Moitree		BM6		HUL57	
	25DAI	29DAI	25DAI	29DAI	25DAI	29DAI
SRC1	20.00	50.00	28.00	42.00	30.00	32.00
SRC2	50.00	50.00	44.00	44.00	50.33	60.00
SRC3	50.00	75.00	20.00	40.00	33.33	35.00
SRC4	42.00	42.00	50.00	50.00	33.33	36.00
SRC5	66.00	66.00	25.00	25.00	25.00	50.00
SRC6	37.00	62.00	30.00	30.50	33.33	75.00
SRC7	65.67	66.00	37.00	37.00	50.00	50.00
SRC8	20.00	30.00	14.33	28.00	12.00	25.00
Control	0.00	0.00	0.00	0.00	0.00	0.00

Table of SEM(±), SE(d). C.D. at 5%

Factors	C.D. at 5%	SE(d)	SE(m±)
Factor (ISOLATES)	0.678	0.341	0.241
Factor (VARIETY)	0.415	0.209	0.148
Interaction (Isolates X Variety)	1.174	0.591	0.418
Factor(DAI)	0.339	0.171	0.121
Interaction (Isolates X DAI)	0.958	0.483	0.341
Interaction (variety X DAI)	0.587	0.296	0.209
Interaction (Isolates X Variety X DAI)	1.660	0.836	0.591

similar type of disease incidence and ultimate mortality of plants except SRC-8 where very mild pathogenicity was resulted caused poor mortality and it was observed in all the three tested varieties (Table 5). On the basis of this basic study in future we may collect more isolates and then can go for pathotype analysis.

In this experiment its indicated that variations among the mycelium growth characters and the sclerotial growth of isolates on PDA media is very clear and easy to compare among themselves, which supported the study of Srividya *et al*., 2018. Surulirajan *et al*. (2007) isolated *S. rolfsii* from root rot disease of lentil and studied the different morphological characters of the pathogen on two percent potato dextrose agar medium as it was found to be the best medium for both vegetative and reproductive growth.

The variability in cultural morphology *i.e*., mycelial growth rate, number of Sclerotium formation with variations in sclerotial colour and their color among *S. rolfsii* isolates have been reported by different scientists on various hosts and media (Almeida *et al*., 2001; Sarma *et al*., 2002; Adandonon *et al*., 2005; Okereke and Wokocha, 2007; Akram *et al*.,2008; Rakholiya and Jadeja, 2011; Sharma *et al*., 2013; and Reddi *et al*., 2014). The differences among the isolates for cultural and morphological traits can be used for the identification of isolates and this form the basis for the study of virulence and genetic basis of variability which will guide for pathotype analysis as well as better management practices for the control of the disease.

But depends on the virulence some isolates are more faster than the others. Kokub *et al*. (2007) isolated 8 fungal strain of *S rolfsii* and investigated the growth behaviour on Potato Dextrose Agar (PDA) media plates at 28ºC ranged between from 0.86 – 1.35 mm/hour. He also found similar kind of results that some are comparatively fast growing and produced highest number of sclerotia than others. All the strains produced round shaped showed sclerotia with average diameter of 0.5 – 2.0 mm. in this experiment we also get different number of sclerotia produced by different isolates which is another parameter to judge their virulence. It indicated that all the isolates are pathogenic and able to cause disease though some are highly virulent and some others moderately. Padole *et al.* (2009) studied incidence of collar rot pathogen in 15 to 45 days old crop which range from 5 to 30% in 51 locations surveyed nearby Jabalpur. Investigation on variations in 51 isolates of *S. rolfsii* showed considerable variations with regards to cultural and morphological characters on PDA nutrient media and grouped into three pathotypes. The pathogenicity test showed the isolates to change in number of days taken to initiate plant mortality and 100% mortality. Iqbal *et al*.1995 said that sowing of lentil during third week of November was found to reduce the incidence of collar rot as compared to early sowing. Artificial inoculation of ten selected genotypes of lentil to collar rot pathogen, *S. rolfsii* showed that all the lines were susceptible to the test pathogen.

CONCLUSION

The isolates from different geographical locations showed differences in their virulence. Among the all isolates SRC2 considered as most virulent as it causes highest disease

incidence as well as mortality percentage in all selected varieties of lentil. All though Maitree ia popular lentil variety of lentil but it showed the most susceptible one as compare to rest two varieties. On the other hand HUL-57 and BM-6 not so much popular among the farmers but can be consider as resistant to collar rot in case of early sowing condition.

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