MORPHOLOGY AND CROSS INFECTIVITY OF *Sclerotium rolfsii* sacc. ISOLATED FROM DIFFERENT HOST PLANTS IN NEPAL

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ABSTRACT

Sclerotium rolfsii Sacc. is prevalent in leguminous and solanaceous crops but over the last five years, its severity has increased in several crops such as rice, onion and chilli in Nepal. A study on cross infectivity of S. rolfsii was carried out in March, 2019 at Agriculture and Forestry University, Chitwan. S. rolfsii were isolated from eight crop species viz. rice, lentil, rajma, onion, chickpea, rapeseed, soybean, and chilli. Cross infectivity of the eight isolates was done on the seven crop species in artificially inoculated soils in a screen house. Morphological characters such as mycelial growth rate, number of sclerotia formed, and size of sclerotia were studied. Morphological characters of the S. rolfsii varied among the isolates. All crop species tested were found to be susceptible to all isolates except onion isolate. Germination percentage was greatly reduced (80%) in rajma. Post emergence seedling mortality ranged between 10%in rice and chilli and 100% in chickpea, mustard lentil and rajma. The results of the present study indicate that management strategies of this pathogen should incorporate selection of non-host crops such as maize for crop rotation which helps to prevent build-up of inoculum.

Keywords: Crop rotation, germination percentage, leguminous crops, non-host crop

INTRODUCTION

Sclerotium rolfsii Sacc. is an emerging and destructive soil borne plant pathogen having a wide host range. *S. rolfsii* commonly occurs in the tropics and warm regions, causing root rot, stem rot, wilt and foot rot in crops (Farr *et al.*, 1989). Profuse mycelial growth, production of persistent sclerotia (Kokub *et al.*, 2007)are the distinguished characters of *S. rolfsii* that contribute in survival and disease development in plants. Advancing mycelia form spherical sclerotia which darken as they mature and become tan to dark brown in color. These sclerotia serve as a survival structure of the pathogen(Money, 2016).During favorable weather conditions, sclerotia germinate by producing hypha and penetrate susceptible plant parts when come into contact with plant. The fungus secrets oxalic acid and tissue degrading enzymes such as polygalacturonase and cellulase, which cause death of host cells (Mullen, 2001). Within few days after infection, symptoms of soft rot, yellowing and wilt are visible(Mullen ,2001).A wide range of symptoms such as seedling blight, seed rots, stem rot, collar rot and wilt in different host plants have been reported (Arunasri *et al.*,2011).

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Over 270 host genera have been reported to be infected by this pathogen in the U.S.A. alone (Farr & Rossman,2006). It has been reported that when legumes, cucurbits and other vegetable crops are grown in rotation with beans incidence and severity of *S. rolfsii* is high and severe(IITA,1996). Between1988 and 1994, the United States encountered a significant economic loss of $36.8 million due to *S. rolfsii* infection in peanut (Franke *et al.*, 1998).Diversity in pathogen population results in variation in virulence towards host. This often can be used for evaluation of disease resistance in crop breeding program (Paparu *et al.*, 2020). Monitoring pathogen population for virulent races is important as it provides us the knowledge of evolution in pathogen in response to crop breeding against the pathogen (Peever *et al.*, 2000).
In Nepal, *S. rolfsii* causes southern blight in vegetables, seedling blight in rice, collar rot in lentil and other diseases in different crops. In recent years, incidence and severity of the disease has been increased. *S. rolfsii* has infected rice in Sunsari, Jhapa, Morang and Udaypur districts during 2016/17(RARS,2017), onion in Dhading district during2018/19(PPD,2018), lentil, rajma, chickpea and mustard in Lumbini and Sudur Paschim province in 2015 (NGLRP,2015) till now and chilli in Chitwan. Despite the growing threat, knowledge on the morphology and pathogenicity of *S. rolfsii* from different crops and geography in the country is lacking, which are required for developing management strategies, including resistant varieties of crop plants. Thus, the objectives of this study were to determine the morphological differences in *S. rolfsii* isolated from different crops and to assess their cross infectivity.

METHODOLOGY

ISOLATION OF *S. rolfsii*

Sclerotium rolfsii was isolated from diseased root samples of six crop species collected from different locations (Table 1). Plant roots were washed and cut into small sections. The cut root samples were surface sterilized by dipping in 1 % sodium hypochlorite for 1 min, rinsed with sterile distilled water, and plated on moist chamber. The plates were incubated at 25°C in an incubator until sclerotia were produced (5-7 days) and the sclerotia produced were inoculated onto PDA and cultured. SRON isolate of onion was collected from National Plant Pathology Research Centre (NPPRC), Khumaltar, and SRRI isolate of rice was collected from RARS, Tarahara.

Table 1. List of *Sclerotium rolfsii* isolated from different crop species and locations

Crop species	*S. rolfsii* isolates	Location
Rice	SRRI	Tarahara, Sunsari
Chickpea	SRCK	Khajura, Banke
Chilli	SRCI	Bharatpur, Chitwan
Soybean	SRSY	Bharatpur, Chitwan
Onion	SRON	Naubise, Dhading

Crop species	*S. rolfsii* isolates	Location
Rapeseed	SRMD	Ghorahi, Dang
Lentil	SRLN	Ghorahi, Dang
Rajma	SRRJ	Khajura, Banke

MORPHOLOGICAL CHARACTERIZATION OF *S. rolfsii* ISOLATES

Eight *S. rolfsii* isolates were assessed for colony growth rate, color of mycelia, number of sclerotia produced, shape and size of sclerotia, and days to sclerotial initiation in completely randomized design (CRD) with three replications. Five mm diameter agar plugs from 3-day-old culture of the fungus were placed at the center of 90mmdiameter Petri dishes upside down for the better contact of the pathogen to the media and incubated at 25°C. Mycelial growth of the fungus was assessed after 24 hours of incubation by measuring colony radius .Measurements were made for 5 to 7 days, based on culture growth beyond the Petri dish base. The size of sclerotia was measured and numbers of sclerotia formed were counted at 28 days after inoculation (Mahato and Biswas, 2017).

CROSS INFECTIVITY OF *S. rolfsii* ISOLATES ON DIFFERENT HOSTS

Eight isolates of *S. Rolfsii* and seven crop species viz. rice (Hardinath-1), soybean (farmers cultivar), Rapeseed (farmers cultivar), chickpea (BG), lentil (Simrik), rajma (farmers cultivar), and chilli (Jwala) were used for the study. Mass culture of each isolate of *S. rolfsii* was prepared in the mixture of rice bran and rice husk in 1:5 proportion. Twenty-five gram of the mixture was autoclaved (121^0C at 15 PSI for 60 minutes) twice on two consecutive days and allowed to cool under sterile conditions. Then, the mixture was inoculated with 5 mm diameter sized agar plugs of 3-days-old pure culture of *S. rolfsii* and incubated for 10 days at 25^0C in an incubator.

For potting, sandy loam field soil was sterilized by using formaldehyde (4%) at the rate of 2 ml per kg soil. Sterilized soil was inoculated with them as culture of *S. rolfsii* at the rate of 2g per kg soil in pots. Sterilized soil without *S. rolfsii* was used as control. Seeds of each crop were sown in pots after 3 days of inoculation. Ten seeds were sown in a pot for small grain crops viz. rice, chilli, lentil, and mustard, whereas for large seeded crops viz, rajma, chickpea, and soybean, six seeds were sown. Completely randomized design with 3 replications was used in the experiment. The pots were placed under greenhouse conditions (29^0 C and 51 % relative humidity). Emergence of seedlings was recorded at 7 days after sowing and post emergence mortality was recorded at 10 days of planting. The reaction of the crop species to the isolates of *S. rolfsii* was categorized as follows (Prabhu and Patil, 2005):
Resistant: 0-10 percent seedling mortality
Moderately resistant: 11-30 percent seedling mortality
Moderately susceptible: 31-70 percent seedling mortality
Susceptible: 71-100 percent seedling mortality

STATISTICAL ANALYSIS

R studio was used for data analysis. To determine the effect of *S. rolfsii* isolates on seed germination percentage, and mortality percentage, values were subjected to arcsine square root transformed before analysis. Mean separation was carried out by Duncan's multiple range test.

RESULTS

MORPHOLOGICAL CHARACTERISTICS OF *S. rolfsii* ISOLATES

Morphology of eight isolates of *S. rolfsii* isolated from the different hosts were studied based on mycelial and sclerotial characters (Table 2). The highest radial growth was observed in SRLN (0.9cm) isolated from lentil in 24 hours and also covered petridish on the third day of inoculation. The mycelial growth rate on PDA medium was relatively fast for the isolates SRLN, SRRI, and SRCI isolated from lentil, rice, and chilli, respectively. While the isolates SRON and SRSY isolated from onion and soybean, respectively had slow radial growth. They completely covered the plate on the fifth day of inoculation. All isolates had bright white-colored cultural growth on PDA. The highest number of sclerotia per plate was observed in SRLN(890) and SRRI (812). While the lowest number of sclerotia per plate was observed in SRON (340), SRRJ (375), and SRMD (390). The sclerotial size was significantly larger in SRON (1.61 mm),and SRSY (1.59 mm) as compared to other isolates. Similarly, days to the formation of sclerotia varied among isolates. It took seven days to initiate sclerotia formation in SRSY while sclerotia formation was initiated within three days in SRRI.

Table 2. Mycelial and sclerotial characteristics of *S. rolfsii* isolates isolated from different crops

Sclerotium isolates	Radius (cm)				Number of sclerotia/ plate	Size of sclerotia (mm)	Days to initiation of sclerotia formation
	Day 1	Day 2	Day 3	Day 4			
SRCK	0.57^{b}	1.66^{bc}	3.04^{cd}	4.50^{a}	410^{b}	1.48^{b}	5^{b}
SRON	0.20^{d}	0.84^{d}	2.03^{f}	3.77^{c}	340^{b}	1.61^{a}	5^{b}
SRRI	0.81^{a}	2.39^{a}	3.84^{b}	4.50^{a}	812^{a}	1.14^{d}	3^{d}
SRCI	0.82^{a}	1.76^{b}	3.41^{bc}	4.50^{a}	556^{b}	1.14^{d}	4^{c}
SRSY	0.37^{c}	0.77^{d}	2.21^{ef}	3.17^{d}	546^{b}	1.59^{a}	7^{a}
SRRJ	0.48^{bc}	1.33^{c}	2.68^{de}	4.10^{b}	375^{b}	1.17^{d}	4^{c}
SRMD	0.62^{b}	1.74^{b}	3.07^{cd}	4.50^{a}	390^{b}	1.35^{c}	4^{c}
SRLN	0.94^{a}	2.16^{a}	4.50^{a}	4.50^{a}	890^{a}	1.18^{d}	5^{b}
Mean	0.56	1.58	3.09	4.19	539.87	1.33	4.62
LSD (<0.05)	0.14	0.36	0.52	0.21	250.41	0.08	0.001
F-test	***	***	***	***	**	***	***
CV(%)	14.04	12.97	9.62	2.91	26.48	3.69	0.02

S. rolfsii isolates named after host crop i.e. SRRI- rice, SRCK-chickpea, SRCI- chilli, SRSY- soybean, SRON- onion, SRMD- mustard, SRLN-lentil and SRRJ- rajma.

EFFECT OF *S. rolfsii* ISOLATES ON GERMINATION OF DIFFERENT CROPS

Soil application of *S. rolfsii* had significant effect on germination of the crop species (Table 3). Germination of the crops varied significantly with different isolate of the pathogen.

Table 3. Effect of *Sclerotium rolfsii* isolates on germination percentage of different crop species in pot culture

S. rolfsii isolates	Crop species						
	Rice	Chickpea	Chilli	Soybean	Mustard	Lentil	Rajma
SRCK	76.67 $^{a-i}$(1.08)	38.89 $^{g-k}$(0.66)	63.33 $^{a-j}$(1.01)	55.56 $^{d-k}$(0.84)	70.00 $^{a-j}$ (1.01)	93.33 ^{ab}(1.41)	26.67 ^{k}(0.38)
SRCI	46.67^{e-k} (0.75)	27.78^{h-k} (0.55)	53.33^{d-k} (0.82)	77.78^{a-g} (1.16)	76.67^{a-g}(1.15)	96.67^{ab} (1.45)	20.00 ^{k} (0.39)
SRON	100.00^{a} (1.55)	83.33^{a-g} (1.15)	90.00^{a-e} (1.30)	72.22^{a-h} (1.10)	66.67^{b-j} (0.98)	90.00^{a-e} (1.30)	53.33 $^{d-k}$ (0.82)
SRSY	86.67^{a-d} (1.33)	38.89^{g-k} (0.66)	26.67^{jk}(0.46)	38.89^{g-k} (0.67)	66.67^{b-j} (0.96)	80.00^{a-g} (1.12)	33.33^{i-k} (0.53)
SRMD	86.67^{a-d} (1.33)	55.56^{c-k} (0.85)	83.33^{a-g} (1.22)	83.33^{a-g} (1.22)	53.33^{d-k} (0.82)	90.00^{a-f} (1.25)	40.00^{g-k} (0.68)
SRLN	93.33^{ab} (1.41)	55.56^{d-k} (0.84)	80.00^{a-g} (1.12)	72.22^{a-i}(1.03)	46.67^{e-k} (0.75)	86.67^{a-f} (1.27)	20.00^{k} (0.39)
SRRJ	73.33^{a-i}(1.04)	44.44^{f-k}(0.73)	86.67^{a-d} (1.33)	72.22^{a-h} (1.10)	83.33^{a-g}(1.22)	93.33^{a-d} (1.35)	46.67^{e-k} (0.75)
SRRI	73.33^{a-i}(1.05)	44.44^{f-k} (0.73)	36.67^{h-k} (0.56)	83.33^{a-g} (1.22)	76.67^{a-i}(1.08)	90.00^{a-e} (1.30)	20.00^{k} (0.39)
Control	100.00 ^{a}(1.55)	94.44^{ab}(1.42)	96.67^{ab}(1.4 5)	94.44 ^{ab}(1.42)	93.33^{ab}(1.4 1)	100.00^{a}(1. 55)	93.33 $^{a-c}$(1.40)
Mean	1.029163						
LSD ($p<0.05$)	0.4378						
F test	*						
CV (%)	26.32						

Figures followed by the same letter in column are not significantly different by DMRT. Figures in the parentheses are arc sin transformation values. S. rolfsii isolates named after host crop i.e. SRRI- rice, SRCK-chickpea, SRCI- chilli, SRSY- soybean, SRON-onion, SRMD-rapeseed, SRLN-lentil and SRRJ- rajma.

Germination of Chickpea and rajma were greatly reduced (up to 27.78% and 20%, respectively) compared with the crop species(Table 3). Germination of rapeseed, chilli and lenti lseeds were reduced ($p< 0.05$). Germination of rice was not significantly for the isolates of *S. rolfsii*, except for SRCI (46.67%).

MORTALITY OF CROP SPECIES DUE TO *S. rolfsii*

All the isolates of *S. rolfsii* were cross infective on the tested crop species (Table 4). There were differences in seedling mortality among the crop species ranging from 0% to 100% (Table 4).

Table 4. Cross infectivity of different isolates of S. rolfsii on different crop species

S. rolfsii Isolates	Crop species						
	Rice	Chickpea	Chilli	Soybean	Mustard	Lentil	Rajma
SRCK	26.46 $^{i-o}$ (0.46)	100.00 $^{a-c}$ (1.53)	10.00 $^{m-o}$ (0.21)	70.00 $^{a-i}$ (1.06)	90.74 $^{a-f}$ (1.31)	63.33 $^{a-l}$ (0.92)	83.33 $^{a-f}$ (1.28)
SRCI	63.33^{a-j} (1.00)	50.00^{d-n} (0.79)	95.24^{a-e} (1.43)	45.56^{e-n} (0.74)	74.17^{a-j} (1.04)	57.78^{c-m} (0.87)	100.00^{a-c} (1.53)
SRRJ	68.25^{a-k} (0.97)	88.89^{a-f} (1.34)	44.44^{f-n} (0.72)	47.22^{e-n} (0.75)	70.24^{a-j} (1.01)	71.48^{a-j} (1.01)	88.89^{a-f} (1.34)
SRSY	20.00^{j-o} (0.37)	100.00^{a-c} (1.54)	75.00^{a-g} (1.20)	100.00^{a-c} (1.54)	100.00^{ab} (1.55)	62.96^{a-l} (0.92)	50.00^{a-f} (1.28)
SRMD	14.44^{k-o} (0.32)	56.67^{a-l} (0.93)	12.86^{k-o} (0.30)	31.67^{g-o} (0.59)	100.00^{a-c} (1.55)	100.00^{a} (1.55)	61.11^{a-k} (0.97)
SRLN	10.00^{l-o} (0.27)	46.67^{e-n} (0.76)	27.31^{h-o} (0.47)	44.44^{f-n} (0.73)	100.00^{a-c} (1.55)	92.96^{a-f} (1.34)	100.00^{a-c} (1.53)
SRON	10.00^{m-o} (0.20)	60.00^{a-l} (0.89)	35.56^{g-o} (0.55)	25.00^{g-o} (0.52)	64.35^{a-j} (1.01)	55.65^{d-m} (0.84)	50.00^{d-n} (0.79)
SRRI	96.30^{a-d} (1.44)	55.56^{d-m} (0.84)	51.19^{b-m} (0.87)	46.67^{e-n} (0.75)	76.39^{a-h} (1.14)	77.22^{a-i} (1.08)	83.33^{a-f} (1.28)
Control	13.33 $^{j-o}$ (0.37)	5.56 ^{no} (0.15)	0.02 ^{o} (0.00)	0.00 ^{o} (0.02)	11.67 $^{l-o}$ (0.29)	3.33 ^{no} (0.12)	0.00 ^{o} (0.02)
Mean	0.8852						
LSD (p<0.05)	0.539						
F test	***						
CV (%)	27.07						

Figures followed by the same letter in column are not significantly different by DMRT. Figures in the parentheses are arc sin transformation values. S. rolfsii isolates named after host crop i.e. SRRI- rice, SRCK-chickpea, SRCI- chilli, SRSY- soybean, SRON-onion, SRMD-rapeseed, SRLN-lentil and SRRJ- rajma.

Seedling mortality was significantly higher in lentil due to SRMD (100%) (P <0.0001). Rice was susceptible to *S. rolfsii* isolate SRRI (96.30 %), and moderately susceptible to SRCI (63.33 %) and SRRJ (68.25 %)while resistant to other isolates. 100 % seedling mortality was observed in chickpea due to SRCK and SRSY. Chickpea was moderately susceptible to other isolates. Similarly, soybean was susceptible to SRSY with100 % seedling mortality. Isolate SRCI caused 95.24 % seedling mortality in chilli. Chilli was resistant to SRCK (10 %) and moderately resistant to other isolates. Mustard and

rajma were susceptible to all isolates of *S. rolfsii* and has high seedling mortality than other crops. Similarly, lentil was susceptible to SRMD (100%), SRLN (92.96 %)and SRRI (77.22 %), while moderately susceptible to other isolates of *S. rolfsii*. All isolates of the pathogen were virulent towards the crop species with an average mortality greater than 50 %. However, average seedling mortality due to SRON was only 42 %. While SRSY caused highest average seedling mortality (72.56 %).

Seedling mortality was found positively correlated with numbers of sclerotia with significant correlation coefficient, r = 0.369 ($p<0.05$) (Figure 1).

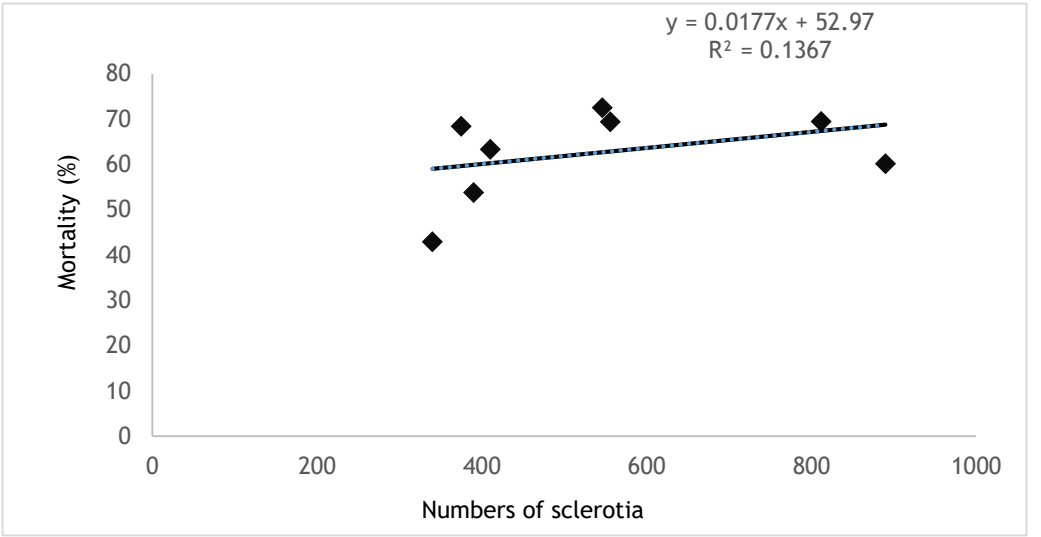

Figure 1. Relationship between seedling mortality and numbers of sclerotia produced

DISCUSSIONS

S. rolfsii is difficult to control due to its wide host range and persistent sclerotia in the soil. Knowledge on the diversity of the pathogen help develop management strategies. The diversity of *S. rolfsii* has been studied by different researchers in different countries such as in peanut in the USA and Japan (Franke *et al.*, 1998;Okabe & Matsumoto, 2000), in cantaloupe, tomato, eggplant, peanut, pepper, snap bean, sweet potato, water- melon, and several ornamental species in USA (Xie *et al.*,2014), in sweet potato in Korea(Paul *et al.*, 2017),in rice and tomato in India (Biswas & Mahato,2017).

In the present study we examined the variations in mycelial growth rates, number and size of sclerotia produced, number of days required to sclerotia formation by the isolates of*S. rolfsii* isolated from eight crop species. Similar results was reported by Le *et al.* (2012) they observed 79 to 1,080 number of sclerotia per plate and their size ranging from 0.88 to 2.24 mm. Similarly, Paul *et al.* (2017) observed 2 to 248

sclerotia per plate size ranging from 0.5 to 2 µm. It was observed that those isolates which require a longer duration to form sclerotia had a slow mycelial growth rate and had larger sclerotial size than the fast growing isolates. Kokub *et al*. (2007) and Manu *et al*. (2018) reported similar results. They found some isolates were comparatively fast growing and produced the higher number of sclerotia than the others. In the present study, the size of the sclerotia varied with isolates. The average size of sclerotia was 1.33 mm. Similar size of sclerotia had been reported for *S. rolfsii* in different crops in Korea(Paul *et al.*,2017).

In this study, we observed a significant reduction in germination percentage of crops due to infection by *S. rolfsii* isolates. Germination was reduced upto 80 % in rajma, 66% in chick pea, and about 40 % in chilli, while rice, lentil, and rapeseed were least affected. This difference in germination among crops by *S. rolfsii* may be due to the reduction of germination speed of different crops. Lentil, rice and mustard have smaller seed size and germinate rapidly as compared to larger seeded crops. Research carried out on different crops such as bean (Al- Rifaee *et al*., 2004; Alngiemshy *et al*., 2020), iron tree(Dera *et al*., 2019), canola (Hwang *et al*.,2014), chilli (Sanjuan-Martínez *et al*.,2020)showed that smaller seeds germinated faster than larger seeds. Although chilli has a small seed size it took longer time to germinate and hence has low germination percentage. According to Kirkpatrick & Bazzaz (1979) rapid emergence of seeds reflect a short period of susceptibility to infection while late emerging seeds would have longer period of susceptibility. This could increase a period of exposure to infection resulting in higher frequency of infection and increased mortality of seeds.

In our study, the infectivity of eight isolates varied among the seven tested crops, ranging from 10 to 100%.The post-emergence mortality caused by all isolates was high in mustard and rajma compared to the other crops. Similar pathogenic variability among *S.rolfsii* isolates on different crops was demonstrated by several earlier workers, on pepper and tomato (Xie *et al*., 2014),on tomato (Biswas & Mahato, 2017), on common bean (Paparu *et al*., 2020).Seedlings are very susceptible to *S. rolfsii* and die quickly once they become infected(Gawande *et al*.,2020). In this study, we found that all the isolates caused infection to all studied crops but, with varied levels of infection and were mostly virulent on their crop species of origin. Similarly, isolates from leguminous crops were more virulent towards the legume crops. However, the isolate from ricewas virulent to all crop species, except soybean.

In the present study, we observed a positive correlation between the number of sclerotia formed and seedling mortality. Similar results were reported by Paparu *et al*. (2020) in their study where they observed that those isolates which produced high numbers of sclerotia on PDA media exhibited a high degree of aggressiveness.

CONCLUSIONS

S. rolfsii is now becoming a major threat to different crops in Nepal. It is essential to understand the variability in the isolates of *S. rolfsii* causing different diseases in various host crops. The isolates from different crop species showed diversity in their morphology and virulence. This pathogen is difficult to control as the host crop species are large in number. For successful management of this pathogen, the sources of disease inoculums should be reduced which can be achieved by removing the affected plant parts and adopting the crop rotation with non- host crops wisely.

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