**Reprint** **ISSN 1923-7766 (Web Version)**

# International Journal of Experimental Agriculture

*(Int. J. Expt. Agric.)*

**Volume: 4** **Issue: 4** **November 2014**

*Int. J. Expt. Agric. 4(4): 29-35 (November 2014)*

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**ISSN-1923-7766 (Online)**
***Int. J. Expt. Agric. 4(4):29-35(November 2014)***

# PATHOGENICITY AND CHARACTERIZATION OF SOME *Sclerotium rolfsii* ISOLATES

A.T.M.S. ISLAM[1], B. MEAH[2], S.M.E. HASSAN[1] AND S.M.M. HOSSAIN[1]

[1]Professor, Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Dinajpur;
[2]Professor, Plant Pathology, Bangladesh Agricultural University, Mymensingh.

Corresponding author & address: Dr. Sk. Md. Mobarak Hossain, E-mail: mobarakhstu@gamil.com
Accepted for publication on 28 October 2014

**ABSTRACT**

Islam ATMS, Meah B, Hassan SME, Hossain SMM (2014) Pathogenicity and characterization of some *Sclerotium rolfsii* isolates. *Int. J. Expt. Agric.* 5(3), 29-35.

Ten isolates of *Sclerotium rolfsii* causal organism of foot rot of vegetables were collected from different hosts and locations of Bangladesh. Morphological characters like mycelial growth, colony colour, colony consistency, sclerotia formation in the PDA were studied. The isolate spinach showed the highest radial mycelial growth from 2 DAI to 6 DAI (1.97 cm to 9.00 cm). At 6 DAI the highest average linear mycelial growth rate was found in isoalte spinach (1.5 cm/day) which was statistically similar to the average linear mycelial growth of isolates lentil-4, eggplant and tomato-2. The highest number of sclerotia was found in the isolate lentil-1 (8.79/cm$^2$). Pathogenicity of 10 isolates of *S. rolfsii* was tested and the isolate spinach was found to be highly virulent. It had significant effect on seed germination, pre-emergence death, damping off and plant stand over other isolates.

***Key words:*** *Sclerotium rolfsii, pathogenicity, characterization, isolates, vegetables*

## INTRODUCTION

*Sclerotium rolfsii* is an economically important pathogen on numerous crops worldwide (Aycock 1966). It is an omnivorous and destructive soil borne pathogen of many plants. It has a very extensive host range which includes more than 500 plants particularly in tropical and subtropical warm temperate areas (Mordue 1974; Singh and Allen, 1979; Wydra 1996). Its growth is optimal at 27-30ºC and rarely occurs where average winter temperatures fall below 0ºC. Growth of *S. rolfsii* on all organic-based and inorganic synthetic media is accompanied by forming of spherical, brown to tan colored sclerotia measuring 0.3 to 3.0 mm in diameter (Edelstein *et al*. 1983). They are initially developed as white aggregates or knots of mycelium and then it differentiates to form the mature sclerotium within 2-3 weeks. Sclerotia form abundantly on potato dextrose agar (PDA) and can also be produced on a substrate such as autoclaved oat kernels moistened with 1.5% water agar (Punja and Grogan, 1981a). The linear growth rate of hyphae on agar media at 27°C ranged from 0.85-0.97 mm per hour, depending on the isolate. Mycelial growth is progressively less with increasing moisture content (Mustafee and Chattopadhyay, 1971) and disease incidence is greater in well-drained, sandy soils (Weerapat and Schroeder, 1966) and at soil water contents below saturation (Ramarao and Raja, 1980). Punja and Grogan (1981b) suggested that the optimum pH range for mycelial growth is 3.0-5.0, and sclerotial germination occurs between 2.0-5.0. Germination of sclerotia is inhibited at a pH above 7.0 (Sharma and Kaushal, 1979). Although percentage of germination is lower at soil depths below 2.5 centimeters than it is at the soil surface and its nil below 8 centimeters, results from controlled gaseous studies suggest that the inhibition is not due to oxygen depletion or ethylene and carbon dioxide buildup (Punja and Jenkins, 1984). The aim of the investigation therefore was to evaluate the differences in growth and other morphological characters of *Sclerotium rolfsii* isolates on PDA media and also to investigate the pathogenicity of the isolates on different vegetable crops.

## MATERIALS AND METHODS

*S. rolfsii* were isolated from diseased plant samples collected from different locations (Table 1). The samples showing typical root and collar rot symptoms were brought to the IPM Lab, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and washed initially with tap water to remove sand and soil particles. Then specimens were cut into small pieces (1.0 cm) along with healthy and dead tissues (inocula). The pieces were surface sterilized with 1% chlorox, washed thrice in sterilized water and placed on filter paper to remove excess water adhering with the pieces. Thereafter 5 pieces were plated in PDA plates aseptically maintaining equal distances (Begum *et al*. 1999). The plates were incubated at 28±1°C for 7 days and observation was taken regularly to see the growth of fungi from the inocula. The fungus was purified by hyphal tip culture technique (Tuite 1969). The isolated fungi were identified following the keys outlined by Aycock (1966) and Barnett (1960). The pure cultures of the isolates of *S. rolfsii* were preserved in PDA plates at 5±1°C in refrigerator as stock culture for future use (Begum 1997).

Table 1. Isolates of *S. rolfsii* collected from different hosts and locations

| Isolates of *Sclerotium rolfsii* | Host plant | Location |
|---|---|---|
| Spinach ($S_1$) | Spinach | Thakurgaon |
| Lentil-1 ($S_2$) | Lentil | BINA farm, Mymensingh |
| Lentil-2 ($S_3$) | Lentil | BINA farm, Mymensingh |
| Lentil-3 ($S_4$) | Lentil | BAU farm, Mymensingh |
| Lentil-4 ($S_5$) | Lentil | Ishurdi, Pabna |
| Lentil-5 ($S_6$) | Lentil | Ishurdi, Pabna |
| Eggplant ($S_7$) | Eggplant | BAU farm, Mymensingh |
| Tomato-1 ($S_8$) | Tomato | Thakurgaon |
| Tomato-2 ($S_9$) | Tomato | Dinajpur |
| Orchid ($S_{10}$) | Orchid | Bhaluka, Mymensingh |

Radial mycelial growth of the isolates of *S. rolfsii* in PDA was studied following the method of Begum *et al*. (1998). A 5 mm block of each *Sclerotium* isolate was placed at the middle of petri plate containing PDA with three replications. After 1, 2, 3, 4, 5 and 6 DAI (Days after inoculation) the radial mycelial growth was measured. Mean of three replications was taken as growth of each isolate.

The colony and sclerotial characters of *Sclerotium* isolates in PDA were observed. The PDA plates were inoculated by mycelial block (5mm) at the centre of the plate. The inoculated plates were placed in incubator at 25±1°C for 15 days and regular observation was done. Observations were made on morphological characters.

To test the pathogenicity of different isolates, inoculum was cultured on barley grains. To prepare the inoculum, barley grains (100 g) were soaked in sterilized water for 12 hours. The water was drained off, water soaked grains (100 g) were taken into 500ml conical flask and autoclaved at $121^0$C with 15 psi for 20 minutes (Yaqub and Shahzad, 2005). The conical flasks containing autoclaved barley grains were brought out and allowed to cool at room temperature for two hours. Then 10 mycelial discs (5 mm dia) cut from the edge of 3 days old culture of *S. rolfsii* were added to each conical flask and incubated at $25^0$C for 20 days. For even colonization, the conical flasks containing inoculated barley grains were shaken thoroughly by hand at every 3 days interval. The colonized barley grains were air dried for 3 days and stored at $4^0$C temperature for further use.

Ten isolates of *S. rolfsii* were tested for their pathogenicity in tray soil in the nethouse. Tray soil was prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2:1:1 and sterilized with 5 ml formalin (40%) diluted with 20 ml water for 4 kg soil (Dashgupta 1988) and the prepared soil was heaped in square block. Soil heap was covered by a polyethylene sheet for 48 hrs. After 4 days of treatment, surface sterilized plastic tray was filled up with the sterilized soil. Tray soil was inoculated with *S. rolfsii* barley culture @ 10 g/kg soil before 3 days of seed sowing. Seeds of eggplant *var*. Dohazari, tomato *var*. pusa rubi and indian spinach var. Local were sown in separate sets @ 50 seed/tray with three replications and the control trays were sown using sterilized soil without inoculation of the pathogen.

Disease incidence was observed regularly and recorded at 7, 14 and 30 days after sowing to estimate the effect of *S. rolfsii* on pre-emergence and post emergence death of seedling. Plants were uprooted after 30 days growth to assess colonization of roots by *S. rolfsii*. The data recorded were seed germination, pre-emergence death of seedling, damping off of seedlings, foot rot and seedling stand. The experiment was laid out in Completely Randomized Design (CRD) with three replications. Data was analyzed following MSTAT-C computer package program and means were separated using DMRT at 5% level of significant.

## RESULTS AND DISCUSSION

All the isolates of *S. rolfsii* produced cottony white mycelia and regular shaped colony. In most isolates, mycelial growth was fluffy**.** Dense fluffy rings were produced in isolates spinach, lentil-1 and lentil-2. Scattered fluffy growth pattern was observed on isolates lentil-3, lentil-4, tomato-1, and orchid. Mycelial growth was embedded and compact in isolates lentil-5, tomato-2 and eggplant (Table 2). Two types of growth habit were observed in the isolates of *S. rolfsii* – very fast and fast. Growth habit of the isolates spinach, lentil-4, eggplant and tomato-2 were very fast and rest of the isolates was found fast.

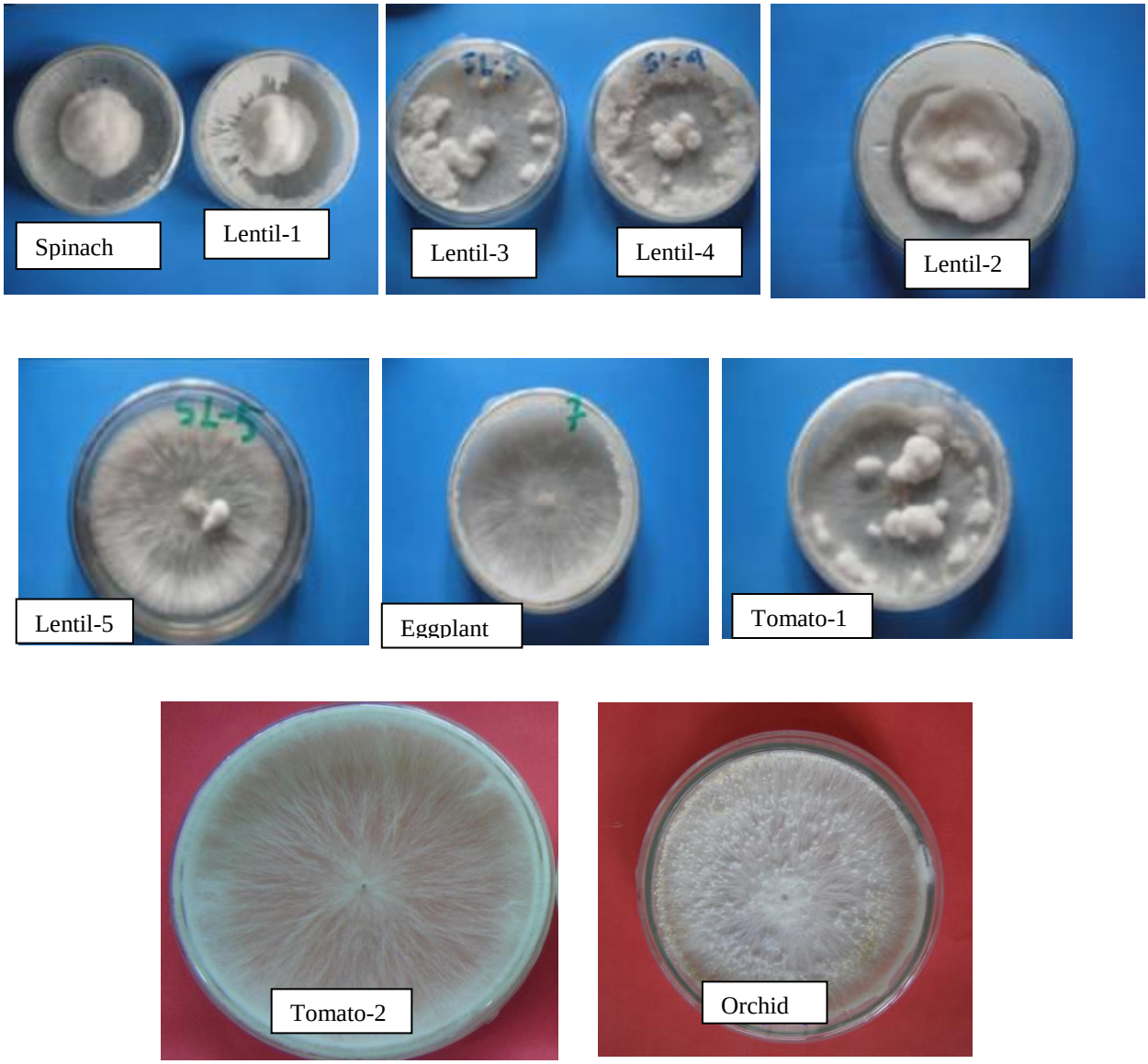


Plate 1. Growth pattern of *Sclerotium rolfsii* isolates on PDA plates

Sclerotia were formed by the fungus at the edges of the plates from 7 days after inoculation when the agar media were completely covered by the mycelia. After 7 days of inoculation the sclerotia was formed first in spinach. After 9 days of inoculation, initiation of sclerotia was observed in lentil-1, lentil-3, lentil-4, eggplant and tomato-1. It was 10 days for lentil-2 and tomato-2 to form sclerotia while it took about 11 days to form sclerotia in isolate orchid. At first white roundish sclerotium was formed mostly at the periphery of petriplate. Subsequently, the sclerotia turned off white, became brown, finally it looked as brownish mustard seed. Number of sclerotia varied from one isolate to another. The highest number of sclerotia was found in the isolate lentil-1 (8.79/cm$^2$). Isolate spinach, lentil-2, lentil-5 and eggplant produced the statistically similar number of sclerotia. The lowest number (3.12 and 3.45/cm$^2$) of sclerotia were produced by isolates lentil-3 and orchid, respectively. Diameter of sclerotia also varied in different isolates. The maximum diameter of sclerotia was found in the isolate orchid (2.50 mm) and the minimum diameter of sclerotia was found in the isolate lentil-2 (1.15 mm) (Table 2).

Table 2. Colony and sclerotial characters of *S. rolfsii* isolates in PDA

| Isolates of *S. rolfsii* | Colony characters | | | Sclerotial characters | | | |
|---|---|---|---|---|---|---|---|
| | Colour | Growth pattern | Growth habit | Colour | Initiation (DAI) | Nunmber of Sclerotia/ $cm^2$ | Dia. of sclerotia (mm) |
| Spinach | Whitish | Fluffy, compact at the centre | Very fast | Brownish | 7 | 6.34 b | 1.84 bc |
| Lentil-1 | Whitish | Fluffy, compact at the centre | Fast | Brownish | 9 | 8.79 a | 1.75 b-d |
| Lentil-2 | Whitish | Fluffy, compact at the centre | Fast | Brownish | 10 | 6.54 b | 1.15 f |
| Lentil-3 | Whitish | Scatteredly fluffy | Fast | Brownish | 9 | 3.12 d | 1.30 ef |
| Lentil-4 | Whitish | Scatteredlyfluffy | Very fast | Brownish | 9 | 5.34 bc | 1.60 c-e |
| Lentil-5 | Whitish | Embedded, compact | Fast | Brownish | 10 | 5.97 b | 2.03 b |
| Eggplant | Whitish | Embedded, compact | Very fast | Brownish | 9 | 5.65 b | 1.75 b-d |
| Tomato-1 | Whitish | Fluffy, scatteredly fluffy | Fast | Brownish | 9 | 3.88 cd | 1.35 d-f |
| Tomato-2 | Whitish | Embedded, compact | Very fast | Brownish | 10 | 5.42 bc | 1.55 c-f |
| Orchid | Whitish | Fluffy, scatteredly fluffy | Fast | Brownish | 11 | 3.45 d | 2.50 a |
| **LSD≥ 0.05** | | | | | | **1.478** | **0.3746** |

Isolates were studied for their mycelial growth on PDA. The radial mycelial growth of the isolates of *S. rolfsii* varied significantly from each other at 1, 2, 3, 4, 5 and 6 DAI. At first 24 hours later the fungus grew out from the edge of mycelial block and mycelial diameter ranged from 0.13 to 0.47 cm. After 4 days, the isolates covered above 50% of the surface area on petriplate. Consequently, after five days of inoculation the prolific growth of fungus almost filled the petriplate and at $6^{th}$ day highest mycelial growth (9 cm) was observed on all plates in most of the isolates. *S. rolfsii* exhibited white cottony mycelial growth. The mycelia were silky white at early stage of growth but after 10 days of inoculation, the pathogen lost its luster and became dull in appearance. The statistical analyses of mycelial growth revealed that there was no significant difference among the isolates spinach, lentil-4, eggplant and tomato-2. The isolate spinach showed the highest radial mycelial growth from 2 DAI to 6 DAI (1.97 cm to 9.00 cm). At 6 DAI the highest average linear mycelial growth rate was found in spinach (1.5 cm/day) which was statistically similar to the average linear mycelial growth of lentil-4, eggplant and tomato-2. The lowest mycelial growth was observed in lentil-1, lentil-2 and lentil-5.

The highest mycelial growth rate was found in the isolate spinach followed by statistically similar growth rate in tomato-2 and eggplant respectively. The lowest growth rate was found in the isolates lentil-1, lentil-2 and lentil-5 (Plate-1). The findings of present investigation are partially in agreement with the report of Darakhshanda-Kokub *et al*. (2007) who found the growth arrangement of mycelia on PDA plate was variable. Initiation of sclerotia was found first in the isolate spinach after seven days of inoculation whereas maximum days required for sclerotia formation in the isolate orchid (11 days). Sarma *et al*. (2002) reported the existence of variability among 26 isolates of *S. rolfsii* collected from various hosts/soil samples and localities in India.The isolates varied in colony morphology, mycelial growth rate, sclerotium formation, and sclerotial size and color. The findings are in agreement with the present study. Harlton *et al*. (1995), Nalim *et al*. (1995), and Okabe *et al*. (1998) also found the geographical variability among the isolates of *S. rolfsii.*

**Pathogenicity of *Sclerotium rolfsii* isolates on different hosts**

The pathogenic effect of 10 isolates of *S. rolfsii* on germination, pre-emergence death, damping off, foot rot and plant stand of eggplant, tomato and indian spinach are showed in figures 1 to 5.

**Germination**

Percent germination of eggplant, tomato and indian spinach was significantly lower due to infection of different isolates of *S. rolfsii*. Percent germination was the lowest in trays inoculated with the isolate spinach followed by the isolates lentil-1 and lentil-2 in all the three crops. Among the isolates lentil-3 had the least effect on germination of eggplant and tomato and isolate orchid had less effect on tomato and indian spinach compared to rest of the isolates (Fig. 1).

**Pre-emergence death**

Like the effect on germination, similar trend of infection was observed in case of pre-emergence death of seedling. The isolates spinach was the most aggressive causing pre-emergence death in all the three crops (Fig. 2).

**Damping off**

In case of damping off the isolate lentil-5 was the most pathogenic to tomato and eggplant and the highest damping off of Indian spinach was observed in trays inoculated with lentil-4. The isolates spinach, lentil-2, lentil-3, eggplant and orchid were less pathogenic to indian spinach but moderately pathogenic to eggplant and tomato (Fig. 3).

**Foot rot**

Percent foot rot was nil in control trays in all three crops. The highest percentage of foot rot inspinach was observed in trays inoculated with the isolate spinach followed by the isolates lentil-5 and tomato-2. In case of tomato, the highest percent foot rot was observed in trays inoculated with the isolate tomato-2 and eggplant. In eggplant the highest percent foot rot was observed in trays inoculated with lentil-5 followed by lentil-2 and eggplant and lentil-1 (Fig. 4).

**Plant stand**

Plant stand of eggplant, tomato and spinach was the lowest in trays inoculated with the isolate spinach followed by lentil-1, lentil-2 and eggplant. Percent plant stand of all the three crops was higher in trays inoculated with the isolates lentil-3 and orchid other than the control trays (Fig. 5). Isolate spinach was found to be highly virulent causing foot rot disease. The findings of Begum (1999) supported the present findings who observed the variable disease response in different isolates of *S. rolfsii*. Spinach isolate showed the highest mycelial growth from 2 DAI to 6DAI. The highest number of sclerotia was found in isolate lentil-1.

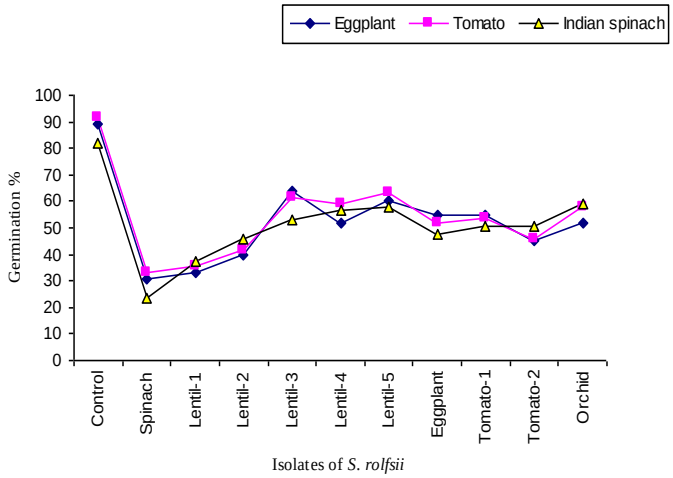


Fig. 1. Germination of eggplant, tomato and spinach after inoculation with 10 isolates of *S. rolfsii*

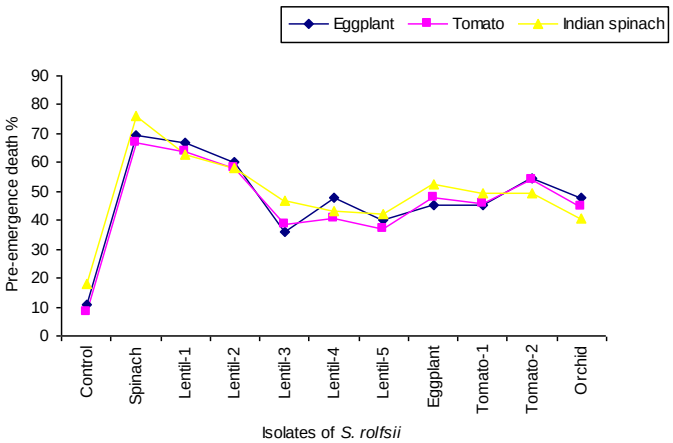


Fig. 2. Pre-emergence death of eggplant, tomato and spinach in pot soil upon inoculation with 10 isolates of S. rolfsii

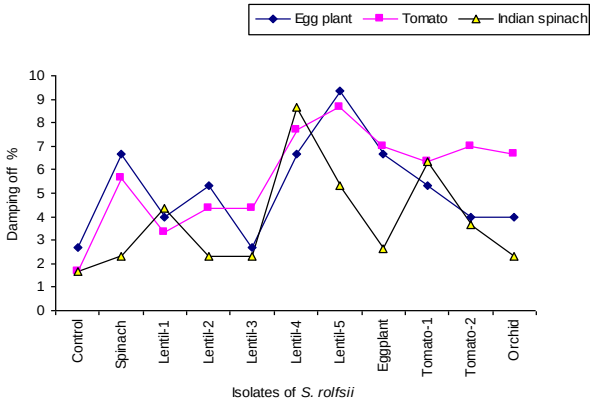


Fig. 3. Damping off of eggplant, tomato and spinach in pot soil upon inoculation with 10 isolates of *S. rolfsii*

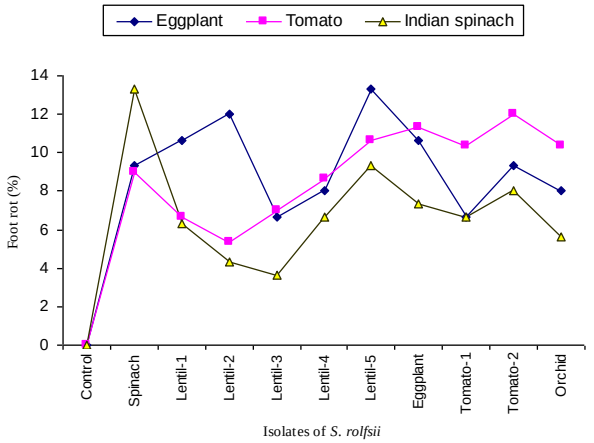


Fig. 4. Incidence of foot rot in eggplant, tomato and spinach when inoculation with 10 isolates of *S. rolfsii*

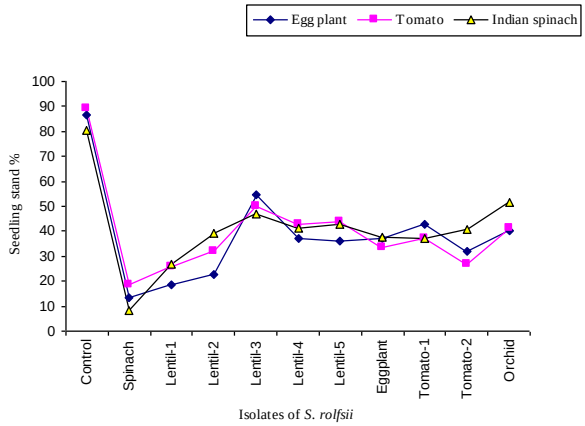


Fig. 5. Seedling stand of eggplant, tomato and spinach in pot soil when inoculation with 10 isolates of *S. rolfsii*

## CONCLUSION

Growth habit of the isolates of *Sclerotium rolfsii* of spinach, lentil-4, eggplant and tomato-2 was found very fast and rest of the isolates was fast. Among the isolates, lentil-3 had the least effect on germination of eggplant and tomato and the isolates spinach was the most aggressive causing pre-emergence death in the crops. The isolate lentil-5 was the most pathogenic on tomato, eggplant and spinach regarding foot rot and plant stand except lentil-1and lentil-2.

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