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EFFECT OF TEMPERATURE AND PH ON THE GROWTH OF *SCLEROTIUM ROLFSII* CAUSING COLLAR ROT OF LENTIL

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ABSTRACT

Sclerotium rolfsii is one of the most important soil-borne plant pathogens which cause severe loss at the time of seedling development. It also causes collar rot in several crops and wild plants. In this experiment, exposure of pathogen to different temperature and pH in order to assess the mycelial growth and number of sclerotia of *S. rolfsii* was done. In present investigation, the collar region of diseased plants showed extensive dark rotting accompanied by visible covering of white mycelial growth of fungus on collar region of plant, interspersed with mustard shaped spherical sclerotia. Maximum mycelial growth of *S. rolfsii* and sclerotia production was recorded at 20°C to 30°C temperature and 90.00 mm diameter growth of *S. rolfsii* was recorded at pH level 6.5.

Key words : Lentil, Temperature, pH, *Sclerotium rolfsii* and Collar rot.

Introduction

Pulses are an essential part of the Indian cuisine, providing a key source of inexpensive protein, particularly for the vegetarian population. Lentil is attacked by fungal, viral and bacterial pathogens. *Sclerotium rolfsii* is one of the important soils borne fungal pathogens having a wide host range and world-wide distribution (Punja, 1988), causing collar rot, root rot, stem rot and wilt on more than 500 plant species including almost all the agricultural and horticultural crops. The fungus is a soil borne pathogen of very aggressive nature and causes considerable damage to young seedlings causing collar rot resulting in substantial yield losses. It survives in the soil for years by producing sclerotia. Infected seedlings exhibit damping-off symptoms, while mature plants turn pale, droop, and dry out (Njambere and Chen, 2011). The disease severely impacts on yield, leading to reduced cultivation areas. To restore lentil production, it is essential to manage collar rot effectively. This disease is particularly problematic for farmers in South-Eastern Rajasthan. The fungus infects lentil crops from the seedling stage to flowering, with seedlings being particularly vulnerable. The present study aims to determine which genotypes of lentil are resistant to *Sclerotium rolfsii*. Punja and Rahe (1992) characterized a fungus by small tan to dark brown or black spherical sclerotia with internally differentiated rind cortex and medulla was placed in genus *Sclerotium*. This disease impacts lentil cultivation in warm areas characterized by elevated soil moisture levels (30-40%) and temperatures approximately 25 ºC during the seedling phase. The pathogen endures as sclerotia in the soil, persisting for several years even in unfavorable conditions (Wu *et al*., 2008). The present study was conducted with the aim of evaluating survivality of *Sclerotium rolfsii* for further study under invitro and in vivo keeping in mind the aforementioned facts.

Materials and Methods

Collection of samples

Infected plants which showing typical collar rot symptoms were collected during the month of November from the lentil fields of AICRP on MULLaRP, Agricultural Research Station, Ummedganj, (Kota). Collected samples were brought to laboratory of Plant Pathology for critical

examination of the symptoms, isolation, identification and description of the pathogen.

Isolation and purification

Diseased samples were brought to Plant Pathology laboratory and thoroughly washed in tap water. After washing, the part of collar region showing typical symptoms of disease was cut into small pieces. Then these pieces were surface sterilized with 0.1% mercuric chloride solution for one minute. Such pieces were washed thoroughly in sterile distilled water three times for 30 seconds in each plate filled with distilled water to remove the traces of mercuric chloride solution and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. These plates were incubated at 28±1°C for 7 days for growth of fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions.

Maintenance of pure culture

The *Sclerotium rolfsii* was sub-cultured on PDA slants and allowed to grow at 28±1°C temperature in the incubator for 10 days. The cultures so obtained was stored in a refrigerator at 4°C and sub cultured once in a month. Hyphal tip method was used for sub culture of fungus.

Symptoms and identification of pathogen

The pathogen *S. rolfsii* forms cottony white colonies on PDA. The colonies appeared as dull white to pure white mycelial growth and initiation of sclerotia formation after 8-9 days of incubation. Sclerotia are brown in colour and mustard seed like in shape. On the basis of these characters pathogen was identified as *S. rolfsii*. Further, pathogen was identified from ITCC (Indian Type Culture Collection) Laboratory, Division of Plant Pathology, IARI, New Delhi-110 012, under ID-1200 5.24.

Pathogenicity test through soil inoculation

In order to ensure and confirm identity of the pathogen isolates, pot culture experiment was conducted in glass house. The required quantity of soil was sterilized to make soil free from microorganisms. Ten seeds of lentil were sown in surface sterilized earthen pots (0.1% mercuric chloride) filled with 2 kg sterilized soil. The pots were irrigated with water time to time to maintain moisture in pot. The pathogen *S. rolfsii* was inoculated in soil before 7 days of sowing @ 10 g mass culture of pathogen per pot. Proper control was maintained where the test fungi was not inoculated.

These were examined regularly for recording disease symptoms. The designated pathogen isolates were re-isolated from the artificially inoculated plants and compared with the original isolates of the pathogen, which were found identical with the original isolates. The per cent severity was calculated according to formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants exhibited collar rot}}{\text{Total number of plants observed}} \times 100$$

Effect of variable temperatures and pH on growth of *Sclerotium rolfsii*

The 6 mm discs of fungus grown on PDA of 3 days was cut and placed on the surface of the solidified medium (20 ml for each Petri plate) in a laminar air flow. The inoculated plates were incubated at 15, 20, 25, 30 and 35 ± 1°C in BOD incubator for 7 days, four replications were maintained for each treatment. The colony diameter was recorded by measuring the radial growth of the colony at 4th and 8th days and number of sclerotial formation was recorded at 20 days and analyzed statistically. The inoculated plates were incubated at pH *i.e.*; 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 incubator for 7 days.

Results and Discussion

Isolation, purification, identification and pathogenicity of the pathogen causing collar rot of lentil

Infected samples produced white cottony fungal growth, containing white, spherical resting bodies (sclerotia) covers the affected area as well as drying and shedding of leaves. Symptoms appeared from the seedling stage to flowering, seedling stage being particularly more vulnerable and destructive. Infected plants exhibited a range of symptoms, that includes leaf yellowing, drooping, drying and premature leaf fall. The collar region of diseased plants showed extensive dark rotting accompanied by visible covering of white mycelial growth of fungus on collar region of plant, interspersed with mustard shaped spherical sclerotia (Plate 1).

The mycelium was hyaline, highly branched, and composed of thin-walled, septate hyphae. Colonies exhibited pure white to off-white mycelial growth, producing sclerotia after 6–7 days of incubation. The sclerotia initially appeared as small, round, mustard-shaped white bodies with clamps, which later turned light to dark brown and developed a shiny surface (Plate 2). The sterilized seeds were sown in earthen pots containing the inoculated soil to assess the pathogenic effect of the fungus on germination percentage. Plants in the uninoculated control pots did not exhibit any disease symptoms (Plate 3). Pal *et al*. (2023) they found that white colonies were produced by *Sclerotium rolfsii* and sclerotia were light brown to dark brown in colour and sclerotial produce mustard shaped (Kumar *et al*. 2017).

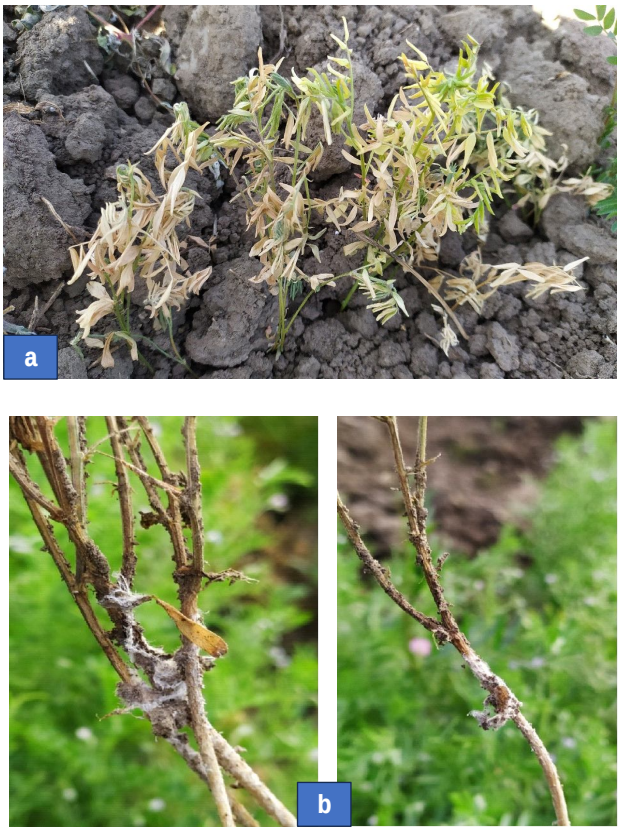

Plate 1 : Symptoms of collar rot disease of Lentil. On above ground part (b) On collar region.

The pathogen *Sclerotium rolfsii* produced cottony white colonies on PDA medium in petri plates. Initially, the colonies exhibited pure white mycelial growth and began forming sclerotia after nine days of incubation. The sclerotia were brown and resembled mustard seeds in shape. Based on these morphological characteristics, the pathogen was identified as *S. rolfsii*. This identification was further confirmed by the Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, under ID-1200 5.24. Meena *et al.* (2024) in collar rot of chickpea caused by *sclerotium rolfsii* the pathogenicity tests were validated by adhering to Koch's postulates and used three inoculation techniques.

Plate 2 : Isolated fungus growth and sclerotia within plate, flask and pure culture slant.

Table 1 : Mycelial growth and sclerotia production of *S. rolfsii* causing collar rot disease of lentil at various temperature level.

Treatments	Temperature (^{0}C)	Colony diameter in mm (4DAI)*	Colony diameter in mm (8DAI)*	Number of sclerotia per Plate (20 DAI)
T_1	15	54.88	85.38	199.75
T_2	20	81.25	90.00	328.75
T_3	25	89.13	90.00	514.25
T_4	30	87.00	90.00	452.50
T_5	35	64.63	71.38	244.50
	SEm±	0.81	0.44	3.03
	CD (p=0.05)	2.45	1.34	9.14

*Mean of four replications; DAI: Days after inoculation.

Growth of *Sclerotium rolfsii* on different temperature levels

The optimum temperature range for growth differs among microorganisms and also influences host–pathogen interactions. The pathogen exhibited significant variation in mycelial growth when tested on PDA at different temperatures, namely 15, 20, 25, 30, and 35°C. Observations recorded on mycelial growth were analyzed statistically and presented in (Table 1) exhibited that Maximum mycelium growth (89.13 mm) of *S. rolfsii* was recorded at 25ºC on 4th days after inoculation which was at par with 30ºC (87.00 mm). However, 90 mm mycelium growth was recorded at 20ºC, 25ºC and 30ºC after 8th days of inoculation. Whereas, minimum mycelium growth (71.38 mm) was observed at 35ºC. Maximum number of sclerotia (514.25) per plate was recorded after 20

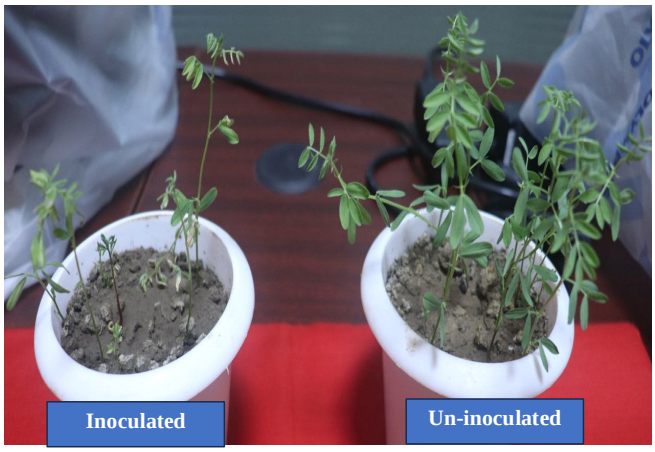

Plate 3 : Pathogenicity test (i) *Sclerotium rolfsii* inoculated (ii) Un- inoculated.

Plate 4(a): Mycelial growth of *S. rolfsii* on various temperature. T_1: 15°C, T_2: 20°C, T_3: 25°C, T_4: 30°C, T_5: 35°C

days of inoculation at 25ºC, followed by 30ºC (452.50). The lowest sclerotia production (199.75) was observed at 15/ °C, which was significantly lower than at all other tested temperature levels (Plate-4 a and b). The results are in corroboration with Zape *et al*. (2013) observed that *S. rolfsii* exhibited rapid mycelial growth at 30°C, while maximum sclerotial production occurred at 25°C. They reported that the optimal temperature range for vigorous radial growth of *S. rolfsii* was between 25°C

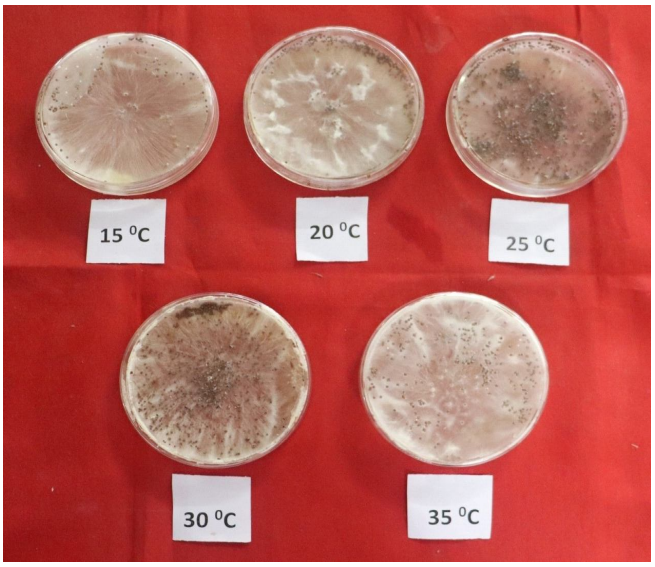

Plate 4(b) : Sclerotia production at different temperature by *S. rolfsii* 20th days after inoculation. T_1: 15°C, T_2: 20°C, T_3: 25°C, T_4: 30°C, T_5: 35°C.

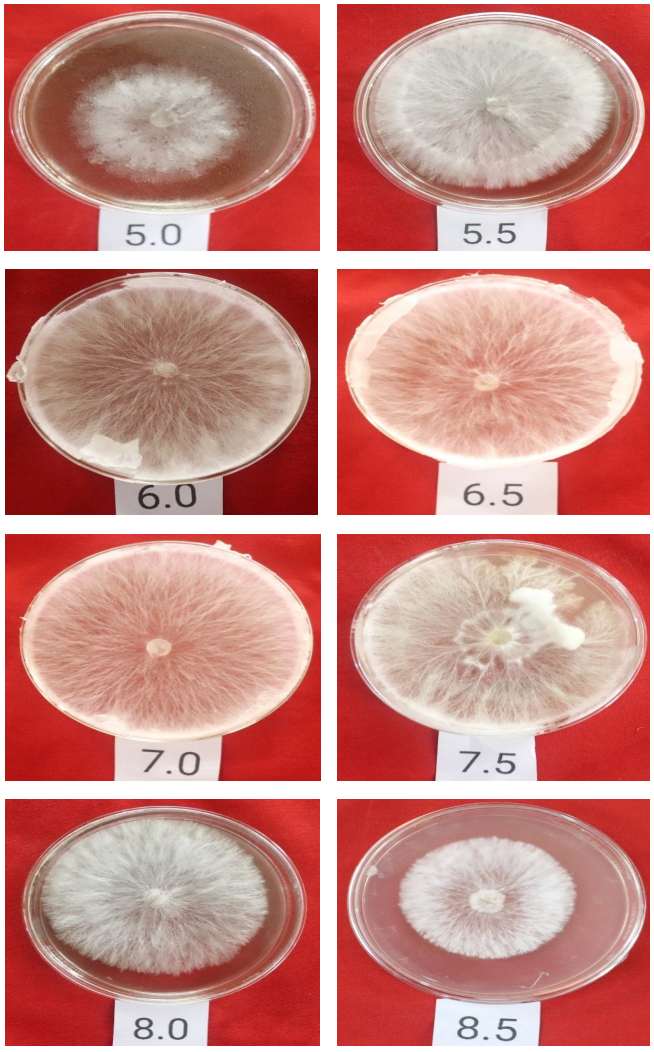

Plate 5(a): Mycelial growth of *S. rolfsii* on different pH. T_1: 5.0, T_2: 5.5, T_3: 6.0, T_4: 6.5, T_5: 7.0, T_6: 7.5, T_7: 8.0, T_8: 8.5

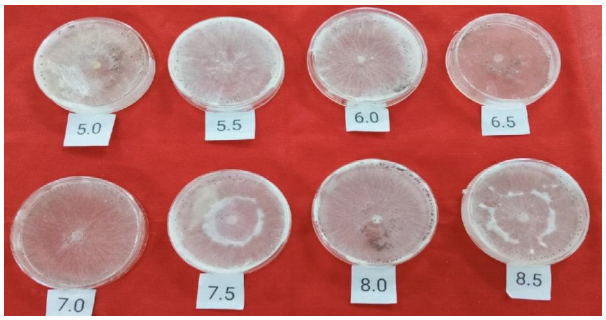

Plate 5(b): Sclerotia production at different pH by *S. rolfsii*. T_1: 5.0, T_2: 5.5, T_3: 6.0, T_4: 6.5, T_5: 7.0, T_6: 7.5, T_7: 8.0, T_8: 8.5

Table 2 : Colony diameter and sclerotia formation by *S. rolfsii* causing CRD of lentil at different pH.

Treatments (pH level)	Colony Dimeter in mm (4DAI) *	Colony diameter in mm (8DAI)*	Number of sclerotia per Plate (20 DAI)
T_1: 5.0	50.63	81.13	89.50
T_2: 5.5	60.25	83.88	86.50
T_3: 6.0	88.38	90.00	108.50
T_4: 6.5	90.00	90.00	98.50
T_5: 7.0	85.75	90.00	80.50
T_6: 7.5	77.25	90.00	121.75
T_7: 8.0	64.50	87.38	152.50
T_8: 8.5	49.80	78.40	102.50
SEm ±	0.85	0.34	0.78
CD (p=0.05)	2.48	1.02	2.30

*Mean of four replications; DAI = Days after inoculation.

and 35°C, whereas the highest sclerotial production was recorded within the 20°C to 30°C range. Kushwaha *et al*. (2019) reported that *S. rolfsii* exhibited maximum growth at 30°C, while its growth declined significantly at temperatures below 25°C and above 35°C.

Mycelial growth and sclerotia formation of *S. Rolfsii* at various pH levels

The observations on mycelial growth, analyzed statistically and presented in Table 2, showed that the maximum colony diameter (90.00 mm) was recorded at pH 6.5. This pH level was found to be significantly superior to all other pH levels tested and considered optimal for the growth of the pathogen. This was followed by pH level 6.0, 7.0, 7.5. and 8.0, 5.5, 5.0 and 8.5 with colony diameter 88.38, 85.75, 77.25 mm and 64.50, 60.25, 50.63 and 49.80 mm of the *S. rolfsii*, respectively. Result indicate that both increase and decrease in the level of pH significantly alter mycelial growth of Pathogen.

Table 2 and Plate 5 a & b indicates that the highest number of sclerotia (152.50 per plate) was recorded at pH 8.0, which was significantly higher than all other pH level tested in the study. The lowest sclerotia production (80.50 per plate) was observed at pH 7.0, which was statistically lower than all other pH levels evaluated. These results were in confirmation with Zape *et al*. (2013), who reported that the maximum radial growth of *S. rolfsii* was observed at pH 6.5. Tushar and Patel (2019) Mycelium growth was observed at all the pH levels tested but it was maximum at pH 6.5 (87.00 mm) after 72 hrs of inoculation. pH 6.0 (85.25 mm) and pH 5.5 (83.75 mm) were also found favourable.

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