**Pathogenic characterization of *Sclerotium rolfsii* isolates causing collar rot of chickpea in Telangana**

**ABSTRACT**

Chickpea (*Cicer arietinum* L.) is a vital legume crop widely cultivated for its high nutritional value, particularly as a rich source of plant-based protein. However, its productivity is significantly affected by various soil-borne pathogens, among which *Sclerotium rolfsii* Sacc. is one of the most destructive pathogens causing collar rot disease. Pathogenicity test with six isolates was carried out on five cultivars of chickpea. The results of Pathogenicity testing of six isolates on the susceptible chickpea cultivar JG 11 revealed significant differences in disease severity. ANOVA showed highly significant effects (p < 0.05) of isolate, host genotype and their interaction on collar rot incidence. Among the isolates CSR 9 caused the highest mean disease severity (88.31%), followed by CSR 11 (83.74%), while the lowest was recorded for CSR 3 (79.65%). Among chickpea cultivars, JG 11 was the most susceptible (90.96% disease index) and RGV 204 the least (61.08%). CSR 9 isolated from Neredigonda village of Adilabad district was the most virulent on JG 11. The results indicate low vegetative diversity but significant pathogenic variability among *S. rolfsii* isolates infecting chickpea.

***Keywords****: Chickpea, Disease severity, Pathogenicity, Sclerotium rolfsii and Variability*

**1.INTRODUCTION**

 Legumes are widely available and affordable sources of protein, vitamins, minerals and energy [1]. Among them chickpea (*Cicer arietinum* L.) is a vital legume crop widely cultivated for its high nutritional value, particularly as a rich source of plant-based protein [2]. It enhance soil fertility by boosting nitrogen levels through a symbiotic relationship with nitrogen-fixing bacteria which benefits later crops in crop rotation systems [3]. However, its productivity is significantly affected by various soil-borne pathogens, among which *Sclerotium rolfsii* Sacc. is one of the most destructive pathogen causing collar rot disease. This pathogen is prevalent in warm and humid agro-ecological zones and is known to cause seedling mortality ranging from 10% to nearly 100% depending on environmental conditions and host susceptibility [4].

 *Sclerotium rolfsii* is a necrotrophic fungus with a wide host range affecting more than 500 plant species [5]. Symptoms include collapse of infected seedlings showing clear signs of rotting at the collar region and below. As the disease advances, all leaves turn brown, dry out and often remain attached to the dead stem. The pathogen produces dense white mycelial growth over the infected plant parts and surrounding soil, eventually forming its characteristic mustard seed-sized sclerotia which range in color from light to dark brown [6, 7].

One of the critical factors influencing the survival, spread and severity of collar rot in chickpea is the genetic and pathogenic variability among *S. rolfsii* isolates. Differences in virulence and host specificity suggest the presence of diverse pathogen populations in different regions [8].

The pathogenic variability studies are majorly carried out understands the distribution of various pathotypes of a pathogen in the crop growing regions. The present study aims to assess the pathogenic variability among *S. rolfsii* isolates collected from chickpea-growing regions, which can provide insights into the population structure and assist in developing targeted control measures.

**2. Materials and methods**

**2.1 Isolation of *S. rolfsii* isolates**

In the present investigation chickpea infecting 13 *S. rolfsii* isolates were obtained from the Department of Plant Pathology, Agricultural College, Jagtial, which were isolated from diseased chickpea samples during *Rabi* 2021-22 and 2023-24 from different chickpea growing regions representing three agricultural zones of Telangana *viz*., Northern Telangana region (Adilabad, Nirmal, Nizamabad and Jagtial districts), Southern Telangana region (Jogulamba Gadwal and Vikarabad districts) and Central Telangana region (Warangal district). These 13 chickpea isolates are purified through hyphal tip method and maintained on PDA medium for pathological characterization.

**2.2 Pathogenic variability of *S. rolfsii* isolates using different chickpea lines**

 Pathogenic variability among six *S. rolfsii* isolates was studied on five different chickpea cultivars as given in Table 1. Differential hosts were used to distinguish the pathological reactions of the pathogen. Five chickpea cultivars NBEG-47, NBEG-452, RGV-204, ADBG-45 including susceptible JG-11 cultivated at different locations were selected for pathological variability studies. Nine healthy and surface sterilized seeds of the each chickpea cultivars were sown in 20×15 cm diameter plastic pots filled with sterilized soil mixture (soil, sand and FYM mixture). Three pots of replicates were maintained for each pathogenic isolate and then placed in poly house.

 **Table 1. Isolates used for pathogenicity test**

|  |  |  |  |
| --- | --- | --- | --- |
| Isolate | District | Mandal | Village |
| CSR 2 | Nirmal | Kubeer | Godisera |
| CSR 3 | Adilabad | Boadh | Pochera |
| CSR 8 | Jogulamba Gadwal | Undavelli | Bhairapur |
| CSR 9 | Adilabad | Neredigonda | Neredigonda |
| CSR 11 | Jagtial | Jagtial | RARS jagtial |
| CSR 13 | Warangal | Hasanparthy | Siddapur |

**2.3 Mass multiplication of *S. rolfsii*:**

The pathogen *S. rolfsii* was mass cultured using sorghum grain medium. The sorghum grains were soaked in water for 6 hours, then partially boiled to soften and excess water was drained off. The grains were then air-dried. About 200 grams of the prepared grain mixture were filled into 6 × 11 inch polythene bags, which were closed using non-absorbent cotton plugs held in place by PVC rings measuring 1.5 inches in length and 1 inch in diameter. The bags were sterilized in an autoclave for 25-30 minutes to ensure sterility. Once cooled, each bag was inoculated with 2-3 mycelial discs (5 mm) cut from the growing edge of a 5-day-old *S. rolfsii* culture on PDA. The inoculated bags were then incubated in a BOD incubator set to 27 ± 2°C for 15 days to allow the fungus to grow and completely colonize the sorghum grains.

**2.4 Inoculation of pathogen:**

The pathogen *S. rolfsii* multiplied on sorghum grains was applied @ 15 g per kg of the soil to each bag filled with 2 kg of soil mixture, following the method of Joyce *et al.* (2024). The inoculated soil was incubated for 10 days, with light watering applied to promote pathogen development. The healthy, surface sterilized seeds of five chickpea cultivar were sown in respective bags (9 seeds/ bag). Surface sterilized seeds were sown in un-inoculated sterilized soil served as control. The bags were watered regularly to maintain sufficient moisture needed by the plants. The experiment was conducted in completely randomized design (CRD) with three replications. The experiment was repeated twice to confirm the reaction of the isolates on different chickpea varieties.

**2.5 Estimation of Diseases Index and mortality percentage:** The seed emergence in each pot was recorded after 10 days of sowing.Seeds that did not emerge were taken out of the soil and inspected for symptoms of pre-emergence damping-off, seed decay, or fungal colonization, including the presence of mycelium or sclerotia on germinated seeds that failed to surface. Collar rot disease severity was recorded after 30 days of sowing using 1-6 scale [9] (Table 2 & 3).

 Sum of scores x 100

 Per cent disease index (PDI) = -----------------------------------------------------------------

 Number of observations x highest no. of rating scale

**Table 2. Observations on visible symptoms found on chickpea plants evaluated based on disease severity 1–6 scale**

|  |  |
| --- | --- |
| **Severity score** | **Description** |
| 1 | No symptoms; plant healthy |
| 2 | Gray water-soaked lesions present on stem above soil, but no visible fungal outgrowth |
|  3 | Visible fungal outgrowth on stem base, characterized by silky-white mycelia or sclerotia that gradually darken |
| 4 | Partial wilting, where younger leaves begin to wilt and stems begin to shrivel |
| 5 | Complete wilting, desiccation and browning of leaves and stem; plant collapse and death (rot) |
| 6 | Pre emergence damping-off; complete seed rot, with no sign of germination, or evidence of germination hampered by fungal colonization |

 Based on the per cent disease index on different chickpea cultivars virulence patternof *S. rolfsii* isolates were classified [10].

**Table 3. Virulence pattern of *S. rolfsii* isolates based on Percent disease index**

|  |  |
| --- | --- |
| **Description** | **Percent disease index (PDI)** |
| Highly virulent | More than 66 per cent disease index |
| Moderately virulent | Percent disease index between 33% to 66% |
| Weakly virulent | Less than 33 per cent disease index |

**3.Results and discussion**

**Pathological variability**

The chickpea collar rot pathogenic variability studies consisted of five host differentials from different parentage that were evaluated under poly house conditions for pathogenicity of six *S. rolfsii* isolates. The tested six *S. rolfsii* isolates induced clear collar rot symptoms on the susceptible cultivar JG 11. The data was collected 30 days after sowing and presented in Table 4. The ANOVA revealed highly significant (at 0.05) differences among the isolates, host genotypes and their interaction for collar rot disease severity. The mean collar rot per cent disease index across the isolates was maximum (88.32%) for *S. rolfsii* isolate CSR 9 from Adilabad district, followed by 83.75 per cent of per cent disease index was recorded with CSR 11 from Jagtial district. Mean Minimum collar rot per cent disease index (80.68%) was observed for CSR 3 from Adilabad and it is statistically similar with CSR 8 from Jogulamba Gadwal district which recorded per cent disease index of 80.69 per cent. Whereas the mean maximum collar rot incidence across the chickpea cultivars was observed on JG 11 (94.66%) and mean minimum collar rot per cent disease index was recorded with cultivar RGV 204 (56.13%). The lowest mean per cent disease index indicates relatively more resistance level in these lines against *S. rolfsii.*

Among the different *S. rolfsii* isolates tested on different chickpea cultivars maximum per cent disease index (98.60) was observed on JG 11 for *S. rolfsii* isolate CSR 9 collected from Neredigonda village of Adilabad district followed by CSR 8 which is on par with CSR 3 with 95.83 and 95.14 per cent disease index. On chickpea cultivar NBEG 47 six *S. rolfsii* isolates showed varied percent disease index ranging from 85.41 to 93.76 per cent disease index. The maximum per cent disease index was observed with the isolate CSR 8 (93.76%) followed by CSR 11 (91.68%). Minimum per cent disease index was observed with isolate CSR 3 followed by CSR 2 (89.57%). Whereas *S. rolfsii* isolate CSR 9 which showed maximum per cent disease index on other chickpea cultivars showed only 90.95 per cent disease index on NBEG 47. Per cent disease index on chickpea cultivar NBEG 452 showed significant variation ranging from 78.47 to 94.42 per cent. The maximum per cent disease index (94.42%) was recorded with isolate CSR 9 followed by 85.40 per cent disease index was recorded with both *S. rolfsii* isolates CSR 3 and CSR 13. CSR 11 and CSR 8 isolates showed 84.02 and 80.54 per cent disease index respectively. Whereas minimum per cent disease index (78.47) was reported with isolate CSR 2.

 Among all the isolates, the chickpea cultivar RGV 204 exhibited the lowest Percent Disease Index (PDI), ranging from 45.14% with isolate CSR 2 to 65.98% with isolate CSR 8. The per cent disease index (64.58%) of the isolate CSR 9 showed on par with per cent disease index recorded with CSR 8. The remaining three *S. rolfsii* isolates CSR 13, CSR 11 and CSR 3 exhibited 61.08, 54.16 and 45.82 per cent collar rot per cent disease index respectively.

 Chickpea cultivar ADBG 45 showed per cent disease index varying from 85.40 to 94.44 per cent disease index. Minimum per cent disease index was recorded with isolate CSR 2 (85.40%) followed by isolate CSR 8 (88.20%) and it is on par with the per cent disease index 88.89 recorded with the isolate CSR 13. Whereas the maximum per cent disease index (94.44) was recorded with isolate CSR 11 followed by 93.04 per cent disease index exhibited by the isolate CSR 9. *S. rolfsii* isolate CSR 3 showed 89.56 per cent disease index.

 All the *S. rolfsii* isolates were categorised into highly virulent group with more than 66 per cent of disease reaction based on per cent disease index across the cultivars except moderately virulent with less than 66 per cent disease reaction on chickpea cultivar RGV 204. None of the cultivars were found resistant to all the six *S. rolfsii* isolates tested.

 Our results also indicate that the *S. rolfsii* isolates were virulent on all the chickpea cultivars and aggressive on susceptible cultivar JG 11 but moderately virulent on RGV 204. Similarly Paparu *et al*. [9] observed the aggressiveness and virulence reaction of 45 *S. rolfsii* isolates across five common bean varieties during pathogenic variation studies.

 Singh *et al.* [11] and Kumari and Ghatak [12] studied the pathological variability of *S. rolfsii* isolates on four chickpea cultivars and observed variation in the disease reaction caused by the isolates.

Our results are consistent with the study by Yan *et al*. [13] which demonstrated that though all *S. rolfsii* isolates were pathogenic on peanut, they varied in disease index. Similarly, in the present study all isolates caused disease across chickpea cultivars but differed in their disease index, confirming that while all were virulent there exists variation in their aggressiveness.

**Table 4. Percent disease index (PDI) of *S. rolfsii* isolates on five cultivars of chickpea**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolate/****Cultivar** | **CSR 2** | **CSR 3** | **CSR 8** | **CSR 9** | **CSR 11** | **CSR 13** | **Mean PDI** |
| **JG 11** | 91.91(73.53)\* | 91.13(72.70) | 95.83(78.45) | 98.6(78.51) | 94.44(72.82) | 90.96(72.82) | **93.81(74.80)** |
| **NBEG 47** | 89.15(70.79) | 87.67(69.47) | 94.75(76.77) | 90.95(71.28) | 91.67(65.70) | 85.41(65.70) | **89.93****(69.95)** |
| **NBEG 452** | 78.16(62.12) | 83.73(66.21) | 81.57(64.57) | 94.42(74.16) | 84.01(67.26) | 85.40(67.27) | **84.54****(66.93)** |
| **RGV 204** | 63.65(52.90) | 45.83(42.58) | 43.14(41.04) | 64.58(52.86) | 54.16(47.37) | 61.08(50.22) | **55.40****(47.83)** |
| **ADBG 45** | 85.74(67.82) | 89.89(71.49) | 87.71(69.47) | 93.04(72.95) | 94.44(76.14) | 88.88(69.09) | **89.95****(71.16)** |
| **Mean PDI** | **81.72****(65.43)** | **79.65****(64.49)** | **80.60****(66.06)** | **88.31****(69.95)** | **83.74****(65.86)** | **82.34****(65.02)** |  |
| **C.D. (*P*=0.05)** | Cultivars: 1.32 |
| Isolates:1.205 |
| Cultivars x Isolates: 2.951 |

 \* values in the parenthesis indicate the angular transformed values.

 **Table 5.** **Statistical analysis of fungal pathogenic variation and Chickpea cultivar reactions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source of Variation** | **DF** | **Sum of Squares** | **Mean Squares** | **F-Calculated** | **Significance** |
| CSR isolates | 5 | 286.745 | 57.349 | 17.649 | < 0.001 |
| Cultivars | 4 | 8,113.39 | 2,028.35 | 624.224 | < 0.001 |
| Intraction CSR isolates X cultivars | 20 | 868.394 | 43.42 | 13.362 | < 0.001 |
| Error | 60 | 194.963 | 3.249 |   |   |
| Total | 89 | 9,463.49 |   |   |   |

 **Fig 1. Percent disease index of *S. rolfsii* isolates on five chickpea cultivars**

**4. CONCLUSION**

To our knowledge this is the first study on evaluation of the pathogenic variation among different *S. rolfsii* isolates on five different chickpea cultivars grown across the major chickpea growing districts of Telangana. The data of present research on the pathogenic variation of *S. rolfsii* isolates indicate the chickpea breeding efforts more challenging. Statistical analysis of disease incidence and host response can aid in developing effective disease management strategies for chickpea cultivation. Based on the pathogenic characterization of fugal isolates obtained across the chick pea cultivating locations different management strategies such as planting resistant or tolerant varieties, crop rotation with non-host crops and use of biological or chemical management approaches may be advised to the farmers in different agro-climatic zones of Telangana.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

1. Patra, G. K., Acharya, G. K., Panigrahi, J., Mukherjee, A. K., and Rout, G. R. (2023). The soil-borne fungal pathogen Athelia rolfsii: past, present, and future concern in legumes. *Folia Microbiologica*, *68*(5), 677-690.
2. Singh, A. K., Singh, S. S., Prakash, V. E. D., Kumar, S., and Dwivedi, S. K. (2015). Pulses production in India: Present status, sent status, bottleneck and way forward. *Journal of AgriSearch*, 2(2), 75-83.
3. Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H. D., Krishna, H., Chandra, S., Vadez, V., and Serraj, R. (2005). Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). *Euphytica*, 146, 213-222.
4. Rajput, V. A., Konde, S. A., and Thakur, M. R. (2010). Evaluation of bioagents against chickpea wilt complex.
5. Aycock, R. (1966). Stem rot and other diseases caused by *Sclerotium rolfsii*. North Carolina Agricultural Experiment Station Technical Bulletin, 175, 1-202.
6. Shirsole, S. S., Khare, N., Lakpalme, N., and Kotasthane, A. S. (2018). Detection of resistant sources against collar rot of Chickpea caused by *Sclerotium rolfsii* Sacc. under field conditions. *International Journal of Current Microbiology Applied Science*, 7(1), 502-505.
7. Hasna, M. K., Kashem, M. A., and Ahmed, F. (2020) Use of *Trichoderma* in Biological Control of Collar Rot of Soybean and Chickpea. *International Journal of Biochemistry Research and Review*, 29(9), 25-31.
8. Kumari, M., Sharma, O. P., and Nathawat, B. D. S. (2021). Pathogenicity, host range and influence of temperature, humidity and ph levels on the growth of *Fusarium* *oxysporum* f. sp. *lentis*. Legume *Research-An International Journal*,1-8.
9. Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Nkuboye, A., Musoke, S., and Mukankusi, C. (2020). Morphological and pathogenic characterization of *Sclerotium rolfsii*, the causal agent of southern blight disease on common bean in Uganda. *Plant disease*, 104(8), 2130-2137.
10. Remesal, E., Landa, B. B., Jimenez-Gasco, M. M., and Navas-Cortes, J. A. (2013). Sequence variation in two protein-coding genes correlates with mycelial compatibility groupings in *Sclerotium rolfsii*. *Phytopathology*, 103(5), 479-487.
11. Singh, G., Khare, U. K., Babbar, A., Wasnikar, A. R., Kumar, A., and Amrate, P. K. (2022). Present status of collar rot in major chickpea growing state of India. *Biological Forum – An International Journal,* 14 (2), 1095-1101.
12. Kumari, A., and Ghatak, A. (2018). Variability in Chickpea Rot-causing Soil-borne Necrotrophs, *Sclerotium rolfsii* and *Macrophomina phaseolina*: Variability in chickpea rot-causing Necrotrophs pathogens. *Journal of agriSearch*, 5(4), 247-253.
13. Yan, L., Song, W., Yu, D., Kishan Sudini, H., Kang, Y., Lei, Y., Huai, D., Wang, Z., Chen, Y., Wang, X. and Liao, B. (2022). Genetic, phenotypic and pathogenic variation among *Athelia rolfsii*, the causal agent of peanut stem rot in China. *Plant Disease*, 106(10), 2722-2729.