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ISOLATION, IDENTIFICATION AND PATHOGENICITY STUDIES OF *SCLEROTIUM ROLFSII* SACC. CAUSING COLLAR ROT DISEASE OF CHICKPEA

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important legume crop cultivated predominantly under rainfed conditions during the *Rabi* season in subtropical and temperate regions. Collar rot, caused by *Sclerotium rolfsii*, is a destructive soil-borne fungal disease that spreads rapidly and results in severe losses to plant stand. Seedling mortality due to *S. rolfsii* has been reported to range from 54.7 to 95.0 per cent. During a field survey, fourteen fungal isolates were obtained from collar rot–infected chickpea plants. The pathogen produced dense, white to off-white cottony mycelium with sclerotia that were initially white and later turned brown to black at maturity. Based on morphological characteristics, the pathogen was identified as *Sclerotium rolfsii*. Pathogenicity tests revealed considerable variation in virulence among the isolates, with CSR5 exhibiting the highest disease incidence (90%), whereas CSR14 was the least virulent, recorded with 10% disease incidence.
***Keywords*:** Chickpea, Isolation, Pathogenicity, Disease, *Sclerotium rolfsii*.

Introduction

Chickpea (*Cicer arietinum* L.) is a major pulse crop of global importance and belongs to the genus *Cicer*, tribe Cicereae, family *Fabaceae* and subfamily *Papilionaceae* (Bentham and Hooker, 1972). It is a diploid species with a chromosome complement of 2n = 16. Chickpea seeds are nutritionally rich, containing essential amino acids such as isoleucine, leucine, lysine, phenylalanine and valine (Karim and Fattah, 2006), and thus play a vital role in enhancing the nutritional quality of cereal-based diets and alleviating protein–energy malnutrition. An average per capita consumption of 14 g of chickpea contributes approximately 2.3 per cent of dietary energy and 4.7 per cent of protein, along with appreciable quantities of calcium and iron. The seeds contain 12.4–31.5 per cent protein, 41.0–50.8 per cent starch and 52.4–70.9 per cent total carbohydrates, in addition to moderate levels of sugars, ash, iron and vitamin B_1 (Jukanti *et al.*, 2012).

Chickpea production is constrained by several abiotic and biotic stresses, of which biotic factors such as soil-borne diseases, insect pests, nematodes and parasitic weeds are major contributors to yield instability across chickpea-growing regions worldwide. Losses caused by diseases and insect pests range from 5–10 per cent in temperate regions to as high as 50–100 per cent in tropical environments (Van *et al.*, 1988). The crop is susceptible to numerous diseases, including fusarium wilt, dry root rot, collar rot, ascochyta blight, verticillium wilt, black root rot, phytophthora root rot, wet root rot, foot rot, pythium rot and seed rot. Among these, collar rot caused by *Sclerotium rolfsii* Sacc. is one of the most destructive soil-borne fungal diseases, posing a serious threat to chickpea cultivation across the country. Symptoms of

the disease are seen at the seedling stage (up to 6 weeks after sowing). Affected seedlings turn yellow, upon uprooting seedlings show rotting at the collar region which covered with whitish mycelial strands. A white mycelial coating can be seen on the tap root of completely dried seedlings. Affected seedlings usually occur in small scattered patches in the field. Mustard grain sized light to dark brown structures known as sclerotia, serve as over wintering bodies and may be seen on the mycelium, on diseased tissues above or below ground, on soil surface, or in soil crevices. Under favourable environmental conditions, the disease can result in 55–95 per cent seedling mortality (Gurha and Dubey, 1982; Maurya *et al*., 2008).

Materials and Methods

Survey and collection of diseased samples

A roving survey of farmer's fields was conducted during month of October to December, 2023 from the various locations of Maharashtra, *viz*., Ahilyanagar, Beed, Chhatrapati Sambhajinagar, Dharashiv, Hingoli, Jalna, Latur, Parbhani, Pune and Solapur. Infected chickpea plants showing typical collar rot disease symptoms were collected and their GPS locations were recorded. The collar and root portion of the diseased plants were cut with sharp scissor and packed in polythene bags. The diseased collar/ root samples were brought to the laboratory for isolation of pathogen.

Isolation of fungus

The part of collar region showing typical symptoms of disease was cut into small pieces. These pieces were then surface sterilized with 0.1% $HgCl_2$ solution for one minute. Such pieces were washed thoroughly in sterile distilled water three times to remove the traces of mercuric chloride solution, and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. The plates were incubated at 27±1℃ for five days for growth of fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of fungus was obtained by following hyphal tip culture technique under aseptic condition as method suggested by (Aneja, 2018). The pure cultures were maintained on PDA slants at 4±1°C for further studies.

Maintenance of pure cultures

The isolates were sub-cultured on PDA slants and allowed to grow at 27±1℃ temperature in the incubator for ten days. The cultures obtained were stored in a refrigerator at 4±1℃ and sub cultured once in a month.

Identification of the pathogen

On the basis of symptoms expressed on chickpea plants (naturally and artificially inoculated) and cultural, morphological and microscopic characteristics, the test pathogen was identified by referring standard description of chickpea collar rot and it's pathogen made by earlier workers, identification was done.

Cultural and morphological characters of the isolates

The pathogen was identified on the basis of its cultural and morphological characters such as mycelial and sclerotial characteristics using standard mycological keys (Barnett and Hunter, 1972). The cultural and morphological characters of the isolates were studied on PDA medium. The pure culture of each of the isolate was inoculated in a petri dish containing PDA medium. After 10 days of incubation, slides were prepared for microscopic observations. The isolates based on their cultural and morphological characters were identified as *Sclerotium rolfsii.*

Pathogenicity

The pathogenicity of these isolates was tested by sick soil inoculation technique in the earthen pot under greenhouse condition by using susceptible chickpea cultivar JG-62. The inoculum of each isolate was prepared on sterilized sand-maize medium. Sand maize medium (3 part partially broken maize grains + 1 part sand + distilled water to moisten the medium) was prepared, filled into 250 ml conical flask and autoclaved at 20 lbs pressure for 30 min, for two consecutive days. After cooling at room temperature, the contents of the flasks were thoroughly shaken to prevent clumping. The flasks were aseptically inoculated with mycelial discs (8 to 10) of the test pathogen obtained from a week-old culture and incubated at room temperature for two weeks. The flasks were shaken every alternate day to avoid clumping. This mass multiplied inoculum was used for preparation of sick soil/potting mixture in earthen pots. Sterilized soil was taken in earthen pots of size 45x30cm. Inoculum multiplied on sand-maize medium was added to sterilized soil in earthen pots @ of 30g kg^{-1} soil, mixed thoroughly and moistened with water and pots were incubated for 2 days by covering with polythene sheets. Then the seeds of chickpea cultivar JG-62 (susceptible) surface sterilized with 1 per cent sodium hypochlorite (NaOCl) were sown @ 10 seed/pots. The pots with uninoculated soil were maintained as control. All these pots were watered lightly and kept in a glass house for recording observation. The observation on number of days

required for disease expression and disease incidence were recorded up to 20 days after sowing by maintaining two replications of each treatment. Re-isolation of the fungus was made from roots of artificially inoculated and diseased plant showing typical collar rot symptoms. The fungus growth obtained in re-isolation from artificial inoculated plants was transferred on PDA slant for comparison with original culture. The symptoms of collar rot were observed and recorded from initiation of the disease till complete rotting of the plant.

Results and Discussion

Isolation of pathogen

During survey, infected chickpea plants showing typical collar rot disease symptoms were collected from the various locations of Maharashtra. Applying the tissue isolation method the associated pathogen was isolated on PDA medium from collar rot infected chickpea samples collected during survey. The pure culture of fungus was obtained by further growing culture and following hyphal tip culture under aseptic conditions. The pure cultures were maintained on PDA slants at 4°C±1 for further studies.

A total of 14 isolates were obtained from different districts of Maharashtra and are represented in (Table 1, Plate 1) Among these, two isolates were obtained from Ahilyanagar district, three from Jalna district and two each from Beed, Dharashiv and Pune district and one each from Hingoli, Chhatrapati Sambhajinagar and Parbhani. All isolates were maintained in pure form for further analysis.

Table 1: List of Isolates of pathogen used for present study

Sr. No.	Isolate designation	Village	Tehsil/Taluka	District
1	CSR1	Sonai	Newasa	Ahilyanagar
2	CSR2	Rahuri	Rahuri	
3	CSR3	Beed	Beed	Beed
4	CSR4	Dhamangaon		
5	CSR5	Aashta	Lohara	Dharashiv
6	CSR6	Achler		
7	CSR7	Kautha	Basmath	Hingoli
8	CSR8	Jalna	Jalna	Jalna
9	CSR9	Badnapur	Badnapur	
10	CSR10	Somthana		
11	CSR11	Gopalpur	Chhatrapati Sambhajinagar	Chhatrapati Sambhajinagar
12	CSR12	Parbhani	Parbhani	Parbhani
13	CSR13	Sanswadi	Shirur	Pune
14	CSR14	Shikrapur		

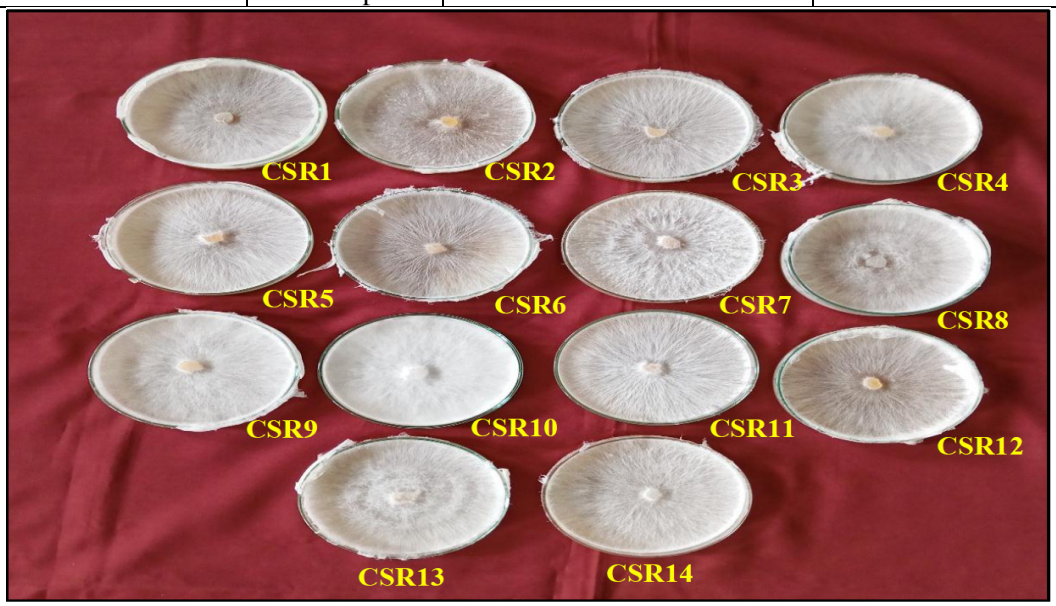

Plate 1 : List of isolates of pathogen used for present study

Identification of pathogen

The pathogen identification was done on the basis of morphological characters produced by the fungus on PDA medium. The fungus produced thick extra white to white, profusely branched, cottony mycelium. At maturity, small mycelial knots were formed in the culture which later converted into sclerotia. In the initial stages sclerotia were white in colour and later turned to light brown/ dark brown/ black on maturity. Sclerotia were shiny, hard rape seed like structures, the surface being finely wrinkled or pitted to smooth, sometimes flattened. The shape of sclerotia was spherical to round and irregular. At maturity sclerotia produced honey dew like liquid on its surface. On the basis of these morphological characters, the pathogen was identified as *Sclerotium rolfsii.*

Similar reports of effective utilization of morphological traits for pathogen identification was given by Prasad *et al.* (2012) who reported that the colony morphology of isolates of *S. rolfsii* was white cottony mycelium with fluffy and woolly strands. The colony diameter varied from 42 to 90 mm among the isolates. Colour of sclerotial bodies was light brown to reddish brown and number of sclerotia per plate ranged from 57 to 306. Similarly, Sangeeta (2019) described the cultural and morphological variability of 15 different isolates of *S. rolfsii* causing collar rot of chickpea. All the isolates varied with respect to days taken for full growth, colour of colony (pure white, dull white and cottony white), margin (regular to irregular), days taken for sclerotia initiation (8-15 days), colour of sclerotia (light to dark brown), shape (round to oval), number of sclerotia per cm^2 of culture (2.2-10) and weight of 100 sclerotial bodies (63.00-264.40 mg).

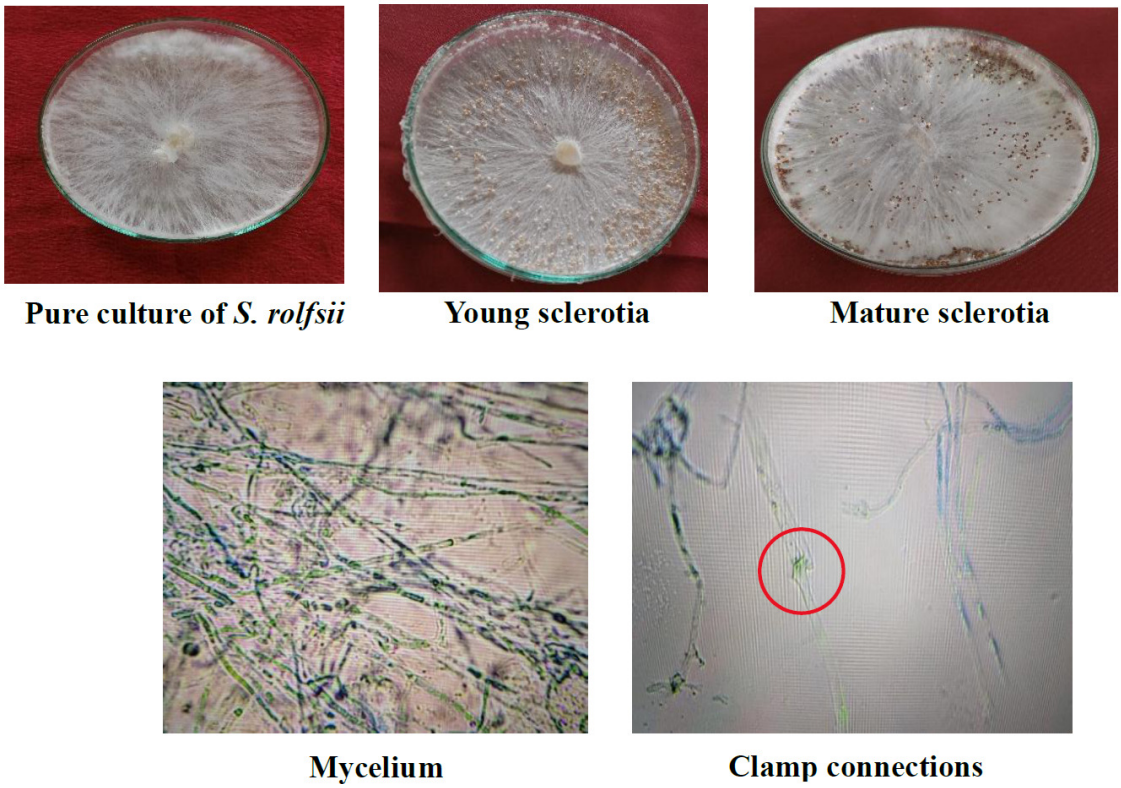

Plate 2 : Identification of pathogen

Pathogenicity

The pathogenicity of each isolates was tested by sick soil inoculation technique in the earthen pot under greenhouse condition by using susceptible chickpea cultivar JG-62. The pathogen was inoculated on sand-maize medium for mass multiplication, the multiplied isolates were mixed with soil in pots. The seedling mortality was recorded up to 20^{th} day of inoculation of pathogen and the per cent of disease incidence (PDI) was calculated.

Results from pot experiment showed variation in pathogenicity among the isolates (Table 2, Plate 3).

The disease incidence values ranged from 10% (CSR 14) to 90% (CSR 5) among the isolates indicating huge variability. The isolates, CSR 7 (80%), CSR9 (80%), CSR1 (70%), CSR 12 (70%) and CSR3 (70%) showed high disease incidence, while the isolates, CSR 2 and CSR 6 recorded 60% disease incidence, followed by the isolates, CSR 3 and CSR 11 which showed 50% disease incidence. The isolates, CSR 14 (10%), CSR 8 (20%) and CSR 10 (20%), recorded less disease incidence. The isolate, CSR 5, took nine days for the disease expression whereas the isolate, CSR 14, took 17 days for disease expression. Thus, the isolate CSR 5, is considered as the most virulent isolate as it produced the symptoms on 9th day with high disease incidence among the isolates, followed by the isolates, CSR 7 (9th day), CSR 12 (8th day) and CSR 2 (10th day).

These observations align closely with the findings of Saileela *et al.* (2020) who studied variation in aggressiveness among the isolates of *S. rolfsii*. The disease incidence values ranged from 14.28% (KCSR 6) to 95.67% (KCSR 16) among the isolates indicating huge variability. The isolates, KCSR 14, (91%), KCSR 4 (90.75%) and KCSR 12 (90.00%), also showed very high PDI values while, the isolates, KCSR 6 (14.28), KCSR 7 (25%) and KSCR 10 (37.78%), recorded less PDI. The isolate, KCSR 16, took eight days for the disease expression whereas the isolate, KCSR 6, took 18 days indicating the less aggressiveness of the isolate in causing disease. Thus, the isolate, KCSR 16, is considered as the most aggressive isolate as it produced the symptoms on eighth day with high PDI among the isolates followed by the isolates, KCSR 14, KCSR 4, KCSR 12 and KSCR 13.

Similar findigs of pathogenicity of isolates was also reported in the studies of Chandra Sekhar *et al.* (2017), Praveen and Kannan, (2021) and Ayyandurai *et al*. (2022).

A) Distinct symptoms of *S. rolfsii* in pathogenicity test

B) General view of pathogenicity test

Plate 3: Pathogenicity test of different isolates of *S. rolfsii*

Table 2: Pathogenicity test of different isolates of *S. rolfsii* on chickpea (variety JG-62)

Sr. no.	Isolate	Days taken for disease expression (DAS)	Disease Incidence (%)
1	CSR1	11	70%
2	CSR2	10	60%
3	CSR3	11	50%
4	CSR4	14	20%
5	CSR5	9	90%
6	CSR6	15	60%
7	CSR7	9	80%
8	CSR8	15	20%
9	CSR9	12	80%
10	CSR10	16	40%
11	CSR11	16	50%
12	CSR12	8	70%
13	CSR13	17	70%
14	CSR14	17	10%

DAS: Days after sowing

Conclusion

Based on field symptoms, cultural and morphological characteristics on PDA, and pathogenicity tests under greenhouse conditions, the collar rot pathogen associated with chickpea in Maharashtra was confirmed as *Sclerotium rolfsii*. Fourteen isolates exhibited considerable variability in virulence, with disease incidence ranging from 10 to 90 per cent. Isolate CSR-5 was identified as the most virulent, showing early symptom expression and highest disease incidence, indicating significant pathogenic diversity among *S. rolfsii* populations in the region.

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