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Collection, isolation and sclerotial germination of *Sclerotinia sclerotiorum* (Lib) de Bary, an arising fungal pathogen causing stem rot disease in chickpea

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Abstract
Chickpea (*Cicer arietinum* L.) is an essential pulse crop that provides protein to the country's undernourished vegetarian population. It has several illnesses. Stem rot of chickpea caused by *Sclerotinia sclerotiorum* (ON831560.1) is a widespread and damaging disease in India. Infected plants first exhibited wilting signs before dying quickly, frequently without yellowing. Later, when the plant dries, the leaves turn straw-colored. Water-soaked patches might occur on the stem and leaves. Affected tissues become a slimy soft rot from which droplets of brown liquid may emerge. The infection persisted on dead plants spread over the area. The pathogen successfully proved the Koch postulates under glasshouse conditions and produced similar symptoms as those produced under field conditions.

Keywords: Chickpea, collection, isolation, purification, *Sclerotinia sclerotiorum* and symptomology

Introduction
Chickpea pea (*Cicer arietinum* L.) is the third most important leguminous plant in the world (San *et al.*, 2022). It is an important legume cultivated and consumed worldwide, especially in Afro-Asian countries (Yegrem, 2021) [10]. Chickpea seeds are a good source of carbohydrates (52.40-70.90%), proteins (12.40-30.60%) and minerals such as Ca, Fe, Mg, K, P, Zn and Cu. It also has vitamin A and B-carotene (Khalifa *et al.*, 2013) [3]. Chickpeas contain significant amounts of all essential amino acids. India produces more than 70 percent of the total world production of chickpea and it is cultivated on 98.5 lakh hectares with a production of 119.9 lakh tons and a productivity of 1217 kg/ha (Anonymous, 2021) [1], but in recent years, chickpea cultivation has started. biotic and abiotic stresses. Crops suffer from various fungal, bacterial and viral diseases during the growing season. Besides Ascochyta wilt and Fusarium wilt, stem rot caused by *Sclerotinia sclerotiorum* (Sharma *et al.*, 2015) [9] has been found to cause major economic loss to the crop (Mehta, 2009). As a legume, it plays an important role in the nutrition of the rural and urban poor in developing countries, as a supplier of protein-rich foods to cereal-based diets. Chickpea productivity is not satisfactory. The main constraints reducing chickpea production are lack of high-yielding varieties, early ripening, adaptation to stress conditions, effective management, adaptation to various climatic conditions and introduction of production technology. As a result, many efforts are made to collect, isolate and purify the isolates of *Sclerotinia sclerotiorum* and its symptoms.

Materials and Methods
The pot experiments were carried out at the Net house, laboratory of the Department of Plant Pathology and field experiments at Students Instructional Farm, College of Agriculture, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during *Rabi* 2020-21 and 2021-22.

Collection, isolation and purification of *Sclerotinia sclerotiorum* isolates
Diseased specimens of chickpea and other crops were collected from different locations in

the various districts of Uttar Pradesh for the isolation of *S. sclerotiorum*. The collected diseased specimens were washed with running tap water to remove the adherent dirt particles and then rinsed with distilled water in the laboratory. The infected plant parts were cut into small pieces. Under aseptic conditions, the infected stem pieces and sclerotia were surface sterilized with 1 per cent sodium hypochlorite for 20-30 seconds and subsequently washed 2-3 times in sterilized distilled water to remove any traces of sodium hypochlorite. Excess moisture was removed by sterilized blotting paper. Surface sterilized pieces or sclerotia were placed on a sterilized Petri plate containing 2 per cent potato dextrose agar (PDA) medium with the help of flame-sterilized forceps at equal distances from each other. Then, inoculated Petri plates, were incubated at 27±1 °C in a BOD incubator. Sub-culturing was done until obtained pure culture.

Plant material

Chickpea cultivar, namely, Pant G-186 was used throughout the study. Chickpea seeds were obtained from Pulse Section, Department of Genetics and Plant Breeding, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya.

Germination of Sclerotium

Sclerotia are placed on a substrate low in nutrients water agar. Myceliogenic germination is observed at 20-25 °C, above 80 per cent relative humidity and initial medium pH of 5. In carpogenic germination, Sclerotia produced at 15 °C and stored for eight weeks at 4 °C and incubated for four to six weeks in sand or vermiculite.

Pathogenicity test

The pure culture of *S. sclerotiorum* was inoculated on a susceptible cultivar (var. Pant G-186) of chickpea to establish Koch's postulates. Then again, pathogen was isolated from the infected plant after symptom development. The obtained pure culture was transferred on a freshly prepared PDA slant and incubated at 27±1 °C. Full growth PDA slant was stored at 20 °C for further use. The stored pathogen was subcultured periodically for longer preservation.

Result and Discussion

Symptomatology

The moderate to severe form of disease symptom of the stem rot of chickpea was observed at Student Instructional Farm, Acharya Narendra Deva University of Agriculture and Technology, Ayodhya. The pathogen survived on dead plants scattered throughout the paddock. Infected plants showed wilting symptoms first and then die rapidly, often without yellowing. Later, as the plant dries out the leaves turn a straw colour. Water-soaked spots may appear on the stem and leaves. Affected tissues develop a slimy soft rot from which droplets of a brown liquid may exude.

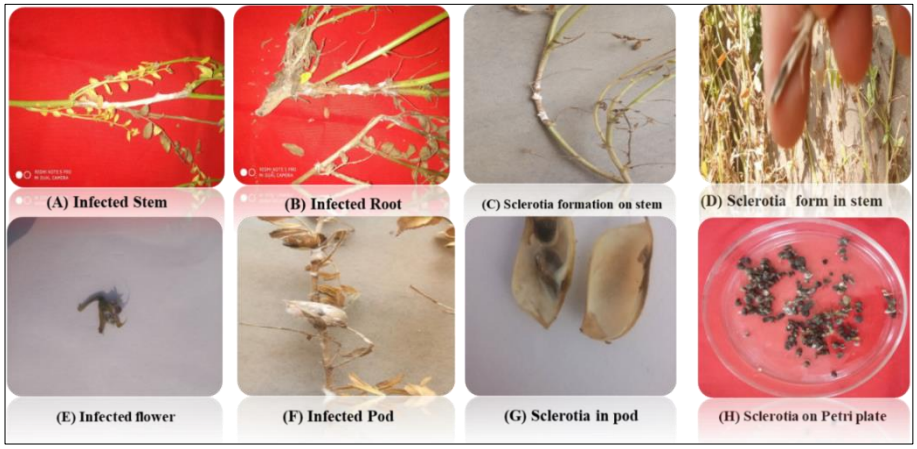

Plate 1: Symptomatology of stem rot of chickpea caused by *S. sclerotiorum*, Infected stem (A), Infected root (B), Sclerotia formation on the stem (C), Sclerotia form in stem (D), Infected flower (E), Infected pod (F), Sclerotia in pod (G), and Sclerotia on petri plate (H)

Plate 2: A visualization of the infected chickpea stem by *S. sclerotiorum* under field condition

Infected plants dry out and may become covered with a web of white mycelium of the fungus. On the surface of the root, or just below ground level, small black fungal bodies called sclerotia can sometimes be seen mingled with white cottony fungal mycelium. Similar symptoms were identified by Saharan and Mehta (2008). Nahar *et al.* (2020) surveyed the incidence of white mould disease in country bean production areas in Bangladesh during 2016-17. He identified and documented the white mould as a disease based on symptoms appear in the field (Plate-1, Plate-2).

Isolation and purification of the pathogen

To obtain a pure culture of the pathogen, the diseased specimens of chickpea collected from different locations were examined by the method accordingly. The fungus was readily isolated from the infected stem portion on 2.0% Potato dextrose agar medium and the isolates were further purified by transferring a bit of mycelial growth to another

fresh medium. For further studies, the pure culture was maintained in a PDA slant and stored at -20°C, Plate 3& 4 (Faruk & Rahman, 2022).

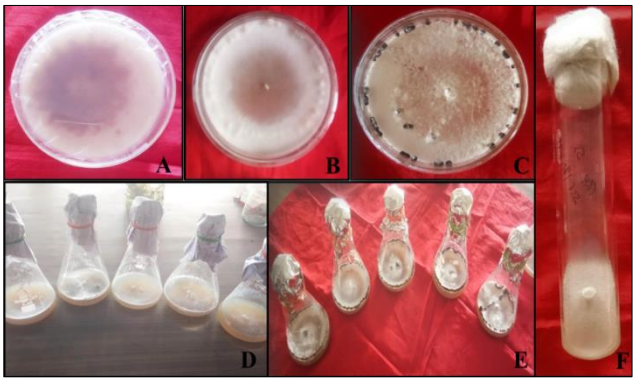

Plate 3: Cultural morphology of *Sclerotinia sclerotiorum* (ON831560.1) on potato dextrose agar (PDA) medium on plate (A, B, C), potato dextrose broth (PDB) medium (D, C) and PDA slant (F)

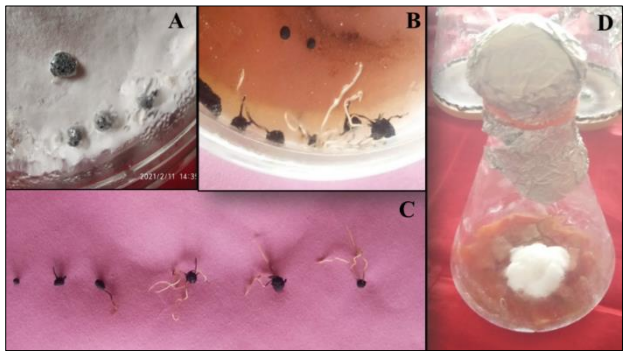

Plate 4: Sclerotia germination on PDA (A), water agar (B), blotter paper (C) and boiled carrot (D)

Sclerotium Germination

Germination of sclerotia occurred in both myceliogenic and carpogenic manner, produces mycelia and apothecia, respectively (Saharan and Mehta, 2008).

Carpogenic germination

Single or multiple stipes were observed insclerotia each with an apothecium. Low osmotic potential inhibitedthe formation of apothecia in some stipes. The sequence of events for apothecium production from sclerotia has been studied by light microscope. Apothecial initials arised in the cortex or medulla as brown to hyaline clusters or nests of interwoven hyphae. Stipes grown in the direction sun light and did not differentiated into disc unless receivedlight. A depression developed at the tip of the apothecia, produced paraphyses within that.

Myceliogenic Germination

After a dormant period, sclerotia placed on a moistened medium, developedmultiple bulges. Thesebulges grew larger, finally ruptured and released a mass of dense myceliumthat was visible to the unaided eye. Sparsed mycelial growth observed and this mycelial growth produces smaller secondarysclerotia.

Pathogenicity test

The Koch postulates successfully proved on the chickpea plant (var. Pant G-186) by three different inoculation methods. Infected plants showed the diseased symptom as described in Table-1. The inoculated pots were observed daily and the development of symptoms was recorded. The maximum per cent disease incidence observed in placement inoculums near axil of plants followed by soil inoculation compared to uninoculated control, Table-1, (Purdy, 1979).

Table 1: Pathogenicity test of *Sclerotinia sclerotiorum* on Chickpea (pot culture experiment)

S. No.	Method of inoculation	No. of seeds Sown	No. of plants obtained	No. of infected plants	Per cent of infected plants
1.	Placement inoculums near axil of plants	10	10	8	80.00
		10	10	7	70.00
		10	10	9	90.00
2.	Soil inoculation	10	10	8	80.00
		10	10	7	70.00
		10	10	6	60.00
3.	Control (Uninoculated soil)	10	10	0	0.00
		10	10	0	0.00
		10	10	0	0.00

Conclusion

The pathogen-caused illness symptoms were thoroughly investigated under natural conditions. The pathogen thrived on the dead plants spread over the fields. Infected plants exhibited wilting signs first and then died quickly, frequently without yellowing. Later, when the plant dries up, the leaves turn straw colored. Water-soaked patches may occur on the stem or leaves. Affected tissues develop a slimy, mushy decay from which droplets of brown liquid may appear. Sclerotia germination occurs in both myceliogenic and carpogenic modes, producing mycelia and apothecia, respectively. The pathogenicity of the fungus was tested under artificial inoculation settings, and it showed that chickpea plants were vulnerable to fungus when inoculums were mixed with soil and placed near the plant's axil. It was found to exhibit wilting signs first before dying quickly, generally without yellowing. The fungus was successfully re-isolated from wilted plants and compared to the primary isolate to prove Koch's postulates.

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