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ISOLATION, IDENTIFICATION AND PATHOGENICITY OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID. CAUSING DRY ROOT ROT OF CHICKPEA

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ABSTRACT

Dry root rot of chickpea caused by *Macrophomina phaseolina* (Tassi) Goid., is a serious yield-limiting disease whose incidence has increased in recent years, particularly under rising temperatures and changing climatic conditions. The present study aimed to isolate, identify and confirm the pathogenicity of the associated pathogen. Chickpea plants showing typical dry root rot symptoms were collected from experimental fields at MPKV, Rahuri. The pathogen was isolated on potato dextrose agar and purified through single-sclerotial isolation. Cultural and morphological studies revealed brown to dark black colonies with profuse aerial mycelium and abundant black microsclerotia. Microscopic examination showed septate hyphae and characteristic sclerotial structures. Pathogenicity was established through artificial soil inoculation using the susceptible chickpea cultivar JG-62. Inoculated plants developed typical symptoms, including yellowing, wilting and root rot with sclerotia formation, whereas control plants remained symptom-free. Re-isolation of the pathogen from infected plants fulfilled Koch's postulates, thereby confirming *Macrophomina phaseolina* as the causal agent of dry root rot in chickpea.
***Keywords*:** Chickpea, Dry Root Rot, *Macrophomina phaseolina*,, Microsclerotia, Koch's postulates.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops cultivated worldwide. It is an annual, self-pollinated crop belonging to the family *Leguminosae* and is predominantly grown in semi-arid and arid regions. It serves as a major source of plant protein, carbohydrates, minerals and vitamins (Hirdyani, 2014; Kaur and Prasad, 2021) and also plays an important role in improving soil fertility through biological nitrogen fixation (Merga and Haji, 2019). Despite its economic and nutritional importance, chickpea productivity is severely constrained by several biotic and abiotic stresses. Among the biotic factors, the soil-borne disease dry root rot (DRR) is particularly destructive and poses a significant challenge to sustainable chickpea production.

Dry root rot of chickpea is caused by *Macrophomina phaseolina* (Tassi) Goid., a necrotrophic fungal pathogen with a wide host range, infecting more than 500 plant species and it survives in the soil as microsclerotia or sclerotia for several years (Gupta *et al*., 2012). The disease is particularly severe under conditions of high temperature and moisture stress (Ghosh *et al*., 2013; Soni *et al*., 2022). Dry root rot symptoms in chickpea usually appear during the flowering and pod formation stages (Singh *et al*., 1990) or seed development stage (Trapero-Casas and Jimenez–Diaz, 1985). Affected plants dry up suddenly and appear scattered across the field, showing drooping petioles and leaflets mainly confined to the upper portion of the plant (Haware, 1990). Infected plants often show straw-colored stems and leaves while in some cases the lower leaves and stems turn brown. The taproot becomes black, decayed and loses most of its lateral and fine roots. Dead roots are brittle and show

bark shredding with small dark sclerotia visible on the surface or inside the root tissues (Sharma *et al*., 2015; Khaliq *et al*., 2020). When the stem near the collar region is split vertically, minute sclerotia or sparse mycelial growth can be observed in the pith (Nene *et al.*, 1991). Yield losses due to DRR are reported to range from about 5% under mild infection to 50% under moderate epidemics, and in severe cases up to 80-100% under hot, dry conditions in susceptible genotypes (Ghosh *et al*., 2013).

Macrophomina phaseolina survives in soil and crop residues for prolonged periods in the form of microsclerotia, making its management difficult (Nene *et al.*, 1991). The pathogen shows considerable variability in morphology, growth behaviour and pathogenicity across different agro-ecological regions (Sharma and Pande, 2013). Accurate identification and characterization of the pathogen are therefore essential for understanding its epidemiology and for developing effective disease management strategies, including host resistance breeding and integrated disease management approaches. Isolation and purification of the pathogen from infected plant tissues are fundamental for accurate identification and further pathological investigations. Pathogenicity testing through artificial inoculation is essential to confirm the causal role of the pathogen and the disease, in accordance with Koch's postulates (Agrios, 2005). Although *Macrophomina phaseolina* has been widely reported as the causal agent of dry root rot in chickpea, region-specific studies focusing on its isolation, identification and pathogenic potential have been reported from different chickpea-growing areas. In view of the increasing incidence of dry root rot under changing climatic conditions and its serious threat to chickpea productivity, the present study was undertaken to isolate, purify and identify *Macrophomina phaseolina* associated with dry root rot of chickpea and to assess its pathogenic potential.

Materials and Methods

Collection, isolation and purification of the pathogen

The chickpea plants showing typical dry root rot symptoms were collected from experimental plots of chickpea at MPKV, Rahuri and the samples were brought to the laboratory and washed under running tap water and blot dried. Infected roots were cut into small bits of size 5-6 mm and were surface sterilized by 1:1000 mercuric chloride solution for 2 minutes. After thorough washing repeatedly thrice in sterile distilled water, the pieces of roots were transferred by using forceps on to sterilized potato dextrose agar (PDA) medium in Petri dishes and incubated at 28±1°C to obtain mycelial growth. After 48 hours of incubation, hyphal tips of the growing mycelium were marked on the underside of the Petri dish with a glass marker by viewing through a light microscope (Singh, 1988). The hyphal tips from margins of resulting colonies were cut with the help of sterilized 2 mm cork borer and transferred to Petri dish containing PDA and allowed to grow at 28±1℃ for seven days. The cultures were purified by single sclerotial isolation and transferred to PDA slants. Such plates and slants were stored in refrigerator at 4±1°C and maintained by sub culturing at 20 to 25 days interval and used throughout the study.

Identification of the pathogen

The test pathogen was identified based on symptomatology, cultural and morphological characteristics, microscopic observations and pathogenicity tests following standard descriptions provided by Dhingra and Sinclair (1977) and Short *et al*. (1978). Cultural characteristics such as colony colour, growth pattern and texture were recorded on potato dextrose agar (PDA) medium. For morphological studies, slides were prepared from actively growing cultures and the structures of hyphae, sclerotia, pycnidia and pycnidiospores were examined under a compound microscope at 40× magnification. Photomicrographs of the fungal structures were captured using an imaging microscope to document morphological features.

Mass multiplication of the pathogen

For mass multiplication, sorghum grains were soaked overnight in water, washed thoroughly and half-boiled. The grains were transferred into 250 ml Erlenmeyer conical flasks up to three-fourths capacity and sterilized by autoclaving at 121°C and 15 lbs pressure for 20 minutes. After cooling, the sterilized grains were inoculated with 8–10 mycelial discs (5 mm diameter) of *Macrophomina phaseolina* and incubated at 28±1°C for 7–10 days. The flasks were shaken on alternate days to ensure uniform fungal growth. The prepared inoculum was used for pot culture experiments at the rate of 15–20 g per kg of soil (Pareek, 1992).

Pathogenicity test

Pathogenicity of the isolated fungus was tested to prove Koch's postulates by artificial inoculation under pot culture conditions. Earthen pots were sterilized using formaldehyde and soil was sterilized by autoclaving at 121°C and 15 lbs pressure for 20 minutes. The pathogenicity of *Macrophomina*

phaseolina was tested under pot conditions using the soil inoculation technique. The pathogen was mass multiplied on sorghum grain medium and mixed thoroughly with sterilized soil at the rate of 15–20 g per kg of soil, except in control pots. Ten seeds of the susceptible chickpea variety JG-62 were sown in each pot. The pots were maintained under regular watering conditions and observed daily for disease development. After typical dry root rot symptoms appeared, the pathogen was re-isolated from infected plants and compared with the original culture to confirm its identity.

Results and Discussion

Isolation and purification of *Macrophomina phaseolina*

The pathogen was successfully isolated from chickpea plants showing typical dry root rot symptoms. Initial fungal growth appeared on Potato Dextrose Agar (PDA) medium within 48 hours of incubation. The colonies developed rapidly, producing profuse white aerial mycelium that later turned brown to black. Black, hard sclerotia were formed at the periphery of the colony after 10–15 days of incubation (Figure 1 A). The culture was purified through hyphal tip and single sclerotial isolation and maintained on PDA plates and slants for further studies.

Identification of the pathogen

Identification of the isolated fungus was carried out based on its cultural characteristics and morphological features of hyphae and sclerotia. On PDA medium, the fungus produced colonies ranging from brown to black in colour, gradually turning darker over time. The colonies showed abundant aerial mycelium with microsclerotia embedded within the hyphal mass. On the reverse side of the Petri plates, numerous dark brown to black microsclerotia were observed.

Microscopic examination revealed septate hyphae that were initially hyaline and later turned honey-coloured to dark brown or black. Hyphal branching occurred predominantly at acute angles, although right-angle branching was also observed. The microsclerotia were irregular to spherical in shape, black in colour and varied in size from 50 to 150 µm depending on the culture conditions (Figure 1 B). These cultural and morphological characteristics were consistent with the descriptions of *Macrophomina phaseolina*.

The similar morphological and cultural characteristics of the pathogen were reported by Lakhran *et al*. (2018), who observed hyaline hyphae branching at right angles that later turned light brown to dark brown and eventually black. They also reported that the sclerotia varied in shape, *viz*. irregular, spherical, oval or oblong, and that pycnidia were formed on the host surface, which were larger than sclerotia, dark brown to black in colour, rough, globose or irregular, beaked and ostiolate. Similar morphological and cultural characteristics of *Macrophomina phaseolina* have also been reported by Gadekar *et al*. (2018), Dhakar *et al*. (2019) and Gaikwad *et al*. (2020).

Pathogenicity test

In artificially inoculated pots, typical symptoms of dry root rot appeared 35–40 days after inoculation in the susceptible chickpea variety JG-62 (Figure 1 C). Infected plants exhibited yellowing, wilting and sudden drying, along with root rot symptoms characterized by brown to blackish lesions, formation of microsclerotia, decayed taproots and loss of lateral roots. No disease symptoms were observed in the uninoculated control plants. The pathogen re-isolated from infected plants showed cultural and morphological characteristics identical to the original isolate, thereby fulfilling Koch's postulates and confirming *Macrophomina phaseolina* as the causal agent of dry root rot in chickpea.

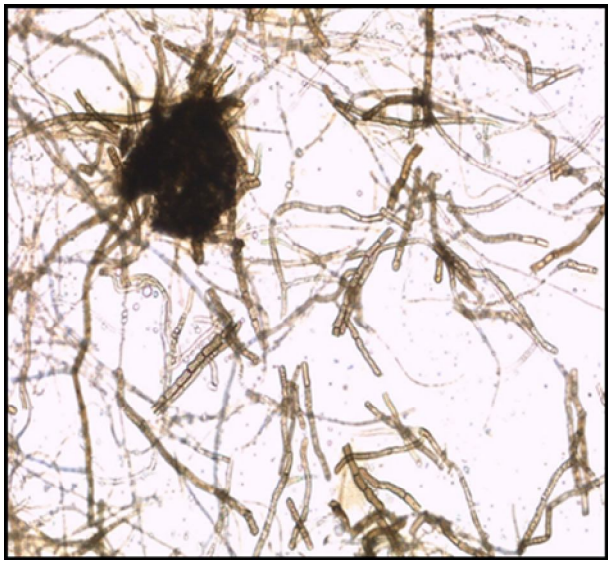
Similar findings were reported by Date *et al*. (2017), who proved the pathogenicity of *Macrophomina phaseolina* using the sick soil inoculation technique in earthen pots under glasshouse conditions with the susceptible genotype JG-62. The incidence of dry root rot was recorded 40 days after sowing. The pathogen was re-isolated from the roots of artificially inoculated diseased plants showing typical dry root rot symptoms. The fungal growth obtained was transferred onto PDA slants and compared with the original culture of *M. phaseolina*. Comparable results were also reported by Lakhran *et al*. (2018), Assfaw *et al*. (2019) and Gaikwad *et al*. (2020), who confirmed the pathogenicity of *Macrophomina phaseolina* through artificial inoculation and re-isolation of the pathogen.

Conclusion

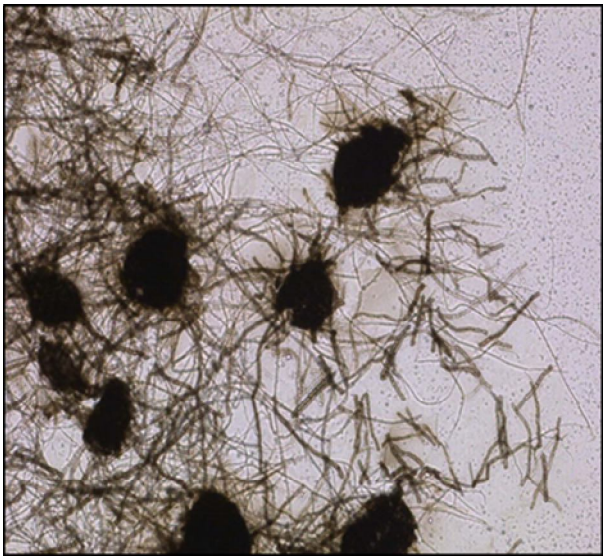
The present study confirmed *Macrophomina phaseolina* as the causal agent of dry root rot in chickpea through isolation, morphological characterization and pathogenicity testing. The pathogen showed characteristic cultural and morphological features on PDA medium, including septate hyphae and abundant microsclerotia. Pathogenicity testing through artificial inoculation on the susceptible chickpea variety JG-62 reproduced typical disease symptoms and re-isolation of the

pathogen confirmed Koch's postulates. These findings provide a reliable basis for further studies on disease management and resistance screening in chickpea.

A. Pure culture of *Macrophomina phaseolina*



B. Fungal mycelium and sclerotia of *Macrophomina phaseolina*



B. Sclerotial formation in *Macrophomina phaseolina*

C. Pathogenicity of *Macrophomina phaseolina* on chickpea variety JG-62
A = Healthy plant B = Infected plant

Fig. 1 : Isolation, identification and pathogenicity of *Macrophomina phaseolina*

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