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Research Article

Isolation and Pathogenicity of *Macrophomina phaseolina* Causing Dry Root Rot of Chickpea

Manjari[1]*, Naresh M Gohel[1] and Raghunandan BL[2]

[1]*Department of Plant Pathology, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India*

[2]*AICRP on Biological Control of Crop Pests, ICAR Unit-9, Anand Agricultural University, Anand, Gujarat, India*

***Corresponding Author:** Manjari, Department of Plant Pathology, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India.

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Abstract

Dry root rot in chickpeas, attributed to "*Macrophomina phaseolina*", is a significant factor that disrupts yields and results in substantial annual losses. Lately, there has been an increasing prevalence in agricultural regions affected by rising temperatures due to climate change. Samples showing typical dry root rot symptoms were collected from infected chickpea fields of Anand Agricultural University, Anand, Gujarat, India. Fungal colonies grown on Potato Dextrose Agar were examined morphologically and microscopically to identify the pathogen. Pathogenicity of the pathogen *Macrophomina phaseolina* was confirmed through Koch's postulates using a soil inoculation method. Inoculated plants showing symptoms of dry root rot were collected to re isolate the pathogen. The isolated pathogen was compared to the pure culture of previous isolate to confirm the identity of the pathogen. The results confirmed the identity of *M. phaseolina* as the causal agent of dry root rot of chickpea.

Keywords: *Macrophomina phaseolina*; Dry Root Rot; Chickpea; Pathogenicity

Introduction

Macrophomina phaseolina is a soil-borne phytopathogenic fungus having a wide host range of about 500 cultivated and wild plant species worldwide [1]. Important diseases caused by *M. phaseolina* include collar rot, damping off, charcoal rot, stem rot, root rot and seedling blight in economically important crops [2]. The plants affected with fungus exhibit necrotic lesions on different parts such as branches, peduncles and stems. A higher temperature and low moisture favour disease development [3]. The microsclerotia of the pathogen can survive on infected plant debris and soil for a long period, i.e., 2-15 years depending on the environmental conditions [4]. Microsclerotia are usually spherical, black and oblong. However, there is a great variation in their shape and size depending on the substrate, isolates and temperature [1]. *M. phaseolina* affects the plant by secreting an array of cell wall degrading enzymes which depolymerize the cell walls components such as cellulose, xylan, pectin, polygalacturonic acid and other proteins [5]. The most significant enzymes secreted by *M. phaseolina* are pectinases, xylanases, cellulases and proteases [6].

Cultural strategies such as crop rotation have been insufficient in controlling *M. phaseolina*. The use of chemical fungicides has been effective and a common practice in controlling *M. phaseolina,* but the rise of a resistant population has been reported throughout. Furthermore, there is an increasing concern over toxicity hazards from accidental exposures to the users, non-target organisms and potential environmental contaminations. Thus, there has been an increasing demand for chemical-free food production and the organic production system has become the fastest-growing sector of crop production.

Taking the aforementioned points into account, a research was conducted on chickpea plants infected with dry root rot to study their infection patterns and develop effective management practices for this disease.

The aim of the study was to investigate the infection patterns of dry root rot in chickpea plants and to devise efficient management strategies for this disease, considering the growing demand for organic food production and the rapid expansion of the organic agriculture sector.

Materials and Methods

Collection of diseased samples

The diseased roots of chickpea exhibiting the typical symptoms of dry root rot were collected from Agronomy farm, Anand Agricultural University, Anand during Rabi 2022-23; placed in brown paper bags and brought to the laboratory for microscopic examination and isolation purposes.

Symptomatology

Visual and microscopic examination of typical dry root rot samples was carried out to confirm the presence of the pathogen. The typical symptoms of dry root rot on roots, shoots and leaves of chickpea under field conditions were visually observed and meticulously documented (Figure 1).

Figure 1: Symptoms of *M. phaseolina* on Chickpea.

Isolation and purification of the pathogen

The pathogen was isolated from root tissues of chickpea displaying characteristic symptoms of dry root rot symptoms. A standard tissue isolation procedure was followed for the isolation of the pathogen [7].

Morphological characters of the causal organism

As shown in Figure (2) identification of pathogen-causing dry root rot of chickpea grown on PDA medium was examined visually as well as microscopically for cultural and morphological characteristics. The cultural characteristics including colony colour, colony topography, colony margin and colony diameter were recorded from the initiation of growth up to 10 days. The photomicrographs of these characteristics were also taken.

Figure 2: Morphological identification of *M. phaseolina*.

Pathogenicity test

As illustrated in Figure (3) the pathogenicity of *M. phaseolina* was demonstrated through artificial inoculation of the pathogen following standard methods of inoculation (Koch's postulates). Prior to the experiment, the soil and earthen pots underwent sterilization as previously described.

The sick pot technique developed by Nene., *et al.* (1981) [8] was employed to confirm the pathogenicity. Autoclaved soil was filled in 30 cm earthen pots at a rate of (2.5 kg/ pot) and inoculated with the pathogen (*M. phaseolina*) multiplied on 100 g of sand maize meal medium at the ratio of (9:1) in 250 ml conical flasks for 15 days inoculated at 25 ± 1 °C. The fungus grown on sand maize meal medium in flasks was added at a rate of 100 g/2 kg of soil. Control pots without inoculums was also maintained. Following a week of colonization in soil, seeds were planted in the pots. Rotted seedlings were examined post germination.

The fungus was subsequently re-isolated from the seedlings exhibiting rotting symptoms in these artificially inoculated pots using the tissue isolation method. The cultures obtained by re-isolation were transferred on PDA plates for comparison with the original culture and further investigations.

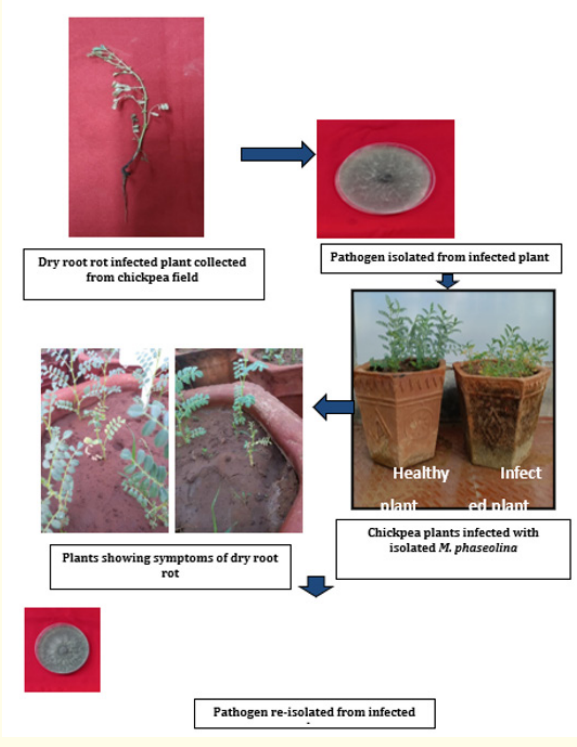

Figure 3: Pathogenicity of *M. phaseolina* on chickpea.

Results and Discussion

The examination of typical dry root rot (DRR) samples visually confirmed the presence of the pathogen. The disease starts to appear after 35-40 days of sowing. It is favoured by high temperatures and moisture stress conditions. Initial symptoms include scattered necrotic lesions on the roots, followed by rotting and withering of the lateral roots, accompanied by prematurely dried, straw-coloured leaves. The foliar symptoms begin with gradual yellowing from the base to the upper leaf and shoots develop a brownish colour. Diseased chickpea plants in the field show premature leaf yellowing and drying. Infected plants die prematurely due to progressive infection in roots, plant development stage and low soil moisture.

Similar observations were also observed by Sharma., *et al.* (2015) [9], Rai., *et al.* (2022) [10] and Mirchandani., *et al.* (2023) [11]. They observed that the dry root rot (DRR) symptoms are most commonly observed in chickpeas during the post-flowering stage which include drooping and chlorosis of petioles and leaflets. The leaves and stems of the affected plants are usually straw coloured and in some cases the lower leaves and stems are brown. The tap root becomes black due to decay and is devoid of the majority of lateral and finer roots.

Infected plants were examined for the isolation of pathogen using the standard agar plate method. Infected roots showing typical symptoms of DRR along with some healthy portions were aseptically transferred to sterilized PDA plates and incubated at room temperature. Fungal colonies emerging from the plates were transferred to fresh PDA plates using the hyphal tip method. In all investigations, the pathogen was preserved in a pure state of culture by periodically transferring it to PDA plates. The fungus colony on PDA expanded quite quickly, reaching a diameter of 90 mm at a temperature of 30 ± 1°C in just seven days.

The mycelial growth was fluffy, linear and coloured from dirty white to light grey at first. However, it eventually turned blackish grey and had brown to black microsclerotia. The microscopic examinations demonstrated that *M. phaseolina* hyphae were aerial, hyaline, light brown or dark brown, with thin walls and septa produced close to the branching and the hyphae branching were at a right angle with a constriction of hyphal branches at their origin. The mycelium's cells had a barrel form. The process of sclerotial development involved the aggregation of 50–200 individual hyphal cells, resulting in multicellular entities known as "microsclerotia". The sclerotia varied in size and shape and ranged in colour from dark brown to black. Based on cultural and morphological characters, the pathogen was primarily identified as *Macrophomina phaseolina* (Tassi) Goid.

The pathogenicity of *M. phaseolina* was proved by artificial inoculation of the pathogen following standard methods of inocula-

tion (Koch's postulates). The disease was successfully produced in the sick pots inoculated with *M. phaseolina* 35 days after sowing whereas in control pots no disease was encountered. Symptoms in inoculated pots included rotting of primary roots followed by withering of lateral roots. Due to blockage in primary roots, the above-ground parts were devoid of water and minerals resulting in chlorosis of leaves and premature death of the plants. On the other hand, uninoculated plants showed no symptoms and remained healthy.

Infected plants were collected from artificially inoculated chickpea plants showing typical symptoms of DRR and reisolated separately on a PDA medium. The reisolation led to a pure culture that was similar to the originally isolated *M. phaseolina*. The pathogenicity of *M. phaseolina* has therefore been confirmed by artificial inoculation methods in accordance with Koch's postulates.

Conclusions

M. phaseolina is a soil-borne phytopathogenic fungus that infects around 500 species of cultivated and wild plants globally. It damages plants by releasing various cell wall degrading enzymes, which break down components like cellulose, xylan, pectin, polygalacturonic acid, and other proteins. In chickpea, it causes a serious disease called dry root rot (DRR) which can cause up to 100% yield loss. The disease is favoured by drought and heat stress. Other environmental conditions that can influence DRR include soil pH, soil type, and high temperatures. *M. phaseolina* infected chickpea plants showed necrotic lesions on the roots, followed by rotting and withering of the lateral roots, accompanied by prematurely dried and straw colored leaves. The pathogen was isolated from infected chickpea plants and were observed morphologically and microscopically to confirm it's identity. Koch's postulates were used to further confirm the association of *M. phaseolina* with chickpea plants.

As a result, the meticulous identification and confirmation of *M. phaseolina* as the causal agent of dry root rot in chickpea plants mark a significant milestone in agricultural research. These findings underscore the urgency of implementing targeted strategies to combat this destructive pathogen, safeguarding crop yields and ensuring food security. Moving forward, further exploration into innovative disease management approaches and the development of resistant crop varieties will be paramount in combating the challenges posed by *M. phaseolina* and preserving the health and vitality of agricultural ecosystems.

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